

UTILIZATION OF INTERNAL TRANSCRIBED SPACER (ITS) AS A MOLECULAR MARKER FOR PHYLOGENETIC RELATIONSHIP OF *Solanaceae* FAMILY

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Solanaceae family is also called 'nightshades' contains more than two thousand species, and are considered as one of the major plant families providing food (Yadav *et al.*, 2016). Only four genera contain economically significant cultivated food crop-species. The most economically important genus of the family is *Solanum*, whilst *Capsicum*, *Physalis* and *Lycium* contribute the remainder of cultivated crop species (Samuels, 2015). The last fifty years witnessed a continual relief in horticultural and agricultural biodiversity of nutritionally important plants, including those of the *Solanaceae* family. So survey of the biodiversity, ethnobotany and taxonomy of subfamily *Solanoideae* was undertaken and presented as an inventory of food species (Samuels, 2015). Developing new cultivars with significantly increased yield and quality is the main target of plant breeder to play an important role in the improvement of *Solanaceae*. Bebeli and Mazzucato (2008) checked out the situation of the most economical species: eggplant, pepper and tomato's germplasm resources, breeding methodology and the implementation of plant breeding in these species.

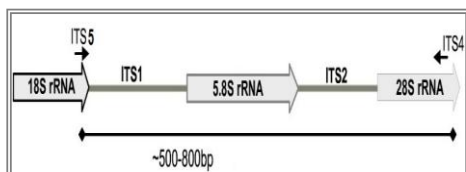
Several molecular markers have been used in recent years to identify and

assess the genetic diversity and phylogenetic relationship in plants. Barchi *et al.* (2011) identified SNP and SSR markers using restriction site associated DNA (RAD) tag sequencing in eggplant. RAPD markers were used to study the molecular diversity of 12 popular potato varieties in Bangladesh (Hoque *et al.*, 2013). Shirasawa and Hirakawa (2013) studied DNA markers benefits and their applications in molecular breeding in tomato, genetic linkage map, QTL and gene mappings, comparative genomics and functional annotations of DNA polymorphism.

Ribosomal DNA (rDNA) consists of one of the largest multigenic families in eukaryotic genomes and is present at one or several locations in arrays of tandem elements. Each unit is composed of three rRNA gene regions (5.8S, 18S, and 28S) that are foregone by an external transcribed spacer, and the two internal transcribed spacers ITS1 and ITS2 separate the genes, respectively (Giudicelli *et al.*, 2016). The rDNA exons are quite conserved across eukaryotic organisms, whereas the ITS regions present length variability due to point mutations and insertions/deletions (indels). ITS sequences have been broadly used in the inference of phylogenetic hypotheses and in molecular

evolution studies of plants at several taxonomic levels (Giudicelli *et al.*, 2016).

The objective of this study was to utilize the ITS as a useful molecular marker to determine the genetic relationship of some *Solanaceae* species.



MATERIALS AND METHODS

Plant materials

Seven different leaf samples of *Solanaceae* (Two of: Tomato, one from each of: Chili pepper, Eggplant, Potato, Ground cherry and Bell pepper) were kindly obtained from the greenhouse of Horticulture Department, Faculty of Agriculture, Ain-Shams university (Table 1).

DNA extraction and ITS amplification

Total DNA was extracted from fresh leaves of each sample using Spin Column Genomic DNA Minipreps Kit for Plant (Bio Basic INC.) following the manufacturer's protocols. The quantity and quality of DNA samples were checked on agarose gel using 100 bp DNA Ladder. ITS rDNA was amplified using the following primer pair: ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS-5 (5'-GGAAGTAAAAGTCGTAA CAAGG-3') (Sharma *et al.*, 2002). The reactions were carried out in 50 μ l mixture containing 24 μ l sterile water, 10 μ l of 5x

PCR reaction buffer (containing $MgCl_2$ and dNTPs), 5 μ l of each primer (0.7 μ M), 1 μ l (5 U) of *Taq* polymerase and 5 μ l (50 ng) template DNA. Techne TC-3000 PCR Thermal Cycler was used with the following PCR steps: an initial denaturation 95°C for 5 min, 35 thermal cycles (95°C for 1 min, 58°C for 1 min and 72°C for 2 min) and a final extension at 72°C for 5 min according to the following diagram:

Gel analysis, fragment purification and sequencing: PCR products were run in 2% agarose gel and image was analyzed using the TotalLab TL120 to determine molecular size of the amplified fragments of ITS region according to 100 bp DNA Ladder. The amplified fragments were purified and sequenced in one direction using ITS-4 primer.

