RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
57 (3) 1264-1274, 2025
http://doi.org/10.54910/sabrao2025.57.3.37
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

PHYLOGENETIC STUDY OF THE *SALVIA* AND *OCIMUM* SPECIES BASED ON MORPHOLOGICAL TRAITS IN NORTHERN IRAQ

A.H. ASKAR and R.H. ALOUSH*

Department of Biology, College of Science, University of Tikrit, Tikrit, Iraq
*Corresponding author's emails: ranaha850@gmail.com, rana_aloush_plant@tu.edu.iq
Email address of co-author: tikritbiophd@gmail.com

SUMMARY

Four species of *Salvia* L., *S. palaestina*, *S.* *spinosa*, *S. compressa*, and *S. multicaulis,* sustained scrutiny by the internal transcribed spacer (ITS) fragments (ITS1, 5.8S rRNA gene, and ITS4) analysis with *Ocimum basilicum* to identify and explore their phylogenetic relationship based on many morphologically investigated properties. The analysis of the phylogenetic tree revealed that *Salvia* L. is a polyphyletic genera, indicating varying degrees of genetic relatedness among *Salvia* spp. and clearly distinct from *Ocimum basilicum*. This genetic differentiation is highly consistent with the obtained morphological data, which provide valuable insights into the evolutionary relationships between both genera and among *Salvia* spp. The observed genetic diversity highlights the reasons beyond morphological modification, including the number of biotic and abiotic environmental factors. Several characters, such as the calyx shape, corolla shape, and stamen features, revealed valuable taxonomic importance of the infrageneric and interspecific differentiating. The newly recorded ITS sequences attained the NCBI GenBank deposit to enrich the species database for more future investigations. The ITS markers represent the most preservative and useful taxonomic tools. Breeding approaches can employ genetic divergence and broad taxonomic phylogeny for Lamiaceae genera.

Keywords: *Salvia, Ocimum*, species, morphological traits, ITS, phylogenetic tree

Key findings: Morphological analysis of four *Salvia* spp. and *Ocimum basilicum* revealed many features of significant taxonomic importance. Molecular analysis based on nuclear ribosomal internal transcribed spacer regions (nrITS) results in the identification of studied taxa, with the new accessions deposited in the NCBI GenBank and construction of a phylogenetic tree.

Communicating Editor: Dr. Kamile Ulukapi

Manuscript received: September 03, 2024; Accepted: December 26, 2024.

Citation: Askar AH, Aloush RH (2025). Phylogenetic study of the *Salvia* and *Ocimum* species based on morphological traits in Northern Iraq. *SABRAO J. Breed. Genet.* 57(3): 1264-1274. http://doi.org/10.54910/sabrao2025.57.3.37.

INTRODUCTION

Lamiaceae Martinov (mint family) is the sixth-largest angiosperm family. It comprises about 236 genera and 7,000 species of shrubs and herbaceous plants commonly distributed in Mediterranean and subtropical regions, with the family well-known to humans as a medicinal, flavor, or fragrance source (Assaf *et al*., 2022). The Lamiaceae family represents an ideal system for evolutionary studies due to their morphological and biogeographical diversity (Rose *et al*., 2022). Both the genera *Salvia* L. and *Ocimum* L. belong to the Lamiaceae family. *Salvia* L., commonly known as dead nettle or sage, had the word Salvia originating from the Latin word 'salvare,' meaning to treat or heal (Li *et al*., 2013). The genus comprises about 1,000 species with a cosmopolitan distribution—about 500 species in Central and South America, 200 species in western Asia, and 100 species in eastern Asia (Özler *et al*., 2011). Although the genus represents about 33 species in the flora of Iraq (Ahmad, 2016). *Salvia* spp. have become known as a group of economical, medicinal, and aromatic herbs (Demirpolat, 2023).

The word Ocimum is originally from a Greek word, which means aromatic, as is basil, also descending from a Greek origin, meaning 'royal' or 'princely' (Kalita and Devi, 2023). It comprises about 150 species (Tangpao *et al*., 2018), distributed mainly in temperate and warm tropical regions, namely, Africa, Tropical Asia, and Central and South America (Enegide, 2021). *Ocimum* is typically the king of herbs due to its enormous uses in cosmetics, pharmacology, and traditional medicines, for instance, serving as tea to cure diarrhea, gas, or nausea or as an antioxidant, and treating cancer and cardiovascular diseases (Kamyab *et al*., 2021). *Salvia* has a floral heterogeneity characteristic, readily identified by the presence of two stamens rather than four, with a significant elongation of appendage separating the anthers' lobes and various micro-morphological synapomorphies (Drew *et al*., 2017).

Given their magnanimous healing properties, both genera attracted considerable attention from biologists. The provision of both genera is necessary for commercial perspective and selecting efficient breeding processes and taxonomic clarity.

Molecular characterization studies carried out in past years provided important ideas about the systematics of medicinal plants because of their preservative nature, even against ecological factors (Khal, 2024). The ITS rRNA genetic marker represents an efficient marker, which is a widely and universally employed in vitro amplification of specific DNA sequences and molecular systematics for variable targeting. As a result of concerted evolution, the ribosomal genes' setup in tandem arrays demonstrated a low intra-genomic diversity (Dover *et al*., 1982).

