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RESEARCH ARTICLE

POLLEN MORPHOLOGICAL STUDIES ON TWO SOLANACEOUS GENERA: *BRUGMANSIA* PERS. AND *DATURA* L.

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Abstract

The exomorphological features of the pollen grains of five taxa of *Datura*L. and six taxa of *Brugmanisa*Pers. (Solanaceae) from Kerala and Tamil Nadu, India, have been studiedin detail. The two genera are closely related with*Brugmansia*being formerly included under *Datura* and later given a generic status. The pollen of both plant groups are also similar being 3-zonocolporate,large or medium andstriateor striato-reticulate, occasionally rugulate. The transverse or longitudinal alignment of the mural subunits in the exine has been considered to be the major distinguishing feature between pollen of the two genera. But in the present study, longitudinal and/or diagonal orientation of mural subunits has been observed in two varieties of *B. sanguinea*. Hence mural subunit alignment cannot be depended upon as a unique distinguishing character between pollen of the two genera. Although microscabrae were reported earlier from some members in both genera,adequate attention seems to be lacking with regard to these bodies. In the present study,microscabrae were observed in all the eleven members studied and showed distinction between the two genera, being large, nodular and dense in single or more rows in *Brugmansia* and small, granular, sparse and irregularly scattered in members of *Datura*. On the basis of the present observations, two pollen types are proposed here for the two genera studied viz. *Brugmansia* -type and *Datura* –type.

Introduction:-

Palynology has moved beyond the realms of classical exine characteristics relating to the aperture, exine ornamentation, exine strata, pollen size and pollen shape to in-depth microstructural analysis of the delicate patterns of exine surface ornamentation employing highly sophisticated tools such as the Scanning Electron Microscope. The ‘micro palynological markers’ so unearthed are often characteristic to particular plant groups, and are now increasingly depended upon as supplementary tools in deciphering the complex patterns of taxonomic interrelationships and species delimitations.

Datura L. and *Brugmansia*Pers. belong to the deadly nightshade family Solanaceae, comprising of over 94 genera and 2950 species worldwide (Mabberly, 2005; Shultes and Raffauf, 1991). *Datura* L., is native to Northern

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America, and comprises of 10-12 species distributed in the tropical and temperate regions of the world, of which four have been reported from South India (Sasidharan, 2004). The genus includes several medicinal plants and some weedy species. *Brugmansia*Pers. (formerly included under *Datura* and later given a generic status by Lockwood, 1973), is native to South America and includes six to nine species of small ornamental trees, of which only one has so far been reported from South India.

The earlier palynological literaturelargely focuses on the economically more valuable genus – *Datura*, and includes the study on the pollen grains of eleven taxa of Chinese Datura by Guang-Fang *et al.* (1985), *Daturastramonium*from Jordhanby Al-Quran (2004),three species of *Datura* from Pakistanby PerveenandQaiser (2007)and *Datura metel* from Nigeria (Adedeji and Akinniyi, 2015). Although Persoon*et al.* (1999) compared the pollen morphology of five species of *Brugmansia* and eight species of *Datura* from America,palynological studies on the taxa recorded from India are scanty. The present study focuses on the comparative evaluation of the fine exomorphological features of the pollen of six taxa of *Brugmanisa*and five taxa of *Datura*from Kerala and Tamil Nadu, India using Light Microscopy and Scanning Electron Microscopy.

Materials and Methods:-

The details regarding the taxa selected for the present study from Kerala and Tamil Nadu are furnished below in Table -1.

Table 1:- Details of thetaxa of *Brugmansia*Pers. and*Datura*L. collected for the present study form Kerala and Tamil Nadu