Alignment and phylogenetic tree: The obtained sequences were aligned on line using the NCBI (<https://www.ncbi.nlm.nih.gov/>) web site through Basic Local Alignment Search Tool (BLAST). Then multiple alignment and phylogeny tree of nucleotide were done using the Clustal Omega free web site: (<http://www.ebi.ac.uk/Tools/msa/clustalo/>) for both phylogenies; one between seven sample's nucleotide sequences and the second between the samples and other related sequences for *Solanaceae* family from NCBI.

RESULTS AND DISCUSSION

The ITS region has been found to be an effective universal region for molecular identification of plants (Samsuddin

et al., 2012). Total genomic DNA was extracted from seven different sample species of *Solanaceae*, and amplified through PCR using the universal primers ITS-4 and ITS-5. Specific single fragment was obtained for each sample after running in 2% agarose gel (Fig. 1), sizes were determined by TotalLab TL120 analysis as follow: 744 bp, 729 bp, 744 bp, 750 bp, 759 bp, 759 bp and 798 bp with Tomato_1, Chili pepper, Eggplant, Tomato_2, Potato, Ground cherry and Bell pepper, respectively.

Fragments were purified and sequenced; each sequence was aligned individually at BLAST to confirm each species identity and to get the other related sequences as shown in Table (2). Two samples of tomato were confirmed with the *Solanum lycopersicum* (accession ID: [KF668233.1](#)), Chili pepper and Bell pepper samples were confirmed with the *Capiscum annuum* (accession ID: [GU944973.1](#)), Eggplant sample was detected with the *Solanum melongena* (accession ID: [AF244726.1](#)), Potato sample was aligned with *Solanum microdontum* (accession ID: [AY875805.1](#)) and Ground Cherry was assured with *Physalis peruviana* (accession ID: [AY665914.1](#)). Multiple sequence alignments were done using Clustal Omega program for the seven sequences (Fig. 2), which illustrates the orthologous variants that occurred between the species. Small stars under the alignment indicate identical nucleotide sequences, whereas the hyphens refer to the indels (insertion/ deletion) mutation as

a gap with multiple alignments. Also, there were a lot of base substitutions as pointed in Fig. (2) with narrow columns.

Phylogenetic relationship tree for seven samples of *Solanaceae* (Fig. 3) showed that Potato was closely related to Tomato as they were grouped in one main cluster. Whereas, the Eggplant was related to both types of pepper (Chili and Bell) and was grouped in another main cluster. The Ground Cherry was separated alone in the third main cluster. Previously, taxonomy of *Solanaceae* family relied on morphological variability like number of carpels that form the gynoecium, number of locules in the ovary, type of ovules and their number, type of fruit and their reproductive characteristics. However recent studies are based on DNA and RNA molecular markers to obtain the relationships between species is more precise, reliable and powerful even between genera in the same family.

Molecular markers were analyzed in three backcross generations in order to verify the occurrence of recombination between homeologous chromosomes and the efficiency of introgressing useful genes which set up to overcome interspecific barriers existing between the cultivated *Solanum tuberosum* and the wild species *Solanum commersonii* (Barone *et al.*, 2001). Bebeli and Mazzucato (2008) reviewed the status of tomato, pepper and eggplant germplasm resources, breeding methodology and proved that marker-assisted selection (MAS) is an application that can be efficient for plant breeding,

which have been successful in tomato; few examples exist in pepper and still in its infancy in eggplant. Comparative genomics investigations can be used to transfer genome information from tomato to pepper and eggplant. Genetic diversity of 49 accessions of the hot pepper species *Capsicum chinensis* was estimated through analyses of 12 physicochemical traits of the fruit, with eight multi-categorical variables using 32 RAPD primers (Finger *et al.*, 2010).

Poczai *et al.* (2010) illustrated the importance of the sequence-based IT (Intron Targeting) markers within different populations of potato and related species in the genus *Solanum*, due to their polymorphism and potential for breeding studies in different taxa and their transferability to a related species (*Solanum nigrum* L.). DNA sequences between *Physalis* and tomato were compared to analyze genetic diversity in *Physalis* using tomato markers, where 38 accessions from at least six species of *Physalis* were subjected to genetic diversity analysis using 97 tomato markers and 25 SSR markers derived from *Physalis peruviana* (Wei *et al.*, 2012). The results indicated similarity between *Physalis* and tomato at both molecular markers and DNA sequence levels. Therefore, the molecular markers developed in tomato can be used in genetic study of *Physalis*.

