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DNA-based assessment of genetic diversity of olive genotypes using RAPD molecular markers

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Abstract

Objective

The olive tree (Olea europaea L.), a cornerstone of Mediterranean agriculture, is one of the oldest cultivated fruit species, belonging to the Oleaceae family. Global request for olive oil continues to rise, necessitating enhanced production through the investigation and conservation of genetic diversity. Random Amplified Polymorphic DNA (RAPD) markers offer a simple, rapid, cost-effective, and reproducible method to evaluate genetic variation at both intergeneric and intrageneric levels. This investigation aimed to evaluate the genetic diversity and relationships among olive genotypes in Iraq applying RAPD molecular markers to support breeding and conservation efforts.

Materials and methods

Managed in 2023 at the Biotechnology Laboratories, Faculty of Agriculture, University of Baghdad, this investigation analyzed ten olive genotypes; Arbequina, Ashrasy, Zaity, Efreny, Leccino, Gemlik, Koroneiki, Dahkan, Alshamy, and Frantoio gathered from diverse geographical regions of Iraq. Genomic DNA was extracted from fresh leaf samples, and genetic diversity was evaluated applying ten RAPD primers: OPA1, OPA8, OPB8, OPC3, OPC7, OPJ5, OPR2, OPV1, OPX4, and OPY3. Polymerase chain reaction (PCR) amplification products were analyzed via gel electrophoresis to identify polymorphic bands.

Results

The ten RAPD primers generated a total of 98 bands, with OPX4 producing the highest number of bands (13) and OPA1 and OPC3 the lowest (7 each), averaging 9.8 bands per primer. Genetic similarity analysis revealed the closest relationship between Dahkan and Koroneiki, with a similarity coefficient of 0.714, denoting low genetic distance. Cluster analysis, based on the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), categorized the genotypes into

three main clusters: Cluster 1 (Arbequina, Alshamy, Dahkan, Koroneiki, Ashrasy), Cluster 2 (Leccino, Zaity, Efreny), and Cluster 3 (Gemlik, Frantoio). These groupings reflect distinct genetic profiles among the genotypes.

Conclusions

This investigation underscores meaningful genetic diversity among Iraqi olive genotypes, providing critical insights for breeding programs aimed at improving yield and resilience. The identified genetic relationships and clustering patterns support targeted germplasm conservation strategies to preserve valuable genetic resources for sustainable olive production in Iraq.

Keywords: genetic diversity, germplasm conservation, Olea europaea, olive genotypes, RAPD markers

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Introduction

The olive tree, *Olea europaea* L., is an evergreen species of profound economic, cultural, and ecological significance in temperate and subtropical regions worldwide. As a member of the Oleaceae family, which comprises 30 genera and approximately 600 species, the genus *Olea* is distinguished by the olive's prominence as a fruit-bearing tree (Brake et al., 2014; Genaidy et al., 2015). Globally, an estimated 2,629 olive varieties exist, with over 40 documented in Iraq, reflecting the species' extensive genetic diversity. Historical and genetic evidence points to the Mediterranean basin—spanning northwestern Iraq, southern Turkey, Syria, Lebanon, and Palestine—as the likely origin of the olive, supported by the presence of wild and natural orchards in the mountainous regions of northwest Iraq (Belaj et al., 2007; Brake et al., 2014). The olive's capability to thrive in challenging climates is attributed to its deep root system and a thick waxy

