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ANALYSIS OF GENETIC DIVERSITY OF SOME OLIVEGENOTYPES USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS

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ABSTRACT

This study aimed to use the Random Amplified Polymorphic DNA (RAPD) markers based on the PCR technique in the analysis of genetic variation among seven olive grown cultivar *Olea europaea* L. cultivars in Saladin province in Iraq Eighteen primers were used in RAPD reactions ,Results of RAPD experiments showed different loci of the bands. The total number of loci defined by the primer was 99 loci, of which 18 were general sites for all samples and 81 different locations. (OPP-01, OPM-15, OPK-13,1700bp) highest, and the lowest partial size in the primers [100 bpOPH-14, OPG-05] The variance between the samples studied was highest, as OPZ-08 (9.0), and less than in OPM-03 (2.1) The ability to discriminate for the primers was characterized by the OPZ-08 primer with a maximum discriminating higher of 9.0. The OPM-03 primer was characterized by the least discriminating of 2.1A total of 429 total bands bundles were produced, of which 18 were general main bands and 411 were polymorphic bands, The total number of distinctive bands resulting from in this study was 49. 21 absent band and(28) Unique bands. The sample Santacatrina 5 received the highest number of missing bands, reaching (16 bands) Shamlaily is a sample 2 with the highest number of unique bands 7. The genetic distance values ranged from (0.769-0.089), the lowest value of the genetic distance (0.089) and the difference between the four types (Frantoryo and 3 photo), and the highest genetic distance was between 5 (Santacaterina and 6) 0.769.While the analysis of the genetic relationship revealed that there are three main groups, including the first sample (5) Santacaterina, the second group including subgroups B1, B2 included the first B1 sample only one sample Krodsoo, while the second group B2 has included the remaining items Shamali, Sorani, Frantoyo Duhkan, Qaisi.

Introduction

Olive *Olea europaea* L. belongs to the Oleaceae family known since ancient times for the fame of olive oil, leaves, and oil used in food for the prevention of many diseases, which do not combine with any other types of olives produce fruits of nutritional value and economic, and is the only type that belongs to this family where it gives 30 genera and 600 species, including olives[1]. The cultivation of olives spread in warm temperate regions of the world and the Mediterranean region is home to the original [2]. The area of cultivated land with olive trees in the world reaches 10,800,000 hectares, in thes arab States The area cultivated with olives (66,44233 ha) [3]. In Iraq there are about 10985 thousand trees, annual production reaches 25 thousand tonsand average productivity 228 kg per tree[4]. The olive plant is the Mediterranean region, because the top ten olive producing countries are located on the coasts of the Mediterranean Sea, and they grow widely, especially in the eastern parts of the Mediterranean basin, Lebanon and the marine parts of Asia Minor and northern Iran and the southern end of the Caspian Sea , Where the Mediterranean beaches of Syria and Palestine are

home to the establishment of the olive tree and spread to other countries of the world[5].*Olea europaea* L. is a medicinal plant that has been an important and successful method of treatment for wise men and women because it has a large role in human life and its uses are increasing and the demand for it is increasing worldwide, due to the increase in the side effects of medicine And is the main source for the production of medicinal medicinal drugs and as a source of active substances used in the preparation of many pharmaceuticals[6].The olive tree (*Olea europaea* L.) was known in the Koran seven times and was mentioned in the Noble Prophetic Sunnah, as adopted by Arab doctors in the treatment of diseases, Ibn Sina [7]. The diversity of global interest in this plant from the preservation of old varieties and the improvement of their production to serious attempts to find new varieties of them, which requires the finding of modern means to identify those and distinguished precisely to preserve its identity and of special components as well as the rights of States and researchers wishing to develop it adopted the latest technology for Analysis of genetic content through the identification of the genetic DNA of their varieties and determine the genetic dimension and genetic relationship among them, using several markers of DNA distance, including Random Randomization polymorphic (RAPD).This marker is widely used. It analyzes the diversity of genome using a group of random, primers consisting of ten base pairs under conditions that allow these initiators to bind [8]. This marker has been used increasingly to distinguish between different species of olives [9] .

Mareials and methods

Total Genomic DNA Extraction [10] .