Gramazio *et al.* (2016) applied RNA-Seq technique with next-generation sequencing (NGS) from pooled RNA of young leaves, floral buds and young fruit

tissues, generating more than one hundred million raw reads per species, which were assembled into 83,905 unigenes for *Solanum incanum* and in 87,084 unigenes for *Solanum aethiopicum* with an average length of 696 and 722 bp, respectively. The single nucleotide variant calling analysis (SNPs and INDELs) was performed by mapping *Solanum incanum* and *Solanum aethiopicum* reads, as well as reads from *Solanum melongena* and *Solanum torvum* available on NCBI database (National Center for Biotechnology Information), against the eggplant genome.

The obtained results from this study proved the usefulness and the importance of molecular investigation using rDNA regions (ITS) to assess the relationships of some species and genera not only depending on morphological characters as reported by Sharma *et al.* (2002) who assessed the genetic diversity in species like barley (*Hordeum spontaneum*) and wheat (*Triticum aestivum*) through the length and sequence of ITS region of ribosomal DNA. Furthermore, to substantiate our results, 10 related sequences from each alignment obtained from BLAST were picked up (Table 2) to make the multiple alignments for seven samples with other species of *Solanaceae* family. Figure (4) illustrate the status of seven samples within the big *Solanaceae* family, that clustered in small groups, one restrain seven sample from this study, followed by another group contain *Capsicum sp*, while *Solanum melongena* and *Physalis* occupied in separate groups. Such studies based on molecular verification between

different taxa could lend a valuable reliable and environmental free tool to assess biodiversity and guard against taxonomic errors in classification studies.

SUMMARY

Solanaceae family is considered one of the major plant families providing food. Studies based on DNA and RNA molecular markers are more precise, reliable and powerful to assess the relationships between species also between genera in the same family. ITS sequences have been broadly used in the inference of phylogenetic hypotheses and in molecular evolution studies of plants, because it is variable, represent point mutations and insertions/deletions (indels). Specific ITS fragments were produced using the universal primer through seven sample of *Solanaceae*. Purified fragments were sequenced and each sequence was aligned individually at BLAST to confirm each species and to determine its relation to other sequence. Multiple Sequence alignment was done using Clustal Omega program for the seven sequences, and phylogenetic relationship showed that Potato was closely related to Tomato as they were grouped in one main cluster. Whereas Eggplant was related to both type of pepper (Chili and Bell) and was grouped in another main cluster. The Ground Cherry was separated alone in the third main cluster. Finally, ten related sequences picked from each alignment were deduced from BLAST to make the multiple align-

ments for the seven studied samples with other species of *Solanaceae* family.

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Table (1): List of the seven different samples, species and genus of *Solanaceae* family

Sample Number	Name	Species	Genus
1	Tomato_1	<i>Solanum lycopersicum</i>	<i>Solanum</i>
2	Chili pepper	<i>Capsicum annuum</i>	<i>Capsicum</i>
3	Eggplant	<i>Solanum melongena</i>	<i>Solanum</i>
4	Tomato_2	<i>Solanum lycopersicum</i>	<i>Solanum</i>
5	Potato	<i>Solanum tuberosum</i>	<i>Solanum</i>
6	Ground cherry	<i>Physalis peruviana</i>	<i>Physalis</i>
7	Bell pepper	<i>Capsicum annuum</i>	<i>Capsicum</i>

Table (2): List of sequence similarities obtained for each sequences and accession number for 10 sequences.

Sample Number	Plant	Confirmed Species	10 related sequences producing significant alignments
1	Tomato_1	<i>Solanum lycopersicum</i> 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence (KF668233.1)	KF668233.1 & JN713142.1 & AB373813.1 & AB373816.1 & AB373815.1 & AJ300201.1 & AJ300202.1 & AY552528.1 & AB373811.1 & AB373812.1
2	Chili pepper	<i>Capsicum annuum</i> Internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence (GU944973.1)	GU944973.1 & HQ705989.1 & AY665841.1 & HQ705990.1 & AF244708.1 & HQ705988.1 & KP006660.1 & DQ314158.1 & KP006657.1 & DQ314160.1
3	Eggplant	<i>Solanum melongena</i> Internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence (AF244726.1)	JQ638857.1 & JQ638834.1 & JQ638898.1 & JQ638896.1 & JQ638908.1 & EU176114.1 & JQ638897.1 & JF978787.1 & JQ638813.1 & AF244726.1
4	Tomato_2	<i>Solanum lycopersicum</i> 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence (KF668233.1)	KF668233.1 & JN713142.1 & AY552528.1 & AB373813.1 & AJ300201.1 & GQ221566.1 & AJ300202.1 & AB373811.1 & AF244747.1 & AB373812.1

