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Analysis of Genetic Diversity of Some Olive Cultivars Olea Europoea L. Using ISSR, SSR

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Abstract: ***In the current investigation, the genetic relations, and the genetic dimension of seven olive varieties (Olea europaea L.) were identified using, ISSR, and SSR markers. The study involved uses leaves from different samples for DNA extraction. The DNA amount ranged from 150 to 400 micrograms with purity ranging between 1.6 to 1.9. Agarose gel electrophoresis is applied to evaluate PCR reaction success. The ISSR marker, which used 4 primers, showed different results for the multiplication. Various bands were observed that differed from each other, resulting in a total of 93 bands - 2 general and 91 different. The primer also distinguished unique bands, with 4 unique bands and 7 absent bands. The primer with the highest molecular size (1500bp) was UBC-817, while the lowest molecular volume (200bp) was observed in the UBC-826 primer. The marker results indicated that the two types, Suranie (3) and Frantoio (4), had the lowest hereditary dimension of (0.030). The Frantoio and Santacatrina varieties showed the highest genetic difference, with a value of 0.622. According to the genetic relationship analysis, there were three main groups. The first group consisted of a single category (5), while There were two subgroups in the second group, B1 and B2. Subgroup B1 had only one category, while the rest of the categories were in Subgroup B2.. Even though these markers use different mechanisms to detect variance and genome coverage, they complement each other. Moreover, five SSR markers were used in this study to describe seven olive cultivars and analyze their genetic relationship. Four out of five primers showed positive results, while one was not present. The SSR markers were effective in identifying the similarity of collected species, as they are specialized indicators ISSR markers.***

Keywords: ***ISSR, SSR, PCR Technique, Olive, Unique Bands, Absent Bands.***

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1. INTRODUCTION

The olive plant, scientifically known as Olea europaea L., is a medicinal plant that has been used by experts and wise men as a successful means of treatment. It has numerous uses and plays a significant role in human life. Due to the risks associated with using chemical drugs and their increased collateral damage, there has been a surge in demand for olives as a source of natural medicine. Olive is the primary source of phytosanitary production and provides active substances to prepare many pharmaceuticals [2]. The origins and domestication history of the first cultivars are still a matter of debate. However, based on archaeological and molecular data, it is believed that they first originated in the Levant region, which is currently located at the border of southwestern Turkey and northwestern Syria, approximately 6,000 years ago. [40].
Most of them have been used extensively in the field of folk medicine in different parts of the world, including the Mediterranean, Arab countries and the tropical regions, as a diuretic, a pressure suppressant, a softener, an intestines, an intestines, a colonizer, a fever hunter, a skin cleanser and a yellow teacher. Gallstones, bronchial asthma, urinary tract infections, colic, diarrhea, alopecia, rheumatism, and sciatica pain [18][38]. The olive plant has attracted a diverse range of global interest. From sustaining old varieties and improving their production to serious attempts to find new varieties, there is a need to create modern means of accurately identifying these items. This is important in order to preserve their identity and the special components that are unique to the regions and countries that wish to develop them. To analyze genetic content, DNA varieties must be found, and DNA markers used to determine genetic dimensions and relationships. [14][34].

2. RELATED WORK

The Inter Simple Sequence Repeat (ISSR) is a powerful, fast and inexpensive marker that identifies the variance within and between DNA regions that have a specific frequency. This marker uses a single initiator and has many applications, including the genetic variance [16] and DNA analysis [1]. Simple sequence (SSR) or microsatellites were also used in this area. The common DNA marker above RAPD, ISSR, SSR are in several stages of development, where this technique is based on specific sites and is spread over the genome of high organisms. These sites include Tandem repeats in different numbers and thus become different lengths. These regions have a unique sequence (Flank) of each type, so they are detected by adding primers to those edges and to the DNA series and in opposite directions (Forward & Reverse) The PCR products are separated on the agaros gel and pigmented to document their results [15.]These markers RAPD, ISSR, SSR have become the best choice for many studies of different olive species[31][32], used to select the interrelationships between different species and within species, as well as between cultivars and other species, Invested in the breeding processes of diverse species of animals and plants [33].
Because of the industrial and nutritional benefits, for example, the contents of wheatseeds from vegetable oils are the main goal of plant breeding and biotechnologies .It has also been used to detect the genetic variability of many plant species such as peaches [6]. These markers have been

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used in many different studies and objectives such as the creation of genetic correlation maps of several important traits for several plant species [13][24].And the study of plant genetic diversity [19][28] , as well as the distinction between species and the clarification of evolutionary relationships and the classification of plant genetic groups[3][11]

3. MAREIALS AND METHODS

DNA Extraction

The genomic DNA was extracted from 0.5-1 gram of fresh leaves taken for each sample by CTAB-based method according to (29).

