RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
57 (6) 2684-2690, 2025
http://doi.org/10.54910/sabrao2025.57.6.41
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

MOLECULAR STUDY OF THE OLIVE (*OLEA EUROPAEA* L.) GROWN IN CENTRAL AND SOUTHERN IRAQ

F.H.R. AL-MIAHY[1]* and I.H.M. AL-JAF[2]

[1]Faculty of Agriculture and Marshes, University of Thi-Qar, Nasiriyah, Iraq
[2]Department of Horticulture, College of Agriculture, University of Al-Anbar, Ramadi, Iraq
*Corresponding author's email: Falah-ha@utq.edu.iq
Email address of co-author: idresshussein@uoanbar.edu.iq

SUMMARY

The research dealt with olive (*Olea europaea* L.) cultivars cultivated in the central and southern regions of Iraq by using the ISSR technique. It aimed to determine the degree of relatedness and draw the phylogenetic tree of the studied cultivars using nine primers. The study also used ISSR indicators in recognizing the relationships among the cultivars. The primers used gave 68 bands, showing the 56 formed had multiformats with an average of 82% for the multi-banding format. Moreover, the primers used succeeded in giving polymorphism among the resulting bandings, as the highest percentage of polymorphism reached 90% in the primers CT7CG, while the primer AG10G did not give more than 71.3% of polymorphism. It indicated that the highest percentage of similarity corresponding to the least genetic dimension (0.80) was between the two types—Shami and Qaisi, and Shami and Satakatrina—which were the most distant genotypes from the rest of the varieties. In conclusion, the study successfully utilized the ISSR technique to assess the genetic relationships among olive cultivars in central and southern Iraq. Hence, it provides useful information in understanding the genetic structure of olive cultivars in the region and could benefit as a guide for future breeding programs and the conservation of genetic resources programs.

Keywords: Olive (*O. europaea* L.), cultivars, genetic structure, ISSR technique, phylogenetic tree, primers CT7CG, polymorphism

Key findings: The ISSR technology effectively distinguished olive (*O. europaea* L.) genotypes and revealed their genetic relationship and the degree of genetic kinship between the genotypes.

Communicating Editor: Dr. A.N. Farhood

Manuscript received: August 04, 2023; Accepted: August 03, 2025.

Citation: Al-Miahy FHR, AL-Jaf IHM (2025). Molecular study of the olive (*Olea europaea* L.) grown in Central and Southern Iraq. *SABRAO J. Breed. Genet.* 57(6): 2684-2690. http://doi.org/10.54910/sabrao2025.57.6.41.

INTRODUCTION

Olive (*Olea europaea* L.) is one of the evergreen subtropical fruit trees that belong to the olive family, Oleaceae. The olive tree has become one of the important and blessed trees, being mentioned well in all monotheistic religions. It has been a symbol of goodness and peace since the beginning of humanity. This tree lives for hundreds of years (Hashmi *et al.,* 2015).

Most studies indicate that the original home of this tree is the Mediterranean Basin Region, with an imaginary line representation passing through Northwestern Iraq, Southern Turkey, Syria, Lebanon, and Palestine. Confirming this was the presence of wild and natural orchards in the mountainous region in Northwestern Iraq (Barazani and Dunseth, 2023; Besnard *et al*., 2015). Olive cultivation is widespread. In the warm temperate world, its characteristics comprised a root system deep in the soil and the presence of a thick waxy layer, which helped it to resist harsh climatic conditions (Fabbri *et al.,* 2024).

An estimated 2,629 olive cultivars usually exist worldwide, and because of the differences in their sources and growing conditions, Iraqi olives have a high level of genetic variety (Haouane *et al*., 2011). This is because of their numerous cultivars, their genotypes, and the problem of naming them given that many of them are similar in morphological characteristics (Motaghi *et al.,* 2012). These characteristics, as affected by the prevailing environmental factors, lead to difficulty in the outward distinction between cultivars (Wjhani, 2004), requiring these taxonomic studies to have the support of biotechnology use (Riccardo *et al.*, 2002).

