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# Molecular characterization of the *Olea europaea L.* by using matK sequences in Iraq

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**Abstract**

Genetic diversity inherent to *Olea europaea L* is remarkably extensive, identified by a plethora of varieties that ultimately facilitate considerable intergenotypic hybridization. Consequently, the categorization and finding of the genetic variability inherent among this cultivars represent a critical challenge of substantial significance. This undertaking facilitates the optimal utilization of olive resources distributed across the globe and contributes to the breeding and genetic improvement of diverse olive varieties. The technique of DNA barcoding acts as a methodological framework for the evaluation and classification of species, employing short, variable, and standardized segments of DNA.This investigation signifies the first application of DNA sequencing data aimed at the DNA barcoding of Iraqi olive cultivars., while also evaluating the genetic affiliations among three indigenous cultivars cultivated in Iraq and their corresponding sequences within the existing database. The efficacy of the matk barcoding loci was appraised through PCR amplification and sequence characterizationupon the execution of multiple alignments of all sequences derived from the scrutinized loci, it was ascertained that matk manifested the most extended sequence length of 1368 base pairs, thereby augmenting the efficacy of differentiating between of cultivars selected. The DNA sequences procured any cultivars concatenated with the barcode loci were utilized to phylogenetic analysis based on the maximum likelihood tree effectively facilitating the distinction of the selected olive cultivars.

**Keywords:** analyses, matk sequences, mutation, *Olea europaea.*, polymerase chain reaction (PCR)

## Introduction

Olea europaea L. Commonly referred to as olives, represent the most prominent arboreal species within originating from the Mediterranean basin, with their intricate intertwined with the cultural narrative of the region. The genetic diversity inherent in olive trees all over the Mediterranean basin is notably extensive, distinguished by a excess of varieties (Zohary *et al.*2012). Over the course of history, as the civilizations of the Mediterranean proliferated, the invaluable heritage associated with the olive tree has regrettably diminished (Kaniewski *et al.* 2012). In recent decades, the distribution of the olive tree has transcended its indigenous Mediterranean confines, now extending to regions as distant as Australia, South Africa, China and Japan (Besnard *et al*. 2018). Moreover, the systematic substitution of native varieties and local genotypes with those demonstrating superior productivity and robustness against biotic and abiotic pressures presents a considerable risk to the future availability and utilization of these essential assets (Selvara *et al.* 2013). The decline in genetic diversity may impede the capacity to identify pathogens, climatic variations and employ novel sources to confront forthcoming challenges, such as emergent pests, and (Koehmstedt *et al,* 2011). The existence of olive groves in Central and Northern Iraq. The existence of numerous olive varieties globally presents significant challenges pertaining to their classification. DNA barcoding constitutes a methodological approach employed the evaluation and classification of biological species, utilizing concise, variable, and standardized DNA sequences. This technique capitalizes subtle nucleotide discrepancies observed within specific genetic loci among a variety of organisms to facilitate the differentiation of genetically distinct species (Savolaien *et al.* 2005). Due to its inherent simplicity and remarkable precision, particularly when juxtaposed with the intricacies


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and subjectivities that characterize morphology-based taxonomic classification, DNA barcoding has experienced a notable surge in popularity (Kress 2017).

In the past ten years, four DNA barcode markers pertaining to plant species have been formulated, assessed, and applies ITS, matk, rbcl, and trnh-psba and trnahisgug gene. These markers effectively address pivotal challenges within the realms of systematics, evolutionary biology, ecology, and the networks of species interactions (Mi *et al*. 2012; Pei et al. 2015in addition to enabling priority evaluations in endangered ecosystems, indigenous populations, and vast geographical areas (Shapcott *et al.*, 2015). The simplicity and superior accuracy of DNA barcoding, relative to the intricate and subjective nature of morphology-based taxonomic identity, continues to contribute to its rising prominence (Kress 2017). Notwithstanding the extensive diversity of olive germplasm, the genetic relationships among cultivars remain inadequately understood (Besnard *et al.*, 2018). Numerous investigations into olive cultivars have been conducted utilizing various combinations of molecular markers, including random amplified polymorphism DNA (Besnard *et al.* 2001), fragment length amplified polymorphism (Khelaghi *et al.* 2017), the issr (Isk *et al.,* 2011), ssr (Pérez-Jimnez *et al*. 2013; Rieenzo *et al.* 2018) and,single-nucleotide polymorphism (Reale *et al.*, 2006). Nevertheless, to this point, there has been a notable scarcity of research concentrating on the delineation of olive cultivars through the utilization of DNA barcoding techniques. As a result, the current study utilized DNA sequencing information for the pioneering implementation of DNA barcoding in relation to Iraqi olive cultivars. To evalute the Hereditary relationships among three indigenous olive cultivars spread in Iraq by use chloroplast gene region matk was utilized as a DNA barcoding markers.

