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Molecular Taxonomy of some Species of Genus *Salvia* L. (Lamiaceae) in Kurdistan Region, Iraq

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ABSTRACT

Genus Salvia, commonly known as sage, is the largest genus in the Lamiaceae family. A phylogenetic analysis was conducted using sequences that were studied. The current study was reached to the identification of *Salvia* L. taxa family (Lamiaceae) in Kurdistan Region, Iraq. The study concluded that the current diagnosis of (5) taxa in different areas of Iraq these taxa were (*Salvia spinosa*, *Salvia palaestina, Salvia viridis, Salvia multicauli*s and *Salvia syriaca*). The objectives of this study were to explore the phylogenetic relationships and molecular taxonomy of five genus species Salvia, I studied the neighbour-joining tree of the nuclear ribosomal DNA internal transcribed spacer (ITS) region of the genus Salvia. The outcomes demonstrated the running of the achieved nucleotide of the Salvia in Mega X to show the similarity with other species of *Salvia*. The result shows the similarity with these five species and the tree divided into four clades, which are (clade 1,2,3, and 4). Which clade 1 consists of (*Salvia spinosa* and *Salvia palaestina*). Then the relationship between *Salvia spinosa* with the *Salvia palaestina* is about 79% in the same group in clade1 and the relationship between them. This group is also a sister relative by 99% with the species of *Salvia syriaca* in the clade 2. The clade 3 and clade 4 consist of (*Salvia viridis* & *Salvia multicauli*s) respectively, and the similarity of these two clades with the clade 1 and 2 is 79%. They were grouped into a different trichotomy in the surface of leaves, stems, and flowers. Most species were different and occurred in separated clades. The phylogenetic relationships of *Salvia* L. taxa consider the first study in Iraq.

Keywords: Lamiaceae, Salvia genus, Molecular systematics, ITS, Iraq.

INTRODUCTION

The genus *Salvia* belongs to the Salvinae tribe, the Mentheae tribe, the epetoideae family, the Lamiaceae family, and the Lamiales family from the two-star astrology group (Walker & Sytsma, 2007). The Lamiaceae family returns to the rank of Lamiales, which occupies about 12% of the Eudicot, comprising 23 families, 1059 genera, and 23,275 species (Watson & Dallwitz, 1992; Stevens, 2001). This family is described as one of the crucial botanical families with more than 250 genera. The genus has 2700 species and the genus Salvia L. is the largest of its genera and includes more than 900 species (Richard, 2004; Simpson, 2006; Kahraman, *et al*., 2009).

Several studies have recorded *Salvia* species in countries neighboring Iraq including Iran (Hedge, 1982b), Turkey (Hedge, 1982a), and Kuwait (Daoud, 1985). The Lamiaceae family It has been reported that in Iraq there are about (31) genus and more than 150wild species (Al-Rawi, 1964). In Iraq, recent studies have recorded more than 30 species of gender Salvia (Al -Musawi & Al- Musawi, 2001; Hussain, 2009). In this reference Hussain (2009), some studies did not depend on what was collected and diagnosed from the Salvia race in Iraq, which raises suspicion and requires further research and investigation to reveal the types of this gender and evaluation of her condition in the country. In this regard, Hedge (1972), describing the genus Salvia, noted that the assessment of the species status, endemic and rare species is an important

step towards protecting it from human activities (like city building, overgrazing, pollution, road construction, and dams) that have led to a deterioration of the gender condition. Salvia species gain their importance from their various uses in the pharmaceutical, medical, industrial, nutritional and aesthetic fields and are often mentioned in Pharmacopoeia's drug constitutions in many countries of the world (Kintzios, 2000; Garcia *et al*., 2016). Despite the above-mentioned images of the importance of taxa, Salvia, many aspects of his life (including phenotypic, anatomical, physiological, and chemical) did not receive the attention required by researchers (Shirsat *et al*., 2012), especially in Iraq and its neighbouring countries, which can be discerned from scarcity studies on this gender in these countries and their insufficien assessment of the status of its types and its importance in the applied field as well as the absence of accurate surveys, accurate diagnosis and refinement of information from time to time (Celep *et al*., 2010). *S. spinosa* was used before the discovery of antibiotics as a herbal mixture for people with tuberculosis to prevent sudation. The plant is still used as a condiment flavoring, for preserving meat and in the manufacture of spirits (Akaberi *et al*., 2015). The Chinese folk medicine known as Dan-shen (*S. multicaulis*) is prescribed as a sedative pain reliever, antimicrobial, antioxidant, antispasmodic, anti-inflammatory, and this medicine is mentioned in the Chinese pharmacy encyclopedia to treat problems associated with the heart and circulatory system as well as
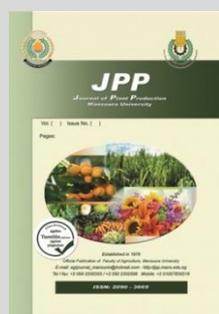
to treat rheumatic pain (Lemle, 2018). Flavonoids extracted from the leaves of type *S. palaestina* showed high efficacy against several bacterial species such as *Staphylococcus epidermis*, *Proteus vulgarie*, *Klebsiella pneumoniae* (Miski *et al.*, 1983) and *S. viridis* showed high efficacy against bacteria causing tuberculosis (Ulubelen *et al.*, 1997; Fournomiti *et al.*, 2015).