The phylogenetic taxonomy had a better combination of information collected from all the sources. It also supports the morphological characteristics in approaching systematic challenges (Zhao *et al*., 2024). Genetic specification studies in *Salvia* spp. have also demonstrated a larger level of genetic variation and diversity (Demirpolat, 2023). The classification of *Ocimum* L. is jumbled up due to its wide morphological divergence and the same species being named by more than one author, which resulted in an inaccurate delamination of *Ocimum* spp. A comprehensive investigation based on morphological and molecular examinations of *Ocimum* L. was successful with many scientists, which provided accurate corrections for misidentified species and their nomenclature (Kalita and Devi, 2023; Bazzicalupo *et al*., 2024). A molecular study focusing on species native to Northern Iraq is an essential first step for elucidating the broader taxonomy of the subfamily Nepetoideae in Iraq. The presented study sought to describe the genetic diversity of four *Salvia* spp. compared with the out-group species of *Ocimum basilicum* utilizing the ITS region. Moreover, this work hopes to assess the efficiency of the ITS sequence in constructing the phylogenetic relationship within *Salvia* spp. and *O. basilicum* due to its highest sensitivity, specificity, and rapidity, in addition to an investigation of morphological characteristics.

Table 1. Four *Salvia* species and *Ocimum basilicum* with their localities and accession numbers.

Taxa name	Region	Longitude E	Latitude N	Altitude (meter)	Accession Number	Percentage of matches
S. palaestina	Sulaimmania -Mawat mountain	45°25'4.02	35°54'3.94	500-1000	PP794578	91.99 %
	Sulaimmania–Saidsadiq	*27.6'51°45*	*23.39'21°35*			
	Erbil-Koye	*47.12'13°44*	36° 6'10.59			
S. spinosa	Kirkuk-Tuz	44°31'30.91	35° 1'19.02	500-700	PP794576	62.81 %
	Sulaimmania -Rania-Shaqlawa	44°28'41.54	36°17'31.78			
	Sulaimmania -Dukan lake road	44°59'13.86	35°53'14.46			
S. compressa	Diyala-Mandali	*26.84'32°45*	33°45'29.97	500-900	PP794575	78.50 %
	Kirkuk-Sulaimmania road 3 km	*6.90'29°44*	*51.23'28°35*			
	Karahingeer	*37.26'36°44*	*8.34'29°35*			
S. multicaulis	Sulaimmania :Zewi	45°14'21.52	*37.86'45°35*	1000-1200	PP794577	67.43 %
	Sulaimmania :Azmar-mountain	45°27'58.25	35°35'32.50			
	Sulaimmania :Sitak	45°31'13.53	35°38'24.72			
O. basilicum	Kirkuk :Kopri	44° 7'31.42	35°43'49.41	160-500	PP800210	71.81 %
	Kirkuk: Taza	44°19'43.26	35°16'53.44			
	Kirkuk	*15.41'25°44*	*7.74'30°35*			

MATERIALS AND METHODS

Taxa sampling and choice of markers

Specimens collected representing *Salvia* spp. (*S. palaestina, S. spinosa, S. compressa, and S. multicaulis*) and *Ocimum basilicum* were at the flowering stage during April to August 2024 in several localities in Northern Iraq (Table 1). Plant specimens of the *Salvia* spp. and *Ocimum* identification used the flora of Turkey and Iran because the part describing the Lamiaceae family in the flora of Iraq is unpublished yet. Terminology in this study followed that stated in Khal (2020). The in-group sampling comprised accessions representing four *Salvia* spp. of the tribe Mentheae, while the out-group comprised one accession of the *O. basilicum* of the tribe Ocimoideae, with voucher specimens deposited in the Iraq National Herbarium (BAG). Plant nuclear ribosomal internal transcribed spacers 'ITS' bore selecting for the phylogenetic reconstruction of *Salvia* L. based on previous phylogenetic studies (Ferrer *et al*., 2001).

DNA extraction, amplification, and sequencing

According to the Sigma-Aldrich (GenElute™) DNA Extraction Kit, plant specimens used for DNA isolation incurred dissolving in double distilled water for storing at -20 °C. Primers for nrDNA and PCR thermal cycler settings followed (Ferrer *et al*., 2001) in the standard PCR. Each reaction contained 25 µl of Promega PCR Master Mix containing *Taq* DNA polymerase, dNTPs, $MgCl_2$, and reaction buffer; 1 µl of each primer at 10 µM of each primer, primer sequences ITS1 (TCC GTA GGT GAA CCT GCG G) and ITS4 (TCC TCC GCT TAT TGA TAT GC) (Ferrer *et al*., 2001); and 10 µl of diluted template DNA approximately 20 ng per 10 µl.

