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MOLECULAR PHYLOGENETIC RELATIONSHIPS IN GENUS SOLANUM (SOLANACEAE) USING NON-CODING DNA SEQUENCES

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ABSTRACT:
Genus *Solanum* is the most species rich genus in the family Solanaceae with many economically important crop plants like eggplant (*Solanum melongena*), potato (*Solanum tuberosome* L.) and tomato (*Solanum lycopersicum*). Within *Solanum*, subgenus *Leptostemonum* (Dunal) bitter is the most species rich clade. Use of molecular data has greatly transformed the approach of plant systematic within this complex genus. In present study molecular phylogenetic study of subgenus *Leptostemonum* was done on the basis of sequence data of trnL-trnF non-coding region. The molecular data set revealed two major clades in the dendogram. First clade was represented by species from subgenus *Leptostemonum*, whereas second clade clustered all the Potatoe species forming a clear out-group. trnL-trnF sequence proved to be supportive in inferring the species level relationship within genus *Solanum*.

Keywords: *Solanum, Leptostemonum, trnL-trnF*

INTRODUCTION:

Amongst over 90 genera under Solanaceae (Vorontsova and Knapp, 2012) genus *Solanum* is the largest genus within this family. Nearly half of the species contributed under this family are from the genus Solanum and show phenotypic variation particularly in vegetative features. Giant and diverse genus containing more than 1400 species distributed in temperate and tropical zones in the world (Aubriot, et. al., 2016), has always remained as an important study area for scientists as it includes economically important crop plants like eggplant (*Solanum melongena*), potato (*Solanum tuberosome* L.) and tomato (*Solanum lycopersicum*). This genus is often referred as "spiny solanum" because of its distinguishing characters of presence of sharp epidermal prickles on leaves and stem in most of the species. Within *Solanum*, subgenus *leptostemonum* (Dunal) bitter is the most species rich clade and have been identified since the time of Linneaus, (1753). This group contains nearly 350-450 species (Bohr, 2005) comprising almost one third of the genus *Solanum* (Levin *et al.*, 2006).

Use of molecular data has greatly revolutionized the approach of plant systematics and has led to new insights of phylogenetic studies between closely related species. Non-coding regions have faster rates of evolution; hence these loci can be used as markers to resolve phylogeny at lower taxonomic level, and to study evolutionary relationship (Taberlet, et al., 1991; Holt, et al., 2004). Among the non-coding regions studied in chloroplast genome, the intron of the trnL (UAA) and intergenic spacer of the trnL-trnF (GAA) are suitable for phylogenetic study from intra-species to interfamily level (Bakker, et al., 2000; Fukuda, et al., 2001). trnL-trnFintergenic spacer was used many times to resolve phylogeny amongst closely related species. A combined data set of trnL-F intergenic spacer and trnN intron was used to infer species level phylogeny from of species belonging to section lasiocarpa of genus *Solanum* (Bohs, 2004). Using a revised molecular data from waxy gene and chloroplast trnT-F region, including the trnT-L and trnL-F intergenic spacer regions it was confirmed that African species *S. vespertilio* and *S. lidii* species are phylogenetically associated with *Solanum lineages* (Anderson *et al.*, 2006). Present study deals with phylogenetic analysis of some species from genus *Solanum* on the basis of trnL-F intergenic spacer sequence collected within Maharashtra State.

METHOD AND MATERIAL:

Sampling: Species included under genus *Solanum*, subgenus *Leptostemonum* were taken into account for present study. It included wild species such as *Solanum anguivi, Solanum khasianum, Solanum macranthum, Solanum virginianum* and cultivated

species *Solanum melongena* (Sungro No. 801). All these species were collected from various geographical regions of Maharashtra state. Outgroup taxa included are from the subgenus Potatoe. Four taxa from this outgroup are *Solanum muricatum, Solanum seaforthianum, Solanum tuberosum, Solanum bulbocastanum* and their sequences were obtained from Genbank database (Table 1). Outgroups were chosen to represent a variety of diverse *Solanum clades*.

DNA analysis: Total genomic DNA was extracted from fresh plant leaves and was further used for amplification reaction. trnL-Fintergenic spacer region was amplified using primers trnL- 5'-GGTTCAAGTCCCTCTATCCC 3' and trnF-5'ATTTGAACTGGTGACACGAG 3'. Total 20µl of PCR reaction mixture contained 3 µl template DNA (30-50 ng), 3µl of 10X DreamTaq Green Buffer with 20mM MgCl2 (Fermentas), 1 µl of 25mM MgCl2 (Fermentas), 0.8 µl of 10 mM each dNTP mix (Fermentas), 0.25 µl of 5U/µl of DreamTaq DNA polymerase (Fermentas), 1 µl (10 ρmoles) of each of forward and reverse primer and 9.95 µl of nuclease free water. Amplified PCR product was then quantified on 1.2% agarose gel. Amplified fragments of non-coding trnL-F region were sequenced from commercial sequencing service 'Chromous Biotech Private Limited', Bangalore, Karnataka. Sequence editing and contig generation was completed using Bioedit tool. Sequence alignment and phylogenetic tree construction based on Parsimony analyses of all the species was conducted using MEGA X (Version 10.0.4).