**Materials and methods**

*Plant materials*

The investigation focused on a selection of three indigenous olive cultivars from Iraq, which were studied on mature olive trees cultivated at the karbala city in Iraq.

*DNA Extraction and PCR Reactions*

To procure the complete genomic DNA, fresh young leaves were employed from three representative specimens using the dneasy Plant Mini Kit of each olive cultivars. The evaluation of the purity and concentration of the DNA was conducted through agarose gel electrophoresis and spectrophotometric analysis. The polymerase chain reaction (PCR) analyses

The total volume 25 µl. The polymerase chain reaction amplification process incorporated 40 ng of DNA Extraction, 0.2 mm (dntps), 1.5 U of dreamtaq DNA polymerase and 1 × Taq buffer, and 0.1 µm from the each primer. The primer pairs used in this study were crafted according on the DNA sequences accessible in the genbank database, specifically of matk R: CGT ACA GTA CTT TTG TGT TTA CGA G and matk F: ACC CAG TCC ATC TGG AAA TCT TGG TTC, The amplification of the gene was executed utilizing the following PCR protocol: an initial denaturation phase at 94 °C for 4 minutes, succeeded by 35 cycles, each comprising a denaturation step at 94 °C for 30 seconds, an annealing phase at 48 °C for 45 seconds, and an elongation phase at 72 °C for 2 minutes, culminating in a final extension phase at 72 °C for 8 minutes. Ultimately the reaction was terminated by sustaining the temperature at 4.5°C. Electrophoretic analysis on a 1.8% agarose gel containing Tris-borate-EDTA (TBE) buffer (ph 8.0) and stain ethidium bromide a was conducted Utilizing Molecular Imager, gel was meticulously analyzed and archived.

*PCR Purfication and the sequencing*

The PCR product was carefully subjected by thegel electrophoresis and subsequently underwent purification utilizing acommercially available PCR cleanup by kit Promega. Furthermore, the ABI 3730xl automated DNA sequences of Bio systems was employed for the direct sequencing of purified PCR products.

The products were derived from a singular source at Corporation situated in the United States. The accession numbers associated with the sequences presented in manuscript archived complete Gen Bank of nucleotide sequence repository.

*DNA barcoding of matk in plastid gene*

The dataset analyzed encompassed three nucleotide sequences. Initially, the forward sequences were adjusted utilizing the polymerase chain reaction products were exposed to gel electrophoresis and subsequently underwent purification, was employed for the direct sequencing of the purified PCR products.

The sequencing products were sourced from a singular entity, specifically the GATC Corporation, located in the United States. Accession numbers corresponding to the sequences detailed in this manuscript have been archived within a Gen Bank nucleotide sequence repository. And using the MEGA11, evolutionary assessments were conducted (Tamure *et al.* 2021).

**Results and Discussion**

Consequent to the polymerase chain reaction (PCR) assays, the amplification products derived from specific barcoding region, namely matk, in the selective cultivars demonstrated a singular band for each Maturase K sequence. A successful the PCR amplification was achieved for every olive cultivar under investigation, the resultant amplified fragment exhibiting a size 1368 base pairs (**Figure 1**).

