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POLLEN MORPHOLOGY AND PALYNOTAXONOMICAL STUDIES OF COMMON GARDEN PLANTS OF APOCYNACEAE, IN KOLKATA, WEST BENGAL, INDIA

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Abstract

Taxonomy and identification of angiosperms are mainly based on external morphological characters and traits of the plants; however palynological data sets are remotely used for the study. Pollen characters are genetically regulated that can be used as an ideal tool for establishing taxonomic groups. This type of integrated study can be useful in redefining the status of families in higher group of plants. Apocynaceae, a Eurypalynous family exhibits variation in possessing more than one type of pollen grains from simple porate to compound colporate apertures. Pollen attributes has been utilized in building a pollen key that would enable us to distinguish genera solely on the basis of pollen characters.

Keywords: Apocynaceae, Taxonomy, Palynology, Pollen, Eurypalynous

Introduction

Every man is a taxonomist from the cradle to the grave. He is surrounded by plants and trees all around him and therefore they can be considered as the primary companion in the biotic community. Classification of the plants can be dated back with the onset of human civilization, when he classified plants under groups based on their economic potential to him. The scientific approach of classification was recognized by A. P. de Candolle and he introduced the formal subject of "Taxonomy" in his legendary work *Theorie elementaire de la botanique* (Candolle, 1813). Taxonomic identification of Angiosperms is mainly done based on visible morphological aspects and characters of the plants. Phenetic system of

classification encompasses characters and data from all available sources including morphology, anatomy, phytochemistry, palynology, cytology and others to establish relationship amongst taxa.

The scientific study of pollen grains and spores, both living and fossils make up the subject "Palynology". It was introduced by Hyde and Williams in the journal of Pollen Analysis Circular (Hyde and Williams, 1944). The term is derived from the Greek word '*palunō*' meaning to sprinkle, fine meal. Pollen grain forms the first cell of the male gametophytic generation of the spermatophytic group of plants. It consists of two wall layers: the outer sculptured, ornamented exine and the inner intine. Characters of pollen grains are genetically labeled and display unique traits in wall ornamentation, aperture and reticulation. Pollen grains are produced in profuse number per anther to ensure successful pollination followed by germination that eventually led to seed-set production (Chatterjee et al., 2014). This makes it a potential tool for taxonomic study as it overcomes the major drawback of other features namely, flower, fruits, seed, etc. which are produced in limited number, a necessary handicap with these parameters. Study of pollen morphology is highly significant as it can be useful in establishing relationship amongst various taxa, resolving disputed taxonomical problem, building phylogenetic tree, tracing ancestry of a particular clade. Besides, this discipline gives us a sound knowledge on pollination ecology, constructing phylogenetic classification as well as in palynotaxonomic studies.

In case of angiosperm, pollen grains are produced from the sporogenous tissue of the anther of flower. Morphologically angiospermic pollen grains exhibit highest level of variation that can be utilized in plant taxonomy (Nair, 1964). They display unique traits of apertural variation right from the simplest inaperturate type recorded from the Valanginian of the Lower Cretaceous of Israel (Brenner and Bickoff, 1992) to the highly advanced echinate form as exhibited by the Asteraceae.

Taxonomists strive to establish evolutionary relationships between plant populations and classify them into particular level of organization. Based on the similarity index in pollen characters, various workers have attempted in drawing the evolutionary divergence and history of plant groups (Guinet, 1966; Tryton, 1986). But attempting in taxonomic identification of plants at generic level based on pollen character is a novel deed that has been attempted in the present work.

Pollen characters are constant and are guided by the genetic makeup of the parent plant, hence providing us with very convincing data that can be utilized in building a systematic classification of angiosperms. Comparative studies of palynological attributes have revealed that angiospermic families can be broadly categorized into two groups:-

- **Stenopalynous**- With respect to size, aperture and stratification of exine, characters of pollen grain types is more or less constant within the family level as exemplified by Poaceae, Crucifeare, Asclepediaceae, Lamiaceae, etc.
- **Eurypalynou**s- Within the family level grains shows considerable variation in attributes like size, exine ornamentation, apertures. Solanaceae, Asteraceae, Apocynaceae, Rubiaceae, etc are some common examples of this group.

Apocynaceae or "dogbane family" was instituted by Antoine Laurent de Jussieu (Jussieu, 1789). On a global scale, there are *c.* 5100 species distributed under 357 genera (Meve, 2002; Meve and Liede-Schumann, 2007; Nazar *et al*., 2012). The family is represented by 24 genera and 52 species, in India (Karthikeyan, 2000). The members have successfully established themselves right from the Southern tropical rainforest to the temperate forest of the Himalayan foothills. In West Bengal, this family is represented by 16 species which are placed under 21 genera (Sur, 2004). The members are widely used for their economically important nature; some of them provide us with crude drugs like cardiac glycosides, reserpine, vincristine, vinblastine, etc. from members like *Rauvolfia serpentina*, *Catharanthus roseus* and others. They are also widely used as ornamental plants like *Nerium*, *Vinca*, *Carissa*, *Allamanda*, *Plumeria*, *Thevetia*, *Mandevilla*, etc.