Sl. No	Name of species	Locality	Voucher No.
1	*Brugmansiaxcandida*Pers. 'Charleston'	Kodaikanal, Tamilnadu	S015 SNCH
2	*Brugmansiax cubensis* (V.R.Fuentes) V.R.Fuentes 'Dr.Seuss'	Kodaikanal, Tamilnadu	S016 SNCH
3	*Brugmansiasanguinea*(Ruiz &Pav.) D. Don 'MishaTora'	Ootacamund, Tamilnadu	S013 SNCH
4	*Brugmansia sanguinea* (Ruiz &Pav.) D. Don 'Oroverde'	Ootacamund, Tamilnadu	S014 SNCH
5	*Brugmansiasuaveolens*(Humb.&Bonpl.exWilld.) Bercht&J. Presl. 'Remembrance'	Kottayam, Kerala	S012 SNCH
6	*Brugmansiasuaveolens*(Humb.&Bonpl.exWilld.) Bercht&J. Presl. 'Valley White'	Kodaikanal, Tamilnadu	S018 SNCH
7	*Datura innoxia* Mill.	Coimbatore, Tamilnadu	S019 SNCH
8	*Datura metel* L.var. *fastuosa* (L.)Saff.	Thiruvananthapuram, Kerala	S020 SNCH
9	*Datura metel* L.var. *metel*	Kollam, Kerala	S010 SNCH
10	*Datura metel* L.var.*rubra*Bernh.	Palakkad, Kerala	S011 SNCH
11	*Datura stramonium*L.	Ootacamund, Tamilnadu	S017 SNCH

Polliniferous materials were collected from live plants and fixed in glacial acetic acid, followed by acetolysisafterErdtman (1952) and Nair (1970). Morphological features relating to the pollen aperture, exine ornamentation, pollen size and shape were studied from LM and SEM observations. Pollen measurements were made using an ocular micrometer, from a random sample of 30 pollen grains from each taxon studied. The terminologies suggested by Punt *etal.* (1994) have been usedto describe aperture types and exine ornamentation pattern.

Results and Discussion:-

The pollen were 3-zonocolporate and large (*B. suaveolens* and *D.inoxia*) or medium in size. *Brugmansia*pollen were in general medium or large-sized, prolate-spheroidal or oblate-spheroidal to suboblate, 3-zonocolporate and striate (Table-2; Fig. 2), while those of *Datura* were mostly medium-sized, 3-zonocolporate, striato-reticulate, sometimes rugulateand possessing variable forms (Table-3; Fig. 15). Endocingulum was a common feature in both genera. Acolpate and monocolpate pollen and thin exine were observed in the three varieties of *D. metel*. The polar outline was rounded with apertures in the obtuse angles,while the equatorial outline was rounded or elliptic, and occasionallyslightly rectangular as in some members of *Brugmansia* (Fig.1).

Table 2:- Pollen morphological characters in species of *Brugmansia* Pers. collected fromKerala and Tamil Nadu

Characters	*B.* x *candida* 'Charleston'	*B. cubensis* 'Dr.Seuss'	*B. sanguinea* 'MishaTora'	*B. sanguinea* 'Oroverde'	*B. suaveolens* 'Remembrance'	*B. suaveolens* 'Valley White'
Quantitative characters						
Polar diameter - P (μm)	34.20±2.53	31.20±3.23	35.40±1.26	26.40±3.10	57.00±4.00	73.20±6.51
Equatorial diameter. E (μm)	32.40±2.37	34.80±1.55	34.80±3.22	25.20±1.55	69.00±5.29	78.00±5.66
P/E	1.06±0.14	0.90±0.10	1.02±0.10	1.06±0.17	0.83±0.11	0.94±0.04
Exine thickness (μm)	3.00±0.00	3.60±1.26	3.60±1.26	3.60±1.27	5.40±1.26	5.40±1.26
Aperture no.	3	3	3	3	3	3
Qualitative characters						
Pollen type	3-zonocolporate	3-zonocolporate	3-zonocolporate	3-zonocolporate	3-zonocolporate	3-zonocolporate
Pollen size-class	Medium	Medium	Medium	Medium	Large	Large
Acolpate / monocolpate grain presence	Absent	Absent	Absent	Absent	Absent	Absent
Polar outline	Rounded with apertures in the obtuse angles	Rounded with apertures in the obtuse angles	Rounded / slightly trilobed	Rounded with apertures in the obtuse angles	Rounded with apertures in the obtuse angles	Rounded with apertures in the obtuse angles
Equatorial outline	Rounded/ slightly rectangular	Rounded/elliptic	Rounded/elliptic/ slightly rectangular	Rounded/elliptic	Rounded/elliptic	Rounded/elliptic/ slightly rectangular
Pollen shape	Prolate-spheroidal	Oblate-spheroidal	Prolate-spheroidal	Prolate-spheroidal	Suboblate	Oblate-spheroidal
Exine thickness in optical CS	Thick	Thick	Thick	Thick	Thick/thin	Thick/thin
Endocingulum presence	Present	Present	Present	Present	Present	Present
Exine ornamentation	Striate	Striate	Striate	Striate	Striato-reticulate	Striato-reticulate
Tectum at mesocolpium	Striate	Striate	Striate	Striate	Striato-reticulate	Striato-reticulate
Tectum at apocolpium	Striate	Rugulate	Striate	Rugulate	Striato-reticulate	Striato-reticulate
Lumina nature	Not clearly visible	Not clearly visible	Not clearly visible	Not clearly visible	Reticulate	Reticulate
Mural nature	Long parallel	Long parallel	Long parallel	Long parallel	Long parallel	Long parallel
Mural height	Distinctly raised	Distinctly raised	Distinctly raised	Less raised	Distinctly raised	Distinctly raised
Mural length	Long	Long	Long	Long	Long	Long
Mural alignment	Long parallel	Long parallel	Long parallel	Long parallel	Long parallel	Long parallel