Table (2): Cont'

5	Potato	<i>Solanum microdontum</i> Internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence (AY875805.1)	AY875805.1 & AY875836.1 & AY875835.1 & AY875796.1 & AY875838.1 & AY875810.1 & AY875815.1 & AY875802.1 & AY875813.1 & AY875818.1
6	Ground cherry	<i>Physalis peruviana</i> 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence (AY665914.1)	AY665914.1 & AY665879.1 & AY665900.1 & AY665883.1 & AY665912.1 & AY665887.1 & AY665913.1 & AY665895.1 & AY665894.1 & AY665891.1
7	Bell pepper	<i>Capsicum annuum</i> Internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence (GU944973.1)	GU944973.1 & AY665841.1 & HQ705989.1 & AF244708.1 & HQ705990.1 & HQ705988.1 & KP006660.1 & DQ314158.1 & AY665843.1 & AY665842.1

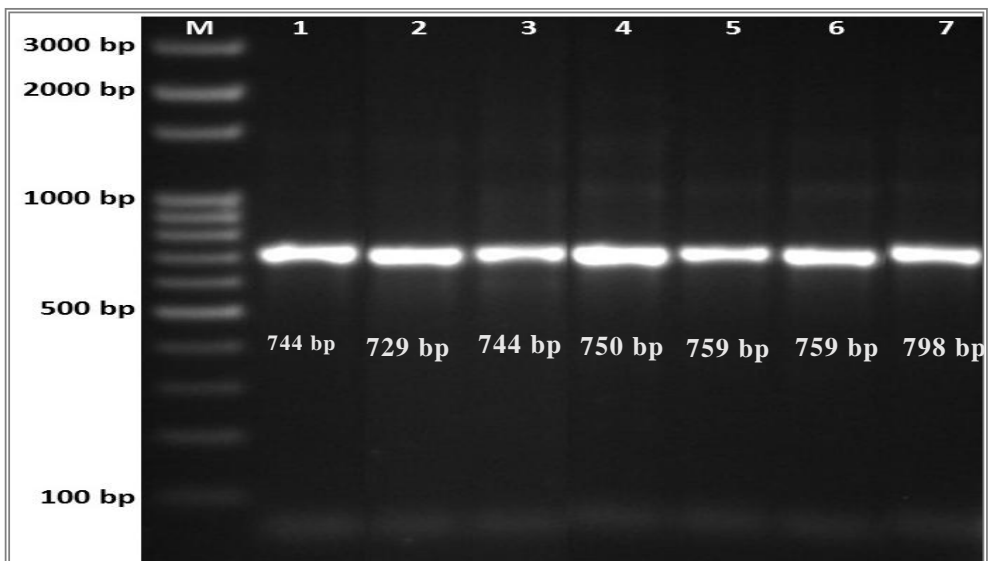


Fig. (1): Specific ITS amplified fragments for the seven samples of *Solanaceae* family run in 2% agarose gel, M; 100 bp DNA ladder, 1; Tomato_1, 2; Chili pepper, 3; Eggplant, 4; Tomato_2, 5; Potato, 6; Ground cherry and 7; Bell pepper.

