MOLECULAR CHARACTERIZATION OF OLIVE CULTIVARS IN IRAQ USING AFLP MARKERS

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Abstract

Amplified fragment length polymorphism (AFLP) analysis was used to study the genotype relation among ten different olives varieties from al- zafrania and al-mosel station ministry of agriculture/Iraq Shami, Sorani, Manzanilla ,Kaissy, Arbequine ,Jlot, Baaskika ,Dahkan, Nabali, Khodeir , Nine primers combinations were used which produced about 250 amplified fragment. . One hundred and forty fife of these loci (59.8%) were polymorphic over all the genotypes tested. Dendrogram and matrix of similarity were obtained by the Unweighted Pair-GroupMethod analysis (UPGMA).study showed four group 1 : Jlot , Dahkan , Kiassy, Basskika 2 : Manzanilla, Khodeiri, Sorani, 3 : Nabali, Arbequine 4 : Shami . AFLP has a powerful tool for detecting genetic relationship among cultivars , and help in known the pedigree of relatives and ancestors .

Key word : Olive , PCR , AFLP , Molecular Markers ,

التوصيف الجزيئي لبعض أصناف الزيتون المزروعة في العراق باستعمال المؤشرات الوراثية AFLP

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الخلاصة

استعملت تقنية AFLP لدراسة علاقة النمط الوراثي بين عشرة أصناف مختلفة من الزيتون من محطتي الزعفرانية والموصل التابعة لوزارة الزراعة العراقية وهي : شامي ،صوراني ، منزانيلا ، قيسي اربكوين ، لبيب ، بعشيقي ، دهكان ، نيبالي ، خضيري . كما استعملت تسعة توليفات من البادئات أنتجت ٢٥٠ حزمة ١٤٥ منها متعددة الأشكال وهذا التعدد يمثل ٥٩.٨% من جميع الأصناف المدروسة . اظهر المخطط البياني للتشابه بين الأصناف والمعتمد على تحليل (UPGMA) اربعة مجاميع شملت الأولى الأصناف : لبيب ، دهكان ،قيسي ، بعشيقي والثانية :الأصناف منزانيلا ،خضيري ، صوراني والثالثة : نيبالي ، اربكوين والرابعة : صنف الشامي . خلصت الدراسة إلى ان تقنية AFLP مفيدة جدا في تحديد العلاقات الوراثية بين الأصناف المتقاربة ومعرفة أصولها .

Introduction

Olive *(Olea europaea* L.) is one of the most ancient cultivated fruit tree species in the Mediterranean basin. Olive cultivars are quite diverse both in external and internal fruit characteristics such as size, shape, color, texture, oil ratio, oil composition, etc., Plant characteristics are also very diverse, ranging from shrubs to large trees, extending to upright, and having small to large leaves [1] , It is a predominantly allogamous species showing a high degree of outcrossing which leads to considerable levels of heterozygosity and DNA polymorphism among individuals [2]. , most morphological traits are influenced by environmental factors, plant age and phenology [3]. but DNA-based markers are not influenced by environmental conditions, and they allow direct scanning of the plant genome [4,5].knowledge of the genetic relationships between wild olives and their cultivated relatives is necessary to improve genetic resources and our understanding of their evolutionary background [6,7]. Presently, DNA-based markers very useful tool for plant scientists in establishing phylogenies determining similarities among cultivar [8]. give us a chance to make direct comparison of the organisms at the molecular level, as the use of DNA-based molecular markers has become popular in plant breeding as well as olives along with the other agriculturally important plants [9]. Genetic polymorphism of the plants can be detected by many different DNA-based marker methods such as , AFLPs [10,11], The AFLP technique is based on the detection of genomic restriction fragments by PCR amplification, the advantages of use this technique that generate reproducible fingerprinting profiles and allows the amplification of a high number of DNA fragments per reaction and enabling the detection of specific amplified fragments and it doesn't require previous knowledge of the DNA sequence [12] The aim of of this study is to determine genetic relationships between olive cultivars natively grown in Iraq.

Material and method

Plant material: Healthy leafs of olive tree were collected from ten local cultivars, these cultivars were be in al- zafrania and al-mosel station ministry of agriculture / Iraq as in table(1).