The extracted DNA samples served as a matrix for multiplication of the ITS rRNA gene area. The PCR had a three-step cycling procedure—initial denaturation at 95 °C for 5 min, 1 cycle; denaturation at 95 °C for 30 s; annealing at 55 °C for 1 min; extension at 72 °C for 0.5–2 min based on size product, 35

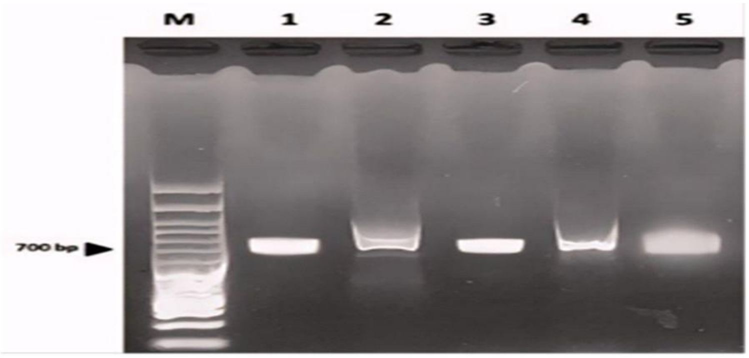

Figure 1. ITS PCR product gel electrophoresis for studied taxa, Lanes: 1000 bp DNA ladder, lanes: (1, 2, 3, 4, and 5): 612 -740bp ITS rRNA of *Salvia* species. 1- *S. palaestina,* 2- *S. spinosa,* 3- *S. compressa,* 4- *S. multicaulis, and* 5- *O. basilicum*.

cycles; and a final extension at 72 °C for 6 min, 1 cycle utilizing the Thermal Cycler (Bio-Rad C 1000, USA).

The end products of amplified PCR reached separation on agarose gel with 1X TBE buffer, stained with ethidium bromide, and visualized under UV light DNA amplicons before extracting from the agarose, utilizing GenElute™ Gel Extraction Kit (Merck KGaA Darmstadt, Germany) (Figure 1). The DNA sequencing succeeded at the Psomagen sequencing company (Maryland, USA) based on Sanger dideoxy sequencing alignments technology, utilizing BLAST all sequences and matching with a public database. After checking all the sequences, the new accessions' addition to the NCBI database ensued. This study listed the GenBank accession numbers for all taxa recovered (Table 1). The comparison of DNA fragment sequences with definite DNA sequences formerly documented in the NCBI gene bank proceeded by utilizing the bioinformatics software. All obtained accessions underwent the construction of a neighbor-joining tree based on the Kimura 2-parameter distance format (Kimura, 1980).

Taxonomic examination

Specimen examination and organographical (habit, root, stem, leaf, petiole, inflorescence, bract, reproductive organs) investigation continued based on the National Herbarium of Iraq collections of *Salvia* spp. and *Ocimum*. Taxonomic literature reviews, especially the protologues of all accepted names of both *Salvia* spp. and *Ocimum* transpired. The study followed the terminology of Khal (2020) for the morphological explanation of examined taxa.

Morphological data

In investigating their taxonomic importance, the morphological features (lifestyle, habit, root, stem, leaves, and reproductive organs) reached scrutiny in the field and herbarium for the classification of the *Salvia* and *Ocimum* species. Using a digital Canon camera helped take all images, with a ruler used during photography. Finally, the mapping of selected morphological data for each taxon continued on to the tree to identify the character evolution within the genera of *Salvia* spp. and *O. basilicum*.

Molecular data analysis

The Clustal W application in Molecular Evolutionary Genetic Analysis (MEGA-10) within *Salvia* spp. and *O. basilicum* proceeded for the alignment of multiple sequences. The phylogenetic analysis occurred by applying the MEGA-10 program and 1000 bootstrap replicates for preliminary accuracy investigation. A genealogy diagram was successful using a MEGA-10 neighbor-joining design to align nucleotides for the 5.8S rRNA gene area. For pair distance analyses, the use of the maximum likelihood (ML) format

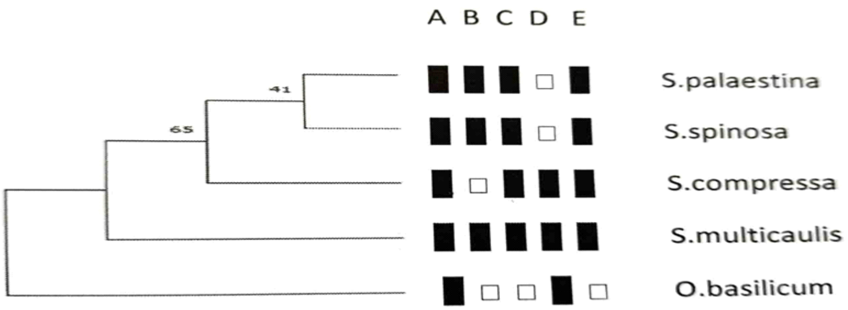

Figure 2. Neighbor-Joining diagram for four *Salvia* spp. and *Ocimum basilicum*. The MEGA-X was utilized as the nucleotide substitution format. The lengths of branches are correlative to numbers of nucleotide substitutions expected. Selected morphological features mapped on to the Maximum likelihood tree, with morphological topologies. Symbols are open squares (O) or filled squares (F). Traits include: A. Habit: shrub = O or herb = F; B. Life style: Annual = O, Perennial = F; C: Basal leaves: present = F, absent = O; D: Calyx shape: tubular = O, campanulate or infundibular or scarcely exceeding = F; E: Number of stamen: 2 = O, 4 = F.

ensued. Some vegetative characteristics (habit, lifestyle, presence/absence of basal leaves, and three floral characteristics, viz., calyx shape and number of stamens) succeeded in mapping on the simplified ML tree from the ITS spacer dataset (Figure 2).