Characters	*B.* x *candida* 'Charleston'	*B. cubensis* 'Dr.Seuss'	*B. sanguinea* 'MishaTora'	*B. sanguinea* 'Oroverde'	*B. suaveolens* 'Remembrance'	*B. suaveolens* 'Valley White'
Mural width	Broad	Broad	Broad	Narrow	Less broad	Broad
Mural packing	Closely packed	Closely packed	Closely packed	Very closely packed	Less closely packed	Less closely packed
Mural subunit nature	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical
Mural subunit alignment	Transverse, perpendicular to muri	Transverse, perpendicular to muri	Transverse, perpendicular to or diagonal; sometimes longitudinal, parallel to muri	Transverse, diagonally oriented	Transverse, perpendicular to muri	Transverse, perpendicular to muri
Mural subunit thickness	Very thin	Very thin	Thin	Thin	Thin	Very thin
Mural subunit distinctiveness	Less distinct	Less distinct	Distinct	Distinct	Distinct	Distinct
Mural subunit packing	Closely packed	Closely packed	Closely packed	Closely packed	Closely packed	Closely packed
Microscabrae presence on muri	Present	Present	Present	Present	Present	Present
Microscabrae density	Dense	Dense	Dense	Dense	Dense	Dense
Microscabrae rows when present	Single/occasionally two or more	Single/occasionally two or more	Single/occasionally two or more	Single/occasionally two or more	Single/occasionally two or more	Single/occasionally two or more
Microscabrae size	Small	Large	Large	Small	Large	Large
Microscabrae shape	Nodular	Nodular	Nodular	Nodular	Nodular	Nodular

Table 3:-Pollen morphological characters in species of *Datura* L. collectedfrom Kerala and Tamil Nadu

