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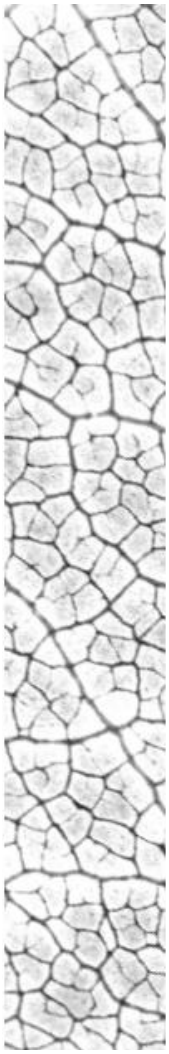

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Phylogenetic relationship among some species of Solanaceae in Iraq

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Abstract
A phylogenetic analysis was conducted using sequences were studied. Sequences were obtained from six genera: (1) *Datura metel*; (2) *D. innoxia*; (3) *Solanum nigrum*; (4) *Hyosyamus reticula*; (5) *Capsicum annum*; (6) *Physalis alkakengy*; and (7) *Lycopersiconesculentum* and included seven species of the Solanaceae family. The taxonomy and phylogenetic relationships among these taxa were used to assess the genetic diversity of eight Solanaceae species using one nuclear internal transcribed space (ITS). Analysis of DNA sequence data from the three (ITS) regions yielded a high level of genetic variability (polymorphism) among the studied samples. Results indicate that six major clades within Solanaceae are supported by high bootstrap values. The sequences of *D.innoxia* and *D.metel* are almost 100% similar and have a close relationship with *S. nigrum*. Morphological characters such hairs, number of corolla and corolla color different between similar species. They were grouped into a different trichotomy in surface of leaves, stem, and flowers. Most species were different and occurred in separated clade.

Keywords: ITS, phylogenetic relationship, PCR, Solanaceae

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INTRODUCTION

The family Solanaceae is one of the largest and most important economic families of angiosperms, including important food, spice, and drug plants (D'Arcy, 1979). This family contains 83-90 genera and 2671 species, but the most recent estimate includes more than 3000–4000 species (Gebhardt, 2016; Ganaie *et al*., 2018). Approximately half of the species in the family are included in the widespread, morphologically diverse, and economically important genus *Solanum*. The family is nearly cosmopolitan in distribution and found throughout tropical and temperate regions, but with a concentration of diversity in Australia and Latin America. (Ganaie *et al*., 2018; Ande et al, 2017).

Based on amplified fragment length polymorphism (AFLP) data, a new classification for the tribe Datureae was proposed, and the arborescent species was placed into a separate genus Brugmansia. Within *Datura* three sections have been recognized, namely Stramonium, Dutra and Ceratocaulis. In addition, *D. discolor*, previously placed in the section Dutra was reported to be an intermediate between sections Dutra and Stramonium (Mace *et al*., 1999).Plastid sequences are less plagued by problems of among-site and lineage rates of heterogeneity, which have often compromised the accuracy of phylogenetic inference in animals. Comparative studies of cpDNA are particularly relevant to Solanaceae because the chloroplast genome of *Nicotiana tabacum* is completely sequenced and could serve as a reference genome within the family. Many of the systematic advances based on extensive cpDNA studies have been reviewed by several researchers(Palmer *et al*., 1988),phylogenetic relationships of *Solanum* (Spooner *et al*., 1993), *Nicotiana* (Olmstead *et al*.,1997), *Jaltomata* (Mione *et al.,* 1994), and *Physalis* and related genera (Mione *et al*., 1994; Wei *et al*., 2012; Marghali *et al*., 2015; Zhao *et al*., 2015; Zamora-Tavares *et al*., 2015). Based on the molecular phylogeny of chloroplast and mitochondrial genes, Kulcheski *et al*. (2006) separated the genus *Petunia* into two complexes while Livingstone *et al*. (1999) showed that *Capsicum* species differed by at least one reciprocal translocation. Rodriguez and Spooner (1997) and Bohs and Olmstead (1997) used a chloroplast DNA study for taxonomic analysis of *Solanum*. Mione *et al.,* (1994) and Olmstead and Palmer (1997) explored systematic implications of chloroplast DNA variations in taxonomy of *Jaltomata* and *Solanum*,

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Table 1. The ITS of nuclear ribosomal DNA, including the 5.8S RNA gene were amplified by PCR using the forward primer AB101 (5)