RESULTS

Morphological characterization

Studied taxa shared many common characteristics. They are all aromatic perennials, except for *O. basilicum* as an annual; they have taproots and quadrangular erect stems, simple, opposite leaves, and cylindrical petioles. Inflorescences are paniculate, bracts are seated, calyxes are bilabiate, and the upper lip is straight, consisting of three lobes. The lower lip has two lobes, a corolla, and bilabiate; the upper lip is falcate bilobed, and the lower lip consists of three lobes with a straight ventricose tube. The flowers have two epipetalous stamens, and the fruits were smooth with four glabrous nutlets (Table 2).

Salvia palaestina

Plant length is 39–105. The root is 10–105 cm, and the stem is about 30–70 cm, which is many-branched above, hirsute, and glandular. The leaves are lyrate below, 10–14 cm × 5–7 cm; on the stem oblong, 5–6 cm × 2–2.4 cm, tomentose, rugose, with erose margins, acuminate apexes, hastate bases, and petioles about 15–20 cm in length. Verticillasters bear six flowers; bracts have caudate apices and cordate bases with entire margins and are purple; pedicels are about 0.2–0.3 cm in length, calyxes about 1.3–1.7 cm in length, with a greenish violet shade, tubular-campanulate, with moderate glandular and eglandular hairs. The corolla is whitish lilac, about 2.7–3 cm in length. The filament is about 1.55–1.75 cm, type B, with anthers about 3.1–3.5 mm in length, and the style is about 1.4–1.6 cm. The fruits are spherical, smooth, about 3.3–3.6 mm in length, and light brown (Table 2).

Salvia multicaulis

Plant is about 48–93.5 cm tall, with the mat forming herbs. Roots are about 10–105 cm in length. The stem is about 50–60 cm, many unbranched above, glandular-pilose, with few dendroid hairs. Basal leaves’ dimensions are about 10–14 cm × 5–7 cm, broadly ovate; stem leaves’ dimensions are about 5–6 cm × 2–2.4 cm, elliptic–oblong, with simple and satellite eglandular hairs. Surfaces are rugose, margins are crenulate, apices are acute-

Table 2. Quantitative morphological characteristics of four *Salvia* species and *Ocimum basilicum.*

No.	Taxon	*S. palaestina*	*S. multicaulis*	*S. spinosa*	*S. compressa*	*O. basilicum*
1	Plant length	39–105 cm	48–93.5 cm	38.7–107.6 cm	13.65–46.8 cm	47.3–69.2 cm
2	Root length	10–38 cm	50–60 cm	9.5–40 cm	4–12.5 cm	15–20 cm
3	Stems	30–70 cm	10–35 cm	30–70 cm	10–35 cm	30–50 cm
4	Leaves	6-14 × 2-7 cm	5-7.9 × 3.2-3.9 cm	6-9 × 4-7 cm	12.5-14 × 9-11.5cm	3.5-3.8 × 1.7-1.8 cm
5	Petiole	15–20 cm	7–9 cm	5–8 cm	6.3–10 cm	1–1.8 cm
6	Verticillasters	6 flowered	12–13 flowered	6 flowered	12–13 flowered	6 flowered
7	Bracts	2-14 ×3-5 cm	1.5-4 × 0.5-1 cm	2-7 × 5-8 cm	1.4-9 × 1.4-4 cm	0.5-3 × 0.3-2 cm
8	Calyx length	1.3–1.7 cm	1.2–1.6 cm	2.1–2.3 cm	1.5–2 cm	0.75–0.95 cm
9	Pedicel	0.2–0.3 cm	0.2–0.3 cm	0.17–0.28 cm	0.18–0.3 cm	0.3–0.4 cm
10	Corolla length	2.7–3 cm	2–2.5 cm	2.7–3.2 cm	1.4–1.55 cm	0.9–1.16 cm
11	Filaments	1.55–1.75 cm	1–1.2 cm	1.8–2.3 cm	0.5–0.7 cm	0.8–0.9 cm
12	Fertile anther length	3.1–3.5 mm	2.2–3.2 mm	1.3–1.6 mm	1.4–1.6 mm	1–1.7 mm
13	Style	1.4–1.6 cm	1.3–2 cm	3.2–3.6 cm	1.3–1.4 cm	0.7–0.8 cm
14	Nutlet length	3.3–3.6 mm	3.3–3.6 mm	3–3.7 mm	2.5–2.6 mm	2.3–2.45 mm

obtuse, and bases are truncate rounded. Petioles are about 7–9 cm in length. Verticillasters bear six flowers; bracts are cordate, with broadly-acuminate apex and cordate base. Margins are denticulate and light green, with pedicels length about 0.2–0.3 cm. The calyx is about 1.2–1.6 cm in length, green-purple, campanulate, with sparsely glandular pilose indumentum, purplish violet corolla, ranging between 2–2.5 cm in length, and a straight tube. The filament lengths' are about 1–1.2 cm, the fertile anthers' length is about 2.2–3.2 mm, and the style lengths are 1.3–2 cm, spherical, about 3.3–3.6 mm in diameter, and light brown (Table 2).

