ISSN: 2790-5306 (Print), 2790-5314 (Online)
IRAQI JOURNAL OF AGRICULTURAL RESEARCH - Ministry of Agriculture
Available online at: www.ijarmoa.gov.iq
VOL. 28 NO. (1) 2024
IJAR
IRAQI JOURNAL OF AGRICULTURAL RESEARCH

STUDY GENETIC DISTANCE OF OLIVE CULTIVARS USING MICROSATELLITE MARKER

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ABSTRACT

The genetic distance between ten cultivated olive was determined through Microsatellite, method at DNA level from Baghdad, and Al-Mosel, position. The genetic distance matrix, UPGMA, and NJ dendrograms, and PCA were performed. The Assessment of genetic relation among olive varieties cultivated in Iraq was ensured exactly through these markers. The study showed Fifteen, Simple, sequence, repeat loci were studied, and produced, 243 amplified, fragment 243 of these loci (99.16%) were polymorphic. Five primers generated sufficient polymorphic bands to figure out the genetic diversity among the cultivars., the results showed that cultivar Shami is a hybrid of two cultivars Arbqween and Nepali while the two cultivars Sorani and Manzenllo with 100% similarity. Which indicates due to belong to the same origin. Establishing the genetic relationship of those varieties would be suitable to use for future genetic improvement studies in olive cultivars.

Keywords: Microsatellite, PCR, pedigree, polymorphic, Olive

INTRODUCTION

Olive (*Olea europaea* L.) is an essential tree used as a source of food and oil in the world, which is acclaimed as a ‘‘precious fruit’’ [6]. The destination , of olive can be traced to the eastern Mediterranean Coast, and the expansion of the Roman Empire favorites the spread of olive all around the Mediterranean basin [21]. where computation for more than 90% of the world’s olive oil production [1].

Olive cultivars are completely diverse both in external and internal Plant characteristics such as color, oil ratio, size, texture, shape, oil composition, etc. Fruit characteristics are also very diverse, ranging from bushes to large trees, extension to upright, and having small to large leaves. There is a high degree of self incompatibility is reported for many olive genotypes, and to the strong environmental leverage, results of compatibility tes often conflicting [27]. Cultivated olive (2n = 2x = 46; genome size 1800 Mb) is an evergreen, high longevity high genetic variability of cultivated olive It is a mostly allogamous

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Received: February 26, 2023.
Accepted: July 2, 2023.
Available online: July 25, 2024

species showing a high degree of out crossing which leads to major levels of heterozygosity and Polymorphism at the DNA level among individuals [28]. Most morphological traits are influenced by environmental factors, plant age and phenology [16]. But DNA-based markers are not affected by environmental conditions, and they authorize direct scanning of the plant genome [25,15]. knowing of the genetic relationships between wild olives and their cultivated relatives is needful to improve genetic resources and conception of their evolutionary background [3,2]. Presently study shows Microsatellite markers very useful tools for plants' scientists in establishment phylogenies and finding out similarities among cultivars [23]. Give a chance to make a direct comparison between the organisms in molecular levels, as the use of DNA-based molecular markers has become common in plant breeding as well as olives along with the other agricultural important plants [14]. Genetic polymorphism of the plants can be detected by many different DNA-based marker methods, such as RFLP, RAPD, AFLPs [4,31,22]. Nowadays Microsatellite has been confirmed to be very appropriate markers for cultivar identification and identity typing in olive as they are transferable, highly polymorphic and co-dominant markers [8]. Microsatellite or Simple sequence repeat (SSR) is one of the most important categories of molecular markers. It comprises the essence marker system of the PCR based molecular markers and is widely used for genetic mapping, DNA fingerprinting, and studies of genetic diversity also studies of population genetics. Simple sequence repeat markers are profuse, highly polymorphic, and co-dominant and differentiate multiple alleles in a plant species owing to variation in the number of motif (repeat units), which are composed of 1-6 bp short deoxyribonucleic acid sequences, such as dinucleotide repeats [(CT)n and (AT)n] and trinucleotide repeats (TTT), and separate mainly in the un-coding regions and regions between genes throughout genomes [6]. The aim of this study is to determine Genetic distance between olive cultivars natively grown in Iraq and use the extremely ratio polymorphism in breeding studies in the future.