Results and Statistical Analysis: ISSR

The process of ISSR interactions (4) , which involves amplification of DNA using ISSR-PCR, was carried out using the Accu Power PCR premix kit. This kit, supplied by Bioneer Korea, contains all the necessary components for the PCR, One unit of Taq DNA polymerase, 250 µM dNTPs mix, 10 mM Tris-HCl (pH 9), 30 mM KCl, and 1.5 mM MgCl2. To initiate the process, 10 Pico mole from the initiator (uL1) was added, along with 25 ng of DNA (1 ul), as per the instructions provided with the kit.

Add distilled water to each tube until it reaches 20 µl, then transfer the tubes to the thermocycler and perform the reaction using the following program: 1 cycle of 30 sec at 94°C, followed by 45 cycles of doubling, with each cycle consisting of 30 sec at 94°C, 45 sec at 52°C, and 90 sec at 72°C. Finish with a final cycle of 5 min at 72°C. Amplify the products by placing them in a 2% agarose gel with Red Safe dye for 90 min (5 V/cm) under UV light. Use a Gel Documentation System to take images of the gel and count the bands produced with their molecular sizes for each primer.

Table (1): The primers used in ISSR reactions

No.	Primer code	RIMERS sequence
1	UBC 817	CACACACACACACACAA
2	UBC 826	ACACACACACACACACC
3	UBC 846	CACACACACACACACART
4	UBC 855	ACACACACACACACACYT

SSR Results and Statistical Analysis:

To amplify DNA, we used the Accu Power PCR premix kit (Bioneer, Korean) and followed the attached instructions. Each tube contained the essential components of the PCR, including Taq DNA polymerase(one unit), dNTPs mix (250 µM), Tris-Hcl (pH 9, 10mM), KCL (30mM), and MgCl2 (1.5 mM). We added 10 Pico moles of the first initiator F (uL1), an equal amount of the second initiator R, and 25 ng of DNA uL1 l) to each tube. Finally, we added distilled water to the

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reaction volume to make it 20 µl per tube. Transfer the tubes to the thermocycler and perform the reaction after the program was programmed according to the program: One cycle for 5 minutes at a temperature of 95 m for the primary strainer of the DNA tape followed by 35 doubling cycles. Each cycle includes 45 seconds at 94 m for the mold burner and 45 s for 57 m for connecting the molded mold primers and 45 seconds For elongation, perform 8 cycles of 72 ° for 1 minute each. The amplification products were loaded onto a 2% agarose gel containing the Red Safe dye and run for 90 minutes at 5 volts per centimeter under UV light. The resulting bands were analyzed using a Gel Documentation System, and the number of bands produced and their corresponding molecular sizes were recorded for each primer

Table (2): The primers used in SSR reactions.

1	Forword	5`-AAA AAC ACA ACC CGT GCA AT
	Reverse	5`-AAA TTC CTC CAA GCC GAT CT
2	Forword	5`-TCA CCA TTC TTA ACT TCA CAC CA
	Reverse	5`-TCA AGC AAT TCC ACG CTA TG
3	Forword	5`-GGA TTT ATT AAA AGC AAA ACA TAC AAA
	Reverse	5`-CAA TAA CAA ATG AGC ATG ATA AGA CA
4	Forword	5`-AAT TAC CAT GGG CAG AGG AG
	Reverse	5`-CCC CAA AAG CTC CAT TAT TGT
5	Forword	5`-TCG GCT TTA CAA CCC ATT TC
	Reverse	5`-TGC CAA TTA TGG GGC TAA CT

4. RESULTS AND DISCUSSION

Results of ISSR Markers

In the interactions of the ISSR markers, four primers consisting of several nusequences ranging from 16 to 20 nucleotides were used, all of which showed multiplication outcomes as shown in Table 4.4, The ISSR markers are used to detect a high level of genetic variance and to detect multiple sites and thus determine the relationship between species. ISSR is also suitable for finding DNA, the optimal conditions for the ISSR interactions were obtained after several experiments. The study found that the most important factor leading to optimum conditions in the ISSR markers is thermocycler, namely the temperature of the primer,

For each of the primers of optimal correlation. These values were taken from previous studies of the ISSR interactions in the olive field. Thus, multiplication outcomes were obtained in all the primer in this study.