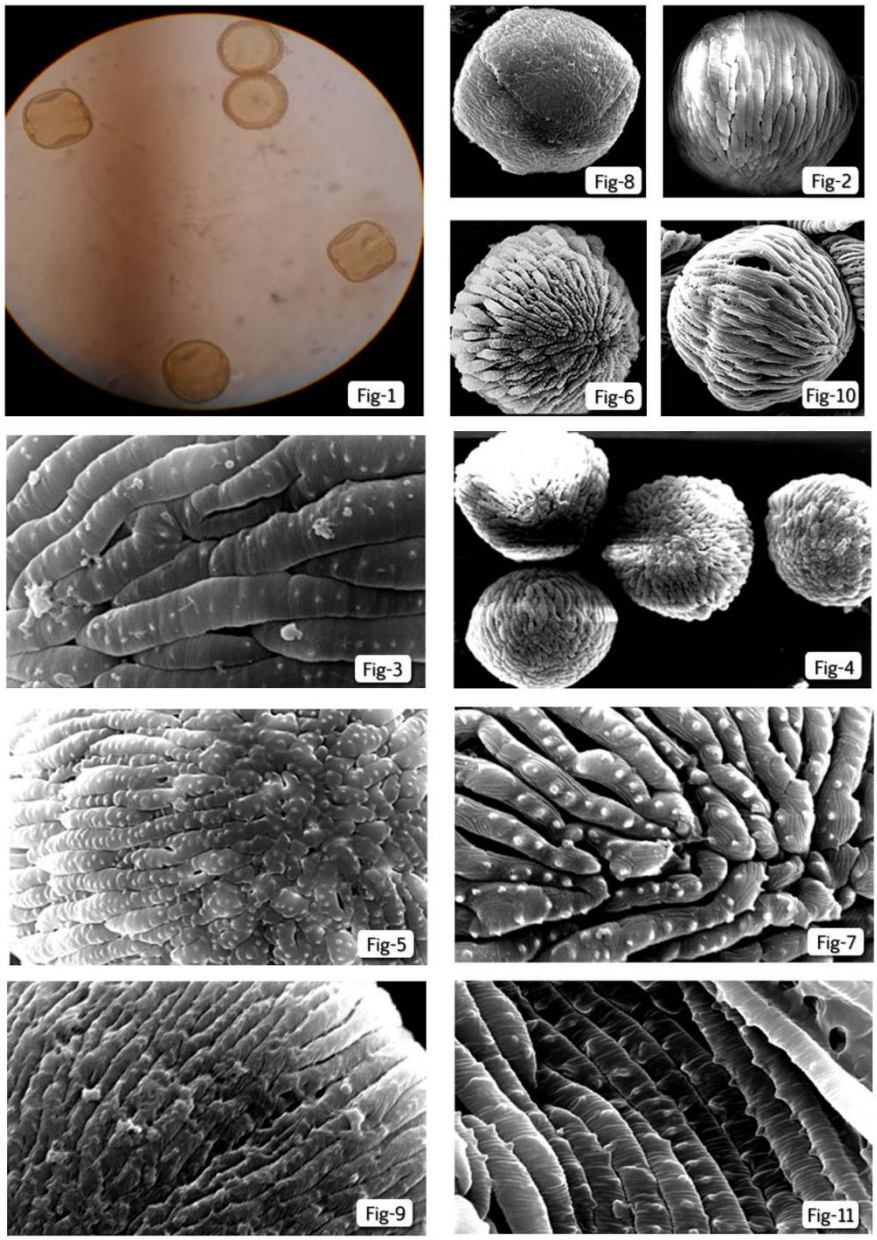
Characters	*D. innoxia*	*D. metel* var. *fastuosa*	*D. metel* var. *metel*	*D. metel* var. *rubra*	*D. stramonium*
Quantitative characters					
Polar diameter - P (μm)	46.80±1.55	33.60±3.69	33.00±3.46	37.20±1.55	30.60±4.20
Equatorial diam.- E (μm)	50.40±3.69	25.80±1.55	32.10±3.48	30.00±2.00	46.20±1.55
P/E	0.93±0.07	1.31±0.22	1.03±0.05	1.24±0.06	0.66±0.10
Exine thickness (μm)	6.00±2.00	3.60±1.27	3.60±1.27	3.60±1.27	4.50±1.58
Aperture number	3	3	3	3	3
Qualitative characters					
Pollen type	3-zonocolporate	3-zonocolporate	3-zonocolporate	3-zonocolporate	3-zonocolporate
Pollen size-class	Large	Medium	Medium	Medium	Medium
Characters	***D. innoxia***	***D. metel* var. *fastuosa***	***D. metel* var. *metel***	***D. metel* var. *rubra***	***D. stramonium***
Acolpate / monocolpate grain presence	Absent	Present	Present	Present	Absent
Polar outline	Rounded with apertures in the obtuse angles	Rounded with apertures in the obtuse angles	Rounded with apertures in the obtuse angles	Rounded with apertures in the obtuse angles	Rounded with apertures in the obtuse angles
Equatorial outline	Rounded/elliptic	Rounded/elliptic	Rounded/elliptic	Rounded/elliptic	Rounded/elliptic