Microsatellites are excellent markers and have been used for the identification of olive cultivars. However, the limited number of Simple sequence repeat markers and the occurrence of blending on the names of cultivars, as well as the possible appariation of clonal variation make it difficult to identify cultivars and explicate relationships among olive cultivars [24].

MATERIALS AND METHODS

Subjects

Healthy leafs of olive tree were collected from ten local cultivars, these cultivars were be in Baghdad and Al-Mosel station as in Table – 1

Table 1: olive varieties, origin

Cultivar	Khodeiri	Qaysi	Manzenllo	Baashiqi	Arbqween	Nepali	lot (Labeeb	Dahkan	Sorani	Shami
Origin	Syrian	Syrian	Spain	Local	Spain	Jordanian	Syrian	Local	Syrian	Syrian

DNA Extraction

Genomic DNA was extracted from young leaves by CTAB according to Ref. Besnard et al. [5]. The estmination of DNA quality and concentration in samples was outright by both spectrophotometric analysis and running on 0.8% agarose gels. Optical density ratios analysis were evaluated and only good-quality DNA samples were used in PCR [11].

Molecular Marker

Fifteen microsatellites primer sequence (Table -2) were used to genotyping ten local olive varieties cultivating in Iraq [8,9,32]. were performed in a 10 µL volume consisting of 25 ng of DNA, 0.5 unit of Fast Start Taq DNA Polymerase (Invitroge n, Brazil), 1 × PCR buffer (Invitrogen, brazil), 2.4 mM MgCl2, 201 µM of each dNTP (Invitrogen, Brazil), 0.2 µM of tailed forward primer (Integrated DNA technology, USA), 0.5 µMtailed labeled with IRD700 fluorophore (Integrated DNA technology, USA), 0.6 µM of reverse primer (Integrated DNA technology, USA), The forward primer was "tailed" by the inclusion of 18 extra nucleotides at the 5' end, which simplified the labeling of the products, The reactions were carried out in a thermo cycler Perkin-Elmer 9700 (Applied Biosystems) with the following profile: 94 °C for 5 min, 6 cycles at 94 °C for 20 s, annealing temperature Table- 2 for 30 s, decreasing 1 °C/cycle, extension temperature 72 °C for 30 s; followed by 29 cycles at 94 °C for 20 s, annealing temperature 50 °C for 30 s, 72 °C for 30 s with a final extension at 72 °C for 5 min. Microsatellite markers were profiled using a LI-COR Bioscience 3100 DNA Analyzer, 1 µL of the product was loaded onto a 6% polyacrylamide gel, and electrophoreses at 1500 V. Molecular Marker standards were loaded on the gels to determine the size to each allele, were scored manually [30].

Data Analysis

Polymorphic Information Content (PIC) measured to know the in formativeness of each loci, that depended on allele frequency, if the PIC > 0.7 represent highly differentiation marker and suitable for mapping, if PIC > 0.5 it will be classified as informative marker [13].

RESULTS AND DISCUSSION

Microsatellite analysis of ten cultivated olive accessions using fifteen SSR loci provided a total of 243 bands as shown in Table 3. Two hundred forty-one of these "loci" (99.16%) were polymorphic over all the genotypes tested. The average number of detected alleles per locus was 3.93 while the average number of detected bands per loci was 15.93. Loci EMOL, ssrOeUA-DC A9, ssrOeUA-DC A11, ssrOeUA-DC A14, ssrOeUA-DC A16 and GAPU101 were suitable for mapping the genome, while the other studied loci were informative markers to olive and that agreed with the result detected in study of Tunisian olive varieties [32]. The highest number of polymorphic bands detected by locus GAPU101 this difference between loci bands productivity because of the differed of the primer combinations in their ability to matching with compatible sequence in all over genome and detection of the polymorphism of the populations as the result [18]. These results confirmed a high degree of polymorphism in the olive germplasm with an average of 99.16% as in (Table - 3), while (59.8%) were polymorphic in the same cultivar when used AFLP technique [26]. Cluster analysis of the SSR markers are explained in "Fig.1" that shown two main distinct groups were observed in the dendrogram. Group A divided in two sub group cultivar Arbqween in sub groupA1 and Nepali, , Shami sub groupA2 . Group B: other cultivar and divided in two sub group B1,B2 . Group B1 divided in two sub group cultivar Manzenllo, Sorani in sub group B1a and Jlot (Labeeb) in sub groupB1b, while sub group B2 divided in two sub group B2a and B2b. Sub group cultivar Qaysi and Baashiqi in sub group B2a. While Dahkan in sub group B