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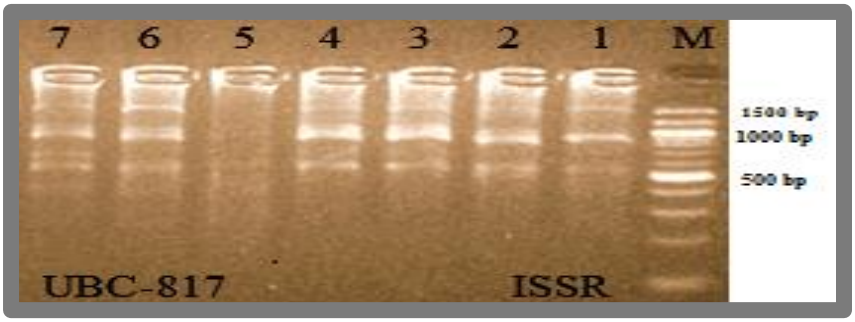

Fig.1. The UCR-817 seed multiplication results of the ISSR marker with DNA represent the 7 olive varieties Olea europaea L. and the 2% agarose gel concentration

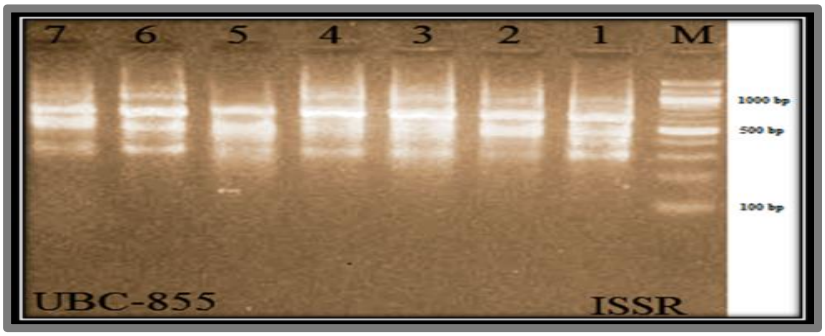

Fig.2. The outputs of the UCR-817 initiator of the ISSR index with DNA represent the seven samples of olives. Olea europaea L. and the phase on the 2% karose gel

Discussion of ISSR Results

The ISSR markers are used to detect a high level of genetic variance and to detect multiple sites and thus determine the relationship between species. ISSR is also suitable for finding DNA, This study showed that the use of ISSR markers was able to distinguish between olive varieties, as the primers used in this study showed double products of the olive varieties included in the study, And gave a high level of variation between these varieties and a difference in the products of the multiplication due to the difference in the target areas of the genome as well as the difference in the sequence of primers used, as it is known that the change in the base of one nitrogen change in the result of multiplication.The total number of bands (total of 93 bands), of which 2 (general bands) and 91 different bands, as well as the differences shown by these primer among the olive varieties, were also distinguished, (4bands) and the number of missing bands (7 bands). The unique and absent bands are the marker of these items from the rest of the items in the study. The number of sites reached four primer (20 sites) (2 public sites) and 18 different locations. The UBC-817 primer had the highest molecular size (1500bp).The lowest molecular size of (200bp) appeared in the UBC-826 primer . This study agreed with previous studies [6], where ISSR was used to determine the genetic characterization of olive varieties in Jordan and the study conducted by the researcher[24] found the DNA of the olive varieties in Portugal.

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Estimation of Genetic Dimension Based on ISSR Results

The genetic dimension of the seven varieties of olive trees was estimated in the same manner as in the RAPD markers. Table (3) shows the values of genetic dimensions among the seven cultivars.

Table (3): represents the values of genetic dimensions based on ISSR markers

	1	2	3	4	5	6	7
1	0.000						
2	0.201	0.000					
3	0.136	0.134	0.000				
4	0.106	0.164	0.030	0.000			
5	0.476	0.458	0.592	0.622	0.000		
6	0.076	0.112	0.112	0.081	0.452	0.000	
7	0.130	0.112	0.112	0.142	0.452	0.051	0.000

(0.030-0.622), the lowest value of the genetic dimension is (0.030) between the types of (3) images and (4) Frantoyo, which means the least difference between the genome of the two varieties compared to other subjects in the study, Frentoyo, and 3 Sourani), this indicates that they have more.The genus is classified in the Arab varieties of olives, an Egyptian olive origin and cultivates this breed in Syria because of adaptation to drought conditions and tolerance of salt and extreme heat, Farantoyo is a foreign cultivar, an Italian cultivar grown in Iraq's high-altitude demographics [27]. This is the same result of the RAPD marker, which is a support for results. This is evidence of the accuracy of the results that reflect the convergence of genetic content between them.