DNA extraction: Genomic DNA was extracted from young leaves by CTAB according to[13]. The determination of DNA quality and concentration in samples was performed by both spectrophotometric analysis and running on 0.8% agarose gels. Optical density ratios from spectrophotometric analysis were evaluated and only good-quality DNA samples were used in PCR [14].

AFLP procedure:The AFLP procedure was performed as in [12].

DNA Digest : Olive DNA (250ng) was double digested with *PstI* and *Tru9I*, by used: 10 unit of each restriction enzyme and 1 X reaction buffer and up to 10 µl with de-ionized distal water (D.D.W.) per reaction, incubate in thermal cycler machine on 37Cº for 3 hours , then electrophoresis on 1% agarose to check the digestion [15].

Adapter ligation : double standard adaptors were ligated to the ends of DNA fragments, to generating DNA template to the amplification (pre-amplification). ligation reactions were carried out by adding 50 pmol of each forward GACGATGAGTCCTGAG and reverse TACTCAGGACTCAT adapters of *PstI* and 50 pmol of each forward CTCGTAGACTGCGT and CATCTGACGCATGT reverse of *Tru9I* adapter ,10mM ATP,1 X reaction buffer 0.3 Unit of T4-DNA Ligase and up to 2.5 µl D.D.W. then adding 5 µl of digested DNA and incubating in thermal cycler at 37 ºC overnight [12].

Pre amplification: The ligation reactions were diluted 1: 5 with D.D.W, then used in pre amplification step .The mix was prepared by adding 50 pmol of each primers *P00*-GACTGCGTACATGCAG and *T00*-GATGAGTCCTGAGTA, 1x PCR buffer with $MgCl_2$, 0.2mM dNTPs ,1 unit DNA polymerase and 3 µl diluted DNA from the restriction-ligation reaction, the amplification was carried

Table 1: olive varieties, origin, distribution, morphological characterization that used in this study

Code	Cultivar Name	Origin	Distrib-Ution	Leaf shape	Fruit shape	Seed shape	% Oil cont.	Productivity	Salt tolerance	Pest resistance		
										Verticilliu m Wilt	Mite	Insects
1	Khodeiri	Syrian	S,J,I	Spear	Oval	Sharp end with spine	26-28	High	Untolerant	Unknown	Unknown	Susceptible
2	Kaissy	Syrian	S,J,I	Spear	Spherical	Oval with spine	18-21	High	Tolerant	Unknown	Unknown	Susceptible
3	Manzanilla	Spain	I,E,S,J, AS, AM,S	Spear to tapered ends	Oval to Spherical	Oval smooth	16-20	High	Tolerant	Susceptible	Unknown	Susceptible
4	Baaskika	Local	North and middle of Iraq	Spear	معقوفoval with end	Elongated with spine	19-21	high	Tolerant	Resistance	Susceptible	Susceptible
5	Arbequine	Spain	S,AS,S, I	Spear	Spherical	Small spherical smooth	17-22	high	Tolerant	Resistance	Susceptible	Unknown
6	Nabali	Jordanian	P,J,I,S	Spear	Oval	Oval to sharp ends	17-28	Mid	Tolerant	Resistance	Susceptible	Susceptible
7	Jlot	Syrian	S,I,J,P	Spear	Elongated oval	Elongated with spine	10-12	High	Tolerant	Susceptible	Susceptible	Susceptible
8	Dahkan	Local	North of Iraq	Spear to tapered ends	large oval elongated معقوفة	Elongate with spin معقوفة قليلا	19-22	high	Tolerant	Resistance	Unknown	Susceptible
9	Sorani	Syrian	S,I,J,P, L	Oval	Oval	Elongated	26-28	High	Tolerant	Susceptible	Unknown	Resistance
10	Shami	Syrian	S,J,P,E, I	Spear	Spherical	Oval with spine	17-20	High	Tolerant	Unknown	Unknown	Susceptible

S =Syria, I= Iraq J= Jordan , P=Palastain L=Lebanon E =Egypt ,AM= America ,AS=Australia ,S=Spain