CLUSTAL O(1.2.4) multiple sequence alignment

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Chili-pepper      --ACACCCCTGGTATCCCGCCTGACTGGGGTCCGCGGTCCGAGCGCC-----TGAGCGCGA
Bell-pepper      -----TCCCTGGCATCCGCTGACCTGGGGTCCGCGGTCCGAGCGCC-----TGAGCGCGA
Potato            TATAACTGGTAATCCGCCCTGACCTGGGGTCCGCGG---TCCGGAGCGCCTAGGCGCAAT
Ground-cherry    ----TCTCTGGTTCGCCCTGACCTGGGGTCCGCGGTCCGAGCGCCT----AAGCGCCGG
Eggplant         -----ACATCTGTATCCGCCTGACTGGGGTCCGCGGTCCGAGCGCGC---TAGCGCCGG
Tomato_1         -----CACCTGGGCATCCGCCTGACTGGGGTCCGCGGTCCGAGCGCCTGGTGAGGCGCGGT
Tomato_2         ----TCAACTGGCTTCCGCCTGACTGGGGTCCGCGGTCCGAGCGCCTGGTGAGGCGCGGT

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Chili-pepper      AGGGGTCTGGAGTCCGAACGCGCGACGGGCCGTAGCC-GCGACGACAAGAGAGTTGAG
Bell-pepper      AGGGGTCTGGAGTCCGAACGCGCGACGGGCCGTAGCC-GCGACGACAAGAGAGTTGAG
Potato            CAGGGTCTGGGAGTCCGGACGCGACGCGGGCTGTAGCCGCGACAACAAGAGAGAGTTGAG
Ground-cherry    AAGGGTCTGTACAGCCGGACGGGCGAC--GGGTTCCGGCCAGGCAACAGAGAGTTGAG
Eggplant         AGGGGCGTGGAGCGCGCAGCGCGACGGGCTGTGGCCGCG--GCACCAAGAGAGTTGAG
Tomato_1         GAGGGTCCGGGAGTCCGGACGCGCGACGGGCTGTAGCCGCGACAACAAGAGAGAGTTGAG
Tomato_2         GAGGGTCCGGGAGTCCGGACGCGCGACGGGCTGTAGCCGCGACAACAAGAGAGAGTTGAG

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Chili-pepper      TTGCAACCACCACTTGCCGCGACGTCCTGCGACGTGGACTCGCATTTAGGCCAGCCGCGA
Bell-pepper      TTGCAACCACCACTTGCCGCGACGTCCTGCGACGTGGACTCGATTTAGGCCAGCCGCGA
Potato            TTTC AACCACCACTTGCCGCGACGTCCTGCGACGTGGACTCGCATTTAGGCCAGCCGCGC
Ground-cherry    ATTCAACCACCACTTGCCGCGACGTCCTGCGACGCGGGCTCGCATTTAGGCCAGCCGCGC
Eggplant         CTACAACCACCACTTGCCGCGACGTCCTGCGACGTGGACTCGCATTTAGGCCAGCCGCGG
Tomato_1         TTTC AACCACCACTTGCCGCGACGTCCTGCGACGTGGACTCGCATTTAGGCCAGCCGCGC
Tomato_2         TTTC AACCACCACTTGCCGCGACGTCCTGCGACGTGGACTCGCATTTAGGCCAGCCGCGC

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Chili-pepper      GCTCGAGGCGCAACGGGAGGCCAGTATCCGCCCCGCGACGACAGCGCGCCCGTGTATCGGG
Bell-pepper      GCTCGGGGCGCAACGGGAGGCCAGTATCCGCCCCGCGACGACAGCAGCCCGTGTATGGG
Potato            GCTCGGGGCGCAACGGAAAGGCCAGCTTCCGCCCC--GGCCAAAG-----CC
Ground-cherry    GCTGCGAGCGCAACGGGAGGCCAGTATCCGTCCGCCCAAGCCCG-----CG
Eggplant         GCGCGAGGCGCAACGGGAGGCCAGTATCCGCCCCGCGACGCCGA-----GC
Tomato_1         GCTCGGGGCGCAACGGGAGGCCAGCTTCCGCCCCGCGCTAAAGC-----CT
Tomato_2         GCTCGGGGCGCAACGGGAGGCCAGCTTCCGCCCCGCGCTAAAGC-----CT

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Chili-pepper      GCGCGGTGCGAGGGGGGCGACGGGATGCGTGACGCCAGGCGAGACGTGCCCTCGGCCTAA
Bell-pepper      GCGCGATGCGAGGGGGGCGACGGGATGCGTGACGCCAGGCGAGACGTGCCCTCGGCCTAA
Potato            TTGCGGCGTGCAGGGGGGCGACGGGATGCGTGACGCCAGGCGAGACGTGCCCTCGGCCTAA
Ground-cherry    GCGCGGGGCGAGGGGGGCGACGGGATGCGTGACGCCAGGCGAGACGTGCCCTCGGCCTAA
Eggplant         ---GGCGTGCGGGGGGCGACGGGATGCGTGACGCCAGGCGAGACGTGCCCTCGGCCTGA
Tomato_1         TGCGGCGTGCGAGGGGGGCGACGGGATGCGTGACGCCAGGCGAGACGTGCCCTCGGCCTAA
Tomato_2         TGCGGCGTGCGAGGGGGGCGACGGGATGCGTGACGCCAGGCGAGACGTGCCCTCGGCCTAA