The highest genetic dimension was between Frenteo and (5) Santacaterina (0.622) and the highest difference between the two genotypes compared with the other classes of the study. The genetic relationship between olive varieties was investigated by grouping them based on their genetic dimension values. As shown in Fig. 3 olive varieties were divided into two main groups

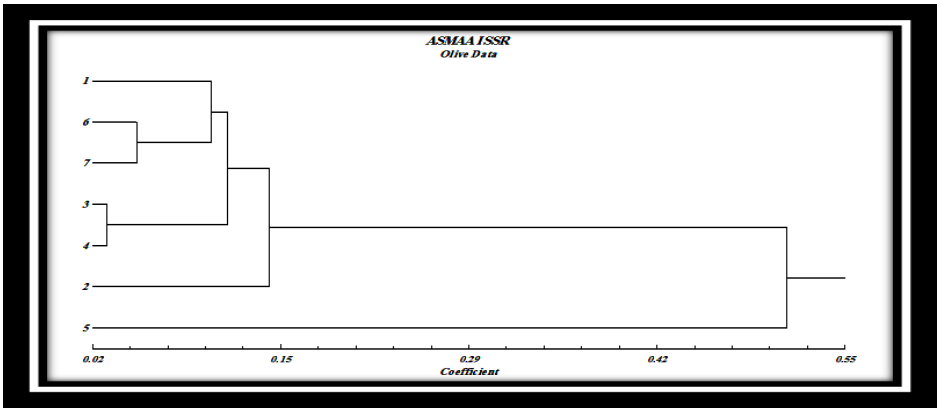

Fig.3. depicts the genetic relationships among seven olive varieties using ISSR indicators.

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First Group

This group included a class of (5) Santacatrina, which separated this species alone in the tree schema Dendrogram, and gave this type a pattern is different from the rest of the groups. The cause of the individual in an independent group of the same reasons mentioned earlier in the results of the genetic dimension of the marker of RAPD.

Second Group

This group consists of two subgroups: B1, B2, B1, 1, 2, and 2, which is classified as Arab Varieties, a Tunisian origin [27].In the second group B2, the remaining varieties included Crodsoo, Sourani, Farantoyo and Dokan Qaisi. The two species were most closely related to both Sourani and Farantoyo, although they differed in the same geographic location. These results were consistent with many studies [4][25].Which combined the correlation between the genotypes of olive varieties and the geographical origin. These findings were agreed with previous studies[7]. The ISSR marker were used to determine the genetic characterization of olive varieties in Jordan, and the study conducted by the researcher[26].The ISSR[16] marker was used to study the genetic differences between 17 species of wheat and the study conducted by[12].

Results of SSR Markers

Analyze the genetic diversity of the seven olive varieties using SSR marker This requires several initial attempts to adapt the factors influencing them and then activate them to optimize the conditions for obtaining reliable results because they are specific in the nature of the DNA regions. Which are being sought, that the results[26] of PCR-based indicators are influenced by several important factors that must be taken into account To obtain accurate results and only among these factors[9].

1. Concentration of the genomic DNA.
2. The suitability of the program implemented on the thermocycler.
3. Primer concentration User.
4. The accuracy of the pipette used in the experiments
5. On this basis, several attempts were made to reach optimal interaction conditions that correspond to the type of study. For the concentration of DNA, the concentration used in the previous markers is 25 ng

As for the second factor, the thermocycler-fed program, the program used in the third chapter was used [33], which consists of (35) cycles of varying docking temperatures depending on the pair of primer used and depending on the components of these primers of nitrogen bases according to the equation: With an increase or decrease of 2.5 ° C from the final product[39], with respect to the other factor, primer conc. It was found by experiments that the optimal concentration was (10) Pico mole, In the fourth factor, the accuracy of the pipettes used. In such experiments, the accuracy of the pipette used should be emphasized. In this study, five pairs of Primer Pairs were used, All the results showed that, except for the primer, there was no result. This is because optimum conditions for this primer do not match the conditions of specific primer primers, which showed results for olive varieties[36].