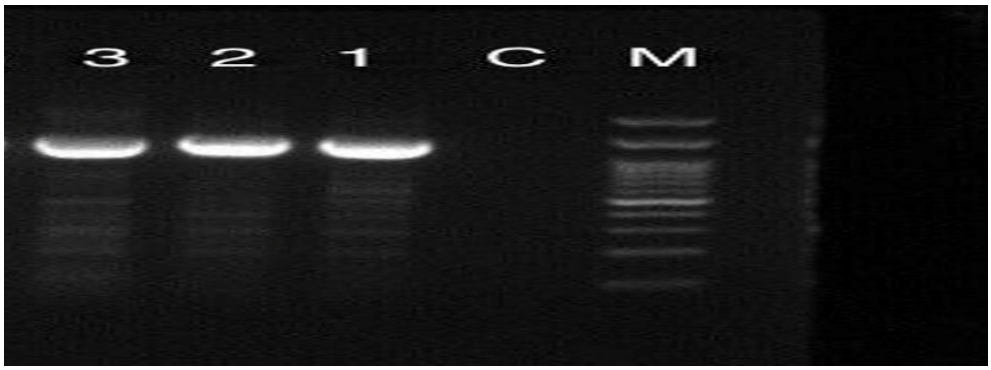


**Figure 1** - Agarose gel of amplification the barcoding (matK) primer of on three Iraqi olive cultivars.

The matk sequences were capable of distinguishing the three olive cultivars These findings underscore the efficacy of two DNA barcoding methodologies in elucidating the phylgenetic relationship among the *Olea europaea L* cultivars investigated. For the any matk loci, three to ascertain the unique sequences that could serve as appropriate barcodes for differentiating Iraqi the olive cultivars derived from various Olea europaea species were subjected to meticulous evaluation and comparison with the sequences cataloged in the NCBI database, specifically focusing on the aligned sequences of the two chloroplast loci matk. Phylogenetic analyses utilizing sequences from the database were conducted, wherein the ML methodology was employed to deduce phylogenetic trees record on the sequences obtained from the matk loci, both separately and in combination.

The DNA analysis of Results sample 1, using matk primers, showed a complete 100% genetic identity with the reference sequence AJ877298.1 available in the NCBI database, corresponding to Olea europaea subsp. Cuspidata (Eastern wild olive) from the Kirstenbosch collection. This perfect match indicates that the examined sample genetically belongs to this taxon with no detectable variations (**Figure 2 and 3**).

Sequence analysis of the examined sample 2 using the matk gene revealed an 89% identity with the complete chloroplast genome of Olea europaea isolate HT5 (MT182984.1) deposited in the NCBI database. This level of similarity indicates a strong genetic relationship between the sample and isolate HT5, suggesting that the sample may belong to a different genetic lineage or a geographically distinct variant of Olea europaea. The analysis also identified two major matching regions with the reference genome, supporting genetic relatedness while simultaneously reflecting natural molecular variations or mutations between different olive populations. (**Figure 4** and **5**)

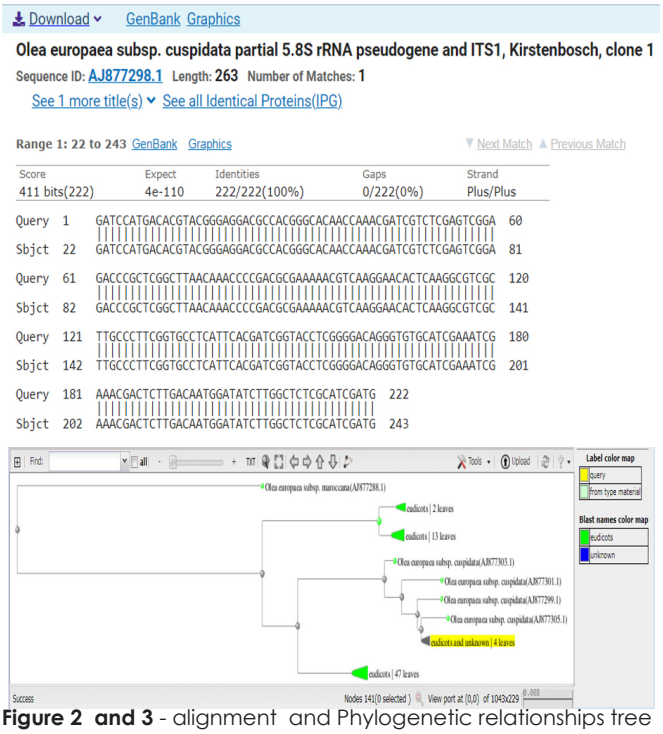


**Figure 2 and 3** - alignment and Phylogenetic relationships tree based on matK sequences of *Olea europaea* L. varieties from the Iraq and from the NCBI GenBank