Table 2: Microsatellite primer sequence for fifteen loci used in the of olive genomic DNA CACGACGTTGTAAAACGAC amplification

Primer Name	Forward 5' 3' with labeled tail	Reverse 5' 3'	Reference
GAPU59	CACGACGTTGTAAAACGACCCCT GCTTTGGTCTTGCTAA	CAAAGGTGCACTTTC TCTCG	[8]
GAPU101	CACGACGTTGTAAAACGACCATG AAAGGAGGGGGACATA	GGCACTTGTTGTGCA GATTG	[8]
UDOO9-4	TTTCCCAGTCACGACGTTTTTGCCCTGGATTGGTACA	AGCTTGAGCATCATCTGTGAG	[9]
ssrOeUA-DCA16	CACGACGTTGTAAAACGACTTAG GTGGGATTCTGTAGATGGTTG	TTTTAGGTGAGTTCAT AGAATTAGC	[32]
ssrOeUA-DCA11	CACGACGTTGTAAAACGACGATC AAACTACTGCACGAGAGAG	TTGTCTCAGTGAACC CTTAAACC	[32]
ssrOeUA-DCA14	CACGACGTTGTAAAACGACAATT TTTTAATGCACTATAATTTAC	TTGAGGTCTCTATATC TCCCAGGGG	[32]
UDOO9-11	TTTCCCAGTCACGACGTTTTGATTTCACATTGCTGACCA	CATAGGGAAGAGCTGCAAGG	[32]
DCA15	GATCTTGTctGTATATCCACAC	TATACCTTTTCCATCTTGACGC	[32]
ssrOeUA-DCA9	CACGACGTTGTAAAACGACAATC AAAGTCTTCCTTCTCATTTCG	GATCCTTCCAAAAGT ATAACCTCTC	[32]
ssrOeUA-DCA8	CACGACGTTGTAAAACGACACAA TTCAACCTCACCCCCATACCC	TCACGTCAACTGTGC CACTGAACTG	[32]
ssrOeUA-DCA7	CACGACGTTGTAAAACGACGGA CATAAAACATAGAGTGCTGGGG	AGGGTAGTCCAACTG CTAATAGACG	[32]
ssrOeUA-DCA5	CACGACGTTGTAAAACGACAACAAATCCCATACGAACTGCC	CGTGTTGCTGTGAAG AAAATCG	[32]
ssrOeUA-DCA4	CACGACGTTGTAAAACGACCTTAACTTTGTGCTTCTCCATATCC	AGTGACAAAAGCAA AAGACTAAAGC	[32]
EMOL(3)	ATGGCACTTTACGGGAAAA	TGCCAATTATGGGGCTAACT	[12]
DCA03	CCCAAGCGGAGGTGtATATTGTTAC	TGCTTTTGTGTTTGAGATGTTG	[8]

Table 3: Monomorphic and polymorphic percentage for olives varieties using 15 SSR

