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RESEARCH ARTICLE

MOLECULAR SYSTEMATIC STUDY OF TWO SOLANACEOUS GENERA DATURA L. AND BRUGMANSIA PERS. BASED ON ITS SEQUENCES OF NRDNA.

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Abstract

Phylogenetic analysis was performed based on ITS 1 and 2 sequences to determine monophyly of *Datura* and *Brugmansia* and to understand their relationships. The results support the splitting of *Datura* and *Brugmansia* into two distinct genera (BS 100 & 91). *D. ceratocaula* earlier considered as the connecting link between the two genera, is nested within the *Datura* clade (BS 57) as a distinct species. Although the three conventional sections of *Datura* viz. *Dutra*, *Ceratocaulis* & *Datura* (*Stramonium*) stand well supported (BS 100, 100 & 83), *D. kymatocarpa*, *D. leichhardtii* and *D. pruinosa* appear shifted from Section *Dutra* to Section *Datura*, corroborating earlier observations. *D. discolor* remains nested within the Section *Dutra*, refuting earlier suggestions as intermediary or ancestral role. But the divergence of *D. discolor* from the remaining taxa within the section *Dutra* as a clearly supported subclade (BS 100) may be suggestive of the subdivision of Section *Dutra* into two subsections. This subdivision of section *Dutra* however remains to be appraised using cladistical studies incorporating both molecular and morphological data. The infrageneric grouping of *Brugmansia* into cold and warm groups viz. sections *Sphaecarpium* and *Brugmansia* exclusively on the basis of fruit shape and habitat lacks adequate support in the present analysis. But fruit shape appears not consistent as the warm climatic inhabitant *B. aurea* with trumpet shaped flowers sports egg-shaped fruits. Hence we propose subdivision of the genus into two sections on the basis of the more stable corolline form, and the proposition is well supported by molecular data viz. section *Brugmansia* (with trumpet and/or funnel shaped flowers, with fruits fusiform or egg-shaped) and section *Sanguinea* (with tubular flowers and consistently egg-shaped or short oval fruits).

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Introduction:-

The Solanaceae forms a monophyletic group comprising approximately 105 genera and 2030 species, and includes many important crop plants such as tomatoes, potatoes, egg-plants, chilli peppers etc (APG III, 2009). The family is traditionally classified into two subfamilies, Cestroideae with straight or bent embryos and capsular fruits, and

Solanoideae characterized by curved embryos and berry-like fruits (D'Arcy, 1979, 1991; Hunziker, 1979, 2001; Olmstead and Palmer 1992). Recently 8 clades have been recognized within the family (APG III, 2009), among which Clade Solanoideae includes two important genera *Datura* L. and *Brugmansia* Pers. Both genera are rich in alkaloids which have been utilized in traditional as well as modern medicines.

Datura, a genus of drug plants with several weedy species, comprises of 12 species distributed in the Tropical and Temperate regions of the world, of which three have been reported from South India (Sasidharan, 2004; The Plant List, 2013; Nandini et al. 2015). The members are glabrous or minutely pubescent shrub-like herbs, with large entire and sinuate or deeply-toothed leaves, large solitary white or purple flowers and armed and globose capsules. *Brugmansia* native to South America, includes 12 taxa of small ornamental trees, of which only two have so far been reported from South India (Sasidharan, 2004; Randhava and Mukhopadhyay, 1986; The Plant List, 2013). The members are profusely flowering with large attractive pendulous flowers and unarmed spindle shaped capsules.

Brugmansia and *Datura* are morphologically similar with hairy leaves that exude a stinking smell when crumpled or injured, and perfumed funnel-shaped flowers. *Datura* differs from *Brugmansia* in having annual or biennial habit with erect flowers and rounded spiny capsules. On the other hand, *Brugmansia* is perennial with pendulous flowers and elongated smooth fruits.

The species diversity of *Datura* is centred on Mexico and southwestern United States in North America (Hammer et al 1983; Mace et al 1999; Jiao et al 2002). The three species of *Datura* (*D. innoxia*, *D. metel* and *D. ferox*) that occur in South America are believed to have been introduced there in pre-Columbian times (Hunziker 1979; Nee 1991). Its occurrence in Australia and India are recent and human-mediated (Symon and Haegi, 1991). However, the Old World origin of *Datura metel* from the Indian Subcontinent has been advocated by Indian researchers on the basis of Indian lexical evidence, Arabic and Indic texts, and Southern Indian iconographic representations (Siklos, 1993; Geetha and Gharaibeh, 2007). The genus *Brugmansia* includes woody species, several of which are considered to be hybrids, unknown in the wild state.