leaf cuticle, which enhance its resilience to drought and other harsh environmental situations (Fabbri et al., 2024). This adaptability has made olive cultivation a cornerstone of agriculture in warm temperate regions, contributing to food security, economic stability, and cultural heritage in Mediterranean and Middle Eastern societies. Conservation of olive genetic resources is essential for sustaining this diversity and supporting breeding programs aimed at improving yield, disease resistance, and environmental adaptability. A global network of 23 national and international germplasm banks, coordinated by the International Olive Council, plays a pivotal role in preserving olive genetic material (Navero, 2000). These repositories are critical for managing germplasm resources and facilitating research to enhance olive cultivation practices (Golmohammadi et al., 2019). For example, Maiuf and Al-Mayahi (2023) managed a comprehensive investigation on 14 olive varieties in Iraq, revealing meaningful genetic and phenotypic differences. Their results were applied to construct a phylogenetic tree, illustrating the genetic relationships among these varieties. Molecular markers, which are unaffected by environmental variables, are the preferred tool for evaluating genetic diversity due to their reliability and precision (Khanam et al., 2012). Genetic diversity is fundamental for developing superior cultivars, preserving existing populations, advancing evolutionary processes, and enabling adaptation to changing environmental situations, like climate variability and emerging pests (Javanmard et al., 2008; Mohammadabadi et al., 2021a). Additionally, the identification of gene polymorphisms is crucial for detecting genetic predispositions to diseases and informing targeted breeding strategies (Mohammadabadi, 2016; Saadatabadi et al., 2023). Molecular techniques are also invaluable for characterizing populations and breeds, providing insights into their genetic structure and supporting conservation efforts (Mohammadifar & Mohammadabadi, 2017; Noori et al., 2017; Mohammadinejad et al., 2022). Effective conservation of genetic diversity needs robust strategies informed by comprehensive data on population structures, containing diversity within and among populations and breeds (Mohammadifar & Mohammadabadi, 2018; Mohammadabadi et al., 2024a). Genetic diversity is a cornerstone of genetic improvement, population preservation, and adaptation to diverse environmental challenges, like soil degradation and climate exchange (Sulimova et al., 2007; Mohammadabadi et al., 2024b). Furthermore, characterizing gene polymorphisms is critical for identifying genotypes related to immune system functionality, disease resistance, or susceptibility, which are vital for developing resilient olive cultivars (Mohammadabadi et al., 2010; Mohammadabadi et al., 2021b; Shokri et al., 2023). These efforts are particularly important in regions like Iraq, where olive cultivation faces challenges from environmental stressors and the need for sustainable agricultural practices. Polymerase chain reaction (PCR)-based methods are broadly utilized for characterizing plant genetic resources due to their precision and versatility. Among these,

Randomly Amplified Polymorphic DNA (RAPD) is a straightforward, cost-effective, and rapid technique that employs short oligonucleotide primers of arbitrary sequence under low-stringency PCR conditions to amplify discrete DNA fragments, which serve as molecular markers (Singh et al., 2006). RAPD analysis needs no prior knowledge of the target genome, relying on the presence of priming sites on both DNA strands close enough to enable amplification. Its advantages include ease of primer apply, random selection, and extensive genomic coverage, making it an effective tool for evaluating genetic heterogeneity based on DNA sequence variation. Despite concerns about reproducibility, RAPD remains broadly applied in molecular biology for diverse applications, containing cultivar identification and genetic diversity investigations (Pan & Chen, 2010). Previous research has demonstrated the utility of RAPD markers in olive investigations. Rawashdseh (2003) employed RAPD markers to detect genetic variation in mutants of Zaity olive cultivars. Similarly, Hassawi and Hadeib (2004) and Hadeib and Hassawi (2006) identified genetic variability in Jordanian olive cultivars applying RAPD markers. Brake et al. (2014) analyzed 13 Jordanian olive (*Olea europaea* L.) cultivars applying RAPD and Inter-Simple Sequence Repeat (ISSR) markers, generating 156 RAPD and 85 ISSR reproducible markers, with polymorphism rates of 55% and 58%, respectively. They identified eight RAPD and three ISSR cultivar-specific markers, with 39 RAPD markers distinguishing 10 cultivars and 12 ISSR markers distinguishing six cultivars. Their results affirmed that RAPD and ISSR markers, when applied in combination, are powerful tools for olive cultivar identification, providing accurate characterization and valuable data for breeding strategies. This investigation addresses a critical gap in the characterization of olive genetic diversity in Iraq, where diverse agroecological situations necessitate robust cultivar identification and conservation strategies. By employing RAPD technology, we aim to characterize the genetic diversity of 10 olive cultivars grown across different regions of Iraq and identify unique molecular markers that serve as genetic fingerprints for each cultivar. These markers will enable precise cultivar identification, support the development of improved breeding programs, and contribute to the sustainable management of olive genetic resources, ultimately enhancing the resilience and productivity of olive cultivation in Iraq and similar regions.

Materials and methods

Plant material and DNA extraction: This investigation was managed in 2023 at the Biotechnology Laboratories, Faculty of Agriculture, University of Baghdad, Iraq. Ten olive (*Olea europaea* L.) genotypes were selected to evaluate genetic diversity and determine genetic relationships among them applying Random Amplified Polymorphic DNA (RAPD) markers.