Preparation of RAPD-PCR reactions: Randomized was amplification uniformed at polymorphic DNA (RAPD-PCR), according to.[8], were performed using the AccruePower PCR premix kit prepared by the Korean company Pioneer and according to the accompany instructions. Each tube contains the basic components of the polymerase chain reaction, which include one unit of Taq DNA polymerase, 250 µM of dNTPs, 10mM Tris-HCl (pH 9), 30mM KCL and 1.5 MgCl2mM. Add Ten Picomole of the primers and 25 ng of DNA and then complete the reaction with distilled water to 20 µL per tube.Mix the tubes well and transfer them to the thermocycler the program was programmed: one cycle for 2 minutes at 94 m for the initial Denaturation of the DNA strip followed by 40 cycles. Each cycle includes one minute at 92 ° C for Denaturing 1 minute on 36 ° C annealing one minute at 72 ° C for extantion with a final cycle of 7 minutes and at 72 °. The amplification products were separated on a 1.2% agarose gel with DNA ladder. The gel was examined after dyeing with the ethedum bromide for 30-45 under UV UV-Light and images using the Gel Documentation System [11].

RAPD results and statistical analysis: To record the random results of the RAPD technique, the images of the gel eletrophoresis patterns for each primer and the recording of bands for each primer in a table were examined to be treated as a binary feature, which represents the existence of the Band [1] and the band (zero) Calculate the total number of RAPD packets shown by each primer in the studied olive genotypes, determine the polymorphic and monomorphic scales and calculate the degree of variation in the RAPD products. The genetic distances coefficient and the similarity coefficient were calculated among the studied samples using Nei's 72 [12], then the cluster analysis The genetic distances plot was plotted among the studied samples using the Un weighted pair group method for the arithmetic average UPGMA[13]. All statistical analyzes were performed using the Numerical Taxonomy and Multivariate Analysis System [14].

Results and discussion

Diagnosis of DNA classifications using RAPD marker means finding fingerprint for each species. The multiplication of the studied cultivars is measured using a specific prefix, i.e., the number and molecular size of those species that are distinctive to that variety without the rest of the varieties. It is the identity of that category that can be used [15]. The method of analysis of the results of the study of the genetic relationship was based on the presence or absence of bands resulting from the multiplication of certain parts of the genome of the plants used and on the molecular weights of those bands that depend on the number and locations of the sequence of prefixes on the DNA template and the very light packets were neglected.

This is consistent with[16]. Variations based on differences in Intensity highlight the beams that are usually caused by the appearance of some beams multiplied together in the same molecular weight and appear in the form of one thick band is actually more than a bands (Co migrating bands) may be the result of homozygotsity Where the same location is multiplied by the other allele, and since it is the same molecular weight, the multiple pieces are combined in these sites together, sometimes increasing the concentration of the DNA template leads to the repetition of the number of DNA copies of the target, which doubles the same site more than once and since the RAPD Is one of the marker that follow full sovereignty. Therefore, the difference in the thickness of the resulting bands cannot be used as a measure of genetic variation, so it is not possible to estimate the number of alleles for a single site [15]. This is consistent with what.,[17]Results of the RAPD marker for 18 primer were significantly different in the number of amplified DNA bands and markedly different in their molecular size depending on the principle of RAPD marker have been able to find unique DNA bands that are able to differentiate among studied olive varieties, ie, these bands were found in a particular species and were absent in other species and can be used as a distinctive genetic