Apocynaceae family has been considerably studied with respect to their morphological characters of pollen (Pichon, 1947; 1948; Leeuwenberg, 1988). Palynologically Apocynaceae depicts eurypalynous nature with variation in apertural characters. The family depicts simple porate aperture to compound colporate one. This palynological variation is helpful in identification of the taxa at the generic level. However this data is yet to be applied for building a pollen key that would be useful for re-categorizing the existing taxonomic position of genera under Apocynaceae.

The present study has been conducted on seven genera under the Apocynaceae family namely; *Allamanda*, *Alstonia*, *Catharanthus*, *Nerium*, *Plumeria*, *Thevetia* and *Tabernaemontana* that are commonly grown as ornamental plants in Kolkata, West Bengal

(Figure 1). The members flowers round the year (except *Alstonia*, a winter bloomer), with pollen output in considerable amount. Species level variation is withheld for the time being. Mature pollen grains were collected soon after anther dehiscence to get optimized result. Pollen grains were collected from freshly opened flower buds preferably of same age to obtain uniformity in result, just before the anthesis stage. Mature anthers were surface sterilized in 10% sodium hypochlorite solution. Grains were pre-hydrated and arrested prior to germination stage when visibility of the apertures is most prominent.

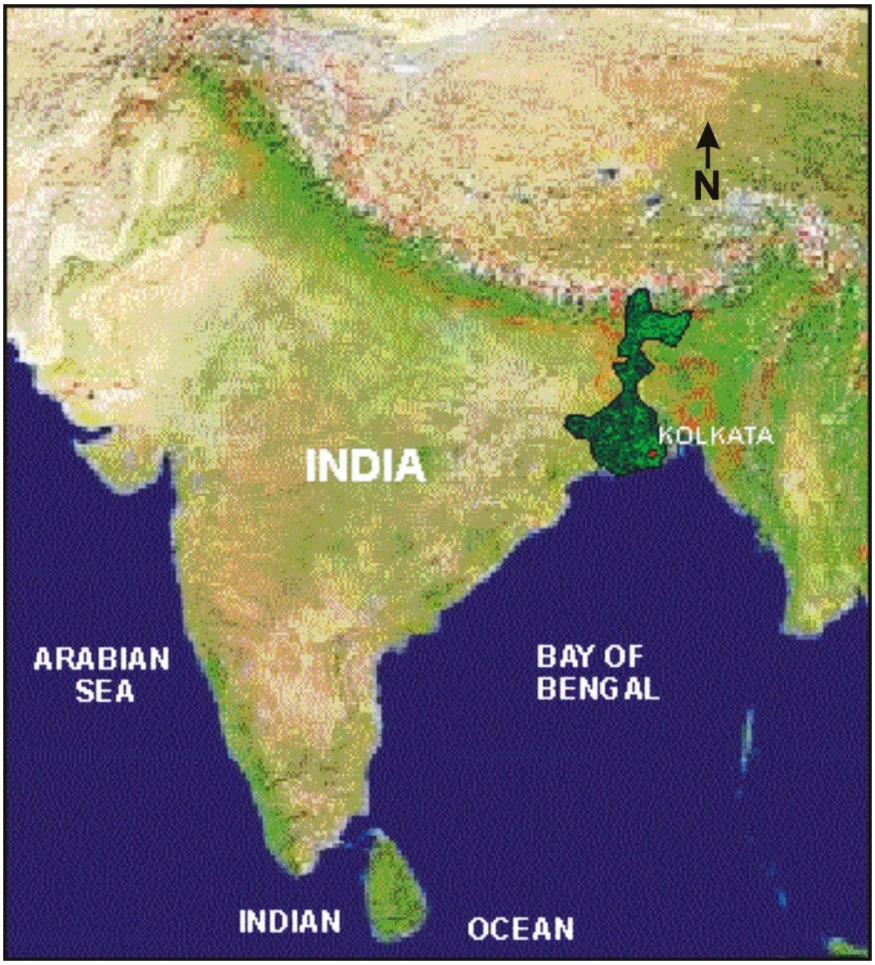

Figure 1. Map of India showing the study area, Kolkata, West Bengal

To minimise error in measurement at least 20 pollen grains were measured randomly per genera from plants growing at different locality in Kolkata. Individual grains were measured using standardized stage and ocular micrometer. Grains were acetolysed following the standard Acetolysis method using Acetolysis mixture (9:1 Acetic Anhydride and concentrated H_2SO_4), followed by several washings with glacial acetic acid and distilled

water (Erdtman, 1969). The acetolysed residue was stored in 50% glycerine with a drop of phenol for microscopic observation. Temporary slides were prepared; shapes of pollen grains are categorized on the basis of the ratio of polar axis (PA) and equatorial diameter (ED) (Erdtman, 1952). Individual microphotographs of the grains were taken after preparing 'single-grain preparation slides' (Figure 2).