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The genotypes were chosen to represent diverse geographical origins within Iraq, ensuring a broad representation of genetic variability. Leaf samples were gathered directly from mature trees in numerous field locations across Iraq to capture regional differences in cultivation practices and environmental situations (Table 1) (Doyle, 1991). The selection of these genotypes was based on their economic importance for olive oil and table olive production, as well as their prevalence in Iraqi agriculture, providing a robust dataset for genetic analysis.

Table 1. Distribution of olive genotypes by country of origin, cultivation purpose, and sample collection sites

No.	English Name	Country of Origin	Purpose of Cultivation
1	Arbequina	Spain	Olive oil
2	Ashrasy	Local (Iraq)	Table olives and olive oil
3	Zaity	Palestine	Olive oil
4	Efreny	Syria	Olive oil
5	Leccino	Spain	Olive oil
6	Gemlik	Syria	Table olives
7	Koroneiki	Spain	Table olives and olive oil
8	Dakhan	Local (Iraq)	Table olives
9	Alshamy	Syria	Table olives and olive oil
10	Frantoio	Italy	Table olives

DNA extraction method: Total genomic DNA was extracted from fresh, healthy leaves applying a modified Cetyltrimethylammonium Bromide (CTAB) method, chosen for its effectiveness in isolating high-quality DNA from plant tissues rich in polysaccharides and polyphenols (Doyle & Doyle, 1987). Approximately 1 g of leaf tissue was ground to a fine powder in liquid nitrogen to ensure cell disruption and minimize DNA degradation. The powder was suspended in 3 mL of preheated extraction buffer (100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 2% w/v CTAB, 2% v/v 2-mercaptoethanol) and incubated at 65°C for 30 minutes to facilitate cell lysis and protein denaturation. DNA was purified applying a chloroform-octanol (24:1) extraction to remove proteins and other contaminants. The aqueous phase was gathered, and DNA was precipitated with 70% ethanol, washed, and resuspended in 200 µL of TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 0.2 mg/mL RNase A) to remove residual RNA. DNA quality and quantity were evaluated applying a spectrophotometer by

measuring the absorbance ratio at A260/A280, with ratios between 1.8 and 2.0 denoting high purity. DNA concentrations were adjusted to 20 ng/µL for PCR, and stock DNA was stored at -20°C to maintain integrity for subsequent analyses.

RAPD analysis: RAPD analysis was carried out applying 10 decamer oligonucleotide primers (Table 2) synthesized by Invitrogen (USA). These primers were selected based on their proven efficacy in detecting polymorphism in olive genotypes, as announced in previous investigations (Tisarum et al., 2020; Brickner et al., 1996; Falakro et al., 2022; Ma et al., 2004; Deng et al., 2006). PCR was managed in a 20 µL reaction volume containing 50 ng of genomic DNA (3.2 µL), 1.2 µL of 2 mM dNTPs (dATP, dGTP, dCTP, dTTP), 2 µL of 5 µM primer, 2 µL of 10X PCR buffer, 0.16 µL of Taq DNA polymerase (5 U/µL), and 11.44 µL of deionized water, following the protocol optimized by Tisarum et al. (2020). Amplification was carried out in an Advanced Primus-96 Thermal Cycler (MWG AG BIOTECH, USA) with the following conditions: an initial denaturation at 94°C for 4 minutes, followed by 40 cycles of 30 seconds at 94°C (denaturation), 1 minute at 35-50°C (annealing, as specified in Table 2), and 2 minutes at 72°C (extension), with a final extension at 72°C for 7 minutes (Table 3). Amplification products were separated on 1.0% agarose gels, stained with ethidium bromide, and visualized under UV illumination. A photographic record was maintained for band analysis. To ensure reproducibility, each PCR reaction was carried out in duplicate, and only consistent bands were scored.