The plants belonging to *Brugmansia* and *Datura* contain tropane alkaloids, mainly atropine and scopolamine. Several members are hallucinogenic used by indigenous people in the new world (Schultes, 1979; Dabur et al. 2004). Some are sources for the commercial production of hyoscyne, an alkaloid of medicinal interest as a spasmolytic, anaesthetic and anti-motion sickness drug. *Datura* has been used to treat skin ulcers, arthritis, rheumatism, hemorrhoids, asthma, lung infections, etc. *Brugmansia* is widely cultivated as ornamentals, because of their attractive large tubular flowers. Bristol (1966, 1969) reported that species of *Brugmansia* are used for medicinal and psychotropic preparation by the Sibundoy Indians.

Brugmansia is closely related to the genus *Datura* in both its morphology and chemistry, and was earlier considered to be synonymous with *Datura*. Persoon (1805) collated all arboreal perennial varieties into a separate genus *Brugmansia*. Bernhardt (1833) opined that the differences recorded by Persoon are not sufficient for separating out the taxon into a separate genus, and included *Brugmansia* as a section within the genus *Datura*. Lagerheim (1895) elevated *Brugmansia* to the status of an independent genus. This uncertainty remained for a long time until Lockwood (1973) transferred all woody members of *Datura* in a separate genus, *Brugmansia*.

Majority of the morphological characters are conserved in both, and differences between the two are mainly confined to the habit and fruit characters (Fig. 1). *Datura ceratocaula* which is restricted to Mexico and Central America, is a semi-aquatic hollow-stemmed prostrate creeping plant. It is considered as the connecting link between herbaceous *Datura* and shrubby *Brugmansia* due to its unique combination of characters (Bernhardt, 1833; Safford, 1921; Griffin and Lin, 2000). The plant is a herbaceous annual with erect flowers, circumscissile calyx, tetralocular ovary and carunculate seeds like *Datura*, but has smooth, pendulous, fleshy fruits characteristic of *Brugmansia*.

Although the chloroplast gene *rbcL* has been utilized for solving plant phylogenetic controversies at higher taxonomic levels (Chase et al. 1993; Qui et al. 1998), the nrDNA ITS region is being increasingly depended upon to resolve inter-generic and inter-specific phylogenetic concerns (Baldwin, 1992; Kron and King, 1996). This region is fast-evolving and serves as a method of direct DNA sequence analysis for detecting reticulate phenomena which would be appropriate for subgeneric or subspecific cladistic studies (Gaut et al. 2000).

Bye and Sosa (2013) studied the molecular phylogeny of the genus *Datura* using *matK*, *ndhF*, *trnH-psbA*, *trnL-F* and morphological markers, and supported the previous recognition of *Brugmansia* as sister of *Datura* in the subtribe *Datureae* by Olmstead et al, 2008. The analyses also supported the recognition of *Datura* as monophyletic with the monospecific section *Ceratocaulis* as the sister to the remaining species of *Datura*. Mace et al. (1999) performed cluster analysis of AFLP data for seven species of *Datura* and six species of *Brugmansia* and considered the genera as sister groups. However both these studies have focused largely on the distinct generic status of *Brugmansia* and the subgeneric classification within the genus *Datura*. The present study while addressing the systematic and phylogenetic relationships between the members of the two genera *Datura* and *Brugmansia* using ITS markers, focuses more on the infrageneric interrelationships between the members of *Brugmansia*.

Materials and Methods:-

Taxon sampling:-

Five taxa of *Brugmansia* and four taxa of *Datura* from Kerala and Tamil Nadu, India, are included in the present study. Author citations are given in Tables 1. The voucher specimens of the taxa collected have been deposited in SNCH herbarium.

DNA extraction, amplification, and sequencing:-

Genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Amsterdam, The Netherlands). DNA amplification and sequencing of the ITS region were performed using the primers ITS1 and ITS2 (White et al. 1990).