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imprint [18]. The total number of sites identified by the primers was 99 loci, of which 18 were general loci for all samples and 81 different locations. Due to several reasons: [1] the result of insertion of a large piece of DNA among the marker link loci, which exceeds the PCR's potential, resulting in a loss of cutting. [3] Delete one of my locus primer anneaing results in either the loss of a primer, or an increase in the size of the piece multiplied.[5]. Replacing nucleotide in one or both of the initiators of the marker, resulting in variation or absence or a change in the size of the multiplier [19].(OPZ-08, OPY-07) was characterized by the highest number of loci produced [10] loci as in figure(1,2). The primer (OPM-03) was the least productive site (2 loci), and the total bands produced from those primer were 429 band, of which 18 were general main band and 411 were polymorphic band The higher variance is a sign of the efficiency of primers in the differentiation among genomes, and these results are consistent with the results of previous studies [9]. 43 band and produced the primer (OPM-03) the lowest number of sample 9 band. The unique or absent band is a specific category of items only. However, the unique band of more than one marker has a profile of the product, Genetic and a very important marker. The total number of distinctive band produced in this study was 49, 21 Absent band and 28 (Unique band). The sample Santacatrina (No. 5) achieved the highest number of missing band, 16 band], and received the Shamalali sample 2 the highest number of unique bands reached 7 The sample was characterized by 1 Krodsoo 6 unique bands and one missing band, and the sample was distinguished 2 Shamalaily 7 unique band. The sample was distinguished 3 Sourani (2 unique band and one band absent) . The sample 4,5 unique band and 16 absent band. The sample 6 was characterized by the Dahkan (3 unique band, one is absent).The sample 7 Qaisi was characterized by 5 unique band and 2 absent]band Variants varied in the size of the resulting beams. Their sizes ranged among 100-1700 bp and showed the lowest molecular size in the primer (100 bpOPH-14, OPG-05) and the highest molecular size in the primer (OPM-01, OPM-15, OPK-13,1700bp). The efficiency of the Proficiency primer also varied in showing the difference between the studied samples. The highest efficiency of the OPZ-08 (9.0) and the lowest efficiency was found in the primer OPM-03 (2.1)The discriminating ability of the primers was characterized by the OPZ-08 primer with the highest discriminating of 9.0, and the OPM-03 primer had the least discriminant of 2.1. These primers, which have the ability to show such diverse bands, are of interest to DNA fingerprinting researchers because they limit efforts and possibilities with minimal number of interactions to reach the target [19]. This opens the horizon not only to find the distinctive bands of other species, But in linking such plots with other field or analytical characteristics to facilitate the task of plant breeders [20].

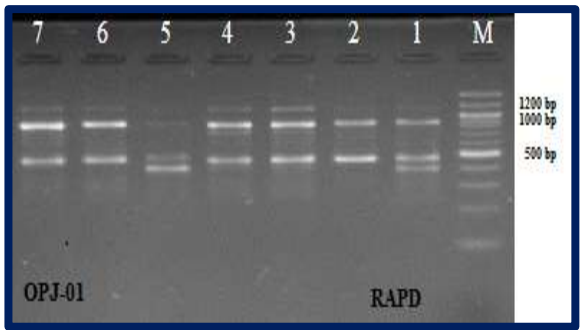


Figure (1)The products of the OPJ_01 primer of the RAPD with DNA are the 7 samples of *Olea europaea*L. and the 2% Agarose gel, 1.Qirdsawi 2_Shemalaly 3_Surani 4_Frantoio5_Santacatrina, 6_Dahican and 7_Qaysi

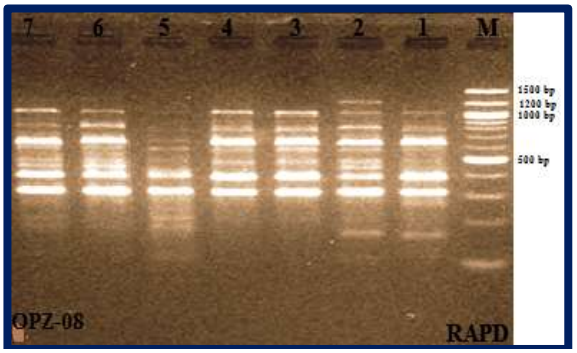


Figure (2) The results of the OPZ_08 primer of theRAPD with DNA are the 7 samples of *Olea europaea* L. and the 2% Agarose gel, 1.Qirdsawi 2_Shemalaly 3_Surani 4_Frantoio5_Santacatrina, 6_Dahican and 7_Qaysi.

Estimation of genetic distance based on the results of RAPD marker: The results of the current RAPD indicators have been invested in estimating the genetic distance between the seven samples of olive varieties using the NTSYS-PC (Version 2.2i) genetic program, which is based on the present of common band among each of the olive genotypes, [13], Table (1) shows the values of the genetic distance of the genotypes of the studied cultivars.