Table 2. RAPD primers applied for analysis of ten olive genotypes

Primer	Sequence (5'-3')	Annealing Temperature (°C)	Reference
OPA1	CAGGCCCTTC	50	Tisarum et al., 2020
OPA8	GTGACGTAGG	50	Tisarum et al., 2020
OPB8	GTCCACACGG	50	Tisarum et al., 2020
OPC3	GGGGGTCTTT	36	Brickner et al., 1996
OPC7	GTCCCGACGA	50	Tisarum et al., 2020
OPJ5	CTCCATGGGG	37	Falakro et al., 2022
OPR2	CACAGCTGCC	35	Ma et al., 2004
OPV1	TGATCCCTGG	36	Brickner et al., 1996
OPX4	CCGCTACCGA	37	Deng et al., 2006
OPY3	ACCGCCTGCT	50	Tisarum et al., 2020

Table 3. PCR reaction conditions for analysis of ten olive genotypes

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Step	Temperature (°C)	Time	No. of Cycles
Pre-Denaturation	94	4 min	1
Denaturation	94	30 sec	40
Annealing	35-50 (Table 2)	1 min	40
Extension	72	2 min	40
Final Extension	72	7 min	1

Cluster Analysis: Reproducible and consistent RAPD bands were scored and converted into a binary matrix, where 1 indicated the presence of a band and 0 indicated its absence (Figure 1). This matrix was applied to construct a phylogenetic tree applying the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) cluster analysis, implemented in the NTSYS-pc software (Swofford, 1990). UPGMA was selected for its robustness in grouping genotypes based on genetic similarity, providing a clear visualization of relationships among the 10 olive genotypes. The resulting dendrogram was applied to evaluate genetic diversity and infer phylogenetic relationships, supporting the identification of unique molecular markers for each cultivar.

Results and discussion

Polymorphism analysis: The Random Amplified Polymorphic DNA (RAPD) markers employed in this investigation effectively generated a high level of polymorphism, enabling the elucidation of genetic relationships among 10 olive (*Olea europaea* L.) genotypes cultivated in Iraq. Understanding genetic similarity is critical for efficient germplasm management, facilitating the selection of optimal cross combinations for breeding programs and complementing agronomic evaluations (del Río & Caballero, 1994). The 10 RAPD primers (listed in Table 2, Materials and Methods) generated a total of 98 scorable markers, averaging 9.8 markers per primer (Table 4). Of these, 64 bands were polymorphic, yielding a polymorphism rate of 64.7%, which underscores meaningful genetic diversity among the genotypes. This level of polymorphism indicates that the selected primers effectively captured genetic variation, permiting differentiation of closely related cultivars. Notably, most genotypes exhibited unique bands not show in others, which can serve as molecular markers for cultivar identification, supporting applications in germplasm conservation and breeding.

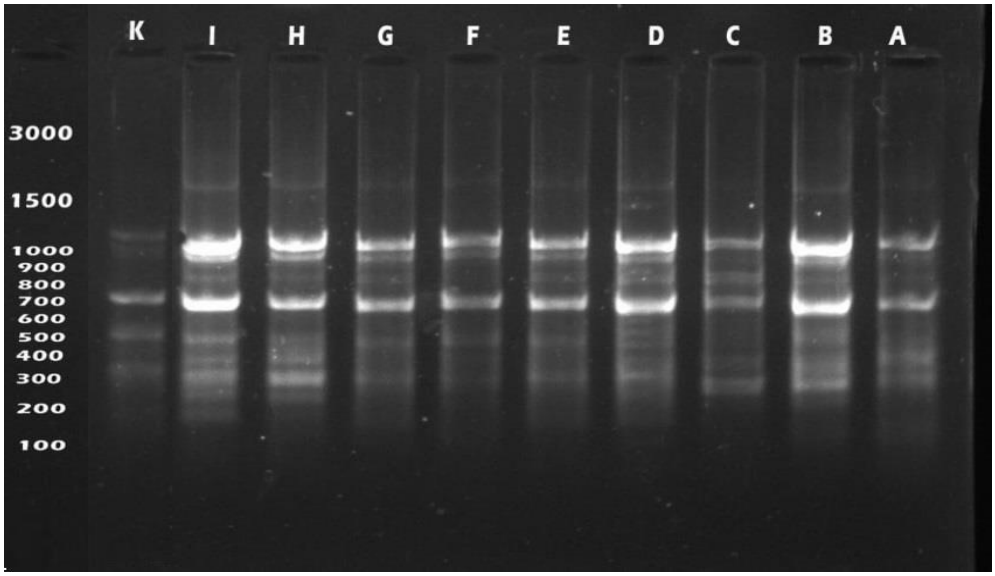

Figure 1. Electrophoretic analysis of RAPD-PCR products on a 1% agarose gel. The gel displays PCR amplification products from Random Amplified Polymorphic DNA (RAPD) analysis of nine olive (*Olea europaea* L.) genotypes, stained with ethidium bromide and visualized under UV illumination. Lane K contains a 100 bp DNA marker ladder for size reference. Lanes A-I represent the nine olive genotypes analyzed, with each lane corresponding to a specific genotype amplified applying one of the 10 RAPD primers listed in Table 2.