Egyptian species of the genus Salvia and the container have a high percentage of Thujone (41.5%) and limonene (14.7%). They have also shown efficacy against bacteria *Sarcina* spp. and *S. viviales* and *S. spinosa* (Farag *et al.*, 1989). Eidi *et al.* (2011) studied the inhibitory efficacy of the alcohol extract of S. aerial parts of type *S. syriaca* as an anti-inflammatory and tested them on laboratory mice which gave significant results. So far, however, several extensive molecular studies have been performed on the genus (Walker *et al.* 2004; Walker & Sytsma 2007; Will & Claßen-Bockhoff, 2014). Nuclear as well as chloroplast molecular markers are widely used to understand the evolutionary relations between taxa (Artyukova *et al.*, 2005; Pleines *et al.*, 2009). Nonetheless, finding an acceptable taxa area remains a problem (Lahaye *et al.*, 2008). Most authors reported that molecular studies have often preferred internal transcribed spacer area (ITS; ITS1?5.8- S?ITS2 sub-units) of 18S–26S nuclear ribosomal DNA (nrDNA) (Wojciechowski 2005; Yao *et*

al., 2010). This region's variation levels are suited for phylogenetic inference at a specific, generic, or even family level (Baldwin 1992; Baldwin *et al.*, 1995; Monsefi, *et al.*, 2015). Apart from the nuclear genome, several chloroplast DNA areas are commonly used for phylogenetic research (Shaw *et al.*, 2005; Kozłowska *et al.*, 2015). The main objectives of this study were to identify and classify the five species of *Salvia* in Kurdistan region-Iraq, which are (*S. viridis, S. spinosa S. syrica, S. multicaulis* and *S. palaestina)* by molecular taxonomy to construct a phylogenetic tree for the species on the basis of the molecular data.

MATERIALS AND METHODS

1-Samples collection

The current study was based on the fresh plant specimens of five species *Salvia* (*S. viridis, S. spinosa, S. syriaca, S. multicaulis,* and *S. palaestina*) was selected for this study, this species under Lamiaceae family, collected in different parts of Iraqi Kurdistan Districts from their natural habitats and directly from the field for the period from March to September 2019. The samples were collected at the flowering stage and were compressed, dried and stored for diagnosis. For each of the taxa studied, habit and flora photos are shown in fig. (1).

Fig. 1. Variation in the shape of the genus *Salvia* taxa.

2- Identification of plant specimens:

After samples were collected, dried and compressed, the samples were identified based on several

books and fluorines including Iraqi, Turkish, Iranian, Chinese, Kuwaiti and Saudi flora. The samples were deposited in the College of Agricultural Engineering

Science / University of Sulaimani after recording the scientific name, place and date of the collection.

Molecular analysis methods:

3-DNA isolation and sequencing:

The leaves of each species were collected directly from the field and leave was dried on silica gel for transportation to the lab where they were ground in liquid nitrogen on it, after that put the leaves in the pestle and mortar and spill some liquid nitrogen and smoothly grind the leaves and put it in the glass tube and protect it in the freezer under (-20 ºC).