Salvia spinosa

Plant length is about 38.7–107.6 cm. Roots are about 9.5–40 cm in length. The stem is 30–70 cm, single, unbranched above, and covered with glandular hairs, and the leaves are ovate, about 6–9 cm × 4–7 cm, glandular pilose, margins are erose, apexes are obtuse, bases are truncate rounded, and petioles are about 7–9 cm in length. Verticillasters bear six flowers; bracts are deltoid, with abruptly acuminate apex and cordate base; margins are entirely yellowish green, with pedicels' length about 0.17–0.28 cm, tubular calyx, about 2.1–2.3 cm in length, yellowish green, with glandular hairs. The white corolla ranged from 2.7 to 3.2 cm in length, and the tube is straight. Filaments are about 1.8–2.3 cm in length; fertile anthers' length is about 1.3–1.6 mm; and the style length is 3.2–3.6 cm, with spherical nutlets, about 3–3.7 mm in length, and light brown (Table 2).

Salvia compressa

Plant size is 13.65–46.8 cm. Roots are about 4–12.5 cm in length. The stem is 10–35 cm, single, unbranched above, and covered with glandular pilose indumentum. The leaves are ovate, about 12.5–14 cm × 15–20 cm, with glandular pilose, crenate margins, obtuse apices, and truncate rounded bases. Petioles are about 6.3–10 cm in length. Verticillasters bear 12–13 flowers; bracts are cordate, with a broadly acuminate apex, rounded base, and entire margins. The pedicel's length is about 1.5–2 cm. The calyx is infundibular, about 1.5–2 cm in length, purple-lilac, with glandular hairs, a purple-lilac corolla ranging between 1.4–1.55 cm in length, and a straight tube. Filaments are about 0.5–0.7 cm; fertile anthers' length is about 1.4–1.6 mm, the style length is 1.3–1.4 cm, and nutlets are spherical, about 2.5–2.6 mm in length, with a brown shade (Table 2).

Ocimum basilicum

Plant length ranged between 47.3–69.2 cm; roots are about 15–20 cm in length, with stems branched above, about 30–50 cm, and inflorescences are verticillaster. The leaves are oblong ovate, 3.5–3.8 mm × 1.7–1.8 mm. The apex is mostly acuminate, with a shallowly dentate margin, subglabrous, glandular punctate, and petiole length is about 1–1.8 cm. Floral leaves were broadly lanceolate, acuminate, with calyx length ranging between 0.75–0.95 cm, and often green-purple, and a six-flowered verticillaster. The calyx length is about 0.75–0.95 cm. The corolla was white or pink, about 0.9–1.16 mm. The plant has four fertile stamens. The nutlets are ovoid, about 2.3–2.45 mm in length, and dark brown (Table 2).

Sequence alignment and phylogenetic tree analysis

The PCR products of amplified ITS rDNA underwent sequencing based on the Sanger dideoxy sequencing alignment technology, utilizing BLAST, with all sequences matched with a public database. After checking all the sequences after nucleotide sequence alignments, the new accessions succeeded in addition to the NCBI database, and the sequences of the recovered taxa ranged from 612 to 740 bp. Based on molecular analysis of four *Salvia* species and *Ocimum basilicum* utilizing ITS sequencing (ITS1-ITS4), the sequences include a small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2. A complete sequence also existed for the large subunit ribosomal RNA gene with partial sequence.

The study revealed the tree comprised four clades, in which *S. palaestina* and *S. spinosa* form a distinct clade, with a bootstrap value of 41%, indicating the highest level of genetic similarity and evolutionary relatedness between these two species. *S. compressa* groups emerged close to the *S. palaestina* and *S. spinosa* clade, supported by a bootstrap value of 65%, suggesting a moderate genetic relationship among these three species in the genus *Salvia*. *S. multicaulis* appears on a separate branch, implying a more distant genetic relationship to the aforementioned *Salvia* species, and still within the same genus. *Ocimum basilicum* served as an outgroup in this analysis. Its placement was on a distinct branch from the *Salvia* species, highlighting the greater genetic divergence between the *Salvia* L. and *Ocimum* L. genera*.* The study contributed to the NCBI database with five sequences, which will facilitate further plant molecular studies.

DISCUSSION

As revealed from the morphological examination, the studied taxa shared many typical characteristics of the Lamiaceae family, including a quadrangular stem shape and bilabiate calyx and corolla shape, with the ovary consisting of four lobes. Contrastingly, many morphological characteristics differed, including stem morphology and length, leaf shape and dimensions, the color of calyx and corolla, and the number and shape of stamen. These showed valuable taxonomic importance, adding to the potential efficiency of the molecular data, differentiating taxa in both infrageneric and interspecific taxonomic levels. These results were consistent with many previous taxonomical studies about *Salvia* and *Ocimum* genera (Bahadori *et al*., 2017; Akinyemi *et al*., 2023). However, no morphological or molecular information was available about the species *S. compressa,* except numerous morphological descriptions reported in the flora of Iran (Rechinger, 1982).