Primers			Sequences (5' - 3')	(Base)
ITS	ITS5	F	5'-GGAAGGAGAAGTCGTAACAAGG-3'	22
	ITS4	R	5'-TCCTCCGCTTATTGATATGC-3'	20

Table 2. Stage of Thermocycler cycle sequencing of PCR

Stage	Temperature	Time (min)
Initial denaturation	95	4
No. of cycles = 32 cycle		
Denaturation	95	1
Anneling	56	1
Extention	72	2
Final extension	72	10

respectively. Melotto-Passarin (2008) studied phylogenetic relationships in Solanaceae and related species based on cpDNA sequences while using an internal transcribed sequence (ITS) as a DNA barcode for unique *Lycium barbarum* studied by Xin *et al.* (2013). The *Petunia* genome sequence is under construction and has been studied by Vandenbussche *et al*. (2016).

The aim of this study was to evaluate phylogenetic relationships with respect to genetic diversity among some species of Solanaceae using sequence data obtained from nuclear ITS.

MATERIALS AND METHODS

In the present study, we collected seven species of Solanaceae species from different places in Iraq. The samples were placed in perforated aluminum foil and sealed tightly and then placed inside polyethylene bags and closed with a little of silica gel for the purpose of absorbing moisture from the leaves.

DNA Isolation and PCR Amplification

DNA extraction was performed on young leaves using the Qiagen DNeasy (QIAGEN, Hilden, Germany) Plant Genomic DNA Mini Kit according to the manufacturer's instructions with some modifications. Extracted DNA samples of 50 µl were stored in the freezer at -20°C.

Polymerase chain reaction (PCR) involved amplification of the (ITS) region of nuclear ribosomal DNA. The ITS region of nuclear ribosomal DNA was composed of ITS 4 and 5 and was amplified using one primer shown in **Table 1**.

During electrophoresis, 9 µL of each PCR product was mixed with 3 µL of bromophenol blue, and the mixture was then spun briefly in a micro centrifuge before loading on a 1.5% agarose gel, which was previously stained with safe view. An aliquot (12.5 µL) from the GoTaq Green Master Mix, 2X was taken and put in a PCR tube. Add Aliquots (1.5 µL) from 10µM of upstream primer and 1.5 µL of 10µM of downstream primer were taken. Five microliters of DNA template were taken, 4.5 µL of nuclease-free water was added. The mixture was vortexed for 30 sec and placed into the PCR machine.

Programmer of PCR

The different stages of cycle sequencing were performed in a Gene Amp PCR System 9700 Thermocycler using the program shown in **Table 2**.

RESULTS AND DISCUSSION

Phylogenetic Analysis

The phylogeny of some Solanaceae species was estimated based on ITS multi-alignments (**Figs. 1** and **2**).