Pollen shape	Oblate - spheroidal	Subprolate	Prolate-spheroidal	Subprolate	Oblate
Exine thickness in optical cross section	Thick/thin	Thin	Thin	Thin	Thick
Endocingulum presence	Present	Present	Present	Present	Present
Exine ornamentation	Striato-reticulate	Striato-rugulate	Striato-reticulate	Striato-reticulate	Rugulate-reticulate
Tectum at mesocolpium	Striato-reticulate	Striato-rugulate	Striato-reticulate	Striato-reticulate	Rugulate-reticulate
Tectum at apocolpium	Rugulate	Rugulate-reticulate	Rugulate-reticulate	Rugulate-reticulate	Rugulate-reticulate
Lumina nature	Reticulate	Granular	Reticulate	Reticulate	Reticulate
Mural nature	Long parallel	Long parallel	Long parallel	Long parallel	Short irregular
Mural height	Distinctly raised	Distinctly raised	Distinctly raised	Distinctly raised	Distinctly raised
Mural length	Long	Long	Long	Long	Short
Mural alignment	Long parallel	Long parallel	Long parallel	Long parallel	Short irregular
Mural width	Less broad	Less broad	Less broad	Less broad	Less broad
Mural packing	Less closely packed	Less closely packed	Less closely packed	Less closely packed	Loosely packed
Mural subunit nature	Striate	Striate	Striate	Striate	Striate
Mural subunits alignment	Longitudinal, parallel to muri	Longitudinal, parallel to muri	Longitudinal, parallel to muri	Longitudinal, parallel to muri	Longitudinal, parallel to muri
Mural subunit thickness	Thin	Thin when present	Thin when present	Thin when present	Thin
Mural subunit distinctiveness	Distinct	Very feeble	Very feeble	Very feeble	Distinct
Mural subunit packing	Closely packed	Not clearly visible	Not clearly visible	Not clearly visible	Closely packed
Microscabrae presence on muri	Present	Present	Present	Present	Present
Microscabrae density	Sparse	Sparse	Sparse	Sparse	Sparse
Microscabrae rows, when present	Irregularly scattered	Irregularly scattered	Irregularly scattered	Irregularly scattered	Irregularly scattered
Micr-scabrae size	Very small	Very small	Very small	Very small	Very small
Microscabrae shape	Granular	Granular	Granular	Granular	Granular