Absolute genomic DNA has been collected with the Cetyltrimethylammonium Bromide (CTAB) process of Salvia leaf tissue (Doyle and Doyle, 1987). NanoDrop 2000c UV–Vis Spectrophotometer then calculated the purity and quantity of the genomic DNA (Thermo Scientific) and using 1% (w/v) agarose gel electrophoresis using a Bio-Rad gel imaging system. Finally, the extracted genomic DNA was diluted to 40 ng.μL^{-1}, after isolation, samples were stored at -20 °C prior to conventional PCR amplification.

A sampling of materials a total of 5 nrDNA ITS sequences in this study was used; in the standard PCR, each reaction contained (MyTaq^{TM} HS Mix-Bioline, USA) master mix (10 μL), 10 pmol of each primer (forward,5`CGTAACAAGGTTTCCGTAGGTGAA -3', Reverse 5'- TTATTGATATGCTTAAACTCAGCGGG-3') and 20 ng template DNA. PCR was performed using a three-step cycling protocol: initial denaturation (95 ºC. 5 min^{-1}) 1 cycle; [(denaturation (95 ºC. 40 sec^{-1}), annealing (60 ºC. 45 sec^{-1}) (extension 72 ºC .1 min^{-1}) 40 cycle] and final extension (72 ºC. 5 min^{-1}) 1 cycle (Bio-Rad C1000 Thermal Cycler, USA) (Zhang *et al.*, 2012). The PCR products were analyzed in 1% agarose gel stained with Syber Safe Dye. To estimate the efficiency and accuracy of the assay, DNA sequencing examined selected PCR-amplified DNA samples. In total samples were being sequenced in Macro gen Company (South Korea).

4-Salvia species, ITS gene sequencing, and sequence alignment:

DNA sequencing was undertaken in Macro gen Company (South Korea) (Genomic Study Center), then the DNA sequences compared with previously recorded DNA sequence in the NCBI database bank using bioinformatics tools.

5-Data Analysis

Clustal W was applied in MEGAX with other species of Salvia for multiple sequences alignment. For pre-trial reliability checking, the phylogenetic analysis was carried out using MEGAX with 1000 bootstrap replicates. A phylogenetic tree was developed with the MEGAX neighbour-joining tree method to provide nucleotide alignment for the 18S rDNA region. The Maximum Composite Likelihood model was used for pair distance analyses (Kumar *et al.*, 2018 and Gharib *et al.*, 2019).

RESULTS AND DISCUSSION

Morphological description of the genus *Salvia*

Salvia species include annual, biennial or perennial herbs and woody subshrubs as well as other families of Lamiaceae. Root taproot in shape, conical, or rhizome.

The stems are typically angled, base leaves are embedded and their blade is oval to sub-cardiac Sub Cordate, pinnately compound feathered edges may be entirely straight edges and sometimes toothed or pinnatisect feathering, flowering stems carry seated bracts can often be colored with sharp or tapered tops or fork or semi-caudal, and Bracteoles may contain flower lights Terminal raceme, compound cluster, panicle, paniculate, or Spiciform fork and containing one or several flowers in a single bracelet in a Verticellate shape, the Calyx tubular or bell-shaped bellows are rarely thorny and sometimes colored. The Corolla is divided into 2-lips, the upper prominent and crescent shape consisting of two lobes, and the bottom in three lobes 3-Lobes are blue or white ... etc. And the coronation tube is slightly dotted Invaginate or swollen in the middle Ventricose towards the bottom and may contain papillae Stamens Long Didynamous shortened to two tiny structures and the joint is usually longer than the thread and has two chambers, the upper fertile and the lower is incomplete imperfect and the stigma is two-fold 2- Clefts are often unequal. The fruits are smooth ovoid or oblong nutlets and sometimes expanding, with a Trigonous or Ovoid smooth Nutlet shape to a suborbicular circular and sometimes covered with a coat Mucilage. Many species of the genus have various trichomes bristles on leaves, stems, and flowers which help reduce water loss in some species. The hairs are often glandular, releasing volatile oils that give the plant a distinctive aromatic character and viscosity. Rarely its bristles are dendroid Trichome. (Al-Hussaini, 2006; Sell & Gina, 2009).

In this study, the sequences included two regions: 21 nucleotides of the 18S rRNA gene and the complete sequences of ITS1, 5.8S rRNA gene, the extracted genome was used as a template for amplification of the ITS rDNA gene, the result showed 600-700 bp amplified ITS rDNA gene. ITS rDNA gene is one of the most repeatedly utilized genes in molecular taxonomy or phylogenetic studies and a substantial marker for random target polymerase chain reaction (PCR) in ecological biodiversity screening.