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out in a thermal cycler programmed at 94ºC for 2min followed by 30 cycles of 94ºC for 30s, 56ºC for 30s and 72ºC for 60s and finally incubated at 4ºC. [16]

Selective amplification : The products of pre amplification was diluted 1:5 with D.D.W. and selective amplifications were carried out by using nine florescent labeled primers as in table 2. below and 50 ng of each were taken,1 x PCR buffer with $MgCl_2$, 0.25mM of dNTPs , 1 unit of DNA polymerase, and carried out in a thermal cycler programmed at, 94ºC for 30s , 65ºC for 30s and 72ºC for 1min one cycle , followed by 11 cycles over which the annealing temperature is decreased by 0.7 ºC per cycle and 94ºC for 30s , 56ºC for 30s and 72ºC 1min for 23cycles then hold it at 4ºC.[15]

Table 2: The primer sequence used in the selective amplification of olive genomic DNA.

1	F-5´GACTGCGTACATGCAGCCT-3´ R-3´GATGAGTCCTGAGTAGA-5´
2	F-5´GACTGCGTACATGCAGCCT-3´ R-3´GATGAGTCCTGAGTAACT-5´
3	F-5´GACTGCGTACATGCAGCCT-3´ R-3´GATGAGTCCTGAGTAAGC-5´
4	F-5´GACTGCGTACATGCAGCCT-3´ R-3´GATGAGTCCTGAGTAAGG-5´
5	F –5´GACTGCGTACATGCAGCGA-3´ R-3´GATGAGTCCTGAGTAGA-5´
6	F-5´GACTGCGTACATGCAGCGA-3´ R-3´GATGAGTCCTGAGTAACT-5´
7	F-5´GACTGCGTACATGCAGCGA-3´ R-3´GATGAGTCCTGAGTAAGC-5´
8	F-5´GACTGCGTACATGCAGCGA-3´ R-3´GATGAGTCCTGAGTAAGG-5´
9	F-5´GACTGCGTACATGCAGCGA-3´ R-3´GATGAGTCCTGAGTAACA-5´

Capillary Electrophoresis:

The fluorescent Amplified products were detected by ABI prism 3100 Genetic Analyser. Samples were prepared by adding 3 µl diluted PCR products to 6.875 µl formamide and 0.125 µl GenSize HD Rox 500. Analyses were performed using the GeneScan 3.1 and Genotyper 2.5 softwares (Applied Biosystems) [17].

Data analysis:

AFLP polymorphic bands were scored as present or absent by (1,0) respectively an estimates of similarity among all varieties were calculated as in [18] definition of similarity Sij= 2a/ 2a+b+c, where Sij is the similarity between two varieties (individuals) i and j, a is the number of bands present in both individuals, b is the number of bands present in j and absent in i, while c is the number of bands present in j and absent in i. The matrix of similarity was analyzed by the Unweighted Pair-Group Method (UPGMA) and the dendrogram was obtained using Past Program [15].

Results and discussion

DNA samples were digested, ligation, and pre amplification as in figure 1, AFLP analysis of 10 cultivated olive accessions using nine combination pairs of primers provided a total of 250 markers as in figure 2. One hundred and forty five of these loci (59.8%) were polymorphic over all the genotypes tested. The highest number of polymorphic bands over all varieties detected by individual primer pair was 26 (for primer combination No. 5) and the lowest was 9 (for primer combination No. 6).