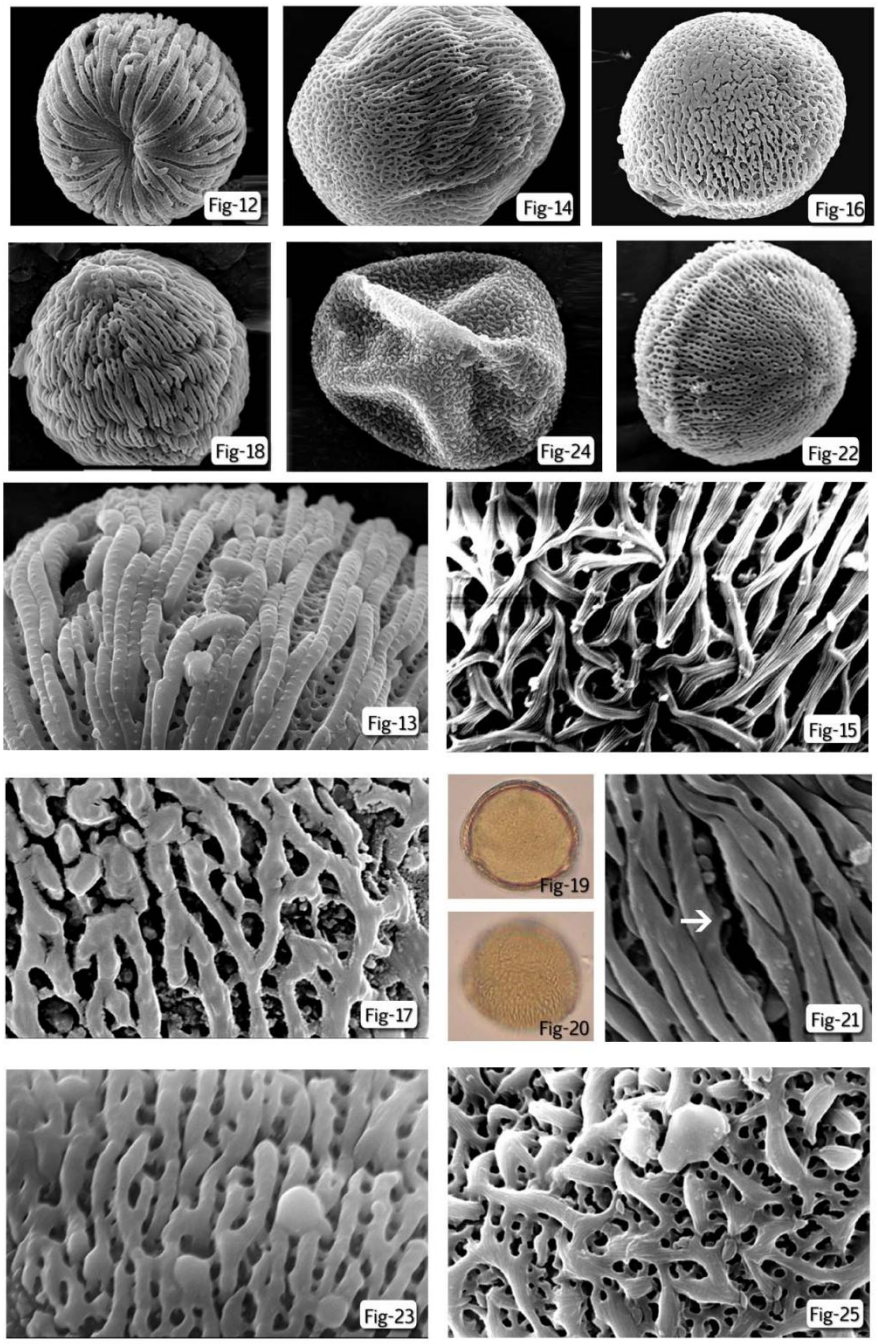
The members of *Brugmansia* had striateor striato-reticulate exines, the latter observed only in the two varieties of *B. suaveolens* (Figs.10 & 12). The exine was striato-reticulate in*Datura* except for striato-rugulate pattern in *D. metel* var. *fastuosa* and rugulate-reticulate exine in*D. stramonium*. The tectum showed slight variation in exine ornamentation at the apocolpium in some members, eg.rugulatein two members of *Brugmansia*andrugulate-reticulate in all five taxa of*Datura* studied.

The lumina were not visible in the striate pollenof *Brugmansia*due to the close packing of the long parallel muri (Fig.2), except inthe two varieties of *B. suaveolens*where it was reticulate. In *Datura*, the lumina were mostly reticulate (Fig.14), except for the granular lumina in *D. metel* var. *fastuosa* (Fig.17). *B. suaveolens*was similar to *Datura* in pollen exine thickness, ornamentation and lumina nature (Tables 2 & 3).

The long muri or liraewere parallelly aligned and distinctly raised in mostmembers (Figs.6 &8), being broaderand more closely packed in *Brugmansia.*In*D. stramonium*, they were short, irregular and loosely packed (Figs.19 & 23). The mural subunits were distinct or less distinct, thin or very thin, cylindrical, transverse and closely packed, perpendicular to the muri in *Brugmansia*(Fig. 3). In *B. sanguinea*, they were occasionally diagonally and/or vertically oriented (Figs.7& 9).On the other hand, the subunits were striate, thin and longitudinally arranged, parallel to the muri in *Datura* (Fig. 15),sometimes very feeble and not clearly visible as in *D. metel*(Figs.17& 21). Large and nodular microscabrae were denselyarranged in single or occasionally two or more rows in*Brugmansia*, in contrast to the small granular ones in *Datura* which were sparsely present and irregularly scattered (Figs. 7 &21).



Figs.1-3: *Brugmansia* x *candida* 'Charleston' pollen- 40x , 3000x, 10,000x; Figs.4-5: *B.* x *cubensis* 'Dr.Seuss' pollen-3000x, 10,000x; Figs.6-7: *B. sanguinea* 'Misha Tora' pollen- 2500x, 10,000x; Figs. 8-9: *B. sanguinea* 'Oroverde' pollen - 3000x, 10,000x; Figs.10-11: *B. suaveolens* 'Remembrance' pollen- 1700x, 10,000x.