Marker	PIC	No. of bands product	No. of Alleles	Alleles Molecular weight (bp)	Mono morphic	%	Polym orphic	%
GAPU59	0.698061	18	4	218,223,228,232	0	0.00	19	100.00
GAPU101	0.800584	33	6	148,205,208,213,230,279	1	2.71	36	97.29
UDOO9-4	0.459184	13	2	127,129	0	0.00	14	100.00
ssrOeUA-DCA16	0.734694	9	4	162,170,174,191	0	0.00	7	100.00
ssrOeUA-DCA11	0.8	15	6	163,167,175,179,182,188	0	0 0.00	15	100.00
ssrOeUA-DCA14	0.745562	14	5	176,194,198,207,210	0	0.00	13	100.00
UDOO9-11	0.8	16	6	163,167,175,179,182,188	0	0.00	15	100.00
DCA15 (10)	0.652778	12	3	145,168,206	0	0.00	12	100.00
ssrOeUA-DCA9	0.75737	20	5	173,186,192,203,213	0	0 0.00	21	100.00
ssrOeUA-DCA8	0.408163	7	2	136,151	0	0.00	7	100.00
ssrOeUA-DCA7	0.592593	15	3	146,161,179	0	0.00	9	100.00
ssrOeUA-DCA5	0.677686	21	4	215,220,223,224	1	4.55	21	95.45
ssrOeUA-DCA4	0.444444	12	2	167,178	0	0.00	12	100.00
EMOL(3)	0.72314	22	5	251,253,256,265,269	0	0.00	22	100.00
DCA03	0.59375	16	3	223,234,251	0	0.00	16	100.00
Total avarge		243	59		2	0.84	241	99.16

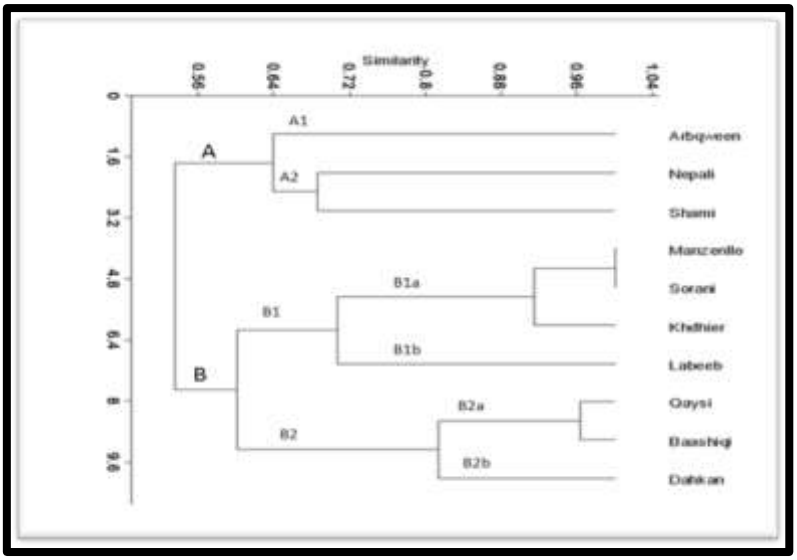

Figure 1: Depict a Genetic distance dendrogram to ten olive cultivars produced by SSR technique using fifteen loci

In the other hand, the results showed that cultivar Shami is a hybrid of two cultivars Arbqween and Nepali."Fig .2" It is becoming clear that gene flow between divergent taxa can create new phenotypic diversity, allow for acclimation to novel environments, and contribute to speciation. Microsatellite has a better molecular marker to authenticate the occurrence of and investigate the outcomes of hybridization in plants. With the growing availability of genomic tools and advancements in genomic analyses [17]. While the two cultivars Sorani and Manzenllo with 100% similarity. due to the belonging to the same origin while the potential possibility of the variation are the hybridization (programmed or natural) and the environmental effect [29]. Microsatellite has a better molecular marker than other molecular technique for differentiate and detecting genetic relationship among cultivars and agreed with [33, 19, 20,10]. Therefore, Microsatellite technique was more specificity and the result depend on Microsatellite marker were consent with [32]. In addition DNA based markers are not influenced by environmental conditions and it allows to directly determining the plant genotype [20]. It is very important to define variety-specific genetic structure; to determine genetic distances and similarities between it and maintain genetic structures of local types foreign to regions and use the highly polymorphism ratio in breeding studies in the in the future.