The inter simple sequence repeat (ISSR) technology has become a highly effective strategy by combining the advantages of the random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR). Its distinction comes from the use of primers designed to target repetitive DNA sequences, granting it superior precision in differentiating genetic structures and greater result consistency compared with the RAPD technique (Verma *et al.*, 2017). The effectiveness of ISSR technology in determining the genetic diversity of wild olive was evident in a study by Kattmah *et al*. (2011) on olive plants using ISSR markers. The results showed wide genetic variation among the studied cultivars, offering important new information about their genetic relationships and adding knowledge of their genetic structure. Using SSR markers, Abozaid and Fattah (2024) found little molecular variation across geographical regions and a high degree of genetic similarity between local and foreign olive cultivars.

This study aimed to investigate the genetic diversity and relationships among six olive cultivars (*Olea europaea* L.) cultivated in specific regions of Iraq, using inter simple sequence repeat (ISSR) markers. Genomic DNA extraction came from leaf samples, with concentrations ranging from 150 to 400 µg and purity values between 1.6 and 1.9. The success of the PCR amplification reached validation through the agarose gel electrophoresis (Al-Obeide and Al-Assie, 2024). These molecular markers provided a reliable approach for generating genetic fingerprints and assessing the genetic distances among the studied cultivars.

MATERIALS AND METHODS

Plant material and DNA extraction

Collection of plant samples came from the leaves of six olive (*O. europaea* L.) cultivars (Qaisi, Khudairi, Nepali, Manzilo, Satakatrina, and Shami), grown in different locations across the Babylon, Najaf, Dhi Qar, and Wasit governorates of Iraq. The collected leaves were free from insect and disease infections. Genomic DNA extraction continued from these cultivars using the CTAB method (Doyle and Doyle, 1987).

DNA amplification

The DNA resulting from the extraction of the studied cultivars reached amplification using nine ISSR primers, where the final volume in the tube was 25 µl. It included distilled water (16.05 µl), polymerase enzyme (0.2 µl), and

the mixture of nucleotide triphosphates (10 mM dNTPs) with a volume of 0.5 µl. Additionally, the primer used (10P g/mL) had a volume of 2.5 µl, MgCl 50 mM in a volume of 0.75, 2.5 volume of 10X buffer, and a volume of DNA 10 ng/mL in a 2.5 µl volume. Afterward, the amplification results underwent separation on a 5.1% agarose gel in a 1XTBE electrophoresis device. The gel staining used a solution of ethidium bromide with continuous stirring for half an hour before being evaluated in a UV apparatus to document the results (Pu *et al.,* 2023).

Statistical analysis

Molecular characterization data collected underwent conversion into digital formats by giving the number '1' if a trait or band is present and the number '0' in the absence of it. The readings entailed submission to the NTSYS statistical program **(**Zietkiewicz *et al*., 1994), with the cluster analysis conducted, which depends on the percentage of genetic similarity through the UPGMA (unweighted pair group method with Arithmetic Mean) method for drawing the kinship tree between categories.

RESULTS AND DISCUSSION

The ISSR indices showed a significant variation in the replication patterns, depending on the primer used, due to differences in the number of complementary binding sites on the genome of each olive (*O. europaea* L.) genotype. These differences influenced the presence and quantity of amplification bands. Analysis of genetic relationships relied on identifying the presence or absence of these bands, which resulted from duplications of specific gene segments (Figure 1).

The molecular weights of the bands, as determined by the number and distribution of complementary primer sites on the DNA strand, provided insights into genetic variation among the selected plants. This approach highlights how variations in primer binding sites among different plants reveal genetic distinctions, as reflected in the unique or shared band patterns generated by each primer. This method is essential for comprehending the evolutionary linkages and genetic variation within the plant species under study.

Polymorphism

The data obtained using nine ISSR primers proved their efficiency in detecting genetic variations between different cultivars, with the variations as non-existent between genotypes belonging to the same group (Table 1). The primers used gave 68 bands, of which 56 were polymorphic, and the percentage of bandings was with a polymorphism of 82%. This indicates the existence of genetic divergence between the olive cultivars used in this study. Bandings (five) appeared during migration of samples on a polyacrylamide gel. This variation in the number of bandings resulting from each