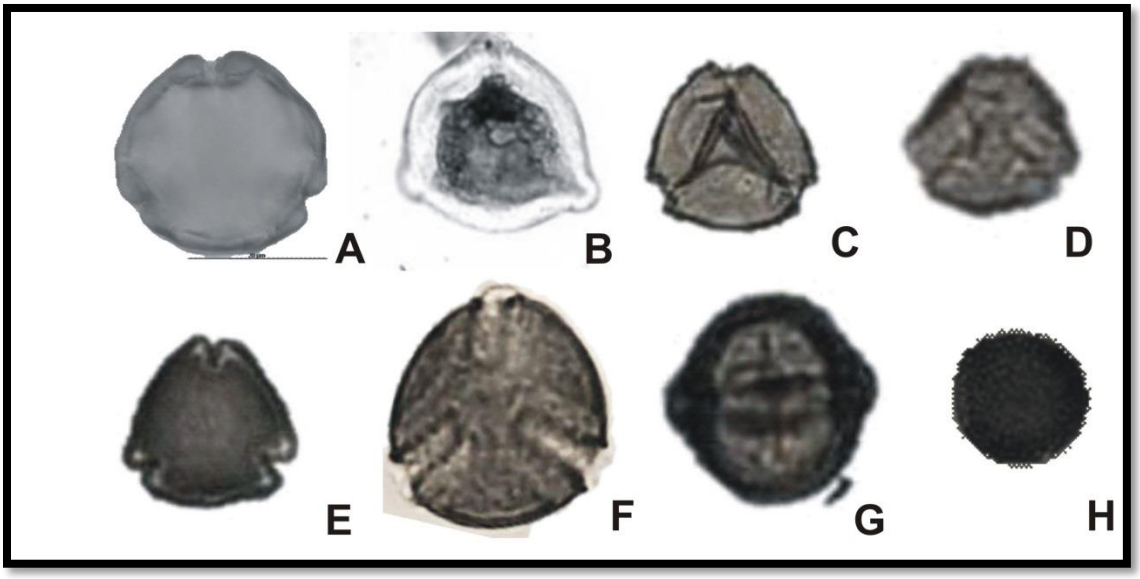

Figure 2. Morphological variation in pollen grains amongst the genera under Apocynaceae

A: *Alstonia*; B: *Allamanda*; C: *Catharanthus*; D: *Plumeria*;
E: *Thevetia*; F: *Plumeria*; G: *Tabernaemontana*; H: *Nerium*.
(All Scale bar = 25 um.)

Since, palynological knowledge has been very limitedly applied in the taxonomical classification of angiosperms. Keeping this view in mind, a Pollen-Key has been drafted for the seven genera of Apocynaceae family that would help for a quick identification of the genera based on the pollen apertural characters. For ease of identification "Bracketed Key" is prepared with number of lead indicated in parentheses. Characters of pollen grains are named following the NPC-System of classification (Erdtman, 1969).

Key to the Genera:

1. Pollen grains monad (single, free).
2. Bladders absent.
3. Pollen grains aperturate.
4. Apertures simple; with pores; porate..... (6)

4. Apertures compound; with ora in colpi; colporate..... (5)
5. Pollen grains with PA/ED > ±75.6/71.4 µm..... (7)
5. Pollen grains with PA/ED < ±75.6/71.4 µm..... (6)
6. Pollen grain porate; 3-porate..... (7)
6. Pollen grain colporate; 3-colporate..... 1. *Alstonia*
7. Pollen grain 3-colporate; apertural pore diameter > ±19 µm..... (8)
7. Pollen grain porate; apertural pore diameter < ±19 µm..... (9)
8. Pollen grain with PA/ED ~ (±159.6 - ±172.2) µm / ~ (±151.2 - ±168) µm; exine psilate; grain prolate-spheroidal.....2. *Catharanthus*
8. Pollen grain with PA/ED ~ (±100.8 - ±126) µm / ~ (±79.8 - ±92.4) µm; exine verrucate; grain sub-prolate..... 3.*Tabernaemontana*
8. Pollen grain 3-porate; pollen grain with PA/ED ~ (±58.8 - ±155) µm / ~ (±50.4 - ±138.6) µm..... (11)
9. Pollen grain 4-porate; pollen grain with PA/ED ~ (±54.6 - ±84) µm / ~ (±50.4 - ±75.6) µm..... (10)
9. Pollen grain sub-prolate..... 4. *Nerium*
10. Pollen grain prolate-spheroidal..... 5. *Plumeria*
10. Pollen grain with PA/ED ~ (±134.4 - ±155) µm / ~ (±113.4 - ±138.6) µm; pore diameter (±29.4 - ±42) µm..... 6. *Thevetia*
11. Pollen grain with PA/ED ~ (±58.8 - ±75.6) µm / ~ (±50.4 - ±67.2) µm; pore diameter (±10.5 - ±16.8) µm.....7. *Allamanda*

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