The amplified ITS rDNA PCR product was subjected to DNA sequencing and the comparison with the public database by BLAST. The PCR product of 18S rDNA was 600-700 bp for genus Salvia (Fig. 2).
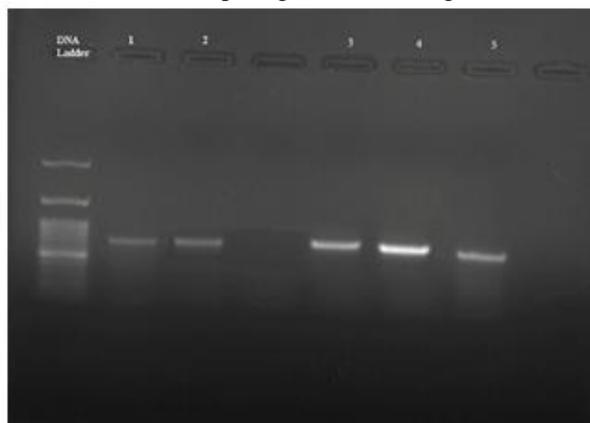

Fig.2. Gel electrophoresis of the amplified PCR product of ITS rDNA gene.

Lane 1: 100 bp DNA ladder, lane 1, 2,3, 4, and 5: 600 -700bp ITS rDNA of Salvia species. 1-*S. palaestina*
2-*S. multicaulis*   3- *S. syriaca*
4-*S. spinosa*   5-*S. viridis*

The result of the aligned ITS rDNA sequence of the (*S. palaestina, S. multicaulis, S. syriaca, S. spinosa,* and *S. viridis*) local isolate showed the ITS rDNA of isolate (No. 1, 2, 3, 4, and 5) were identical to ITS rDNA gene and identical to the internal transcribed spacer 1, 5.8S r RNA gene of (*S. palaestina, S. multicaulis, S.syriaca, S. spinosa* and *S. viridis*) Figure 3 , 4, 5, 6, and 7).

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TGTCGACTGCAAGCAGACCGCGAACATGTGTTTAACACCGACCGACGGCGCAGGCTCGGGGCCGACCCC
GCTCCGTGCCGCCTTCAACCCCGCCCGCGCGTTCCCTCGGGTCGCGCGGCGCGGGCTAACGAACCCCGGC
CCGAAATGCGCCAAGGAAAACTAAACAAAGCGTCCTCCCCCCCGCGCCCCGTTCGCGGAGTGTGCGGGG
GTGTCGAACGTCTATCAAATGTCATAACGACTCTGGGCAACGGATATCTCGGCTCTCGCATCGATAAAAA
ACGTACCAAAATGCGATACTTGGTGTGAATTGCAAAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTT
GCGCCCGAAGCCATTAGGCCAAGGGCACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCCACCATGT
GCGGGGGCGGATACGGGCCCCCCGTGCGCCCCGGCGCGCGGCTGGCCCAAATGCAATCCCTCGGCAACTC
AGGTCACGACAAGTGGTGGTTGAAATCTCAATCTCTTGCGCCGTCGTGCCGCTGCGTCGTCCGTAAGGGC
ATCCATCACGACCA
```

Fig. 3. Alignment of ITS gene of *Salvia palaestina*.

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AAGTCTTGTCGACCTGCAAGCAGACCGCGAACACGTGCCTAACACCAACCGACGGTGCATGGCGTGGGG
GCGACCCCCGTCCTGTTCCCGTCACCCCCGCCCGCGTGTTCCCATCGGGTCACGTCGTGTGGGCTAACGAA
CCCCGGCGCGGAATGCGCCAAGGAAAACCAAATGATGCATCCCCCCCCGTGCCCCGCTCGCGGAACGTGC
GGGGGTGTCGGATGTCTATCAAATGTCAAAACGACTCTTGGCAACGGATATCTCGGCTCTCGCATCGATT
AAAAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCA
AGTTGCGCCCGAAGCCATTAAGCCGAGGGCACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCCTCC
GTGCGCACAGCGCCCGTTGTGGGGGGGGGGGAAATTGGGCTCCCCTGGTCCCCCGCGTGTGGGTGGGCCC
AAAGGGATCCCCCCGGGAAACCCGTGTCGAAAAATGGGGGGTTAAAAAATTTATCTCTCGCGCCCTCTTG
GCCCTGGGTCTTCCGCTTGGGGATCCCTCTAAGAACCCACGGTGTCCGTGCCTCACAACGCCCACCTTTTA
ACGCGAAACCCCGTCAAGCGGGATTATCCCCTGAATTTATCCATTCAAAAAAAAAA
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Fig.4. Alignment of ITS gene of *Salvia multicaulis*.

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AGTCTGTCGAGCTGCAAGCAGACCGCGAACACGTGTTTAACACCGACCGACGGCGCACGGCTCGGGGGT
GACCCCGCTCCGTGCCGCCGTCGACCCCGCCCGCGCGTTCCCTAGGGTCGCGCGGCGCGGGCTAACGAAC
CCCGGCGCGGAATGCGCCAAGGAAAACTAAACGAAGCGTCCCCTCCCCCGCGCCCCGTTCGCGGAGTGC
GCGGGGGTGTCGGACGTCTATCAAATGTCATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGA
TGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACG
CAAGTTGCGCCCGAAGCCATTAGGCCGAGGGCACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCCA
CCATGTGCGGGGGCGGATACTGGCCTCCCGTGCGCCCCGGCGCGGGCTGGCCCAAATGCGATCCCTCGG
CGACTCATGTCACGACAAGTGGTGGTTGAAATCTCAATCTCTTGCGCCGTCGTGCCGCTGCGTCGTCCGTA
AGGGCATCCATCAACGACCCAACGGGCCGGTGCGCCTCACGGCGCCCCGACCTTCGACCGCGACCCCGG
TCAGGCGGGATTACCCGCTGAGTTTAATCATAAAAACCATAAAAA
```