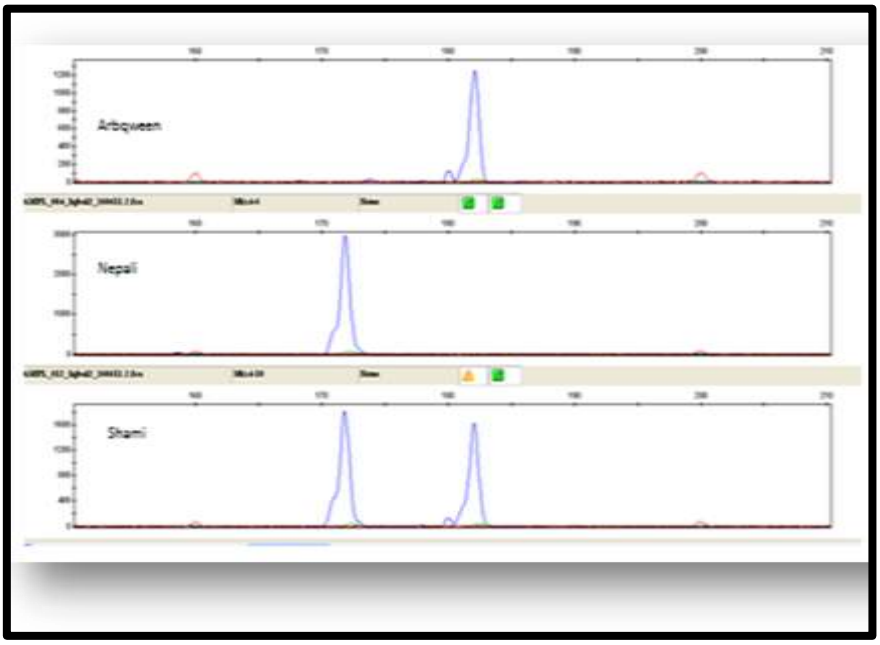

Figure -2: Depict Allelic profiles at the Microsatellite locus GAPU101of DNAs show cultivar Shami is a hybrid of two cultivars Arbqween and Nepali.

The highest Genetic distances and lowest in Microsatellite that shown in (Tables 4). where cultivars Arbqween and Qaysi show highest Genetic distances (0.762) While Sorani and Manzenllo they were lowest Genetic distances (0.000) .

Principal component analysis (PCA).

To support the results of genetic relationships among olive cultivars, data were analyzed by multivariate *Principal component analysis*. based on Nei's genetic distance was shown in "Fig.3". The scatter, diagram of the first two (PC1 and PC2) based on on 15 Microsatellite polymorphic loci which represent 56.84% of the total variation in which PC1 recorded 31.97% and PC2 was 25.57%. The two axes exhibited similar clusters of cultivars as shown in the dendrogram "Fig. 2". The results of PCA for the ten olive accessions was basically in agreement with that of the UPGMA cluster analysis.

Table 4: Genetic distance of ten olive cultivars using fifteen Microsatellite loci

Shami	Sorani	Dahkan	Labeeb	Nepali	Arbqween	Baashiqi	Manzenllo	Qaysi	Khodeiri	OTU
									0.000	Khodeiri
								0.000	0.658	Qaysi
							0.000	0.659	0.312	Manzenllo
						0.000	0.577	0.192	0.609	Baashiqi
					0.000	0.756	0.722	0.762	0.756	Arbqween
				0.000	0.568	0.693	0.659	0.676	0.676	Nepali
			0.000	0.564	0.707	0.707	0.548	0.646	0.540	Labeeb
		0.000	0.456	0.609	0.696	0.430	0.692	0.435	0.637	Dahkan
	0.000	0.637	0.540	0.632	0.717	0.609	0.000	0.658	0.272	Sorani
0.000	0.716	0.569	0.677	0.561	0.632	0.577	0.745	0.637	0.716	Shami