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Chili-pepper      TGGCTTCGGGCGCAACTTGGCTTCAAAGACTCGATGGTTCACGGGATTCTGCAATTCACA
Bell-pepper      TGGCTTCGGGCGCAACTTGGCTTCAAAGACTCGATGGTTCACGGGATTCTGCAATTCACA
Potato            TGGCTTCGGGCGCAACTTGGCTTCAAAGACTCGATGGTTCACGGGATTCTGCAATTCACA
Ground-cherry    TGGCTTCGGGCGCAACTTGGCTTCAAAGACTCGATGGTTCACGGGATTCTGCAATTCACA
Eggplant         TGGCTTCGGGCGCAACTTGGCTTCAAAGACTCGATGGTTCACGGGATTCTGCAATTCACA
Tomato_1         TGGCTTCGGGCGCAACTTGGCTTCAAAGACTCGATGGTTCACGGGATTCTGCAATTCACA
Tomato_2         TGGCTTCGGGCGCAACTTGGCTTCAAAGACTCGATGGTTCACGGGATTCTGCAATTCACA

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Chili-pepper      CCAAGTATCGCATTTGCTACGTTCTTCATCGATGCGAGAGCCGAGATATCCGTTGCCGA
Bell-pepper      CCAAGTATCGCATTTGCTACGTTCTTCATCGATGCGAGAGCCGAGATATCCGTTGCCGA
Potato            CCAAGTATCGCATTTGCTACGTTCTTCATCGATGCAAAAGCCGAGATATCAGTTGCCGA
Ground-cherry    CCAAGTATCGCATTTGCTACGTTCTTCATCGATGCGAGAGCCGAGATATCCGTTGCCGA
Eggplant         CCAAGTATCGCATTTGCTACGTTCTTCATCGATGCGAGAGCCGAGATATCCGTTGCCGA
Tomato_1         CCAAGTATCGCATTTGCTACGTTCTTCATCGATGCGAGAGCCGAGATATCCGTTGCCGA
Tomato_2         CCAAGTATCGCATTTGCTACGTTCTTCATCGATGCGAGAGCCGAGATATCCGTTGCCGA

