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 Brugmansia Pers. (Solanaceae) from Kerala and Tamil Nadu, India, have been studied in 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 and striate or 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...
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 literature largely 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 Pakistan by Perveen and Qaiser (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 *Brugmansia* 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 the taxa of *Brugmansia* Pers. and *Datura* L. collected for the present study from Kerala and Tamil Nadu

| Sl. No | Name of species | Locality | Voucher No. |
|--------|----------------|----------|-------------|
| 1      | *Brugmansia x candida* Pers. ‘Charleston’ | Kodaikanal, Tamilnadu | S015 SNCH |
| 2      | *Brugmansia x cubensis* (V.R.Fuentes) V.R.Fuentes ‘Dr.Seuss’ | Kodaikanal, Tamilnadu | S016 SNCH |
| 3      | *Brugmansia sanguinea* (Ruiz & Pav.) D. Don ‘MishaTora’ | Ootacamund, Tamilnadu | S013 SNCH |
| 4      | *Brugmansia sanguinea* (Ruiz & Pav.) D. Don ‘Oroverde’ | Ootacamund, Tamilnadu | S014 SNCH |
| 5      | *Brugmansia suaveolens* (Humb.&Bonpl.exWilld.) Bercht & J. Presl. ‘Remembrance’ | Kottayam, Kerala | S012 SNCH |
| 6      | *Brugmansia suaveolens* (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. | Tiruvananthapuram, 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 acetolysis after Erdtman (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 *et al.* (1994) have been used to describe aperture types and exine ornamentation pattern.

**Results and Discussion:**

The pollen were 3-zonocolpate 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 rugulate and 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 occasionally slightly rectangular as in some members of *Brugmansia* (Fig.1).
### Table 2: Pollen morphological characters in species of *Brugmansia* Pers. collected from Kerala and Tamil Nadu

| Characters | *B. x candida* ‘Charleston’ | *B. cubensis* ‘Dr. Seuss’ | *B. sanguinea* ‘Misha Tora’ | *B. sanguinea* ‘Oroverde’ | *B. suaveolens* ‘Remembrance’ | *B. suaveolens* ‘Valley White’ |
|------------|-----------------------------|---------------------------|-----------------------------|---------------------------|-------------------------------|-------------------------------|
| 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 |

#### Quantitative characters

- **Polar diameter - P (μm)**
- **Equatorial diameter. E (μm)**
- **P/E**
- **Exine thickness (μm)**
- **Aperture no.**

#### Qualitative characters

- **Pollen type**
- **Pollen size-class**
- **Acolpate / monocolpate grain presence**
- **Polar outline**
- **Equatorial outline**
- **Pollen shape**
- **Exine thickness in optical CS**
- **Endocingulum presence**
- **Exine ornamentation**
- **Tectum at mesocolpium**
- **Tectum at apocolpium**
- **Lumina nature**
- **Mural nature**
- **Mural height**
- **Mural length**
- **Mural alignment**
| 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 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. collected from Kerala and Tamil Nadu

| Characters | D. innoxia | D. metel var. fastuosa | D. metel var. metel | D. metel var. rubra | D. stramonium |
|------------|------------|------------------------|---------------------|---------------------|---------------|
| 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 |

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

| Characters | D. innoxia | D. metel var. fastuosa | D. metel var. metel | D. metel var. rubra | D. stramonium |
|------------|------------|------------------------|---------------------|---------------------|---------------|
| Pollen type | 3-zonocolporate | 3-zonocolporate | 3-zonocolporate | 3-zonocolporate | 3-zonocolporate |
| Pollen size-class | Large | Medium | Medium | Medium | Medium |
| 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 alignment        | Long | Long | Long | Short |
| 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 subunits 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 |
| Microscabrae size      | Very small | Very small | Very small | Very small | Very small |
| Microscabrae shape     | Granular | Granular | Granular | Granular | Granular |

The members of *Brugmansia* had striate or 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. rugulate in two members of *Brugmansia* and rugulate-reticulate in all five taxa of *Datura* studied.

The lumina were not visible in the striate pollen of *Brugmansia* due to the close packing of the long parallel muri (Fig. 2), except in the 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 lira were parallely aligned and distinctly raised in most members (Figs. 6 & 8), being broader and 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 densely arranged 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 eubensis* ‘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.
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* ‘Misha Tora’ 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* ‘Oro Verde’ showed diagonally
orientated mural subunits (Fig.9). Hence mural subunit alignment cannot be considered unique or major distinguishing character between the two genera. Moreover, Persoon et al. 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 \textit{Brugmansia} in contrast to being small, granular, sparse and irregularly scattered in members of \textit{Datura} (Figs 5 & 21). Interestingly, they did not observe these bodies in the American taxa of \textit{B. candida} and \textit{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 \textit{Brugmansia} -type and \textit{Datura} -type as follows:

| 1. \textit{Brugmansia}-type | Exine thick, striate or striato-reticulate, apocolpium same as mesocolpium in ornamentation; \textit{mural subunits} cylindrical, distinct and transverse - rarely diagonal and/or longitudinal; \textit{microscabrae} large, nodular, dense and arranged almost regularly in one or occasionally more rows. |
|---|---|
| 2. \textit{Datura}-type | Exine thin, striato-reticulate or sometimes striato-rugulate or rugulate-reticulate at mesocolpium, apocolpium rugulate-reticulate; \textit{mural subunits} long or sometimes short and less closely or loosely packed; \textit{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 \textit{Datura} and its closely related genus \textit{Brugmansia} are similar being 3-zonocolporate, large or medium and striate or 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 \textit{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 \textit{Brugmansia} and small, granular, sparse and irregularly scattered in members of \textit{Datura}. On the basis of the present observations, two pollen types are proposed here for the two genera studied viz. \textit{Brugmansia} -type and \textit{Datura} -type.

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