Fig. 5. Alignment of ITS gene of *Salvia syriaca*.

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AGTCTTGTCGACCTGCAAGCAGACCGCGAACACGTGTTTAACACCGACCGACGGCGCACGGCTCGGGGGC
GACCCCGCTCCGTGCCGCCGTCGACCCCGCCCGCGCGTTCCCTCGGGTCGCGCGGCGCGGGCTAACGAAC
CCCGGCGCGGAATGCGCCAAGGAAAACTAAACGAAGCGTCCTCCCCCCGCGCCCCGTTCGCGGAGTGTG
CGGGGGTGTCGGACGTCTATCAAATGTCATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGAT
GAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGC
AAGTTGCGCCCGAAGCCATTAGGCCGAGGGCACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCCAC
CATGTGCGGGGGCGGATACTGGCCTCCCGTGCGCCCCGGCGCGGGCTGGCCCAAATGCGATCCCTCGGC
GACTCATGTCACGACAAGTGGTGGTTGAAATCTCAATCTCTTGCGCCGTCGTGCCGCTGCGTCGTCCGTAA
GGGCATCCATCAACGACCCACGGGCTGGGGGCGCCTCGCGGCGCCCCGACCTTCGACCGCGACCCCAGGT
CAGGCGGGATTACCCGCTGAGTTTAAGACATATCAATAAA
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Fig. 6. Alignment of ITS gene of *Salvia spinosa*.

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AGTATTGTCGAACTGCAAGCAGACCGCGAACACGTGTTTAACACCGACCGACGGCGCAGGCTTGGGGGC
GACCCCTCTCCGTGCCGCAGTCGCCCCCGCCGGCGTGTTCCCTCGGGTCACGTCGTGCGGGCTAACGAAC
CCCGGCGCGGAATGCGCCAAGGAAAACCAAACGAAGCGTCCTTCCCCCGCGCCCCGTTCGCGGTGCGCGC
GGGGGGGTCGGACGTCTTTCAAATGTCAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGAT
GAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGC
AAGTTGCGCCCGAAGCCATTAGGCCGAGGGCACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCTCCC
TCCAAGTGCTTAGGCACCGGCGGCGGGGGCGGATATTGGCCTCCCGTGCGCCCCGGCGCGCGGCTGGCCC
AAATGCGATCCCTCGGCGACTCGCGTCACGCACAAGTGGTGGTTGAAATCTCAATCTCTTGCGCCGTCGTG
CCGCCGCGTCGTCTGCACGGGCATCCATAAACGACCAACGGGTGAAGGCGCCCCCTCGTGGGGCGCCCG
ACCTTCGACCGCGACCCCAGGTCAGGCGGGATTACCCGCTGAGTTTAAGCATAAAATCCAATAAAA
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Fig. 7. Alignment of ITS gene of *Salvia viridis*.