Table 1: GeneBank accessions of Potatoe species

RESULT AND DISCUSSION:

Length of amplified region of non-coding trnL-trnF fragments from Leptostemonum species was in the range 500 to 600 bp. Phylogenetic tree of five species based on trnL-trnFintergenic spacer region under Subgenus *Leptostemonum* viz. *Solanum anguivi, Solanum khasianum, Solanum macranthum, Solanum virginianum and Solanum melongena* (Sungro No. 801) and out-group Potatoe species gave a resolved analysis of species (Fig. 1). Parsimony analysis of these species data resulted into generation of phylogenetic tree which was divided into two major clades. Clade I was represented by clustering of all the species of subgenus *Leptostemonum*. Clade I was then further divided into two sub-clades. Sub-clade I was represented by *S. anguivi , S. virginianum, S. macranthum* along with the cultivated variety *S. melongena* (Sungro No. 801), However sub-clade II was represented by *S. khasianum* only forming the basal lineage. Clade II brought all species from Subgenus Potatoe together. Clade II was also further divided into two sub-clades. Sub-clade I was represented by *S. muricata* alone; whereas sub-clade II was uniting *S. seaforthianum, S. tuberosum* and *S. bulbocastanum* together.

Chloroplast genes and non-coding regions have been extensively used to reconstruct the phylogeny of related species. When compared to coding DNA region, variations of non-coding DNA regions was considered to be more helpful for phylogenetic analysis (Borsch and Quandt, 2009). In the present study, sequence data from non-coding trnL-trnF region emerged as a phylogenetically informative character. Two major clades noticeably separated two subgenera. Within clade I of subgenus *leptostemonum*, sub-clade I brought *S. melongena* and *S. macranthum* together. *S. khasianum* was separated from all other four species *S. anguivi, S. macranthum* and *S. virginianum* along with cultivated *S. melongena*, forming a sub-clade II alone. Formation of this sub-clade with separation of *S. khasianum* as a basal lineage is well supported with the findings of Hidayat, *et al.*, (2016) on the basis of ITS sequence data set. Closeness of *S. anguivi* and *S. melongena* in this sub-clade is also in tune with the investigation of Vorontsova, et. al., (2013) as they accounted the same relation on the basis of ITS sequence data. This indicates that *S. melongena* and *S. anguivi* are more closely related with each other; however they are equally distant from *S. virginianum*.

As the species under subgenus Potatoe exhibit the clear separation, this is an indicative of genomic distances between these clearly indicates the separation of species from these two sub-genus under investigation. All the species from subgenus Potatoe were differentiated from the Clade-I of subgenus *Leptostemonum* to form a separate Clade-II grouping all the related species together. This states that molecular data set of trnL-F region determines the genetic closeness. While studying

genus *Solanum*, Olmstead and Palmer (1997) also separated these two subgenera on the basis of chloroplast restriction site data. While studying a three gene phylogeny of Solanaceae species from *Leptostemonum* and Potatoe subgenus, Weese and Bohs (2007) clearly indicated the separation of these two subgenera. Findings of previous research are in good congruence with the data generated by trnL-F intergenic spacer region data in current study.

CONCLUSION:

Although a relatively vigorous understanding of the major genera within Solanaceae exists, it is interested to note that if the sample population is more then phylogenetic inference was found to be poor. As indicated by present investigation after utilizing non-coding trnL-F data, it generate advocacy towards the utilization of this region to discriminate the species under same genus. Moreover, combined data set from coding and non-coding regions of species can also provide robust analysis for separation of closely related species as well as varieties.

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Table 1: GeneBank accessions of *Potatoe* species

Sr. No.	Species	GenBank accession	Subgenus
1.	*Solanum seaforthianum*	DQ180438.1	Potatoe
2.	*Solanum tuberosum*	HM006842	Potatoe
3.	*Solanum bulbocastanum*	DQ180444	Potatoe
4.	*Solanum muricatum*	DQ180469	Potatoe

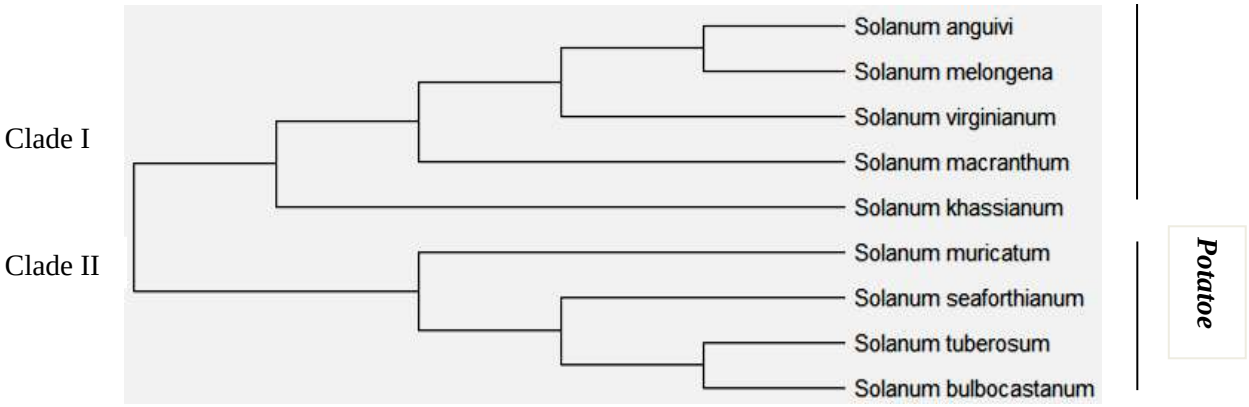

Figure 1: Phylogenetic tree of nine *Solanum* species based on maximum parsimony analysis based of *trn*L-F sequences