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Molecular characterization of some olive cultivars found in Fadak farms in Karbala By Using Internal Transcribed Space (ITS1)

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Abstract

The identification and organization of olive (*Olea europaea L.*) cultivars are crucial for improving olive production, protecting genetic diversity, and ensuring the authenticity of local varieties. This study aims to describe the genetic diversity of cultivars grown in Karbala Governorate, Iraq, using the internal transcribed spacer region of the nuclear ribosomal DNA. Fifteen leaf samples were collected from Fadak farm, and total genomic DNA was extracted. PCR amplification of the ITS1 region was accomplished using specific primers, and the products were sent for sequencing. The resulting sequences will be aligned and compared with those deposited in GenBank to determine the degree of similarity and to construct a phylogenetic tree, thereby enabling cultivar identification and assessment of genetic variation. Results showed that of ITS1-5.8S-ITS2 sequences from five Olea europaea samples collected in Iraq revealed varying degrees of genetic similarity with known cultivars. Four samples showed high sequence similarity (above 96%) to established cultivars such as Olive yellow, Picual-Bakraju-Sulaymani, and Qaysi-Alqush-Musil, indicating close genetic relationships and potential affiliation to these cultivars or their local variants. One sample exhibited a lower similarity (92.3%) with Khoderi-Bakraju-Sulaymaniya, suggesting it may represent a distinct local isolate or a unique genetic lineage within Iraqi olive diversity. These findings highlight significant genetic variation among Iraqi olive populations and emphasize the importance of further morphological and agronomic characterization to clarify their taxonomic status.

Keywords: ITS1, genetic diversity, molecular characterization, olive

Introduction

Olea europaea L. is one of the most important crops in the world, as 95% of the genetic material of olives is found there, meaning that it is the main area for genetic diversity of olives. Olives occupy an important medical and economic position due to their many uses, as they are used in the production of oil known for its benefits as antifungals, antioxidants, antimicrobials and immune boosting. They are also used in the cosmetic and food industries (Baldoni et al., 2006). is one of the important crops in the cultivated in other regions, including Iraq (Al-Rufaye, et al,2003) The olive genus includes 30-40 species and is widely cultivated in the other parts of the world (Green et al,2002). Olives are important for their significant medical, economic and environmental effects. Accurate identification of olive cultivars is critical for breeding programs, conservation of local germplasm, and improvement of oil quality. Morphological methods alone are often insufficient due to environmental influences and phenotypic plasticity; thus, molecular tools offer a more reliable approach (Benchimol-Reis etal.,2023). One of the most active DNA regions used in plant molecular systematics the (ITS). This region, in particular, has shown high variability among species and even within species, making it a suitable marker for distinguishing closely related cultivars (Al-Kilani, et al. 2024). The first internal transcribed spacer (ITS1) region of nuclear ribosomal DNA (nrDNA) is a non-coding among the 18S and 5.8S (rRNA genes). This region is widely used as a molecular marker for recognizing plant species due to its high level of variability compared to coding regions. (Santamaria et al., 2018)

Between the characteristic features of the ITS1 region are It is situated between conserved regions of the genome, enabling the design of universal primers for PCR amplification, such as ITS1-F and ITS4.(Banchi et

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al., 2020) It is characterized by high sequence diversity, even among closely related species, making it perfect for taxonomic studies at the species and taxon stages. (Schoch et al., 2012) It has been successfully used in investigating evolutionary relationships and DNA barcoding studies of plants and fungi.(Sion et al., 2021). intra-individual polymorphism was found in the ITS1 sequence, strengthening the role of this region as an accurate analytical tool in genetic studies (Graper et al. 2021). The ITS one DB database, created by Santamaria et al., 2018, has compiled more than 985,000 ITS1 region sequences from over 134,000 eukaryotic species, making it a reliable reference for molecular taxonomy and biodiversity analysis. In a comparison of the ITS1, ITS2, matK, and rbcL regions in a study on olive cultivars, Sion et al. (2021) demonstrated that ITS1 provides higher taxonomic accuracy at the taxonomic level and is more efficient at amplifying DNA from dried plant samples.

A recent study indicated that choosing the appropriate primers for ITS1 amplification plays an important role in improving the accuracy of metagenomic analysis (metabarcoding), as the new primer ITS1-F-new was shown to yield better results compared to older designs (Espinosa Prieto et al., 2024). This study aimed to employ the variation In the sequences to construct phylogenetic relationships 6 Iraqi olive varieties compare them to the sequences of the some olive varieties

Materials and Methods

Sample Collection

Young leaves were collected from 6 trees planted in the Fadak farm of the Holy Karbala. during January 2025. After collecting the olive leaves, they were washed. Then, DNA was extracted from six samples by the method of (Saghai & Maroofeetal, 1984). According to Genomic DNA Mini Kit of Plant Protocol prepared by Geneaid.