The selection of obvious synapomorphies among *Salvia* species for phylogenetic analyses is rather difficult and challenging to date because *Salvia* L. comprises numerous species with abundant morphological variation (Drew*,* 2020). Nonetheless, the detailed morphological data for both genera of *Salvia* and *Ocimum* were presently insufficient. However, the tubular calyx shape (Figure 3 G) just occurred in *S. palaestina* and *S. spinosa*. In contrast, they were campanulate and infundibular in the remaining species, with the presence of two stamen rather than four and the significant elongated appendages, which separate both anthers' lobes. These characters represent long-standing synapomorphy restricted to *Salvia* spp. and not in other genera, which

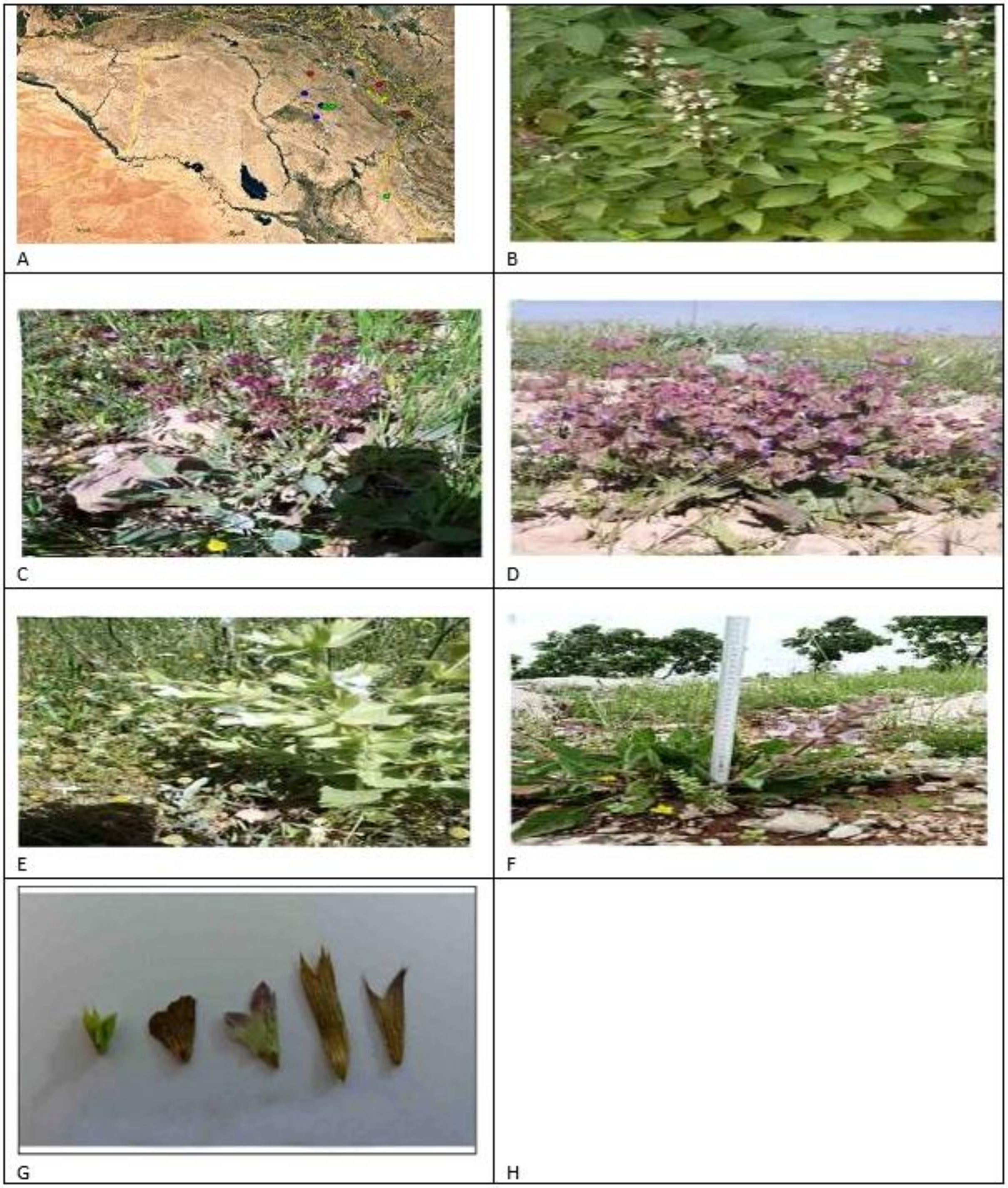

Figure 3. (A) Geographical distribution of studied taxa:-. 🔴 *Salvia palaestina*, ○ *S. spinosa,* 🟢 *S. compressa,* 🟡 *S. multicaulis,* 🔵 *O. basilicum*; (B) Morphology of *O. basilicum*; (C) Morphology of *S. multicaulis*; (D) Morphology of *S. compressa*; (E) Morphology of *S. spinosa*; (F) Morphology of *S. palaestina*; (G) Calyx shape from left to right of the *O. basilicum*, *S. multicaulis*, *S. compressa*, *S. spinosa*, and *S. palaestina.*

