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MOLECULAR STUDY FOR THE GENUS *BUPLEURUM* L. (APIACEAE) IN IRAQ

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Abstract

The current research was conducted to examine the molecular characters of the entire sequences of plastid *trn*L-F gene which were made up for four species of the genus *Bupleurum* L. with in the family Apiaceae in Iraq to infer the phylogenetic relationship among the species under study. *Hohenackeria exscapa* (Steven) Kos.-Pol. was diagnosed as an outgroup for the Dendrogram. Cluster analysis by using mega 6 program gave Maximum parsimony trees with high supported (Bootstraping value in the clade). The tree showed that *B. brevicaule* Schlecht. is the major basal lineage in the dendrogram and sister clade to *B. lancifolium* Hornem. whilst the other species assembled the species *B. gerardi* All. and *B. kurdicum* Boiss. The monophyly of each clade is well supported.

Key words: *Bupleurum* L.; *Apiaceae; plastid trnL-F gene*

Introduction

Apiaceae is one of the families within the Iraqi Flora that includes 3590 species throughout the world which are divided on 440 genera (Singh, 2010). In Iraq includes 130 species divided on 59 genera (Al-Rawi,1964). In U.S.S.R. Flora, (Linchevskii,1973) indicated that 43 species of *Bupleurum* are found. While in Turkey, (Snogerup, 1972) stated 46 species from the studied genus, In Europe, (Tutin,1968) indicated 39 species of *Bupleurum*. In Saudi Arab (Migahid and Hammouda, 1978) stated 2 species of the genus. In Iran, (Ghahreman and Attar, 1999) mentioned 16 species. (Rechinger,1964) in the low land Iraq Flora remarked 4 species. (AL-Rawi, 1964) indicated that 11 species found in Iraq mentioning the districts in which the species distribute, while (Ridda and Daood, 1982) stated that 9 species found. (Ghazanfer and Edmondson, 2013) pointed out 10 species in Iraq. (Khalaf,1980) stated 4 species in Sinjar mountain. (Faris, 1983) and (Ahmed, 2010) mentioned 1 species in Piramagrun mountain and Darband Gomaspan respectively. (Ahmed, 2013) mentioned 4 species in Hawramanregion, while (Fatah, 2003; Hameed, 2016; Darwesh, 2017) didn't mention the genus *Bupleurum* in Haybat Sultan, Hujran Basin and Choman respectively. (Chakravarty, 1976) mentioned 11 species in Iraq and each of *B. brevicaule* and *B. falcatum* Linn. with economic values.

The present study aimed to study some molecular characters of the species *B. brevicaule*, *B. gerardi*, *B. kurdicum*. and *B. lancifolium* to determine the phylogenetic relationships among them in Iraq.

Materials and Methods

Taxon Sampling

The plant specimens used in the current study have been collected from the different districts of Kurdistan region-Iraq that preserved in the Herbarium of College of Education/ Salahaddin University. Four distinct species and one out group (*Hohecnackeria exscapa*) were used in the analysis.

DNA Extraction

The complete DNA has been extracted from the studied specimens. The method of extraction depended on the CTAB protocol of (Doyle, 1990) with some modification (1X CTAB: 10 mL of 1.0 M Tris-HCl, PH 8; 4 mL of 0.5 M EDTA, PH 8; 28 mL of 5 M NaCl; 2% CTAB; 2g PVP; and 158 ddH_2O), the washing process

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Table 1: The primers and their sequences which used in the study.

Primer	Direction	Sequence 5'—— 3'	Resources
trn-L	Forward	CGAAATCGGTAGACGCTACG	(Taberlet *et al.*, 1991)
trn-F	Reverse	ATTTGAACTGGTGACACGAG	(Taberlet *et al.*, 1991)

of the DNA pellet has been conducted twice with 0.5 mL of 80% ethanol, then DNA was dissolved in 25 µl TE-buffer.