DNA concentration and purity are measured after extraction using a bio drop device. This is a very important step in the DNA extraction process.

Steps	Temperatures	Time
Initial denaturation	94°C	5 min
Denaturation	95°C	30 s
Annealing	58°C	30 s
Extention	72°C	1 min
Final extention	72°C	5 min

Primers used in diagnosis

The primer utilize in this research were lyophilized by Pioneer. ITS Forward:5'-AAAAGGTA GACCCAGAACTCG-3' Reverse 5'-TCGCATTTTGCTGCGTTCTTC-3'. The stock solution was prepared using TE buffer to yield 100 picomoles/microliter and stored in at -20°C. From this, this primer used in the polymerase chain reaction experiment was prepared at a concentration of 10 picomoles/microliter.

Polymerase Chain Reaction (PCR)

The steps were performed using AccuPower® PCR PreMix according to the manufacturer's instructions. The ITS primer was used to amplify the nucleic ribosomal DNA (nrDNA) region, and the polymerase reaction was performed according to the following steps.

DNA Sequencing Method

Species were identified using DNA sequencing of targeted genetic regions. PCR products were sent to Microgen (Korea) for sequencing using the AB system, and sequences were compared via BLAST with NCBI databases. Phylogenetic analysis was conducted using MEGA 11, and sequences were submitted to GenBank to obtain accession numbers.

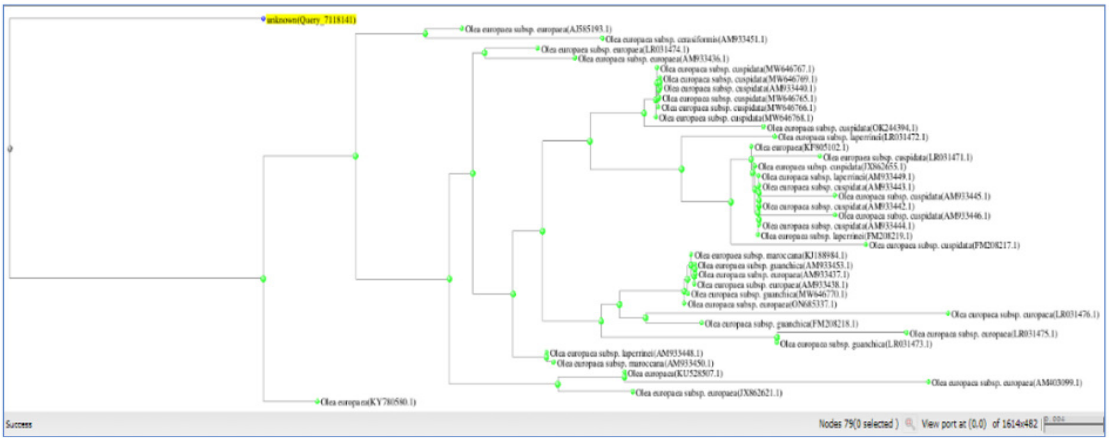

Figure 1. Phylogenetic tree showing the genetic relationship and sequence similarity of the sample1 with related *Olea europaea* entries in the GenBank database.

Results and Discussion

The ITS1 sequence analysis studied sample 1 showed a 96.3% similarity with the sequence of Olea europaea cultivar Olive yellow (GenBank: KY780580.1). This high level of similarity indicates a clear genetic relationship between the sample and the Olive yellow cultivar. However, the similarity being below 98% suggests that the sample does not represent an identical match, which supports the possibility that it may represent a local variant or a closely related new cultivar. Accordingly, we propose to tentatively name the sample Olea europaea isolate Karbala-Fadak - Olive yellow-like, pending further morphological and agronomic studies to confirm its cultivar identity (Besnard, et al. 2011) (**Figure 1**).

While sample 2,3,4, showed a 98.17% match with the recorded sequence of Olea europaea cultivar Olive yellow (GenBank accession: KY780580.1). This high level of similarity indicates a close genetic relationship between the sample and the mentioned cultivar, suggesting that the sample may belong to the same cultivar or represent a local strain thereof. However, the slight divergence (1.83%) may be attributed to environmental factors or local mutations and requires confirmation through a comprehensive morphological study (Khadari, et al., 2010) (**Figure 2**).

The alignment results of sample 5 showed a the ITS1, 5.8S, and ITS2 region sequences of the studied sample revealed a high similarity of 98.54% with Olea europaea cultivar Picual-Bakraju-Sulaymani (GenBank accession: OQ124159.1), and 98.47% with Olea europaea cultivar Qaysi-Alqush-Musil (GenBank accession: OQ134703.1). This high genetic similarity suggests a potential affiliation of the sample to the same genetic group or a very close relationship with these Iraqi local cultivars, indicating a close evolutionary connection. (Mariotti, et al., 2023).