The polymerase chain reaction (PCR) was performed with standard methods using AccuPower HF PCR premix (Bioneer, Daejeon, Korea) in 20 μ l volumes containing 2 μ l of 10X buffer, 300 μ M dNTPs and 1 unit of HF DNA polymerase. To this 1 μ l of a 10 pM solution of each primer, 1 μ l of genomic DNA and distilled water added to make a total volume of 20 μ l. PCR amplification was performed with 40 cycles (Denaturation for 1 min at 94°C, annealing for 1 min at 49°C, and 1 min of extension at 72°C followed by a last cycle of final extension for 5 min at 72°C). PCR products were checked for the presence of appropriate bands on a 0.8 % agarose gel, purified, and sequenced at Sci-Genome, Kochi. Sequences comprised of ITS1, 5.8S, and ITS2 regions. Forward and reverse sequences were edited and assembled using the computer program DNA Baser (vers. 3) (2011). All sequences have been deposited in GenBank (Table 2).

Phylogenetic analyses:-

A total of 45 nucleotide sequences (including all outgroups) were aligned using Clustal X vers. 2.0.11 (Thompson et al. 1997) followed by manual adjustments in Clustal W (Thompson et al.1994). All positions containing gaps and missing data were eliminated. Phylogenetic analyses were done using neighbour-joining (NJ) methods and RAxML (maximum likelihood, ML). ML analyses were done using RAxML-HPC BlackBox 8.0.9 (Stamatakis 2006; Stamatakis et al. 2008). The best-fit model was determined using jModel Test vers. 0.1.1 ascue1 2003) by the Akaike Information Criterion (AIC) and the maximum likelihood method; the lowest AIC score and highest log-likelihood score. Relative support for the clades recovered was assessed via bootstrap analyses using 1,000 replicates in both analyses. The following criteria were used to assess bootstrap support percentages (BP): 50–70 %, low; 71–84 %, moderate; 85–100%, strong. The results of MP and NJ analyses are congruent with those from Bayesian analyses.

Results:-

Sequence analyses: -

45 accessions of 21 species were used for the analysis and out of 21 species 9 were generated new, 9 sequences consists of five *Brugmansia* and four *Datura* species. All the sequences have been deposited in GenBank (Tables 2).

Alignment:-

The ITS alignment comprises of total of 45 accessions of 21 species and has an aligned length of 636 base pairs. The ITS sequences used for analysis were having 362 (56.9%) identical sites and remaining variable and informative sites - Major variations were observed in ITS1 and ITS2, while the 5.8s region remained conserved. Mean pairwise percentage is 89.6%. Of the total sites, 3.1% consists of gap.

Maximum Likelihood analyses:-

Monophyly of both the genera (*Datura* and *Brugmansia*) is proved and is well supported (BS 100 and 91). *Datura ceratocaula* once considered to be a hybrid or connecting link between *Datura* and *Brugmansia* is very well placed in

Datura clade (BS 57) and is grouped along with other species. The genus Brugmansia appears to be split into two with *B. sanguinea* and *B. vulcanicola* diverging out as a distinct cluster (BS 91).

Discussion:-

The results obtained from the present study support the separation of Brugmansia from Datura by earlier taxonomists, and the tree topology obtained using ITS data is also congruent to their study (Text Fig. 2). In the light of the present observations, the systematic inter-relationships between and within the two genera are discussed below.

Elevation of Brugmansia as a new genus:-

Earlier taxonomic studies based on morphological as well as phytochemical data consider Brugmansia to be synonymous with Datura (Bernhardi, 1833; Bristol 1966), the grouping being challenged by several others who advocated its elevation as an independent genus (Persoon, 1805; Lagerheim 1895). The erection of Brugmansia as a separate genus by Lockwood (1973) was generally accepted, yet doubted by some taxonomists despite it being recognized under the International Code of Botanical Nomenclature (Nee, 1991). The protein chemotaxonomy study based on amino acid sequences of ferredoxin supported the conventional classification that included Brugmansia within the genus Datura (Mino 1994, 1995). Phylogenetic study using ITS1 sequences, isozymes and morphology on 12 species of Datura and Brugmansia by Mace et al. (1999) separated the arborescent species of Brugmansia as a genus from the herbaceous species of Datura. Pollen morphological studies on Datura and Brugmansia also separated these two taxa (Persson et al. 1999). Dhanya et al. (2016) proposed two pollen types for the two genera namely Datura-type and Brugmansia-type. Later floristic studies also recognized the distinctiveness of the two genera (D'Arcy 1973; Haegi 1976; Shaw 2000; Hunziker 2001). The present study also revealed two major clades - Datura clade (BS 100) characterised morphologically by herbs, erect flowers and spiny capsules and Brugmansia clade (BS 91) encompassing mostly small trees having pendant flowers and smooth capsules (Text Fig. 1). The split of Datura and Brugmansia is well supported (BS 80).