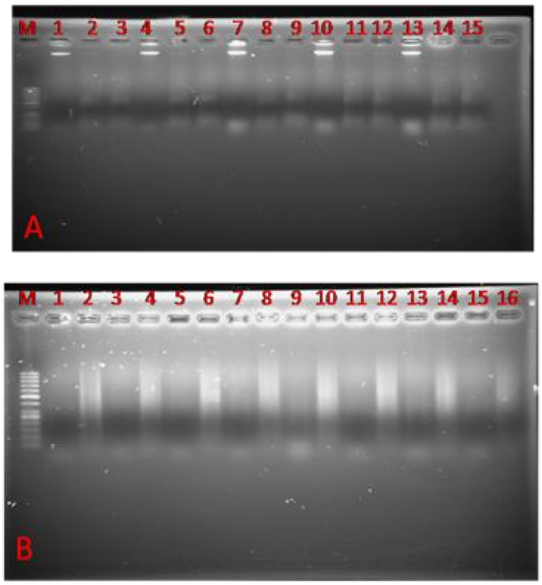

Figure 1: A :lane 1,4,7,10,13= DNA control ,lane 2,3, 5,6,8,9,11,12,14,15=digested DNA, M = marker , B lane 1,3,5,7,9,11,13,15=ligation digested DNA with adapter, lane 2,4,6,8,10,12,14,16 = pre amplification products

this difference between primers bands productivity because of the differed of the primer combinations in their ability to (number of match) matching with compatible sequence in all over genome and detection of the polymorphism of the populations as the result [19]. These results demonstrated a high degree

Table 4: Genetic distances among 10 Olive varieties in Iraq estimated by AFLP analysis using nine primers

	Khdhier	Qaysi	Manzenllo	Baashiqi	Arbqween	Nepali	Labeeb	Dahkan	Sorani	Shami
Khdhier	0.000									
Qaysi	0.527	0.000								
Manzenllo	0.311	0.544	0.000							
Baashiqi	0.592	0.271	0.634	0.000						
Arbqween	0.733	0.761	0.787	0.798	0.000					
Nepali	0.620	0.621	0.682	0.652	0.644	0.000				
Labeeb	0.654	0.613	0.667	0.675	0.702	0.662	0.000			
Dahkan	0.575	0.477	0.671	0.460	0.776	0.735	0.522	0.000		
Sorani	0.357	0.625	0.369	0.650	0.722	0.671	0.659	0.636	0.000	
Shami	0.794	0.857	0.813	0.817	0.781	0.716	0.769	0.849	0.808	0.000