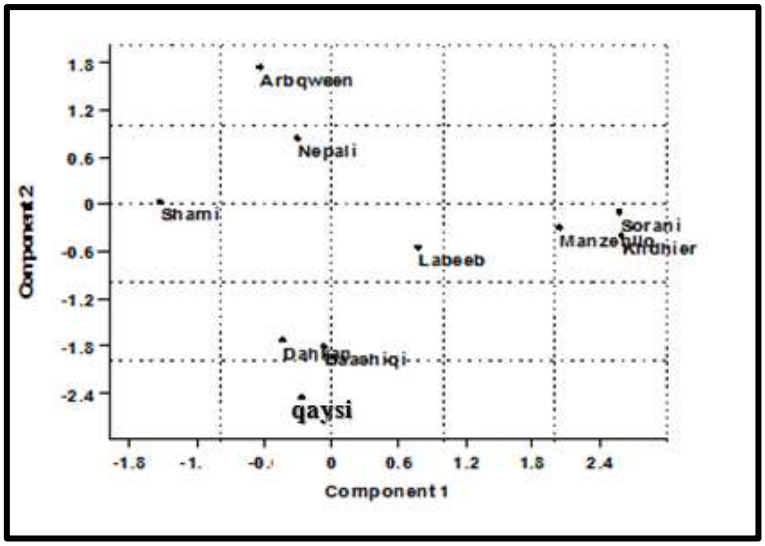

Figure 3:Depict a PCA of 10 olive cultivars as revealed by Microsatellite analysis

Conclusions

Microsatellite markers represent the easiest and cheapest markers for olive genetic fingerprinting and have been the tool of choice for studying the genetic diversity of this crop in Iraq, to resolve cases of homonymy and synonymy among the commercialized varieties, to identify rare cultivars, to improve knowledge about the genetic variability of this crop, toApplications of Microsatellite Markers for the Characterization of Olive Genetic Resources of Iraq.. Some of Microsatellite markers were suitable for mapping the genome, while the other were informative markers to olive genome, a high degree of polymorphism in the olive germplasm with in 99.16%. The dendrogram depend on SSR markers shown two main distinct groups . the similarity and differences between varieties of olives are not related with their geographical origin. Microsatellite has a better molecular marker than other molecular technique for separate and detecting genetic relationship among cultivars and ability to document the occurrence of and inspect the outcomes of hybridization in plants .

Acknowledgement

In this work the authors would like to thank The Ministry of Agriculture, Iraq and ICARDA, Aleppo, Syria. for the excellent technical assistance, and acknowledge Biotechnology Institute for Postgraduate Studies, University of Baghdad for their assistance, guidance, and hosting the research activities within their facilities.

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ISSN: 2790-5306 (Print), 2790-5314 (Online)
مجلة الزراعة العراقية البحثية - وزارة الزراعة
متاح على الانترنت: www.ijarmoa.gov.iq
مجلد 28 العدد (1) 2024
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الملخص

حددت المسافة الجينية بين عشرة اصناف زيتون بواسطة مؤشرات المايكروستيلات على مستوى DNA في محافظتي بغداد والموصل. تم إجراء مصفوفة المسافة الجينية و UPGMA و NJ dendrograms و PCA، تم التأكد من تقدير العلاقة الوراثية بين أصناف الزيتون بدقة من خلال هذه المؤشرات، **اذ درست باستخدام خمسة عشر موقعا من مواقع Simple sequence repeat اذ اظهرت 243 قطعة مضخمة منها مئتان وواحد واربعون من هذه المواقع (99.16%) كانت متعددة الاشكال**. أنتجت خمس بادئات نطاقات متعددة الأشكال كافية لمعرفة التنوع الجيني بين الأصناف. واظهرت النتائج ان صنف الشامي هو عبارة عن هجين من صنفي اربكوين والنيبالي، بينما اظهر صنف الصوراني ومنزنيلوا تشابهاً كبيراً وبنسبة 100% وهذا يدل على انه ينتمي الى الاصل نفسه. سيكون إنشاء علاقة وراثية بين هذه الأصناف مناسبًا لاستخدامها في دراسات التحسين الوراثي المستقبلية في أصناف الزيتون.

الكلمات الدالة: التكرارات المترادفة البسيطة، تفاعل البوليمر المتسلسل، شجرة النسب، متعدد الاشكال، زيتون

[1] **دائرة البستنة، وزارة الزراعة، بغداد، العراق.**

تاريخ تسلم البحث: 26/شباط/2023
تاريخ قبول البحث: 3/تموز/2023
متاح على الانترنت: 25/تموز/2024