The variation in band numbers across primers, ranging from 7 (OPA1, OPA8) to 13 (OPX4), reflects differences in primer binding specificity to the olive genome, influenced by sequence composition and genomic structure (Vos et al., 1995). The primer OPR2 achieved the highest polymorphism rate (88%), while OPA1 and OPA8 exhibited lower rates (57% and 60%, respectively). This variability is attributed to differences in primer binding sites, which are shaped by genetic rearrangements, linkage patterns, genome size, and other molecular factors (Bradeen et al., 2001). The distinct banding patterns observed on 1% agarose gels (Figure 1) highlight the robustness of RAPD markers in detecting fine-scale genetic differences, which are critical for identifying unique genotypes for breeding and conservation purposes. The high discriminatory power of RAPD markers in olive genetic investigations aligns with previous research. Wiesman et al. (1998) demonstrated the efficacy of RAPD markers in distinguishing olive cultivars, noting their capability to detect subtle genetic variations. Similarly, Sanz-Cortés et al. (2001) screened 40 olive cultivars from Valencia, Spain, applying 18 RAPD primers, generating 34 repeatable polymorphic fragments. Their cluster analysis identified three distinct groups, primarily separated by geographical origin, with no clear correlation to morphological traits like fruit size. In our

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investigation, the 64.7% polymorphism rate suggests that RAPD markers are particularly suited for characterizing olive genotypes in Iraq, where diverse agroecological situations necessitate precise cultivar identification. The presence of unique bands in most genotypes further supports the potential of RAPD markers to develop genetic fingerprints, which can be applied to authenticate cultivars, prevent misidentification in germplasm banks, and guide breeding programs aimed at enhancing traits like disease resistance and environmental adaptability. The observed polymorphism also has implications for olive breeding in Iraq. The high genetic diversity detected suggests a rich genetic pool that can be leveraged to develop cultivars with improved yield, oil quality, or resilience to abiotic stresses like drought, which is prevalent in the region. By identifying unique molecular markers, this investigation prepares a foundation for marker-assisted selection, enabling breeders to target specific traits while preserving genetic diversity. Compared to other molecular techniques, like microsatellites or AFLP, RAPD markers offer a cost-effective and rapid approach, making them particularly valuable in resource-restricted settings like Iraqi research institutions (Sanz-Cortés et al., 2001).

Determination of genetic affinity among studied genotypes: Genetic distances among the 10 olive genotypes were calculated applying the formula proposed by Powell et al. (1996), revealing a spectrum of genetic similarities that reflect the diversity of the studied cultivars (Table 5). The highest genetic similarity (0.714) was observed between Dakhan and Koroneiki, denoting a close genetic relationship, likely due to shared regional origins or historical breeding practices in the Middle East. Conversely, the lowest similarity (0.462) was found between Frantoio and Arbequina, suggesting meaningful genetic divergence, possibly attributable to their distinct geographical origins (Italy and Spain, respectively). These results highlight the capability of RAPD markers to detect fine-scale genetic differences, which are essential for identifying unique genotypes for conservation, breeding, and cultivar authentication. The genetic similarity values prepare insights into the evolutionary and cultivation history of these genotypes. For instance, the high similarity between Dakhan (a local Iraqi cultivar) and Koroneiki (a Spanish cultivar) may reflect historical gene flow or selection pressures in similar agroecological environments. In contrast, the divergence between Frantoio and Arbequina aligns with their cultivation in distinct Mediterranean regions, where different environmental and cultural factors have shaped their genetic profiles. These results can inform breeding strategies by identifying genetically distant genotypes for hybridization to maximize heterosis and introduce novel traits, like improved oil quality or pest resistance (Hernández et al., 2001).