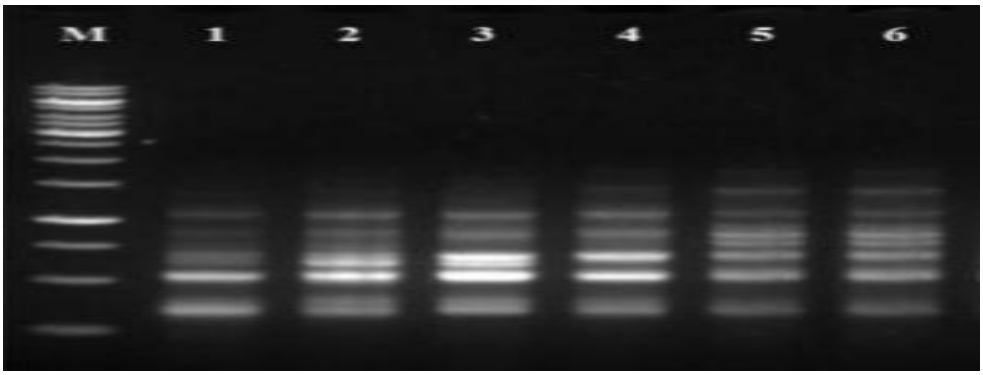

Figure 1. Gelation resulting from migration when using primers.

Table 1. Number of bands and polymorphisms of the primers used.

Primers	Pack number	Polymorphism	Polymorphism (%)
(CGA)5	8	7	87.5
(GACA)4	9	8	88.8
(CA) 8(GT)	7	5	71.4
AG10G	6	5	71.3
CT8AC	5	3	83.3
AG10T	7	5	71.4
CT7CG	10	9	90.0
AGG6	7	6	85.7
CGA10	9	8	88.8
Total	68	56	--
Average	7.55	6.22	82.0

Table 2. Nucleotide sequences and binding temperature of the linked primers.

Primers	Nucleotide sequence	Temperature (°C)
ISSR 1	(CGA)5	51
ISSR 2	(GACA)4	55.3
ISSR 3	(CA) 8(GT)	41
ISSR 4	AG10G	43
ISSR 5	CT8AC	42
ISSR 6	AG10T	40
ISSR 7	CT7CG	41
ISSR 8	AGG6	42
ISSR 9	CGA10	38

pair of primers used depends on the compatibility of the initiator link with the plant genome. Likewise, the components of each initiator of the nitrogenous bases and the different sequence of the bases of the primers used lead to a variation in the number of bandings resulting from the primers used (Vos *et al*., 1995). Despite this discrepancy in the number of bandings, the primers succeeded in giving polymorphism among the resulting bandings, as the highest percentage of polymorphism reached 90% in the primers CT7CG, whereas the lowest was notable with primer AG10G (71.3%). This discrepancy in percentages is due to the different sequences Table 2. The bases in the plant genome, which affect the primer binding sites, revealed this difference is because of the rearrangement, linkage, or genetic crossing over, among others. Researchers saw divergent bandings that appear clearly on the gel or did not appear in certain sites on the gel (Muzzalupo *et al.,* 2018).

Determination of genetic affinity among studied genotypes

The genetic distance between the cultivars under study incurred calculation based on the formula by Powell *et al.* (1996). The results showed the extent of similarity and difference between the pure strains, indicating the highest percentage of similarity corresponding to the least genetic dimension (0.80) was between the two types, Shami and Qaisi. Meanwhile, the lowest percentage of genetic similarity that corresponds to the largest genetic dimension is 0.11 between the two cultivars Satakatrina and Al-Qaisi, followed by 0.12 between the two cultivars Shami and Satakatrina. They were the most distant genotypes from the rest of the varieties, as shown in Table 3. The high genetic similarity between Shami and Qaisi may result from shared evolutionary origins, geographic proximity, or historical breeding practices that selected for similar traits, such as drought

Table 3. Genetic similarity matrix of six olive cultivars using ISSR markers.

Genotypes	Qaisi	Khudairi	Nepaly	Menzilo	Satakatrina	Shami
Shami	0.80	0.33	0.25	0.37	0.12	1
Satakatrina	0.11	0.28	0.37	0.50	1	
Menzilo	0.50	0.22	0.62	1		
Nepaly	0.37	0.25	1			
khudairi	0.50	1				
Qaisi	1					