The taxonomic status of Datura ceratocaula:-

Datura ceratocaula has been considered to be a hybrid of Datura and Brugmansia, owing to its unique combination of traits being a herbaceous annual with erect flowers, circumscissile calyx, tetralocular ovary and carunculate seeds like Datura and bearing smooth, pendulous, fleshy berries similar to Brugmansia. The fruits however dehisce irregularly like some species of Datura. This reticulation of characters from the two major genera in *D. ceratocaula* was raised as a major argument in support of the inclusion of Brugmansia as a section under the genus Datura. Consequently, *D. ceratocaula* was considered as the connecting link between the two genera (Bernhardi, 1833; Safford, 1921). However, later molecular studies revealed that the species is well nested within the genus Datura (Mace et al. 1999; Luna-Cavazos et al. 2000; Jiao et al. 2002; Bye and Sosa, 2013). The present molecular data also suggests that *D. ceratocaula* is a distinct species, nested within the Datura clade (BS 57), amply separated from the Brugmansia clade.

The taxonomy based on fruit morphological characters recognizes three sections in the genus Datura (excluding section Brugmansia in some earlier classifications) viz. Dutra, Ceratocaulis and Datura (Bernhardi 1833; Dunal 1852; Safford 1921; Hammer et al. 1983). Sections Dutra and Datura are characterized by dry dehiscent spiny capsules, pendant in the former and erect in the latter. On the contrary, section Ceratocaulis exhibits spineless pendant fleshy berries that disintegrate irregularly (Knapp, 2002). Avery et al. (1959) found *D. ceratocaula* to be incompatible with all other species of the genus, and hence included it singly under the section Ceratocaulis of the genus Datura. The distinctiveness of section Ceratocaulis is well-supported in the present study also (BS 57).

Subgeneric classification of Datura:-

Datura has been traditionally subdivided into three sections viz. **Section Datura** comprising annual herbs with erect spiny septifragal capsules (*Datura stramonium*, *D. quercifolia* and *D. ferox*), **Section Ceratocaulis** with pendant unarmed foraminicidal capsules including only one species - *D. ceratocaula* and **Section Dutra or Stramonium** encompassing perennial herbs with pendant spiny septifragal or foraminicidal capsules. The latter is the largest and includes 6-8 species - *D. discolor*, *D. inoxia*, *D. kymatocarpa*, *D. lanosa*, *D. metel*, *D. pruinosa*, *D. reburra*, *D. wrightii* and *D. leichhardtii* (Safford, 1921; Avery et al. 1959; Fosberg, 1959; Hammer et al. 1983).

The largest Section Dutra is heterogenous and shows a mixture of characters of the former two sections. Consequently, several researchers have proposed its division into either independent sections or subsections.

Division of genus *Datura* into four sections was proposed by erecting a fourth section either by separating out *D. discolor* alone as Section *Discolor* (Mace et al. 1999) or by subdividing Section *Dutra* into two (Jiao et al. 2002). Luna-Cavazos et al. (2009) also recognized two distinct clades within section *Dutra* from their cladistic analysis of morphological characters. Bye and Sosa (2013) shifted *D. kymatocarpa*, *D. leichhardtii* and *D. discolor* from conventional Section *Dutra* to Section *Datura* and opined that no new section needs to be added, retaining the three conventional sections proposed by Bernhardt (1833).

The three conventional sections of *Datura* stand well supported in the phylogenetic tree from the current study also (BS 100, 100 and 83). *D. kymatocarpa*, *D. leichhardtii* and *D. pruinosa* from Section *Dutra* appear shifted to Section *Datura*, corroborating the observations by Bye and Sosa (2013). The only difference is seen with regard to *D. discolor*, which remained stationed within the Section *Dutra* and yet diverged from the remaining members of the section, forming a clearly defined subclade (BS 100). The nodding, septifragal capsules of *D. discolor* led Barclay (1959) to consider the species as an intermediate between the sections *Dutra* and *Stramonium* (*Datura*). This was supported by Mace et al. (1999 and 2000) from isozyme and ITS-1 analyses, who considered that *D. discolor* might be a recombinant from taxa of both sections or it may represent the ancestral form of the two groups. However in the present study, *D. discolor* was well-nested within the Section *Dutra*, and the molecular data do not support its intermediary or ancestral ranking. The divergence of the species from the remaining taxa within the section *Dutra* observed in the phylogenetic tree (Fig.2) is not sufficient enough for the erection of a new section as was proposed earlier (Mace et al. 1999). All the same it forms a clearly supported subclade (BS 100) which may be suggestive of the subdivision of Section *Dutra* into two subsections and this remains to be appraised using cladistical studies incorporating both molecular and morphological data.