The slight differences in a few nucleotides (less than 2%) may be attributed to point mutations or environmental variations that led to fine-scale genetic divergence, especially considering that these cultivars originate from different geographical regions

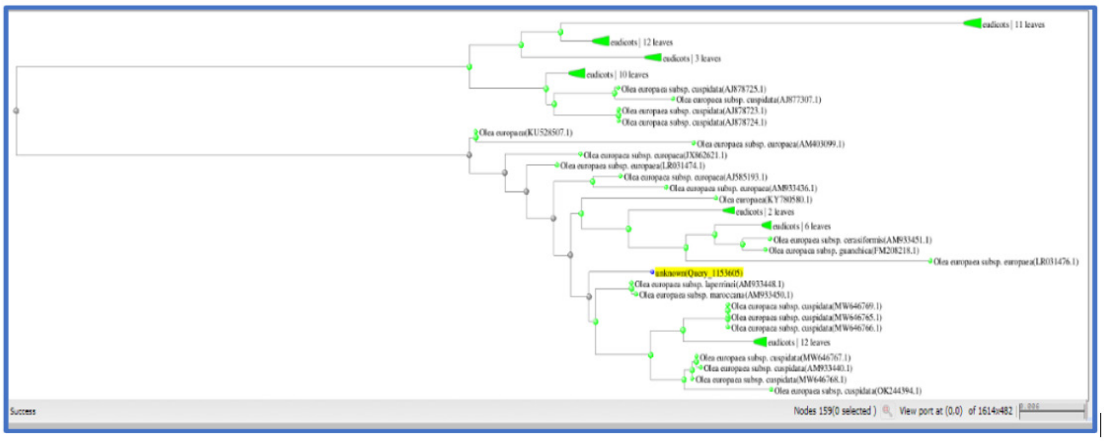

Figure 2. Phylogenetic tree showing the genetic relationship and sequence similarity of the sample 2, 3, 4, with related *Olea europaea* entries in the GenBank database

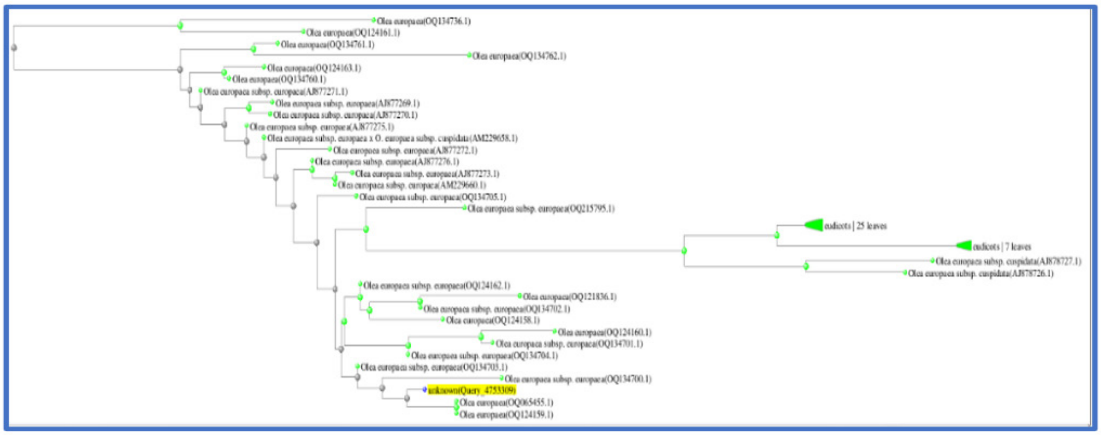

Figure 3. Phylogenetic tree showing the genetic relationship and sequence similarity of the sample 5 with related *Olea europaea* entries in the GenBank database.

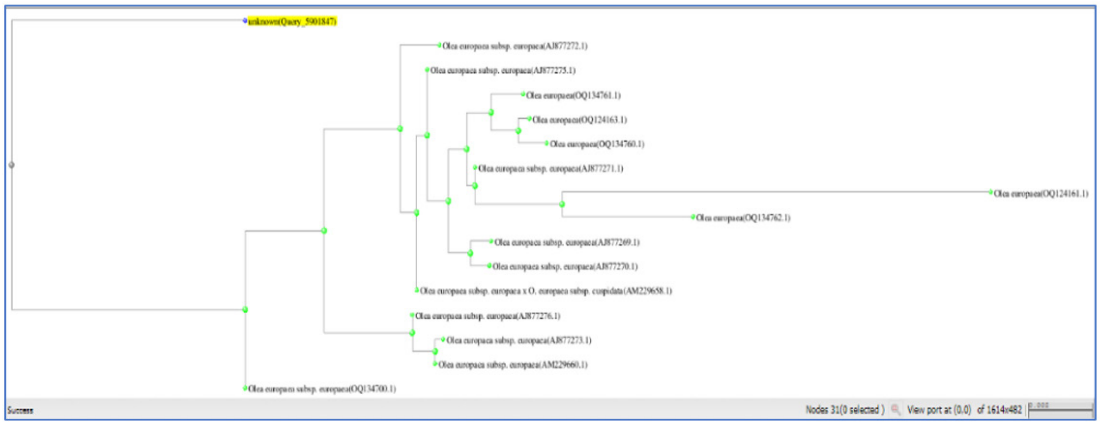

Figure 4. Phylogenetic tree showing the genetic relationship and sequence similarity of the sample 6 with related *Olea europaea* entries in the GenBank database.