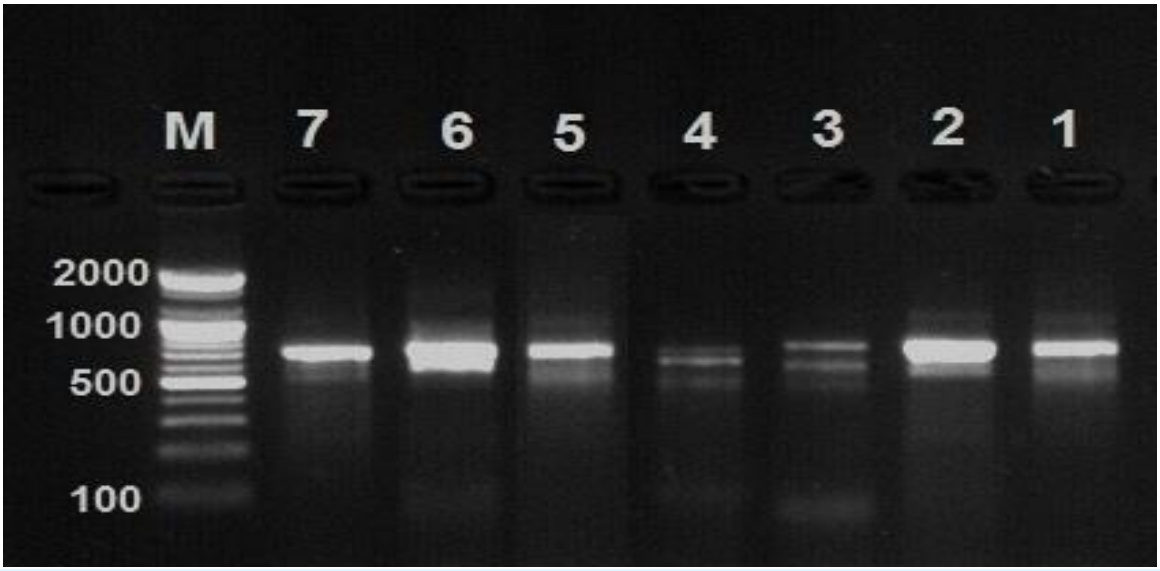

Fig. 1. DNA profile of ITS region, M: 100 bp DNA ladder, 1-7 represents amplicon from samples
1- Capsiccum annuum 2- Datura innoxia 3-Datura metel 4- Hyosamus reticula 5- Lycopersiconesculentum 6-Physalis alkekungi 7-Solanum nigrum

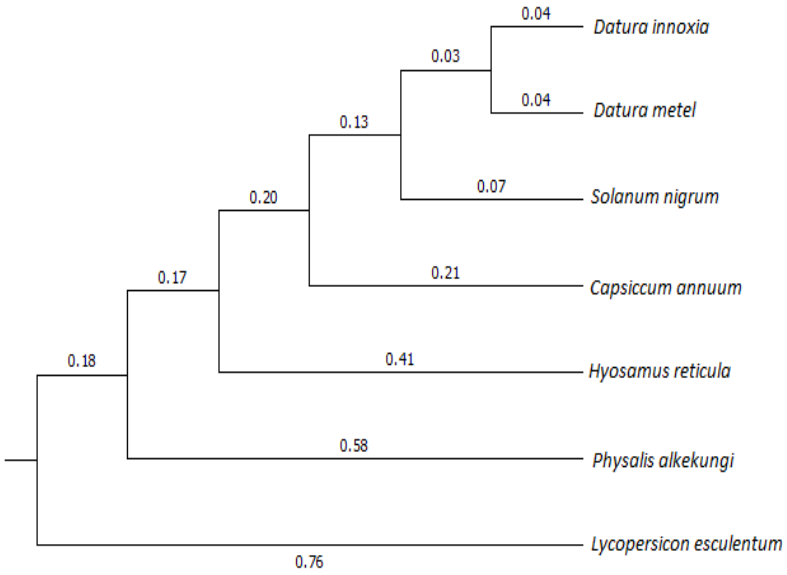

Fig. 2. Phylogenetic relationships among Solanaceae species as the outgroup

Analysis of DNA sequence data from the three ITS regions yielded a high level of genetic variability (polymorphism) among the studied samples. Results indicate that six major clades within Solanaceae are supported by high bootstrap values. The sequences of *D.innoxia* and *D.metel* were almost 100% similar, and they have close relationship with *S.nigrum*. 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 clade.

The first analysis included seven species of Solanaceae. The analysis of this data set identified six main clades, one containing *Physalis*, two with *Capsicum*, the third clade was composed of *Solanum*, *Lycopersicon* species occupies fourth clade, and *Capsicum* formed the sixth clade. The sixth clade included two species of *Datura* (*D. metal* and *D. innoxia*), which were grouped into one clade as a sister group to *Solanum* (**Fig. 2**).

Three major clades were found in Solanaceae species: (1) the *Datura* clade with *Datura* species (0.04 % bootstrap support); (2) the *Solanuum* clade with C (0.07 %);(3),*Capsicum* 0.21 % clade; and (4), *Lycopersicon,* which was 0.75 % in agreement with Borisjuk *et al*. (1994) who indicated that the new world *Solanum* species are more related to *Lycopersicon* than to other *Solanum* species.

One obvious example is the value of placing model organisms in the appropriate phylogenetic context in order to obtain a better understanding of both patterns and evolutionary processes, such as tomato (*Lycopersiconesculentum*) and other species dependent on an anther within the well-marked subclade (Olmstead *et al*. 1999, Spooner *et al*. 1993). This is an important observation for geneticists and molecular biologists.

CONCLUSION

Sequencing some of seven studied species of the Solanaceae family, yielded results in which chloroplast sequences are highly conserved among related species. However, several mutations, such as indels and base substitutions, were observed. This relative conservation of the genome allows for investigations of the phylogenetic relationships among divergent plants to be done. The non-coding ITS intergenic spacer thus showed nucleotide sequence polymorphism and variation in sequence length between Solanaceae and related species. It could be included in analysis with multiple chloroplast regions in order to improve the resolution of phylogenetic studies of the species studied.

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