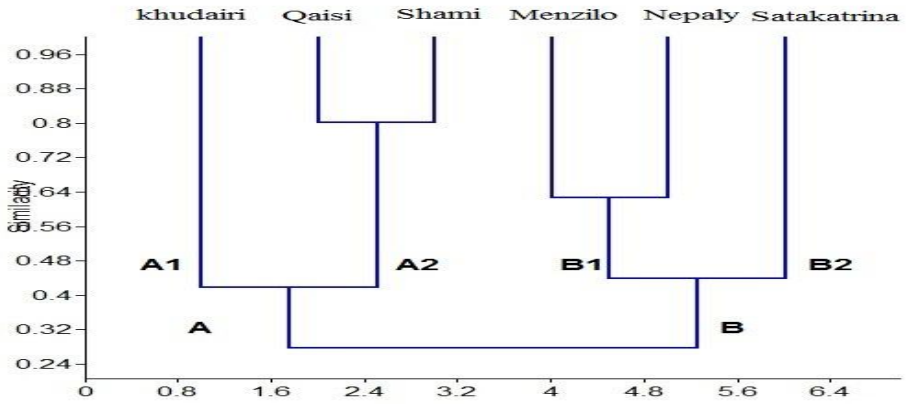

Figure 2. Genetic kinship tree between the studied olive cultivars.

tolerance, disease resistance, or grain quality. Such similarities are often evident in cultivars that have undergone cultivation within similar environmental and agronomic conditions. According to previous studies, cultivars adapted to comparable climatic and soil conditions typically exhibit convergent genetic traits (Chen *et al.,* 2021)**.**

The low genetic similarity between Satakatrina and other cultivars, like Qaisi and Shami, suggests significant genetic divergence. This could refer to distinct evolutionary paths, geographical isolation, or adaptation to unique environmental conditions. Satakatrina might have been coming from a different gene pool or subjected to breeding practices that emphasized distinct traits, such as high resistance to specific pests or adaptability to unique stress conditions. Additionally, hybridization with wild relatives or exposure to different selective pressures may have further contributed to this genetic distance (Kumar *et al.,* 2020; Singh *et al.,* 2022).

Cluster analysis

Cluster analysis allows the studied genotypes to undergo division into groups that reflect the degree of genetic kinship among them. Samples may achieve clustering into one group based on their place of origin or according to their origin and lineage. The dendrogram depended on genetic dimension values using the UPGMA method (Figure 2). The construction relied on the results of indicators among simple sequence repeats (ISSR) showing distribution of the six genotypes into two main groups (A and B). The study noted the division of group A into two main branches that included the first, A1, with the Al-Khudairi variety, while the second branch, A2, included two cultivars (Shami and Al-Qaisi). As for cluster B, it broke into two main branches, including the first, B1 (Nepali and Manzilo), and the second branch, B2 (Satakatrina). A research conclusion reported a molecular study showed the pomegranate genotypes under

study contained high genetic diversity. These results can serve as basic material in evaluating these genotypes, establishing an identity for them, and preserving them in genetic complexes. The results of the study agreed with those of Mhawesh *et al*. (2021), Tanhaei *et al.* (2023), Bornet *et al.* (2023), Abozaid and Fattah (2024), and Gago *et al.* (2024).

CONCLUSIONS

The application of ISSR technology helped in separating and distinguishing the studied olive (*O. europaea* L.) phenotypes and determining the degree of genetic kinship between them. These results will be useful in breeding and improvement programs and in studying these phenotypes to form genetic maps for each of them.

REFERENCES

Abozaid KJ, Fattah YM (2024). Genetic diversity of olive varieties in northern Iraq using microsatellite markers. *Egyptian J. Bot*. 64(1): 419–429.

Al-Obeide AA, Al-Assie AH (2024). Analysis of genetic diversity of some olive cultivars *Olea europaea* L. using ISSR, SSR. *J. Prev. Diagnosis Manag*. Hum. 4(6). https://doi.org/10.55529/jpdmhd.46.33.45

Barazani O, Dunseth P (2023). Genetic diversity and origins of wild and cultivated olive trees in the Mediterranean basin. *Front. Plant Sci.* 14: 1131557. https://doi.org/ 10.3389/fpls.2023.1131557

Besnard G, Bervillé A, Khadari B, Baradat P, Petit RJ (2015). Genetic structure of *Olea europaea* L. (olive) populations: Geographic patterns and conservation implications. *New Phytol*. 206(2): 587–601. https://doi.org/10.1111/ nph.13181

Bornet B, Slobodova N, Sharko F, Gladysheva-Azgari M, Petrova K, Tsiupka S, Tsiupka V, Tsygankova S (2023). Genetic diversity of common olive (*Olea europaea* L.) cultivars from Nikita botanical gardens collection revealed using RAD-seq method. *Genes* 14(7): 2–14.