Subgeneric classification of *Brugmansia*:-

Hay et al (2012) divided the genus *Brugmansia* into two sections - Section *Brugmansia* - the warm group with long, spindle shaped fruits and Section *Sphaerocarpium* – the cold group with short, egg-shaped fruits. They observed that hybridization is not possible across the sectional limits. The former includes *B. aurea*, *B. x candida*, *B. insignis*, *B. suaveolens* and *B. versicolor*, while the latter includes *B. arborea*, *B. x rubella*, *B. sanguinea* and *B. vulcanicola*. This classification based mainly on habitat and fruit shape, ignores corolline features. But the short, egg shaped fruit of *B. arborea* makes it a misfit in Section *Brugmansia* characterized by long spindle shaped fruits. Hence fruit shape alone cannot be depended upon as a distinguishing character for subdividing the genus into sections.

Haegi (1976) observed that in *Brugmansia* large trumpet or funnel shaped corollas are characterized by flared throats and limbs, while they were scarcely flared out in tubular corollas. Morphological observations reveal a close association between tubular corollas and short egg shaped fruits, while large flared out corollas are not linked to any particular fruit form. So when the two traits are considered together, the members of the genus diversify into two clear morphologically distinct groups – (1) a larger group including taxa having large trumpet or funnel shaped and flared out corollas along with long spindle shaped or short egg shaped fruits and (2) a second group with narrow tubular corollas and short egg shaped fruits.

The phylogenetic tree observed in our study shows a clear subdivision of *Brugmansia* Clade into two subclades - a larger subclade with majority of taxa conforming to the larger morphological group based on corolline and fruit characters and a second subclade including the taxa with narrow tubular corollas and short egg shaped fruits. The two subclades stand well supported (BS 100 and 91). Hence it is proposed that the genus *Brugmansia* may be subdivided into two sections based on corolline and fruit characters as Section *Brugmansia* and Section *Sanguinea*, as explained above.

Conclusions:-

The present molecular analysis based on ITS 1 and ITS 2 sequences supports the splitting of *Datura* and *Brugmansia* and also establishes the distinctiveness of the two genera (BS 100 and 91). *D. ceratocaula* earlier considered as the connecting link between the two genera, is nested within *Datura* clade (BS 57) as a distinct species. The three conventional sections of *Datura* viz. *Dutra*, *Ceratocaulis* and *Datura* (*Stramonium*) stand well supported in the phylogenetic tree (BS 100, 100 and 83). *D. kymatocarpa*, *D. leichhardtii* and *D. pruinosa* from Section *Dutra* appear shifted to Section *Datura*, corroborating earlier observations. *D. discolor* was nested within the Section *Dutra*, and the molecular data do not support its intermediary or ancestral ranking, contrary to earlier molecular reports. The divergence of *D. discolor* from the remaining taxa within the section *Dutra* is not sufficient enough for the erection of a new section as was proposed earlier. The egg shaped fruit of *B. arborea* makes it a misfit in Section *Brugmansia*

characterized by long spindle shaped fruits. Hence fruit shape alone cannot be depended upon as a distinguishing character for subdividing the genus *Brugmansia*. The phylogenetic tree observed in the present study shows a clear subdivision of *Brugmansia* Clade into two well supported subclades (BS 100 and 91). Hence we propose the subdivision of the genus *Brugmansia* into two sections based on corolline and fruit characters as Section *Brugmansia* comprising majority of taxa under the genus characterized by large trumpet or funnel shaped and flared out corollas along with long spindle shaped or short egg shaped fruits and Section *Sanguinea* with narrow tubular corollas and short egg shaped fruits including *B. sanguinea* and *B. vulcanicola*.