Table 3. Disparity index of four *Salvia* species and *Ocimum basilicum.*

S. palaestina				
S. multicaulis	0.08511			
S. spinosa	0.00654	0.08567		
S. compressa	0.01309	0.09046	0.01146	
O. basilicum	0.16917	0.20848	0.16257	0.16667

clearly indicates these species developed an adaptation to enhance the pollination success via the pollinators (birds or insects or both) visiting the pollinated flowers to feed on nectar juices (Wester *et al*., 2020). The other explanation for this restriction is the minimum occurrence among *Salvia* spp. due to enforced reproductive isolation because of mechanical exclusion in pollinators and flowers by only bees or hummingbirds (Celep *et al*., 2020).et a

This is the first phylogenetic study of the two genera of *Salvia* L. and *Ocimum* L., proceeding from rRNA ITS sequences in Iraq. This phylogenetic tree allows one to peer into the evolutionary dynamics both at a general level of the Lamiaceae and, specifically, into the studied genera. The clustering of *Salvia* spp. could have a connection to the genetic conservation within this genus due to similar ecological niches or evolutionary constraints. On the other hand, the genetic radiation occurring among *Salvia* spp. can indicate an adaptive radiation probably driven by either ecological diversification or geographical isolation. The tree branching refers to the polyphyletic origin of *Salvia* spp. (Figure 2) meaning the certain traits' evolution was parallel with variance forms (Kriebel *et al.*, 2020) (Table 3).

The study agrees with past findings enunciating that genus *Salvia* is a non-monophyletic group (Will and Claßen-Bockhoff, 2014; Khal, 2020). Here, the relationship among the studied taxa was much more valid based on an evolutionary tree constructed according to ITS sequence, supported by a comparison of selected morphological traits considered typical synapomorphies among the studied taxa. The molecular marker ITS occurred to be crucial for investigating the genetic diversity of various crop plants and, specifically, for classifying species that are difficult to identify utilizing traditional classification approaches (Taib *et al*., 2023). This phylogenetic analysis shows the genetic complexity and diversity within the Lamiaceae family. Thus, it forms a foundation for further research concerning possible application of genetic markers in taxonomy, conservation biology, cultivar specification, germplasm configuration, and parenthood detection of the hybrids due to its simplicity and the least cost.

However, at present, the two genera's comprehensive morphological, palynological, and anatomical studies have not materialized, and the available data is rather insufficient; therefore, the detection of new and valuable taxonomic features is essential for confirming a typical infrageneric classification framework.

CONCLUSIONS

The phylogenetic study based on four species of *Salvia* (*S. palaestina, S. spinosa*, *S. compressa*, and *S. multicaulis*) and *Ocimum basilicum* using nuclear ribosomal RNA internal transcribed spacer (ITS) region morphological characteristics serves as supporting evidence in differentiating the taxa in Iraq. The larger variance was evident between the two genera, while a degree of resemblance was notable among *Salvia* species. The ITS area was the most conserved, proving appropriate for phylogenetic inference at all the taxonomic levels. Additionally, the procedure emerged as rapid, simple, broadly applicable, and inexpensive. The phylogenetic taxonomy can better combine the information collected from all the sources.

REFERENCES

Ahmad SA (2016). *Salvia ali-askaryi* (Lamiaceae), a new species from Kurdistan, Iraq. *Harward Papers in Bot.* 21(2): 227-229.

Akinyemi OF, Ayodele MS, Ayo-John EI (2023). Morphological characterization of some members of *Ocimum* L. (Lamiaceae) in the southwest of Nigeria. *Fudma J. Sci.* 7(6): 23-36. https://doi.org/10.33003/fjs-2023-0706-2053.

Assaf M, Korkmaz A, Karaman Ş, Kulak M (2022). Effect of plant growth regulators and salt stress on secondary metabolite composition in Lamiaceae species. *S. Afr. J. Bot*. 144: 480-493. https://doi.org/10.1016/j.sajb.2021.10.030.

Bahadori S, Sonboli A, Jamzad Z (2017). Anatomical and morphological characteristics of *Salvia candidissima* Vahl. ssp. *candidissima* (Lamiaceae) as a new record from Iran. *Iran J. Bot*. 22(2): 104-111. https://doi.org/10.220-92/ijb.2016.107943.

Bazzicalupo M, Betuzzi F, Frigerio J, Nissim WG, Rapallo F, Ruffoni B, ... & Copetta A (2024). Characterization of the floral traits, pollen micromorphology and DNA barcoding of the edible flowers from three basil taxa (Lamiaceae). *Genet. Resour. Crop Evol*. 1-21. http://dx.doi.org/10.1007/s10722-024-02170-5.

Celep F, Atalay Z, Dikmen F, Doğan M, Sytsma KJ, Claßen-Bockhoff R (2020). Pollination ecology, specialization, and genetic isolation in sympatric bee-pollinated *Salvia* (Lamiaceae). *Int. J. Plant Sci.* 181(8), 800-811. http://dx.doi.org/10.10-86/710238.

Demirpolat A (2023). Essential oil composition analysis, antimicrobial activities, and biosystematic studies on six species of *Salvia*. *Life* 13(3): 634. https://doi.org/10.3390/life13030634.

Dover G, Strachan T, Coen E, Brown S (1982). Molecular drive. *Science* 218(4577): 1069-1069. https://doi.org/10.1126/science.7123257.

Drew BT (2020). Evolution, pollination biology, and species richness of *Salvia* (Lamiaceae). *Int. J. Plant Sci.* 181(8): 767-769. https://doi.org/10.1086/710711.