Chen Y, Li Y, Wang L (2021). Genetic diversity in crop varieties: Mechanisms and applications. *Plant Sci. J*. 58(3): 112–124.

Doyle JJ, Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull*. 19: 11–15.

Fabbri A, Luciana B, Tiziano C, Franco F (2024). The Olive: Botany and Production. CABI Publ. 129 pp.

Gago P, Boso S, Santiago J, Martínez M (2024). Identification and characterization of relict olive varieties (*Olea europaea* L.) in the Northwest of the Iberian Peninsula. *Horticulturae* 10: 175.

Haouane H, El Bakkali A, Moukhli A, Tollon C, Santoni S, Oukabli A, El Modafar C, Khadari B (2011). Genetic structure and core collection of the World Olive Germplasm Bank of Marrakech: Towards the optimised management and use of Mediterranean olive genetic resources. *Genetica* 139: 1083–1094.

Hashmi MA, Khan A, Hanif M, Farooq U, Perveen S (2015). Traditional uses, phytochemistry, and pharmacology of *Olea europaea* L olive. *Evid.-Based Complement. Altern. Med.* 129 pp.

Kattmah G, Hamed F, Makhool S (2011). Studying the genetic relationship of some cultivated wild olive types in Mesiaf region by using ISSR technique. *Jordanian J. Agric. Sci*. 7(1): 139–148.

Kumar V, Mishra S, Reddy K (2020). Advances in plant breeding: Enhancing genetic diversity. *Int. J. Plant Sci*. 75(2): 98–115.

Mhawesh HH, Radi FH, Radhi MN (2021). Response of rosemary plant to the effect of NANO-NPK fertilizer and biological factors and their effect on the active substances. *Int. J. Agric. Stat. Sci*. 17: 1771–1777.

Motaghi L, Mazinani SM, Jabari H (2012). Evaluation of genetic diversity in some Iranian olive cultivars using RAPD markers. *Plant Ecol. Syst*. 33: 91–104.

Muzzalupo I, Muto A, Badolati G, Veizi A, Chiappetta A (2018). Genotyping of Albania olive (*Olea europaea*) germplasm by SSR molecular marker. *Emirates J. Food Agric*. 30(7): 573–580.

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed*. 1(2): 225–238.

Pu Y, Wang P, Xu J, Yang Y, Zhou T, Zheng K, Sun G (2023). Overexpression of the *Caragana korshinskii* com58276 gene enhances

tolerance to drought in cotton (*Gossypium hirsutum* L.). *Plants* 12(5): 3–11.

Riccardo L, Giorgio V, De Giovanni C, Lotti C, Gallotta A, Fanizza G (2002). The genetic of Apulian apricot genotypes (*Prunus armeniaca* L.) assessed using AFLP markers. *Cell Mol. Biol. Lett*. 7(2): 431–436.

Singh P, Sharma R, Gupta S (2022). Role of genetic divergence in crop improvement. *J. Agron. Crop Sci.* 208(4): 567–579.

Tanhaei A, Dadras AR, Sabouri H, Alamdari EG, Sajadi SJ, Moghaddam HH (2023). Evaluation of genetic diversity in olive germplasm (*Olea europaea* L.) using image processing and molecular markers based on physical properties of fruits and stones. *Int. J. Hortic. Sci. Technol*. 10(2): 173–192.

Verma KS, Ul Haq S, Kachhwaha S, Kothari SL (2017). RAPD and ISSR marker assessment of genetic diversity in *Citrullus colocynthis* (L.) Schrad: A unique source of germplasm highly adapted to drought and high-temperature stress. *3 Biotech*. 7: 288.

Vos P, Hogers R, Bleeker M, Reijans M (1995). AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res*. 23: 4407-4414. http://dx.doi.org/10.1093/nar/23.21.4407Wjhani ZY, Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M (2004). Genetic studies on the biodiversity of local and wild Syrian wheat using modern biotechnological techniques. Ph.D. Thesis, Department of Genetics, Cairo Univ., Egypt, 119 pp.

Zietkiewicz E, Rafalski A, Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20: 176–183. http://dx.doi.org/10.1006/geno.1994.1151