(Sulaymaniyah and Mosul), while the studied sample was collected from a central region with distinct climatic conditions, such as Karbala (**Figure 3**).

While the genetic alignment results of the ITS1 region sequence revealed in sample 6 showed a 92.3% similarity between the sample studied and the sequence of Olea europaea cultivar Khoderi-Bakraju-Sulaymaniya (GenBank: OQ134700.1). This level of similarity indicates a genetic relationship between the two samples, yet it does not reach the threshold required for cultivar-level identity. This suggests that the sample may represent a distinct local isolate or a unique genetic pattern within the genetic diversity of olive trees in Iraq. Accordingly, it is proposed to consider the sample as a new isolate with an evolutionary relationship to the mentioned cultivar (**Figure 4**).

The ITS1 region sequences analysis of five Olea europaea samples collected from central Iraq revealed varying degrees of genetic similarity with previously characterized local and regional cultivars. Notably, one sample exhibited a 96.3% similarity with Olea europaea cultivar Olive yellow (KY780580.1), while another showed a slightly higher identity of 98.17% with the same cultivar. These results suggest a close genetic relationship, potentially indicating either membership within the Olive yellow cultivar or the presence of a local variant that has diverged due to microenvironmental factors or point mutations. (Mariotti, et al. 2023, Hamid, et al. 2023).

Two other samples demonstrated high sequence identity with local Iraqi cultivars: 98.54% with Picual-Bakraju-Sulaymani (OQ124159.1) and 98.47% with Qaysi-Alqush-Musil (OQ134703.1). These results imply a strong genetic affiliation and evolutionary proximity among olive trees cultivated in geographically distinct regions of Iraq (Sulaymaniyah and Mosul), despite environmental and ecological variation. The studied sample, collected from Karbala—a region with different climatic conditions—may represent an ecotype or a locally adapted strain within the same genetic lineage. In contrast, a fifth sample revealed only 92.3% sequence similarity with Khoderi-Bakraju-Sulaymaniya (OQ134700.1), indicating a more distant genetic relationship. This level of divergence suggests the possibility of a novel genetic lineage, potentially representing a unique local isolate within the broader genetic pool of Iraqi olives.

Overall, the high but variable levels of sequence similarity among the samples highlight the rich intra-specific diversity of Olea europaea in Iraq and support the hypothesis of ongoing microevolutionary processes driven by ecological, climatic, and anthropogenic factors. (Jalab & Al-Rufaye, 2024).

Conclusion

This study demonstrates that the Iraqi olive samples analyzed through ITS1-5.8S-ITS2 sequencing

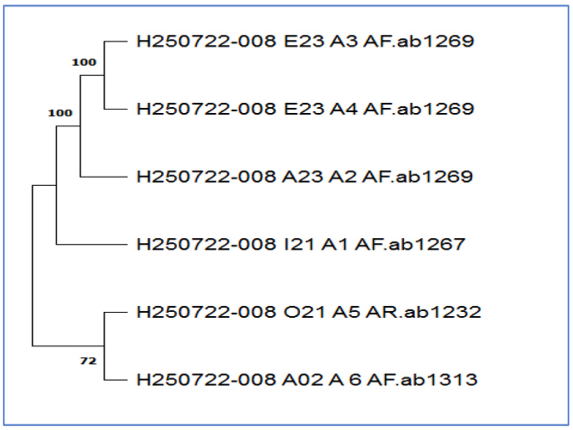

Figure5. Shows the Phylogenetic tree of Iraqi *Olea europaea L.* varieties according to ITS-1 sequences

possess significant genetic diversity and exhibit varying degrees of similarity with known cultivars. Some samples are closely aligned with established cultivars, while others may represent distinct local isolates or variants.

Morphological and agronomic studies are needed to confirm the cultivar identity of genetically similar but non-identical samples. Samples showing less than 98% genetic identity should be further investigated to assess their potential for registration as novel local isolates or cultivars. Expanded sampling across different regions of Iraq is recommended to capture the broader extent of olive genetic diversity. Furthermore, integrating molecular data with phenotypic and ecological information will strengthen conservation strategies and support the development and selection of cultivars adapted to regional environmental conditions.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.