PCR and DNA Sequencing

The noncoding regions of cDNA were magnified by application of primers *trn*L and *trn*F of Taberlet *et al.,* (1991) Table 1. The primers were ordered from (IDT) company-Skokie, Illinois-USA. The entire amplification reactions volume was 25 µl and the Master Mix made up of 10.8 µl of ddH2O, 2.5 µl ThermoPol reaction buffer, 2.5 µl MgCl2, 5 µl dNTPs, 2 µl template, 1 µl from each primer, 0.2 µl DNA polymerase (Taq polymerase). The PCR-Thermal cycler started with 2 min for initial denaturation at 94˚C followed by 30 cycles: 30 sec. denaturation at 94˚C; 60 sec. for extension at 72˚C and 180 sec. for final extension at 72˚C. The resultant PCR products were checked on 1.5% agarose gel run in TAE buffer. The gel was stained with EtBr and photographed under UV transilluminator.

The products of PCR were purified using Kits (Promega company-Madison-USA). The purified PCR products were sent to the National Science and Technology Development Agency (NSTDA) in Thailand for sequencing.

Sequence Alignment

All the DNA sequences were edited and aligned with Mega 6 option available in Mega 6 and manual adjustment, there are 4 accessions for *trn*L-F gene, including the out group species.

Phylogenetic Analyses

Maximum Parsimony Analysis

The reconstruction of the phylogenetic relationships has been depended on Maximum Parsimony (MP) methods. MP analysis was performed by using PAUP* version 4.0a164 (Swofford, 2000). Using heuristic search with 100 replicates of random taxon additions, Tree-Bisection-Reconnection (TBR) branch swapping, Mul Trees on and steepest decent off has been conducted. The maximum numbers of saved trees were 100 for each replicate. The bootstrap values were calculated from 100 replicates.

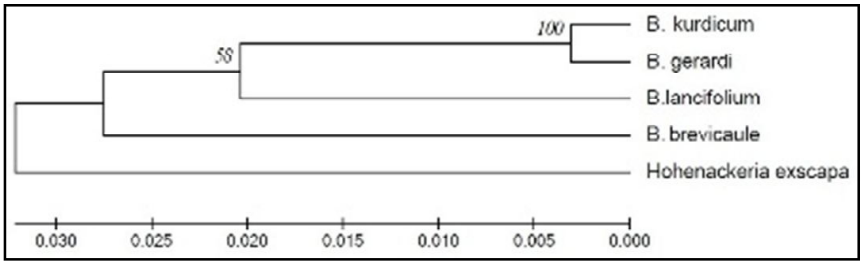

Fig. 1: Strict consensus tree of most parsimonious tree resulting from phylogenetic analysis of Chloroplast gene *trn*L-F. Numbers on the branches indicate bootstrap support.

Results & Discussion

Phylogenetic relationships within Bupleurum species

The result of DNA sequences analysis showed a good tool for inferring phylogenetic interpretation among taxa, topology of *trn*L-F trees represented depending on the Maximum Parsimony trees.

Three major clades were recovered within *Bupleurum* in tree, although the positions of these clades are varied Fig. 1. The analyses was consisted of four ingroups and one outgroup.

The species *B. brevicaule* was the most basal lineages in the base of tree which consider the first species in the tree and consider the sister species or clade to *B. lancifolium* with support value (bootstrap value 58) Fig. 1.

The species *B. gerardi* and *B. kurdicum* as the sister second main clade included the two species of the genus with full bootstrap value 100% and gathered these two species. The importance of use DNA sequencing data in general and especially *trn*L-F data sets is to visualize the best Apiaceae systematics in Iraq and taxonomy of the genus *Bupleurum* L. and analysis of this data demonstrate comparable reliable study in the Apiaceae systematics & plant systematic especially with using modern software in representation Phylogenetic Trees. Similar findings were concluded by (Qader, 2014) in his study of the genus *Cousinia* (AL-Mousawi, 2015) in separation some *Papaveraceae* species in Iraq and (AL-Edhari *at el.,* 2018) on the Genus *Cephalaria* from Caprifoliaceae in Iraq, as well as the study of (Hasan, 2019) on the genus *Potentilla* L. in Kurdistan region-Iraq.

Conclusion

The present study showed three major clades within the genus *Bupleurum* and the species *B. brevicaule* was the main basal lineage in the dendrogram and sister clade to *B. lancifolium*. while the species *B. gerardi* and *B. kurdicum* gathered in

a separate subclade.

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