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Chili-pepper      GAGTCGTTTTTTTCTTACAGACGAAGCACAGGTC
Bell-pepper      GAGTCGTTTTTTTCTTACAGACGAAGCACAGGTC
Potato           GAGTCGTTTTGTGTTAAAGAGCACCGC-GCGTCC
Ground-cherry    GAGTCGTTCTGTGTTTCAAGCGAGGGCGCAGCTCC
Eggplant         GAGTCGTTTTGTGTTTTCGAAAGAAGCACGCATCC
Tomato_1         GAGTCGTTTGTGTTAAACAGAGCAG-CGCGCTCC
Tomato_2         GAGTCGTTTGTGTTAAACAGAGCAG-CGCGCTCC
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Chili-pepper      CGAGGGGACAGGCGCTCGATTCAAGTATTCCTTG
Bell-pepper      CGAGGGGACAGGCTGTCGATTCAAGTATTCCTTG
Potato           G-AGGGGAGGTTCTGCCGATTTTATTCTTG
Ground-cherry    --GAGGGGCGGGCCGTCGGTTAAGTATTCCTTG
Eggplant         --GGGCGGAGGGCTCTCGTTTGAAGTATTCCTT
Tomato_1         GAGGGGAGGGCTGTCGATTGTAGTATTCCTTG
Tomato_2         GAGGGGAGGGCTGTCGATTGTAGTATTCCTTG
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Chili-pepper      CGCCCCGGCGCGCAGCTGGGGCGCGCCCCAGGCC
Bell-pepper      CGCCCCGGCGCGCGC-CCGGGGCGCGCGCCGAGG
Potato           CCACAAAACAAGCGCACAAGCCCGCAGGTCGAGG
Ground-cherry    CGACCGCGACCGCGCACGCGACCGCCGGCCGAC
Eggplant         CCCCCCGAAGAGGGGGCGAGGGGCGAGCGCGGG
Tomato_1         CGCCCGAAGAGCTTGC CGCCTCGGGCGACGG-
Tomato_2         CGCCCCAAGAGCTTGC CGCCTCGGGCGACGG-
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Chili-pepper      ACGCTCTGGACCGCCCCGGCCGCGCCACCGCCG
Bell-pepper      TGGCGGAGTCCGTCGCCGGGCGACTCCTCGGTT
Potato           GCGCA-----ACCCCCAAAAGCTTTTAAACCG
Ground-cherry    GCGCG-----C--CTCCCCGTTTCAAACAGGTT
Eggplant         GCGGG-----C--TCCCCGTTTGAACGTTTTCG
Tomato_1         GCGCC-----G--CCCCGTTTAAACAGGTTTCG
Tomato_2         GCGCC-----G--CCCCGTTTAAACAGGTTTCG
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Chili-pepper      ---GCCAGG-----GGGGGCGGAGCCCCCGGC
Bell-pepper      GCTGTGCAGGGCTCTAGCATGTTTGTGAACGTGT
Potato           GCGTGACACGGGACGCCGAAGAACAGCCAACAAC
Ground-cherry    GCTTCTCAGGTTTTCGACAATGATCCTTCCGCA
Eggplant         GCTGTGCAGGTTTTCGACAATGATCCTTCCGCA
Tomato_1         GCTGTGCAGGTTTTCGACAATGATCCTTCCGCA
Tomato_2         GCTGTGCAGGTTTTCGACAATGATCCTTCCGCA
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Chili-pepper      G-----GGGGGAACAACCCGGC-----CGCG
Bell-pepper      GCTGACTTCCATGAACCATCGAGTCTTTGAACG
Potato           ACAAAAGCAACAAGGGGAAGA--AGGGGGGAAG
Ground-cherry    ACTTTTTAATTCAGTCTT--TGGGG--CGG-
Eggplant         TTTTACTTCCAAGCCGAG--AGCAA--ACA-
Tomato_1         TTTTATATTCTCAA-----
Tomato_2         CTTAAACAATCAAGGTTTG--TGTGT--GGTGGT