Table 1:- Details of the taxa of *Brugmansia* Pers. and *Datura* L. collected from Kerala and Tamil Nadu along with author citations

S. No	Name of species	Vernacular name/ common name	Native range	Locality	Voucher No.	N	E	Altitude
1	<i>Brugmansia candida</i> Pers.	<i>B. candida</i> 'Tutu' x <i>B. versicolor</i> 'Kaskade'	Ecuador, South America – the hybrid variety from France	Kodaikanal, Tamil Nadu	S015 SNCH	10°14'16"	77°29'23"	2091 m
2	<i>Brugmansia cubensis</i> (V.R.Fuentes) V.R.Fuentes	<i>B. aurea</i> x <i>B. suaveolens</i> x <i>B. versicolor</i>	Peru, Chile and Columbia, South America – the hybrid from Columbia	Kodaikanal, Tamil Nadu	S016 SNCH	10°14'09"	77°29'34"	1548 m
3	<i>Brugmansia sanguinea</i> (Ruiz & Pav.) D. Don	-	Peru, South America	Ootacamund, Tamil Nadu	S013 SNCH	11°24'23"	76°41'35"	2248 m
4	<i>Brugmansia sanguinea</i> (Ruiz & Pav.) D. Don	-	Ecuador, South America	Ootacamund, Tamil Nadu	S014 SNCH	11°24'16"	76°41'29"	2263 m
5	<i>Brugmansia suaveolens</i> (Humb. & Bonpl. ex Willd.) Bercht. & J. Presl.	Angel's Trumpet	South east Brazil, South America	Kumili, Kottayam, Kerala	S012 SNCH	9°36'4"	77°10'12"	1048 m
6	<i>Brugmansia suaveolens</i> (Humb. & Bonpl. ex Willd.) Bercht & J. Presl.	Angel's Trumpet	South east Brazil, South America	Kodaikanal, Tamilnadu	S018 SNCH	10°14'13"	77°29'41"	2109 m
7	<i>Datura innoxia</i> Mill.	Ummam/ Downy Thorn Apple/ Indian-Apple/Recurved Thorn	Central and South America, India and	Coimbatore, Tamilnadu	S019 SNCH	11°00'54"	76°57'14"	457 m

		apple / Sacred Thorn Apple	China					
8	Datura metel L. var. fastuosa (L.) Saff.	Ummam/ Black Datura	Asia and Africa / America	Pallippuram, Thiruvananthapuram, Kerala	S020 SNCH	8°36'34 "	76°51'2 0"	34 m

Table 2 :-Voucher information of Brugmansia and Datura used for molecular study employing ITS markers