Table (1) The values of the genetic distance of the genetic composition of the olive varieties included in the study

	7	6	5	4	3	2	1
7	0.000						
6	0.251	0.000					
5	0.760	**0.769**	0.000				
4	0.235	0.184	0.538	0.000			
3	0.283	0.251	0.514	**0.089**	0.000		
2	0.311	0.258	0.521	0.204	0.270	0.000	
1	0.445	0.489	0.562	0.301	0.309	0.360	0.000

Using 18 primers starters from the RAPD marker can find the genetic distance and similarities between the studied models. If the genetic material matches two genotypes, it indicates that the genetic distance between them should be equal to zero. The scale of similarity genetic represents among two individuals or between two groups of individuals or even between species or structures of the same sex [21];It was determined by the values of the genetic distance indicated in Table(1). The genetic distance values ranged between (0.769-0.089), the lowest value of the genetic distance (0.089), separated between the 4 Frantoio and 3Sourani. That they have the largest similarity in the genetic material based on the primer used in this study. This result reflects their status under one variety and another. which found a high degree of similarity between pistachios in a given location Comparison of other loci based on RAPD marker The highest genetic distance was between 5 (Santacatrina species and 6 dahacan), which reached (0.769). This means that there was the least similarity in the genetic material between these two species. They shared the lowest number of bands for other species. The genetic distance of the other varieties ranged between these values. The values of the genetic distance among the seven cultivars were invested in the formation of the genetic relationship between them and in groups [1]. Results of this study agreed with previous studies on olive varieties in different countries, for example in Egypt [18], Iran,[22] Genetic arrangement, depending on the values of the genetic distance, results in a form called the gene of genealogy or the schema of the dendogram, and depends on the genetic range in which the major groups are associated. Thus, the presence of section of the genotypes in a given group indicates the similar genetic extent of those structures In that group [7].

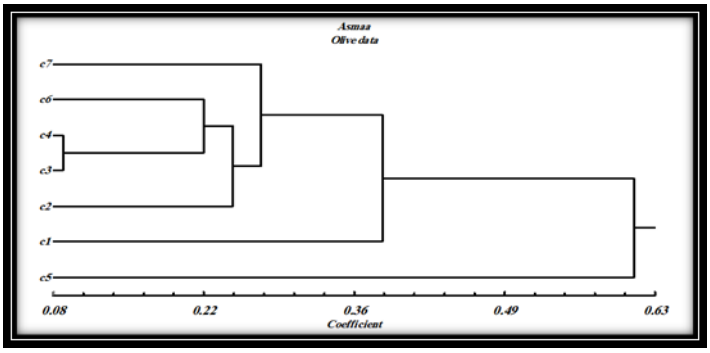


Figure (3) represents the genetic relationship of the seven varieties of olives based on the genetic distance of the RAPD

Marker The dendogram in group 4 formed the seven genotypes of the olive varieties using cluster analysis. Type *Olea europaea* L. variety were divided into two main groups, A and B.: **First main group**: This group included only 5 varieties, since it was associated with all the studied variety except in a few RAPD loci, thus showing a unique number of loci and their lengths, making them the most distant of all genotypes, giving a strange pattern that is completely different from the groups of variety The other foreign varieties (Foreign Varieties),It is an Italian cultivar that is found in the fertile and rainy lands and has been successfully cultivated in the northern and central regions of Iraq [23], one of the earliest variety in production and high-yielding table varieties[24]. Olives in Jordan [25]. And the study conducted by Martins-Lopes[16]. on the identification of the genetic material of the olive varieties in Portugal. This conclusion was also agreed with the results of previous studies of plant species[26]. That the models studied according to the RAPD method are separate by their genetic dimension and their morphological variation.