Neighbour-Joining Tree of genus Salvia

Neighbour-Joining Tree (Fig. 8) is the result of the proceeding the achieved nucleotide of the (*S. viridis, S. spinosa, S. syriaca, S. multicaulis* and *S. palaestina*) in the Mega X to show the similarity. The result shows the similarity with these five species and the tree divided into four clades, which are (clade 1, 2, 3, and 4). Which clade 1 consists of (*S. spinosa* and *S. palaestina*). Then the relationship between *S. spinosa* with the *S. palaestina* is about 79% in the same group in clade1 and the relationship between them. This group is also sister relative by 99% with the species of *S. syriaca* in the clade 2. The clade 3 and clade 4 consist of (*S. viridis* and *S. multicauli*s) respectively, and the similarity of these two clades with the clade 1 and 2 is 79%.
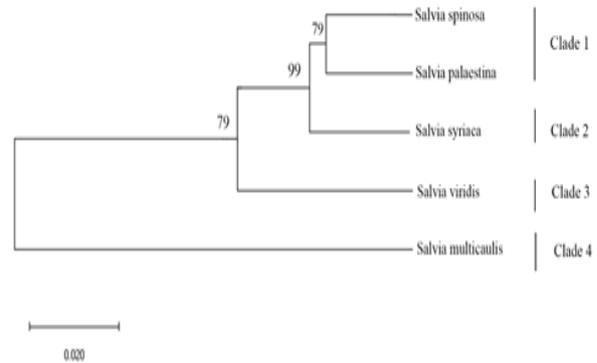

Fig. 8. Neighbor-Joining Tree for five Salvia species. The MEGA-X has been used as the nucleotide substitution model. The branch lengths are proportional to numbers of nucleotide substitutions predicted.

The sequencing was done at Macrogen Company, Korea and the sequence was deposited in GenBank. For phylogenetic analysis, sequences of amplified products were retrieved in FASTA format from GenBank. Phylogenetic analysis (Disparity Index, Pairwise Distances and Nucleotide composition) using the Neighbour-Joining (NJ) method (N. Saitou *et al.*, 1987) was performed with the MEGA v. X computer program (K. Tamura *et al.*, 2011). The phylogenetic tree was constructed using the Kimura two-parameter distance model (M. Kimura *et al.*, 1980) with the 'pairwise deletion of gaps option'. The robustness of branches was assessed by bootstrap analysis with 1000 replicates (Gharib *et al.*, 2019).

Disparity Index

Table (1) displays the disparity index that calculates the difference in substitution patterns observed for a pair of sequences. The maximum and lowest differences of *S. viridis* revealed with the *S. multicaulis* and *Salvia spinosa* and *S. syriaca* by 0.230% and 0.000% respectively.

Table 1. Disparity Index of genus Salvia.

S. viridis			
S. spinosa	0.0000000000		
S. syriaca	0.0000000000	0.0000000000	
S. multicaulis	0.2305433186	0.4526315789	0.3888888889
S. palaestina	0.1698774081	0.1783216783	0.1765734266	0.0755711775

Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution

Table (2) demonstrates the consequence of estimating the maximum composite probability of the nucleotide substitution pattern. The highest transitional substitution revealed between the nucleotides of G and A and C and T by 10.7106 and the lowest between the nucleotides of T and A, C and A, G and T and G and C was 7.1447. The nucleotide frequencies are A = 25.00%, T/U = 25.00%, C = 25.00%, and G = 25.00%.

Table 2. Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution.

From\To	A	T	C	G
A	-	7.1447	7.1447	10.7106
T	7.1447	-	10.7106	7.1447
C	7.1447	10.7106	-	7.1447
G	10.7106	7.1447	7.1447	-

Pairwise Distance

Table (3) shows the pairwise distance which is a measure of the distance between each pair of species. The results show the highest distance of *S. viridis* with *S. multicaulis* by 0.1777 %.

Table 3. Pairwise Distances of genus *Salvia*.