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Chili-pepper      GGGCCCCGCCACGCGGGGCGGGCGGGCGGGGCT
Bell-pepper      AGGGCACGTCTGCCGTAACGCATCGCGTCCGCA
Potato           --AGGGCGAGAAGAAGGGAGGGCGGGACTGAG-
Ground-cherry    -----
Eggplant         -----
Tomato_1         -----
Tomato_2         --TGGTGGTGTGGTGTGGGGATGGGTGTGTG-

```

Chili-pepper	GCAGGC GGG--GAGGCCGCGGGGCGGCGGC GG-----
Bell-pepper	CGGGGCGTGCTGTCGTCGCGGGGCGGATACTGGCCTCCC GTGCGCTCTGAGCTCGCGGCT
Potato	-----
Ground-cherry	-----
Eggplant	-----
Tomato_1	-----
Tomato_2	-----
Chili-pepper	-----
Bell-pepper	GGCCTAAATACGAGTCCACGTCGACGGACGTCGCGGCAAGTGGTGGTTGCAACTCGACTC
Potato	-----
Ground-cherry	-----
Eggplant	-----
Tomato_1	-----
Tomato_2	-----
Chili-pepper	-----
Bell-pepper	TCTTCGTCGTTGCGGCTAAGGGCCGGTAGCGCGTTTGGACTCAAGGACTCCTTTGCGCAT
Potato	-----
Ground-cherry	-----
Eggplant	-----
Tomato_1	-----
Tomato_2	-----
Chili-pepper	-----
Bell-pepper	AGGCGCTCAGTCTGCGTCTCATGGTCTGGCGGGATTTCCGGCTGGGGTGTAGGAATTCAT
Potato	-----
Ground-cherry	-----
Eggplant	-----
Tomato_1	-----
Tomato_2	-----
Chili-pepper	-----
Bell-pepper	TCACCTGGGAGGAGTTGTTTTCGAAGTGT TTTGTTATTACGCTATGCTAATGAATGTGGAG
Potato	-----
Ground-cherry	-----
Eggplant	-----
Tomato_1	-----
Tomato_2	-----
Chili-pepper	-----
Bell-pepper	TTGAACTGTGTCATTTT
Potato	-----
Ground-cherry	-----
Eggplant	-----
Tomato_1	-----
Tomato_2	-----

Fig. (2): Multiple alignment of seven ITS nucleotide sequences for the different samples of *Solanaceae* family using Clustal Omega program

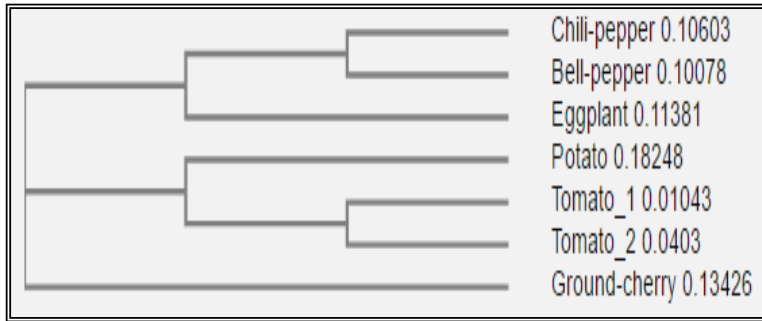


Fig. (3): Phylogenetic tree of seven ITS nucleotide sequences for the different samples of *Solanaceae* Family using Clustal Omega program.

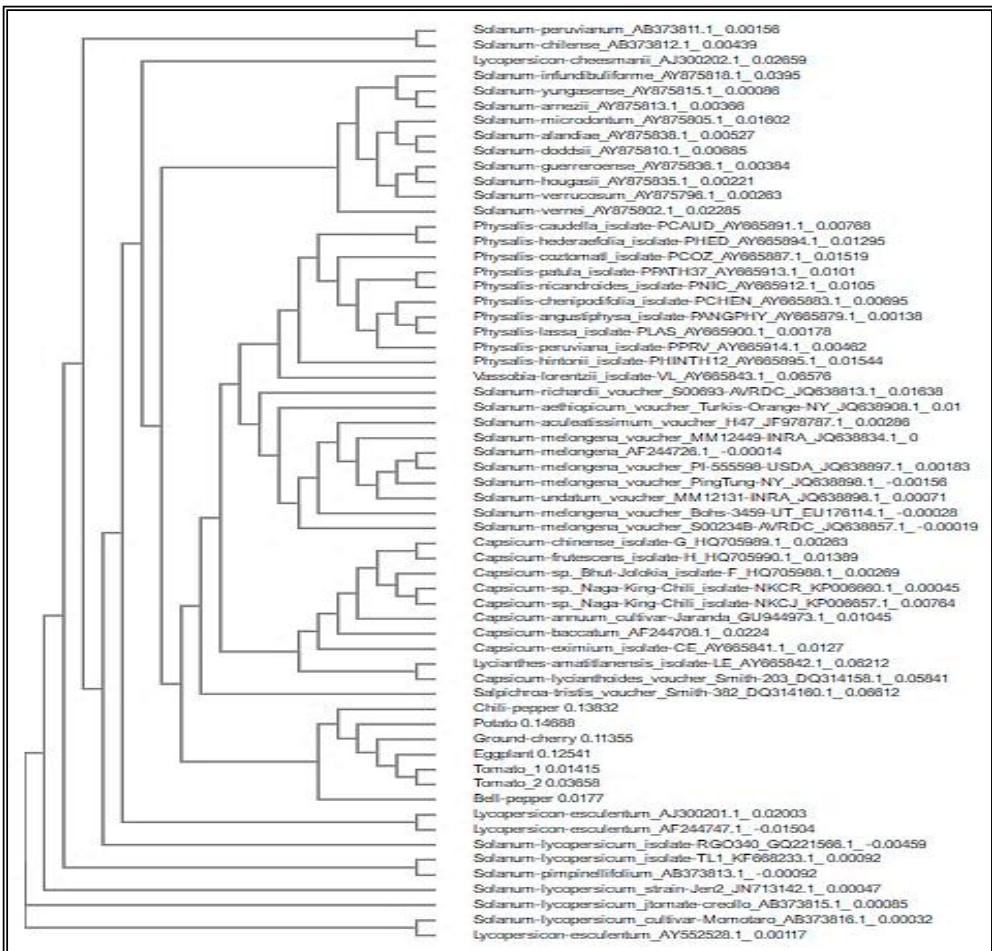


Fig. (4): Phylogenetic relationship and position of the seven studied samples with other species of *Solanaceae* using Clustal Omega program.1.