Second main group: This group consists of two subgroups: B1and B2, which is classified under the foreign varietal category, which is a Spanish class and has spread in northern and central Iraq Mehdi *et al.*,[23]. The second group consisted of the remaining two groups, including Shamlali, Sourani, Farantoyo Dohkan and Qaisi. There was a closer relationship between the two types of Sourani and Farantoyo, although their different types differed from the same geographic location[15]. The degree of genetic similarity based on RAPD indicators was found to be highly dependent on the geographical location and origin of the species under study The study agreed with other studies on olive varieties in Jordan [molecular characterization of a number of olive varieties using RAPD & ISSR indices [25]. This study showed the efficiency of RAPD indicators in distinguishing between the studied olive varieties and in determining the degree of proximity and genetic dimension among them, which contributed to the detection of genetic diversity among some olive cultivars in Iraq which can be exploited and used in the future, Histology in order to preserve it as an important genetically important source.

Conclusions

This study showed the efficiency of RAPD technique in distinguishing between studied olive varieties and in determining the degree of proximity and genetic distance among them. This contributed to the detection of the genetic variation among some cultured *Olea europaea* L**.** cultivars in Iraq which can

be exploited and exploited in the future, In order to preserve it as an important genetic source.

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التحليل الوراثي لبعض أصناف الزيتونOlive oil باستخدام مؤشرات التضاعف العشوائي للدنا المتعدد الأشكال RAPD Markers

أسماء عدنان العبيدي ، عقيل حسين العاصي

قسم علوم الحياة ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

الملخص

حدد في هذه الدراسة العلاقة الوراثية، والبعد الوراثي، وإيجاد البصمة الوراثية لسبعة اصناف من الزيتون (*Olea europaea* L) باستعمال مؤشر RAPD، عزل الدنا DNA المجيني من أوراق الأصناف المستعملة في الدراسة، تم الحصول على كمية من الـ DNA تراوحت بين (150_400) مايكرو غرام لكل 0,65 غم من أوراق النبات، وبنقاوة تراوحت بين (1,65−1,80) ثم اجريت تفاعلات PCR ورحلت العينات على هلام الاكاروز، طبقت مؤشرات الـ RAPD باستعمال18 بادئاً، اظهرت نتائج البادئات مواقع مختلفة من الحزم فكان المجموع العام للمواقع التي تعرفت عليها البادئات 99 موقعا ،كان منها18 موقعا عامة لجميع العينات و 81 موقعا متباينة. تباينت البادئات بأحجام الحزم الناتجة فقد تراوحت احجامها بين (100-1700bp). تباينت كفاءة البادئات في إظهار التباين بين العينات المدروسة فكانت اعلى كفاءة للبادئ OPZ-08 90، واقل كفاءة ظهرت في البادئ OPM-03 (2.1).اما القدرة التمييزية Power discrimination للبادئات فقد تميز البادئ OPZ-08 باعلى قدرة تمييزية بلغت 90 ،وتميز البادئ OPM-03 باقل قدرة تمييزية بلغت (2.1).وكان مجموع الحزم التي انتجت من تلك البادئات 429حزمة Total band ،كان منها 18حزمة عامة main band،و 411 حزمة متباينة Polymorphic band،كان مجموع الحزم المميزة الناتجة عن البادئات المنتجة في هذه الدراسة (49) حزمة مميزة كان منها 21 حزمة غائبة Absent band،و 28 حزمة فريدة Unique band فقد حصل الصنف سانتاكاترينا عينة رقم5 على اعلى عدد للحزم الغائبة فبلغت 16حزمة وحصل الصنف شملالي عينة رقم 2 على اعلى عدد للحزم الفريدة فبلغت 7حزمة وان قيم البعد الوراثي تراوحت بين(**0.769−0.089**)فقد كانت اقل قيمة للبعد الوراثي (**0.089**) ويفصل بين صنفي4صنف فرانتويو ، و3صنف صوراني،أما أعلى بعد وراثي فكان بين (صنف 5 سانتاكاترينا وصنف6 دهكان) وقد بلغت (**0.769**).في حين اظهر تحليل العلاقة الوراثية ان هناك ثلاث مجموعات رئيسة ضمت الاولى صنف 5 سانتاكاترينا، اما المجموعة الثانية فتكونت من مجموعتين فرعيتينsubgroupsB1,B2 ضمت الأولىB1 صنف واحد فقط صنف 1كرودساوي، اما المجموعة الثانية B2 فقد ضمت الاصناف الباقية شملالي ، صوراني، فرانتويو ، دهكان، قيسي.