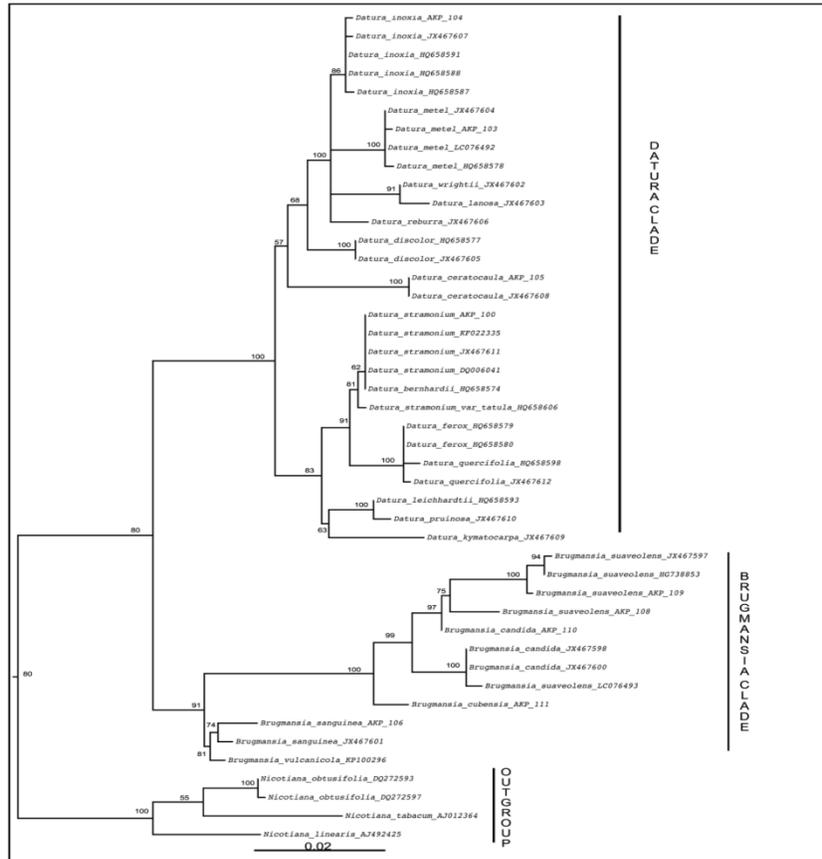
Sl.No	Taxon	Voucher No.	Locality	GenBank
1	Brugmansia candida Pers.	S015 SNCH	Kodaikanal, Tamil Nadu	-
2	B. cubensis (V.R. Fuentes) V.R.Fuentes	S016 SNCH	Kodaikanal, Tamil Nadu	-
3	B. sanguinea (Ruiz & Pav.) D.Don	S014 SNCH	Ootacamund, Tamil Nadu	-
4	B. suaveolens (Humb. & Bonpl. ex Willd.) Bercht. & J. Presl	S012 SNCH	Kottayam, Kerala	-
5	B. suaveolens (Humb. & Bonpl. ex Willd.) Bercht. & J. Presl	S018 SNCH	Kodaikanal, Tamil Nadu	-
6	Brugmansia candida Pers.		Mexico	JX467598, JX467600
7	B.sanguinea (Ruiz & Pav.) D.Don		Mexico	JX467601
8	B.suaveolens (Humb. & Bonpl. ex Willd.) Bercht. & J.Presl		Mexico	JX467597, HG738853, LC076493
9	B. vulcanicola (A.S.Barclay) R.E.Schult.		USA	KP100296
10	Datura ceratocaula Ortega	S07 SNCH	USA	-
11	D. inoxia Mill.	S019 SNCH	Coimbatore, Tamil Nadu	-
12	D. metel L. var. fastuosa (L.)Saff.	S020 SNCH	Thiruvananthapuram, Kerala	-
13	D. stramonium L.	S017 SNCH	Ootacamund, Tamil Nadu	-
14	D. ceratocaula (Ortega) Court ex Gaede	-	Mexico	JX467608
15	D. bicolor Pers.	-	Germany	HQ658577, JX467605
16	D. ferrox L.	-	France	HQ658579, HQ658580
17	D. inoxia Mill.	-	China, UK	JX467607, HQ658591, HQ658588
18	D. lanosa Barclay ex Bye	-	Mexico	JX467603
19	D. leichhardtii Benth.	-	China	HQ658593
20	D. metel L.	-	China, Indonesia	JX467604, HQ658595, LC076492, HQ658578
21	D. pruinosa Greenm	-	Mexico	JX467610
22	D. quercifolia Kunth	-	France	HQ658598, JX467612
23	D. reburra Barclay	-	Mexico	JX467606
24	D. stramonium L.	-	France, Mexico	KF022335, JX467611, DQ006041, HQ658606
25	D. kymatocarpa Barclay	-	Mexico	JX467609
26	D. wrightii Regel	-	Mexico	JX467602

27	Nicotiana linearis Phil.	-	UK	AJ492425
28	N. obtusifolia M. Martens & Galeotti	-	USA	DQ272593,DQ272597
29	N. tabacum L.	-	USA	AJ012364



Figure 1. The diversity of flowers and fruits in *Datura* and *Brugmansia* - a & j - *Datura innoxia* Mill. - Flower & Fruit; b & m - *Datura metel* L. var. *metel*- Fl. & Fr.; c & r - *Brugmansia sanguinea* 'Oroverde'- Fl. & Fr.; d & o - *Brugmansia sanguinea* 'MishaTora'- Fl. & Fr.; e & s - *Brugmansia suaveolens* 'Valley White'- Fl. & Fr.; f & q - *Brugmansia suaveolens* 'Remembrance'- Fl. & Fr.; k & u - *Datura metel* L. var. *fastuosa* (L.) Saffl - Fl. & Fr.; h & p - *Datura metel* L. var. *rubra*- Fl. & Fr.; i & t - *Brugmansia x cubensis* 'Dr.Seuss'- Fl. & Fr.; l & n - *Datura stramonium* L.- Fl. & Fr.; g & v - *Brugmansia x candida* Pers. 'Charleston'- Fl. & Fr.

Figure 2. Best ML tree inferred from analysis of *nrDNA* (ITS), for 45 accessions of 21 species including outgroup analysed under GTRGAMMA and rooted on *Nicotiana* spp. (RAxML bootstrap values ≥ 50) indicated above the branches.



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