Drew BT, González-Gallegos JG, Xiang CL, Kriebel R, Drummond CP, Walked JB, Sytsma KJ (2017). *Salvia* united: The greatest good for the greatest number. *Taxon* 66(1): 133-145. https://doi.org/10.12705/661.7.

Enegide C (2021). *Ocimum* species: Ethnomedicinal uses, phytochemistry and pharmacological importance. *Int. J. Curr. Res. Physiol. Pharmacol.* 1-12. http://dx.doi.org/10.31878/ijcrpp.2021.52.01.

Ferrer C, Colom F, Frasés S, Mulet E, Abad JL, Alió JL (2001). Detection and identification of fungal pathogens by PCR and by ITS2 and 5.8 S ribosomal DNA typing in ocular infections. *J. Clin. Microbiol*. 39(8): 2873-2879. https://doi.org/10.1128/jcm.39.8.2873-2879.2001.

Kalita M, Devi N (2023). A taxonomic review of the genus *Ocimum* L. (Ocimeae, Lamiaceae). *Plant Sci. Today* 10(sp2): 126-137. https://doi.org/10.14719/pst.2552.

Kamyab R, Namdar H, Torbati M, Ghojazadeh M, Araj-Khodaei M, Fazljou SMB (2021). Medicinal plants in the treatment of hypertension: A review. *Adv. Pharm. Bull*. 11(4): 601. https://doi.org/10.34172%2Fapb.2021.090.

Khal LH (2020). Molecular taxonomy of some species of genus *Salvia* L. (Lamiaceae) in Kurdistan Region, Iraq. *J. Plant Prod*. 11(10): 967-974. https://dx.doi.org/10.21608/jpp.2020.124305.

Khal LH (2024). Systematic study in some taxa of the genus *Astragalus* L. (Fabaceae) in District of Kurdistan - Iraq. Faculty of Agricultural Sciences, University of Sulaimani. pp. 171-174. http://dx.doi.org/10.13140/RG.2.2.30128.16645.

Kimura M (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16: 111-120. https://doi.org/10.1007/bf01731581.

Kriebel R, Drew B, González-Gallegos JG, Celep F, Heeg L, Mahdjoub MM, Sytsma KJ (2020). Pollinator shifts, contingent evolution, and evolutionary constraint drive floral disparity in *Salvia* (Lamiaceae): Evidence from morphometrics and phylogenetic comparative methods. *Evolution* 74(7): 1335-1355. https://doi.org/10.1111/evo.14030.

Li M, Li Q, Zhang C, Zhang N, Cui Z, Huang L, Xiao P (2013). An ethnopharmacological investigation of medicinal *Salvia* plants (Lamiaceae) in China. *Acta Pham. Sin.* 3(4): 273-280. https://doi.org/10.1016/j.apsb.2013.06.001.

Özler H, Pehlivan S, Kahraman A, Doğan M, Celep F, Başer B, Bagherpour S (2011). Pollen morphology of the genus *Salvia* L. (Lamiaceae) in Turkey. *Flora: Morphol. Distrib. Funct. Ecol. Plants* 206(4): 316-327. https://doi.org/10.1016/j.flora.2010.07.005.

Rechinger KH (1982). Labiatae in: KH Rechinger, Flora Iranica. *Academische Drucku Verlagsanstalt, Graz-Austria* 150: 403-76.

Rose JP, Xiang CL, Sytsma KJ, Drew BT (2022). A timeframe for mint evolution: Towards a better understanding of trait evolution and historical biogeography in Lamiaceae. *Bot. J. Linn*. 200(1): 15-38. http://dx.doi.org/10.1093/botlinnean/boab104.

Taib TM, Aloush RH, AL-soufi ASM (2023). Taxonomy study of some *Euphorbia* L. species by leaf anatomical and molecular characteristics using *rbcL* and *matK* genes. *SABRAO J. Breed Genet*. 55(6): 1029-7073. http://doi.org/10.54910/sabrao2023.55.6.13.

Tangpao T, Chung HH, Sommano SR (2018). Aromatic profiles of essential oils from five commonly used Thai basils. *Foods* 7(11), 175. https://doi.org/10.3390/foods7110175.

Wester P, Cairampoma L, Haag S, Schramme J, Neumeyer C, Claßen-Bockhoff R (2020). Bee exclusion in bird-pollinated *Salvia* flowers: The role of flower color versus flower construction. *Int. J. Plant Sci.* 181(8): 770-786. http://dx.doi.org/10.1086/709132.

Will M, Claßen-Bockhoff R (2014). Why Africa matters: Evolution of old world *Salvia* (Lamiaceae) in Africa. *Ann. Bot.* 114(1): 61-83. https://doi.org/10.1093%2Faob%2Fmcu081.

Zhao Y, Chen YP, Drew BT, Zhao F, Almasi M, Turginov OT, Xiao JF, Karimi AG, Salmaki Y, Yu XQ (2024). Molecular phylogeny and taxonomy of Phlomoides (Lamiaceae subfamily Lamioideae) in China: Insights from molecular and morphological data. *Plant Divers*. 46(4): 462-475. http://dx.doi.org/10.1016/j.pld.2024.04.011.