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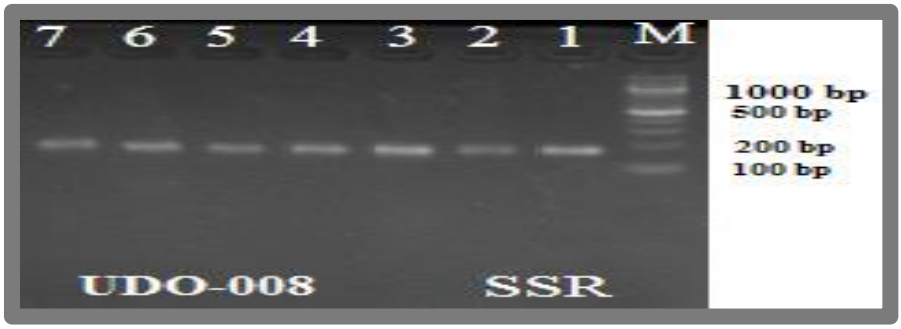

Fig4. The UDO-008 multiplication product by the SSR index with DNA represents the 7 samples of Olea europaea L. and the 2% agaros gel. 1. Krodsoo, 2_shamalali, 3_sourani, 4_Fantoyo, 5_Santakaterina, 6_dakkan and 7_kissi

Fig. 5. The outputs of the UDO-043 multiplication by the SSR index with DNA represent the seven samples of olives. Olea europaea L and the phase on 2% karose gel, 1.crodeo, 2_chemical, 3_color, 4_Frantoyo, 5_Santacaterina, 6_decan and 7_quisi.

Discussion of SSR Indicators

The SSR marker is a specialized type of marker that differs from non-specialized markers such as RAPD and ISSR. The indices of this marker are derived from the olive plant genome and can reveal any heterogeneity within it. Like other plant species, the olive plant has a single genetic material germplasm that inherits and gives uniform characteristics of the species or cultivar. The results of this study were in line with the basic principle of the SSR marker, as all the samples displayed unified bands, except for some variations that indicate a mutation due to various reason ,The strength of these markers in terms of their ability to show the extent of the variance is negligible between any two models no matter how close they are, because they depend on the difference in the number of Tandem Repeats replicated between two sites possessing a unique nucleotide sequence corresponding to the sequences of their primer pairs[15].

This variation exists naturally within the genomes of organisms and the result of inheritance of DNA fragments during the formation of new recombination during the interactions of high organisms and these pieces of DNA are scattered over all areas of the genome[23]. The number of

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bands produced by these markers was consistent and the number of primer pairs used in this study. Scientific sources indicated that fewer or more similar numbers were used in other studies and were able to achieve their objectives. Only a couple of primers were used to analyze 12 genetically modified potato varieties. Solanum tuberosum [30].Five pairs of primers were used to compare the efficacy of SSR markers with RAPD markers for the analysis of two groups of barley, the first of which were 23 spring cultivars and the same number as the winter of agriculture[20].The bands of these markers represent the alleles of the site of integration with the pair of primers, which apply to the Mendelian laws, and thus can be traced back to the ancestors[37]. Differentiated by their status as common sovereign indicators[15].

The high specificity of the bands of these markers has gained from the method of deriving the primers used in them, and at the same time represents the decisive factor in the construction of these markers, The design of these primers depends on the isolation of the clones from the olive plant library specifically, so the chances are to find their complement of sequences on the genome of this plant and in a variation between one model and another[15].However, the absence of this site sometimes in some models is unlikely due to the high privacy, characterized by the sequences of some of these primers because the optimum conditions for this initiator do not fit the conditions of the specific primer, Which may not be owned by all varieties. The SSR markers have become indicators of choice for the study of variation in olive varieties[10]. Germplasma contains the CRA-ISOI (The CRA Istituto Sperimental Per I'Olivicoltura) (a group of high frequency and contrast SSR regions)SSR In this study, The SSR was used to characterize seven olive varieties to analyze their genetic relationships.

The objective was to identify similarities between groups based on germplasm differentiation. Implemented on olive varieties of SSR marker[31][32].By identifying the different gene structure and unique characterization of the gene structure, and these results coincided with the results of a set of recent research[5].

In this study, we can confirm that the SSR marker is an effective marker of the genetic makeup of the genetic material CRA-ISOI germplasm of olive varieties among groups showing common genotypes. To conclude that the seven species have a repetitive area of replicas consisting of equal and similar replicates common to all studied species, These results have been consistent with several studies[21][36]. This study showed that the use of molecular markers such as SSR is very useful for building the germplasm database in olives.

5. CONCLUSIONS

This study showed that the use of ISSR indicators was able to distinguish between olive varieties, and gave a high level of variation between these varieties and a difference in the products of the multiplication, Finding the DNA of a number of olive cultivars by means of the primers that gave a definite or definite bundle in the and ISSR marker, The ability of SSR marker to achieve the objectives of the study, The results of DNA indicator align with the index's basic principle, The indicator identified a repetitive area consisting of equal and similar replications among all items studied.

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