Table 5. Genetic Similarity Values Between 10 Olive Genotypes

	Arbequina	Ashrasy	Zaity	Efreny	Leccino	Gemlik	Koroneiki	Dakhan	Alshamy	Frantoio
Arbequina	1	0.651	0.637	0.643	0.647	0.637	0.623	0.714	0.684	0.578
Ashrasy		1	0.589	0.614	0.625	0.506	0.637	0.621	0.642	0.602
Zaity			1	0.526	0.617	0.468	0.633	0.635	0.554	0.581
Efreny				1	0.591	0.535	0.567	0.569	0.611	0.647
Leccino					1	0.473	0.703	0.550	0.565	0.512
Gemlik						1	0.600	0.625	0.506	0.512
Koroneiki							1	0.621	0.577	0.493
Dakhan								1	0.513	0.500
Alshamy									1	0.462
Frantoio										1

Cluster analysis: The dendrogram constructed applying the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) (Figure 2) illustrates the genetic relationships among the 10 olive genotypes, with the horizontal axis representing genotypes and the vertical axis denoting genetic distance. Smaller distances indicate higher similarity. Ashrasy and Koroneiki formed the closest pair, branching at a node less than 1.0, suggesting a shared genetic background, possibly due to historical cultivation or gene flow in the Middle East. Similarly, Dakhan and Alshamy clustered closely, denoting strong genetic affinity, likely reflecting their adaptation to similar environmental situations in Iraq and Syria. The genotypes were grouped into three distinct clusters (Table 6). Cluster 1 (Arbequina, Alshamy, Dakhan, Koroneiki, Ashrasy) exhibited high genetic similarity, branching from a low node, suggesting a common Middle Eastern origin or local adaptation. This cluster is valuable for investigating regional diversity and identifying traits suited to Iraqi agroecosystems. Cluster 2 (Leccino, Zaity, Efreny) showed moderate similarity, branching from a higher node, potentially reflecting hybridization or dispersal across Mediterranean regions, as Leccino and Efreny are related to Spain. Cluster 3 (Gemlik, Frantoio) was genetically distinct, branching independently, denoting unique genetic diversity that could be leveraged to introduce novel traits, like disease resistance or distinct oil profiles, in breeding programs. These clustering patterns are consistent with previous investigations. Belaj et al. (2007) analyzed wild olive populations in the northwestern Mediterranean applying microsatellite markers, identifying four gene pools influenced by geographical and climatic factors, with evidence of admixture due to hybridization with cultivated olives. Similarly, Belaj et al. (2002) studied 51 Mediterranean olive cultivars applying 190 RAPD fragments, result clustering by geographical origin, affirmed by chi-square distance (Benzkeri, 1970) and UPGMA analysis.

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Table 6. Genetic Similarity Among Olive Genotypes

Group	Genotypes	Genetic Similarity	Notes
Cluster 1	Arbequina, Alshamy, Dakhan, Koroneiki, Ashrasy	High	Local diversity or common Middle Eastern origin
Cluster 2	Leccino, Efreny, Zaity	Moderate	Possible hybridization or Mediterranean origin
Cluster 3	Gemlik, Frantoio	Low	Genetically distant, suitable for breeding