Figs.12-13: *B. suaveolens* 'Valley White' pollen- 1900x, 5,000x. Figs.14-15: *Datura inoxia* pollen-5000x, 10, 000x; Figs.16-17: *D. metel* var. *fastuosa* pollen-2000x, 10,000x; Figs.18-21: *D. metel* var. *metel* pollen-2500x, 40x, 40x, 10,000x; Figs.22-23: *D. metel* var. *rubra* pollen-5000x, 10,000x; Figs. 24-25:*D. stramonium* pollen-1500x, 10,000x.

The most extensive pollen morphological study comparing *Brugmansia* and *Datura* was made by Knapp and Persoon (1999). They recognized two pollen types on the basis of the thickness of exine, height of muri and alignment of mural subunits viz. *Datura inoxia* type and *Brugmansia sanguinea* type. Here the major focus seems to be on the alignment of the mural subunits, a feature distinct to each group being transverse in *Brugmansia* and longitudinal in *Datura* (Figs. 3 &15). But in the present study, *B. sanguinea* 'MishaTora' showed a combination of both longitudinal and transverse alignment of mural subunits occasionally, even though the major pattern was the transverse type characteristic of the *Brugmansia* type (Fig.7). Similarly, *B. sanguinea* 'Oroverde' showed diagonally

oriented mural subunits (Fig.9). Hence mural subunit alignment cannot be considered aunique or major distinguishing character between the two genera. Moreover,Persoon*etal.*observed microscabrae in only some species of both genera and did not consider them significant in distinguishing between the two groups. But in the present study, microscabrae were present in all the eleven members and showed clear distinction in their size, form, occurrence and distribution between the two genera. They were large, nodular and dense in single or more rows in *Brugmansia* in contrast to being small, granular, sparse and irregularly scattered in members of *Datura* (Figs 5 & 21). Interestingly, they did not observe these bodies in the American taxa of *B. candida* and *B. sanguinea*, while the Indian taxa showed very distinct large, nodular microscabrae in large numbers on the pollen muri (Figs. 3 & 7). Hence microscabrae may also be considered as distinctive markers for comparison between the two genera.

In the light of the above discussion, the pollen of the two groups may be classified into *Brugmansia* -type and *Datura* -type as follows :

1.	***Brugmansia*-type**	**:**	**Exine** thick, striate or striato-reticulate, apocolpium same as mesocolpium in ornamentation; **muri or lirae** long and closely packed; **mural subunits** cylindrical, distinct and transverse - rarely diagonal and/or longitudinal; **microscabrae** large, nodular, dense and arranged almost regularly in one or occasionally more rows.
2.	***Datura*-type**	**:**	**Exine** thin, striato-reticulate or sometimes striato-rugulate or rugulate-reticulate at mesocolpium, apocolpium rugulate-reticulate; **muri** long or sometimes short and less closely or loosely packed; **mural subunits** striate, feeble and longitudinal; **microscabrae** small, granular, sparse and irregularly scattered.

Conclusions:-

The exine sculpturing viewed under the Scanning Electron Microscope opens up a treasure trove of pollen microstructural features. These 'micro' traits are most often specific to plant groups and are increasingly used as 'micro' palynological markers in systematic studies and phylogenetic evaluations. The pollen of *Datura* and its closely related genus *Brugmansia* are similar being3-zonocolporate,large or medium andstriateor striato-reticulate, occasionally rugulate. Despite these overall similarities, the two genera have been palynologically distinguished largely based on the transverse or longitudinal alignment of the mural subunits in the exine. But longitudinal and/or diagonal orientation of mural subunits have been observed in two varieties of *B. sanguinea*presently studied. Hence mural subunit alignment cannot be depended upon as a unique distinguishing character between pollen of the two genera.In addition, microscabrae on the muri also showed distinction between the two genera, being large, nodular and dense in single or more rows in *Brugmansia* and small, granular, sparse and irregularly scattered in members of *Datura*. On the basis of the present observations, two pollen types are proposed here for the two genera studied viz. *Brugmansia* -type and *Datura*–type.

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