S. viridis				
S. spinosa	0.0717			
S. syriaca	0.0744	0.0418		
S. multicaulis	0.1777	0.1759	0.1802	
S. palaestina	0.0841	0.0350	0.0455	0.1722

Nucleotide composition

Table (4) shows numbers of nucleotide (A, T, C, and G) within the sequences. The result revealed numbers of cytosine nucleotides (C) is more than other nucleotides in all sequences of species.

Table 4. Nucleotide composition of genus Salvia.

Salvia Species / Nucleotide	*S. viridis*	*S. spinosa*	*S. syriaca*	*S. multicaulis*	*S. palaestina*	Avg.
T(U)	17.27	16.99	16.77	19.21	16.96	17.46
C	33.24	34.13	34.12	32.02	34.44	33.56
A	19.57	18.93	19.73	21.25	20.45	19.98
G	29.93	29.96	29.38	27.51	28.15	29.01
Total	695	671	674	687	572	659.8
T-1	18.88	19.2	18.22	21.65	19.79	19.55
C-1	30.04	30.8	32.44	28.57	31.77	30.68
A-1	20.6	20.09	19.56	22.51	21.35	20.81
G-1	30.47	29.91	29.78	27.27	27.08	28.96
Pos #1	233	224	225	231	192	221
T-2	15.52	14.67	15.11	18.34	13.54	15.5
C-2	34.48	35.56	34.67	34.5	36.98	35.18
A-2	17.24	17.78	19.11	18.34	20.31	18.5
G-2	32.76	32	31.11	28.82	29.17	30.83
Pos #2	232	225	225	229	192	220.6
T-3	17.39	17.12	16.96	17.62	17.55	17.32
C-3	35.22	36.04	35.27	33.04	34.57	34.83
A-3	20.87	18.92	20.54	22.91	19.68	20.62
G-3	26.52	27.93	27.23	26.43	28.19	27.22
Pos #3	230	222	224	227	188	218.2

There were no studies on the phylogeny of Salvia species from Iraq for nrDNA ITS sequences; however, all the Salvia species in Iraq have not been studied on this basis (21-23). According to analysis in the present study, there were obvious differences in molecular phylogeny between Salvia species. The phylogeny of some species of salvia was estimated based on ITS multi-alignments (Figs. 2 and 8). Analysis of DNA sequence data from the two ITS regions yielded a high level of genetic variability (polymorphism) among the studied samples. Results indicate that four major clades within salvia are supported by high bootstrap values.

The sequences of (*S. spinosa* and *S. palaestina*) were almost 79% similar, and they have a close relationship by 99%. with *S. syriaca*. Morphological characters, such hairs, number of corolla and corolla color different between similar species. They were grouped into different trichotomies based on leaf, stem, and flower surfaces. Most species were different and occurred in separated clades. The first analysis included five species of Salvia. The analysis of this data set identified four main clades, one containing *S. spinosa* and *S. palaestina*, two with *S. syriaca*, the third clade was composed of *S. viridis*, *S. multicaulis* species occupies fourth clade (Fig. 8).

The evolutionary evidence refers to the phylogenetic tree as being monophyletic descending. According to (Gao *et al*., 2010) the phylogenetic classification must be a synthesis of evidence obtained from all sources. Comprehensively, the evolution score depends upon the advanced characters that owned by the genus comparatively with other plant families. Salvia species is near the advanced end due to more reduced characteristics (Marghali, 2015). In Subclade A, S. evansiana, S. przewalskii, S. paucifl ora, S. aerea, S. flava, S. cynica, S. digitaloides, S. brevilabra, S. cyclostegia, S. castanea, S. omeiana, S. roborowskii, and S. tricuspis were clustered together; all belong to subgen. Salvia (Zhang *et al*., 2012).

The ITS data set consists of 5 Salvia taxa sequences. The total species area of (600-700) bp was used in the present study. My findings were compatible with the findings of Will & ClaßenBockhoff (2014) as of the sect. "Hymenosphace in Salvia genus is a non-monophyly group" and "Expanding calyces with assorted lips in fruits, not only in *Salvia* but in Zhumeria, has modified many

times in parallel." The phylogenetic ITS tree of this study was established, and the taxa of the group, Will & Claßen-Bockhoff (2014). Old world Hymenosphace is distributed in three areas as Southwest Asia, Turkey, Iran, Afghanistan, and Russia are the first area, the Canary Islands are the second region, and Southern Africa is the third region. It is difficult to specify an appropriate region for phylogenetic studies. For phylogenetic studies, a region highly interspecific will be fruitful as this region can distinguish species of plants in the established phylogenetic tree (Gao *et al.*, 2010). However, genetic divergence parameters, parsimony network analysis, and phylogenetic tree structures show that the ITS area has an advantage over the chloroplast DNA regions.