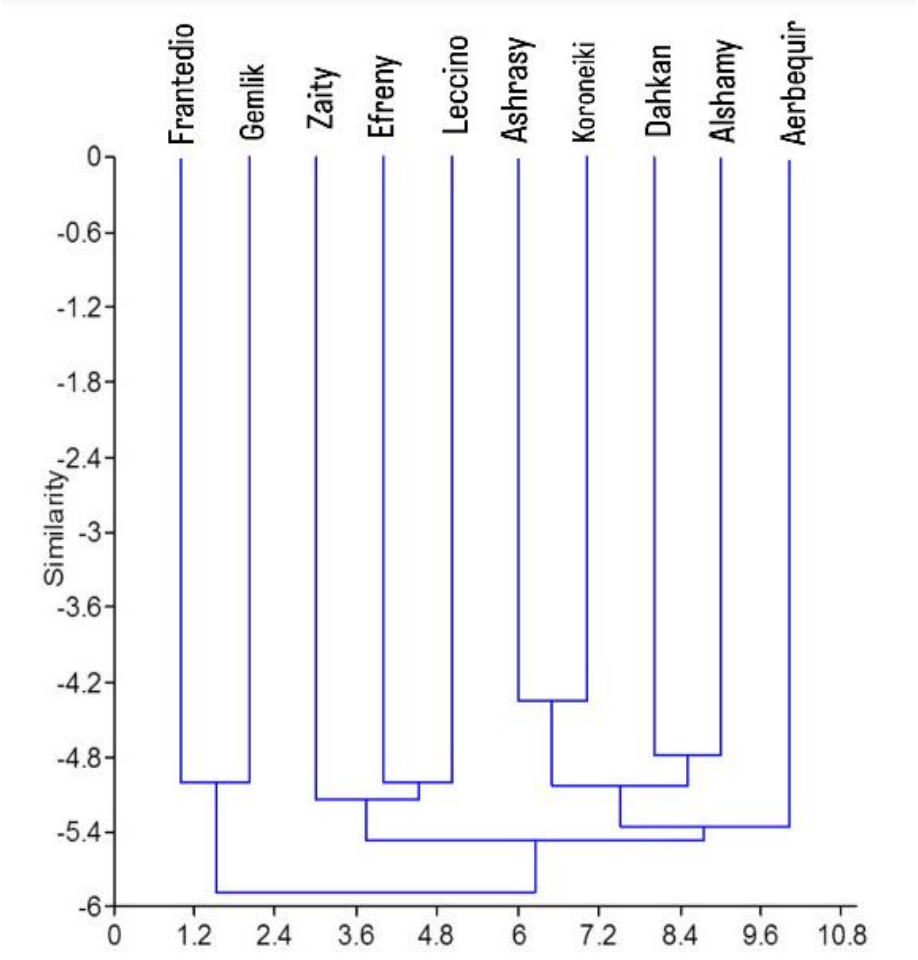

Figure 2. Dendrogram of the Studied Olive Genotypes

Hernández et al. (2001) also announced geographical clustering in olive cultivars, supporting the hypothesis that genetic diversity is shaped by regional evolutionary histories. Our results

extend these results to Iraqi olive genotypes, highlighting the influence of geographical and environmental factors on genetic structure. The clustering patterns have meaningful implications for olive breeding and conservation in Iraq. Cluster 1 genotypes, with high similarity, may share adaptive traits suited to local situations, making them candidates for targeted breeding to enhance yield or stress tolerance. Cluster 3 genotypes (Gemlik, Frantoio), with their genetic distinctness, offer opportunities for hybridization to introduce novel traits, like improved oil quality or resistance to pests prevalent in Iraq. The moderate similarity in Cluster 2 suggests a balance between conservation of existing diversity and potential for crossbreeding with other clusters to enhance genetic variability.

Conclusions: This investigation demonstrated substantial genetic diversity among the 10 olive genotypes, with a polymorphism rate of 64.7% and unique banding patterns that enable precise cultivar identification. These results prepare a robust foundation for evaluating and preserving olive germplasm in gene banks, supporting sustainable cultivation in Iraq. The apply of RAPD markers proved effective in capturing genetic variation, facilitating the construction of genetic maps and informing breeding programs aimed at enhancing traits like disease resistance, oil quality, and environmental adaptability. The clustering patterns and genetic similarity values highlight the potential for targeted hybridization and conservation strategies, ensuring the long-term resilience of olive cultivation in diverse agroecological situations.

Author contributions

F.H.R.A.: Conceptualized the investigation, designed the methodology, and managed the investigation, containing sample collection and RAPD analysis of olive genotypes. Carried out formal data analysis, curated the dataset, and validated the results. Wrote the original draft, reviewed and edited the manuscript, secured resources and funding, and managed project administration.

Data availability statement

The datasets generated and analyzed in this investigation, containing RAPD marker profiles and genetic similarity matrices, are available upon reasonable request. Interested researchers may contact the corresponding author, F.H.R.A., via institutional email for access to the data, subject to institutional data-sharing policies.

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Ethical considerations

This investigation did not involve human or animal subjects; thus, ethical approval was not needed. All plant material was gathered in accordance with local regulations and with permission from relevant agricultural authorities in Iraq.

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Conflict of Interest

The author declares no financial, personal, or professional conflicts of interest that could influence the objectivity or integrity of this research.

