

Molecular Study of the Genus *Cephalonia* Schrader ex Roemer and schultes (Caprifoliaceae) in Iraq

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Abstract

This study conducted to investigate the complete sequences of plastid *TRN* L-F gene, which were determined for five species of *Cephalonia* Caprifoliaceae in Iraq to infer phylogenetic relationships. *Dipsacus laciniatus* used as out groups. Cluster analysis using PhyDE-1 and mega6 yielded Maximum Likelihood trees and UPGMA with high supported (bootstrapping) clade. Both trees showed *C. dictyophora* and *C. setosa* forming the two most basal lineage in dendrogram. The other *Cephalonia* species divided into two clade. The first clade occupied by *C. syriaca* alone, while the last clade gathered *C. stapfii* and *C. paphlagonica*. Monophyly of each clade is well supported. Relationships among *Cephalonia* species clear based on molecular data in Iraq.

Key words: *Cephalonia*, Schrader ex Roemer, schultes, Caprifoliaceae, Molecular Study

INTRODUCTION

From the families in the Iraqi Flora is Caprifoliaceae (Dipsacaceae) family that includes 350 species in the world that distributed on 11 genera [1]. In Iraq involves 24 species within four genera [2]. In Europe, Dipsacaceae called Teasel [1], and the genus *Dipsacus* L. from the same family called Teasel [3]. In the Flora of U.S.S.R., [4]. Indicated 23 species of the genus *Cephalonia*, and in Turkey [5] stated 29 species of the genus. In Europe, [6] mentioned that 14 species of the genus *Cephalonia* found, and in Saudi Arabia, [7] pointed out to 1 species of the genus. In Iran, [8] indicated that eight species of the genus found. [9] The Flora of low land Iraq mentioned two species. While [2, 10-12] indicated that five species found in Iraq. [13] Pointed out to the presence of two species in Sinjar Mountain. [14] Mentioned three species in Piramagrur Mountain, while [15] stated only one species of the genus in Haybat Sultan Mountain. [16 and 17] mentioned one species, which is *C. syriaca* (L.) Schrad. In Darband Gomaspan and Hujran Basin respectively and [18] pointed out to the presence of four species in Hawraman region, while [19] did not mention any species of the genus in Choman. The present study aimed to study sequences of chloroplast *TRN* L-F gene of the species *C. dictyophora* Boiss., *C. paphlagonica* Bobrov, *C. setosa* Boiss. &Hohn., *C. stapfii* hausskn. Ex Bornmuller and *C. syriaca* to add a reliable part to the information about the genus *Cephalonia* in Iraq.

MATERIALS AND METHODS

DNA Isolation , Polymerase Chain Reaction (PCR) Amplification And Sequencing.

Leaf tissue was harvested from fields-collected plants and herbarium specimens from Salahaddin University, Herbarium-College of Education (ESUH) .Total cellular DNA was isolated in CTAB (acetyltrimethyl ammonium bromide) buffer following [20]. We used two primers *TRN* L with sequencing (5'-CGAAATCGGTAGACGCTACG-3 and *TRN* F with sequencing (5'-ATTTGAACTGGTGACACGAG-3) respectively. The PCR products were electrophoresed in 0.8% agarose gels and then the DNA Fragments of desired size excised under U.V light and purified using a Promega Kits, the entire *TRN* L-F gene region was sequenced, Sequencing reaction was carried out using Stable volume Big Dye (1μM) and Primers *TRN* L & *TRN* F and ddH₂O. Then the products sent to the National Science and Technology Development in Thailand to determine the sequencing of the Gene.

Sequencing alignment and phylogenetic analysis.

When the result of sequencing sent from National Science and Technology Development , the result opened in Bio Editor

program , some modification performed in gene bases , Genetic cladogram were draw in Mega 6 program . We used *Dipsacus laciniatus* as out-group according to the Flora of Iraq Vol.5, P.2.

RESULT AND DISCUSSION

In order to knowledge, the History of ancestry or ancestral group for any taxonomic group like tribe, genus, species and variety. We must doing phylogenetic analysis, which is a trustworthy tool for the interpretation of all possible solutions or relationships among large taxa of phenotype and genotype [21].

Result showed that Phylogenetic analysis was a high resolution or accuracy method based on DNA sequencing data obtained in this study. The alignment of the *Trn*L-F data sets for the five species examined range in length from 777bp (*C. stapfii*) – 879 in (*C. syriaca*, *C. setosa* and *C. dictyophora*) and demonstrate good characters in these length.

The Result of phylogenetic dendrogram, topology of the alignment of *Trn*L-F cladogram designed based on Maximum Likelihood Trees and UN Weighted Pair-group Method ariythetic (UPGMA) in the mega 6 program to visualizing evolutionary history among the species of new record *Cephalonia* species in Iraq Fig1and Fig2 respectively.