Figure 4 and 5. Alignment and Phylogenetic relationships tree based on **matK** sequences of *Olea europaea* L. varieties from the Iraq and from the NCBI GenBank

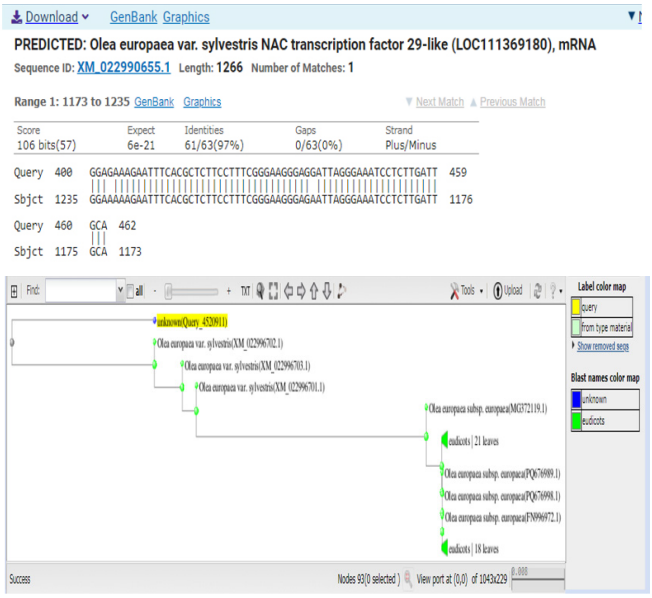


Figure 6 and 7 - Alignment and Phylogenetic relationships tree based on **matK** sequences of *Olea europaea* L. varieties from the Iraq and from the NCBI GenBank

The results showed that the third sample 3 of olive subjected to molecular characterization using the matk sequence exhibited a 96% match with Olea europaea var. Sylvestris NAC transcription factor 29-like (LOC111369180), mrna (Sequence ID: XM_022990655.1, length 1266 bp). This indicates that the sample belongs to the wild olive, considered the natural ancestor of cultivated olive varieties. The match with the NAC transcription factor 29 gene also suggests potential roles of this gene in regulating environmental stress (**Figure 6** and **7**).

The considerable richness and diversity of olive germplasms have facilitated the extensive movement of plant genetic material in various directions. The elevated genetic degradation to the indigenous olive germplasm, attributable to the socio-economic transformations observed in recent decades (Trujill *et al*. 2014). Despite the substantial availability of olive genetic resources, the genetic interrelations among the cultivars remain inadequately elucidated (Besnerd ,2018). Consequently, the examination and classification of the diversity present within olive cultivars represent paramount challenges. As a result, numerous investigations have been undertaken employing a range of molecular markers, including random amplified polymorphism DNA (RAPD), amplified fragment length polymorphism repeat (ISSR), single-nucleotide polymorphism DNA (RAPD), (ISSR), (SNP), and cp-DNA The capacity of specific regions the chloroplast genome to serve of DNA barcodes across a diverse array of plant species (Wue *et al.* 2019). Contemporary investigations have elucidated that matk, serves as efficacious barcodes for a multitude of plant species (de Melo *et al.* 2019). The success rate of sequencing the matk loci achieved in this study is consistent with the findings reported in CBOL (CBOL 2009). The findings of the present study suggest that the utilization the molecular barcodes inherent (Hollingswor 2011). Their applicability in distinguishing the investigated Iraqi olive cultivars is attributed to their adequacy concerning sequence variability.

## Conclusion

The current investigation employed of DNA sequencing data for inaugural application of DNA barcoding to Iraqi olive cultivars. To evaluate the

genetic relationships among three indigenous cultivars cultivated in Iraq and their association with sequences in existing databases, matK, was utilized as molecular barcodes. The findings substantiate the barcoding matK, effectively differentiate the selected Iraqi olive cultivars due to the presence of distinct variable sites. The deoxyribonucleic acid sequences obtained from both singular and composite barcode utilized in the likelihood cultivars method tree of the phylogenetic analysis effectively differentiated selected of olive. This result provides significant advantages for future research projects, consisting DNA barcoding markers intended for the identification of olive species and preservation efforts.

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