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ارزیابی تنوع ژنتیکی ژنوتیپ‌های زیتون بر اساس DNA با استفاده از نشانگرهای مولکولی RAPD

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چکیده

هدف: درخت زیتون (*Olea europaea* L.) که یکی از ارکان کشاورزی مدیترانه‌ای به شمار می‌رود، از کهن‌ترین گونه‌های میوه‌ای کشت‌شده و متعلق به خانواده زیتونیان (Oleaceae) است. با افزایش تقاضای جهانی برای روغن زیتون، نیاز به افزایش تولید از طریق مطالعه و حفاظت از تنوع ژنتیکی بیش از پیش احساس می‌شود. نشانگرهای DNA چندشکلی تکثیرشده تصادفی (RAPD)روشی ساده، سریع، مقرون‌به‌صرفه و قابل تکرار برای ارزیابی تنوع ژنتیکی در سطوح بین‌گونه‌ای و درون‌گونه‌ای فراهم می‌کنند. هدف از این مطالعه، ارزیابی تنوع ژنتیکی و روابط بین ژنوتیپ‌های زیتون در عراق با استفاده از نشانگرهای مولکولی RAPD به منظور پشتیبانی از برنامه‌های اصلاحی و حفاظت ژرم‌پلاسم بود.

مواد و روش‌ها: این مطالعه در سال ۲۰۲۳ در آزمایشگاه‌های بیوتکنولوژی دانشکده کشاورزی دانشگاه بغداد انجام شد. در این تحقیق، ده ژنوتیپ زیتون شامل Arbequina، Ashrasy، Zaity، Efreny، Leccino، Gemlik، Koroneiki، Dahkan، Alshamy و Frantoio که از مناطق جغرافیایی مختلف عراق جمع‌آوری شده بودند، مورد تجزیه و تحلیل قرار گرفتند. DNA ژنومی از نمونه‌های برگ تازه استخراج شد و تنوع ژنتیکی با استفاده از ده آغازگر RAPD شامل OPA1، OPA8، OPB8، OPC3، OPC7، OPJ5، OPR2، OPV1، OPX4 و OPY3 ارزیابی گردید. محصولات واکنش زنجیره‌ای پلیمراز (PCR) از طریق الکتروفورز روی ژل برای شناسایی باندهای چندشکلی تحلیل شدند.

نتایج: ده آغازگر RAPD مجموعاً ۹۸ باند تولید کردند. آغازگر OPX4 بیشترین تعداد باند (۱۳ باند) و آغازگرهای OPA1 و OPC3 کمترین (هر کدام ۷ باند) را تولید کردند. میانگین تعداد باندها برای هر آغازگر ۹.۸ بود. تحلیل شباهت ژنتیکی نشان داد که ژنوتیپ‌های Dahkan و Koroneiki بیشترین نزدیکی را با ضریب شباهت ۰/۷۱۴ دارند که بیانگر فاصله ژنتیکی پایین

بین آن‌هاست. تحلیل خوشه‌ای با استفاده از روش گروه‌بندی بدون وزن با میانگین حسابی (UPGMA) ژنوتیپ‌ها را به سه خوشه اصلی تقسیم کرد: خوشه اول (Arbequina، Alshamy، Dahkan، Koroneiki، Ashrasy)، خوشه دوم (Leccino، Zaity، Efreny) و خوشه سوم (Gemlik، Frantoio). این گروه‌بندی‌ها نشانگر پروفایل‌های ژنتیکی متمایز در میان ژنوتیپ‌ها هستند.

نتیجه‌گیری: این مطالعه تنوع ژنتیکی قابل توجهی را در میان ژنوتیپ‌های زیتون عراقی نشان می‌دهد و بینش‌های حیاتی برای برنامه‌های اصلاحی با هدف افزایش عملکرد و مقاومت فراهم می‌کند. روابط ژنتیکی شناسایی‌شده و الگوهای خوشه‌ای پشتیبانی‌کننده از راهبردهای هدفمند حفاظت از ژرم‌پلاسم برای حفظ منابع ژنتیکی ارزشمند جهت تولید پایدار زیتون در عراق هستند.

کلمات کلیدی: تنوع ژنتیکی، حفاظت ژرم‌پلاسم، ژنوتیپ‌های زیتون، نشانگرهای RAPD، *Olea europaea*

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