According to the cladogram it can be recognized two main clade (major group). Fig (1and 2).

The first major clade (basal lineage) gathered *C. setosa* and *C. dictyophora* with excellent Bootstrap value (100) with the second major clade.

The Second major clade (sister clade to the basal lineage) subdivided in two secondary clade , the first one Unique to the *C. syriaca* alone with good bootstrap 79% with the secondary clade that included the *C. stapfii* and *C. paphlagonica* with 99% bootstrapping .

Conducted analysis by UPGMA model fig2. had the same topology of the Maximum Likelihood model but the difference in bootstrapping value ranging from good (76%) in *C. stapfii* and *C. paphlagonica* clade to high good in *C. syriaca* clade, *C. stapfii* and *C. paphlagonica* clade, while the bootstapping value Soar up to excellent in the *C. setosa* and *C. dictyophora* clade Fig.2. which showed how closely these species are related to each other .

The distribution of *Cephalonia* species genotype in different clade and sub clade, may indicate the separation and diversity of species from each other. While The convergence that shows in some species is due to the fact that these species inhabit in one geographical area is the northern region of Iraq and affected by the same geographical conditions in that region as mentioned by [22] when she studied on some Tomato Genotypes. Moreover,

similar result was founded by [23] on her investigation on diversity of *Zea mays* varieties in iraq. The importance of identify of genetic variation with different markers and in particular DNA sequencing data generally in the taxonomy of different plant families .This method provides a

frame work for facilitate the process of classification of plant groups and this is consistent with what it reported by [24] on her studied on Agrostideae tribe from poaceae. and [25] study on the Family Papaveraceae In Iraq.

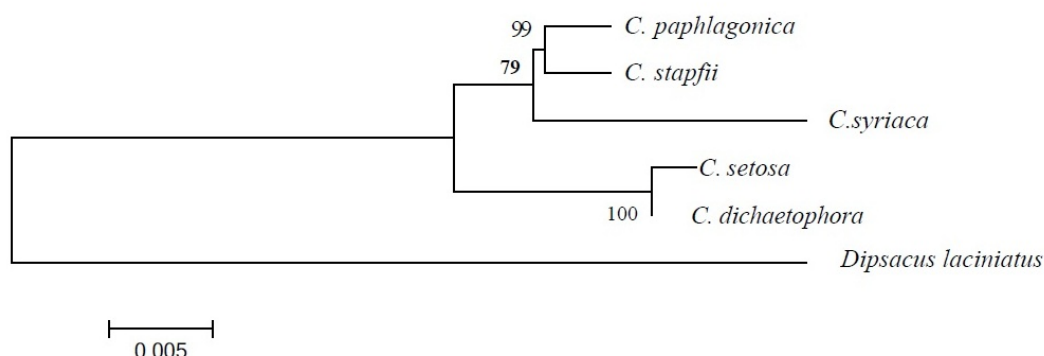


Fig1: Cladogram Tree according to Maximum Likelihood Methods depending on *trnL-F* sequencing. The values are clads represent Bootstrapping.

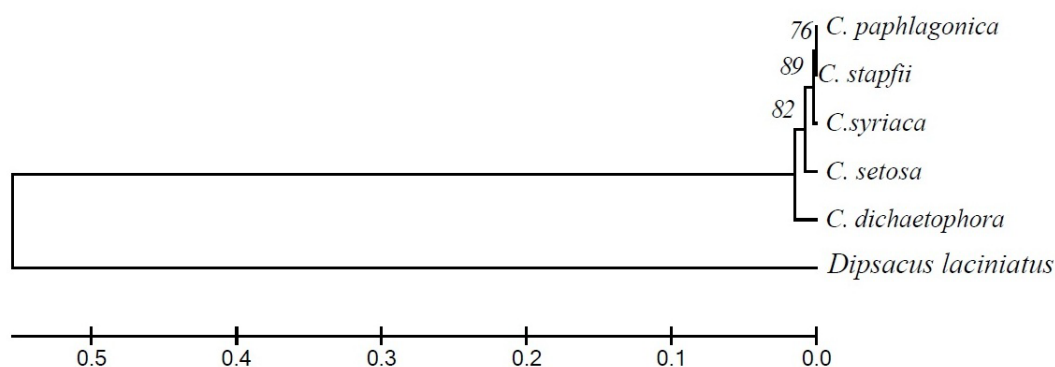


Fig2: Cladogram Tree according to UPGMA Methods depending on *trnL-F* sequencing. The values are clads represent Bootstrapping.

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