The evolutionary tree built on the ITS sequence showed the highest interspecific divergence so that relations between the Salvia taxa were much better solved (Fig. 8). The study shows before a phylogenetic study starts, the most potential region should be found. While our samples were limited to one gene, the regions which could be useful in further studies were sufficient to be clarified. Our study shows that in the nuclear genome ITS has the highest variability and high discrimination efficiency in regions with sufficient variability or marker. Also, many representative sequences are available in a database that would be valuable for understanding Salvia species evolutionary relationships worldwide.

The use of molecular markers has more advantages than morphologically based phenotypic characterization (Jose *et al.* 2013), because molecular markers are generally unaffected by environmental and cultural practice. The classification of five taxa of commercial Salvia growing in the Kurdistan region of Iraq using ITS analysis was highly consistent with previous studies based on different marker systems. Owing to its simplicity and low cost, this marker system can be further employed in cultivar identification, germplasm organization and parentage identification of hybrids.

CONCLUSIONS

The molecular taxonomy and phylogenetic relationships of the genus Salvia has been presented in this study. Five species were noted in Kurdisatan-Iraq region which were (*S. viridis, S. spinosa, S. syriaca, S. multicaulis* and *S. palaestina*). I've analyzed the nuclear ribosomal DNA internal transcribed spacer (ITS) region of the genus Salvia by Neighbor-Joining Tree. The outcomes demonstrated the maintaining the achieved nucleotide of the Salvia in the Mega X to show the similarity with other species of Salvia. The result shows the similarity with these five species and the tree divided into four clades. Then it can be noticed that the relationship between *S. spinosa* with the *S. palaestina* is about 79% in the same group in clade1 and the relationship between them. This group is also sister relative by 99% with the species of *S. syriaca* in the clade 2. The clade 3 and clade 4 consist of (*S. viridis* and *S. multicaulis*) respectively, and the similarity of these two clades with the clade 1 and 2 is 79%.

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التصنيف الجزيئي لبعض انواع الجنس .Salvia L (العائلة الشفوية) في اقليم كردستان ، العراق

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قسم البستنة ، كلية علوم الهندسة الزراعية ، جامعة السليمانية ، السليمانية ، اقليم كردستان ، العراق

تم إجراء تحليل علم الوراثة لجنس سالفيا هو أكبر جنس في (العائلة الشفوية) باستخدام التسلسلات التي تمت دراستها. توصلت الدراسة الحالية إلى تحديد الجنس سالفيا (العائلة الشفوية) في إقليم كردستان العراق. خلصت الدراسة الحالي إلى أن التشخيص لـ (5) أصناف في مناطق مختلفة من العراق كانت هذه الأصناف (Salvia spinosa, Salvia palaestina, Salvia viridis, Salvia multicaulis and Salvia syriaca) من الجنس سالفيا. . أظهرت النتائج تشغيل النيوكليوتيدات المحققة للحمض النووي الريبوزومي ITS النووي من سالفيا في ميجا إكس لإظهار التشابه مع الأنواع الأخرى من سالفيا. تظهر النتيجة التشابه مع هذه الأنواع الخمسة والشجرة مقسمة إلى أربعة فروع وهي (مجموعة 1 و 2 و 3 و 4). يتكون مجموعة من Salvia spinosa, Salvia palaestina,). ثم تكون العلاقة بين Salvia spinosa, Salvia palaestina, حوالي 79٪ في نفس المجموعة في مجموعة 1 والعلاقة بينهما. هذه المجموعة هي أيضًا أخت نسبي بنسبة 99٪ مع أنواع Salvia syriaca في المجموعة 2. يتكون مجموعة 3 ومجموعة 4 من (Salvia viridis و Salvia multicaulis) على التوالي ، وتشابه هاتين المجموعتين مع مجموعة 1 و 2 هو 79٪. تم تجميعهم في ثلاثي مختلف في سطح الأوراق والسيقان والزهور. كانت معظم الأنواع مختلفة وحدثت في مناطق منفصلة. تعتبر الدراسة الاولى حول علاقات النشوء والتطور لجنس سالفيا في العراق.