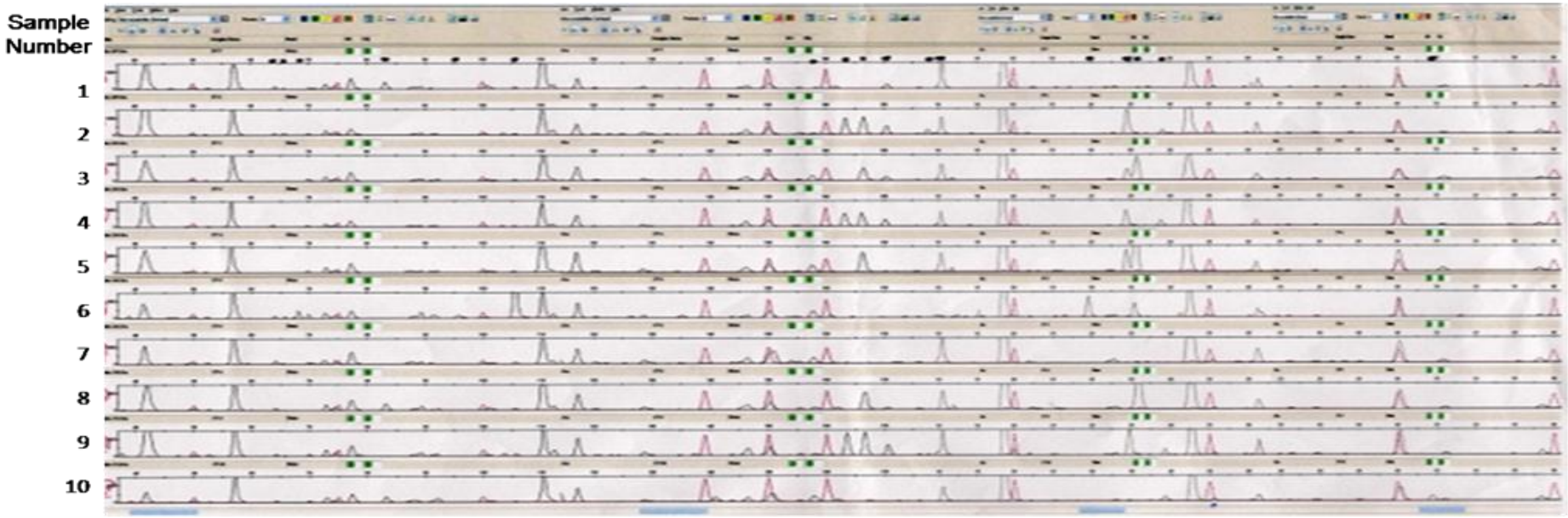

Figure 2: showed the detection of selective amplification by ABI prism 3100 Genetic Analyzer for ten olive variety to primer combination 6 . red pike : marker ,blue pike : amplification product ,when pike appear in all variety in the same size that is mean monomorphic and when appear in some variety and disappear in another in the same size that is mean polymorphic

of polymorphism in the olive germplasm with an average of 58% as in table 3.

Table 3: Monomorphic and polymorphic percentage for olives varieties

Primer No.	Total product	Monomorphic	Percentage %	Polymorphic	Percentage %
1	37	18	49	19	51
2	25	15	60	10	40
3	33	17	52	16	48
4	25	9	36	16	64
5	36	10	28	26	72
6	18	9	50	9	50
7	30	12	40	18	60
8	24	6	25	18	75
9	22	9	41	13	59
Total average	250	105	42	145	58

The dendrogram derived from an UPGMA cluster analysis of the AFLP markers are explain Genetic distances in table 4 and Figure 3. that shown four main distinct groups were observed in the dendrogram. Group 1 consisted of cultivar labib, dehkan, qaissy, bashike Group 2 : minzula, kudire,sorani, Group 3 : nibali, arbeqwen Group 4 : shami ;The cultivar in group 1 was also found in closed area (Syria and north
of Iraq) and they have phenotypic similarity among them ,like fruit weight , fruit shape, leaf shape, salt tolerant , yeild , resistance to verticillium wilt , drought and oil ratio [20,21,22] **.** either group 2 was found in closed area (Syria) except sorani was Spain and as in [23] noted that the similarity and differences between varieties of olives are not related with their geographical origin. High similarity had been noted among varieties of same group, this is due to the belonging to same origin while the potential possibility of the variation are the hybridization (programmed or natural) and the environmental effect [24] .

The AFLP marker system showed ability to separate closely related olive (Olea europaea L.) accessions, and powerful tool to study the related varieties that have the same morphological shape especially when environmental factors affect on morphologic features of cultivated plants[19]. Therefore, it is difficult to determine varieties based on the phenotype because of the association of environmental conditions [25].

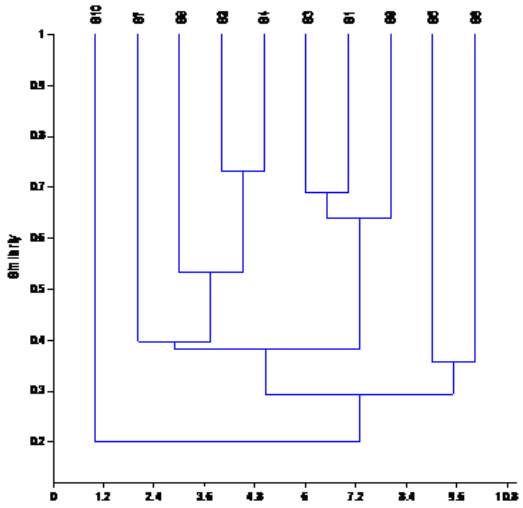

Figure 3: Genetic distance dendrogram produced by AFLP technique using nine primers (S1: Khodeiri ,S2: Kaissy ,S3: Manzanilla ,S4: Baaskika ,S5: Arbequine ,S6: Nabali ,S7: Jlot ,S8: Dahkan ,S9: Sorani ,S10: Shami)

Therefore we used AFLP-PCR to explain the genotype of olive variety that cultivated in Iraq based on genetic continent . DNA based markers are not affected by environmental conditions and it allows to directly determining the plant genotype. It is very important to define variety-specific genetic structure; to determine genetic distances and similarities between it and preserve genetic structures of local types peculiar to regions and use the highly polymorphism ratio in breeding studies in the future.

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