Plant Archives Volume 21, No 1, 2021 pp. 1047-1053 *e-ISSN:2581-6063 (online), ISSN:0972-5210*

Plant Archives

Journal home page: www.plantarchives.org

DOI Url: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.114

PALYNOLOGICAL INVESTIGATIONS ON SOME SELECTED BEE FORAGE PLANTS OF FAMILY FABACEAE USING LIGHT AND SCANNING ELECTRON MICROSCOPY FROM HIMACHAL PRADESH, INDIA

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(Date of Receiving-22-12-2020; Date of Acceptance-04-03-2021)

ABSTRACT

The study aimed to investigate and describe the pollen morphology of six nectariferous and polleniferous members of family fabaceae by using light and scanning electron microscope. Pollen grains of *Albizia lebbeck*, *Bauhinia variegata, Caesalpinia decalpetala, Cassia fistula, Dalbergia sissoo* and *Robinia pseudoacacia* were examined for the morphological characterization. The pollen grains were analysed in terms of aggregation, shape, shape class, size, aperture, polarity, symmetry, surface pattern and exine complexity. All the pollen had solitary grains, except *Albizzia lebbeck* having compound grains. The shape of pollen grains varies from circular-ovoid in *Albizia lebbeck*, triangular in *Bauhinia variegata,* oval in *Caesalpinia decapetala,* oval / round in *Cassia fistula* and triangular/round in *Dalbergia sissoo* and triangular/elongated oval *Robinia pseudoacacia.* The pollens were either prolate-spheroidal or oblate spheroidal. The size of pollen grains studied ranged from small, medium to large sized among different members of family fabaceae. Most of pollens observed were tricolporate, except pollens of *Dalbergia sissoo* which were tricolpate and triporate. Exine ornamentation include psilate/faveolate in *Albizzia lebbeck*, psilate in *Dalbergia sissoo* and *Robinia pseudoacacia,* straite –reticulate in *Bauhinia variegata*, reticulate-rugulate in *Cassia fistula* and *Caesalpinia decapetala*. All the pollens observed were isopolar and radially symmetrical.

The purpose of this study is to increase knowledge of the aspects related to the variability and to characterize the pollen morphology of some members of family fabaceae and to evaluate the utility of pollen features in order to develop an additional alternative plant material identification tool. Moreover, such studies can be of utmost important to students, researchers, and beekeeping industry, horticulture and forest departments.

Keywords: SEM, Pollen morphology, Fabaceae

INTRODUCTION

Fabaceae or Leguminosae is the third largest economically important family of flowering plants with about 751 genera and about 19,000 known species (Christenhusz and Byng, 2010). Fabaceae includes three subfamilies: Mimosoideae, Caesalpinioideae, and Faboideae (or Papilionoideae). Legumes are one of the important plant groups, being the source of numerous pulses, soil rotation plants, oil, timber trees, gums and dyes (Simpson 2006). The Mimosoideae has 80 genera and 3,200 species, Caesalpinioideae has 170 genera and 2,000 species and Papilionoideae has 470 genera and 14,000 species.

Palynology is an emerging field which has attracted the attention of workers of different disciplines on the account of its numerous applications and in solving complicated problems and taxonomic disputes of interrelationships between family, subfamilies, tribes, genera, species and subspecies of angiospermic plants families. It has become integrated part of the multidisciplinary and collaborative approach in plant systematic and evolution (Moar, 1985; Bibi *et al.*, 2008; Zhang *et al.*, 2017). Palynology is the distinct branch of biology deals with the dispersed microscopic tiny living and fossil entities including pollen grains, spores, algal and fungal fragments and others. An important aspect of Palynology is the Pollen morphology. Pollen morphology gives insights about the structural details of pollens and significantly employed in classification of many plants.

Pollen grains are the male reproductive part of the flowering plants. These particles, though very small act as an important marker in various studies. When they become suspended in air they give an idea about the local flora (Randall *et al.*, 1986; Savelieva *et al.*, 2000).When become deposited in strata/sediments of any time period they indicate past flora and climate (Barreto *et al.*, 2012; Zhang *et al.*, 2017).When studied in honey they demonstrate its geographical and botanical origin (Moar, 1985; Bibi *et al.*, 2008)

When pollen stick to the body parts, clothes, shoes or any other article of a suspect, they become evidence in forensic analysis (Mildenhall, 2008, Morgan *et al.*, 2014). When inhaled pollens can cause allergy (Chatterjee, 2016) and also reveal adulteration. (Azzazy, 2016).The morphological features of pollen grains such as shape, size, apertural pattern, exine ornamentation, thickness of exine and intine etc. are helpful in taxonomic identification of plants (Zafar *et al.*, 2006; Khyer *et al.*, 2015). Keeping in view the above the multi-dimensional usages of pollen morphology, recognition and identification of pollen grains becomes valuable.

MATERIALS AND METHODS

In Present investigations (field and laboratory studies were done as a part of Ph.D. Work during 2009 to 2012). However, extensive field work was also done between 2012-2016 in order to study the bee flora from different agro-climatic zones Himachal Pradesh. Himachal Pradesh is a hilly state situated in the heart of Western Himalayas between 30^0 22' 40" to 30^0 12' 40" North latitude and between 75^0 45' 55" and 79^0 04' 20" East longitude. The total area of Himachal Pradesh is 55,673 sq. km. The territory is almost wholly mountainous with altitudes ranging between 350 to 7000 meters above the sea level. To its North lies the state of Jammu and Kashmir, whereas, Uttar Pradesh is in the South-East with Haryana on South and Punjab on West, in the East, it forms India's border with Tibet. Physio-graphically, the territory can be divided into three zones-outer Himalaya or Shivaliks, inner Himalaya or Mid-mountains and the greater-Himalaya or Alpine zone. Average rainfall in state stands at 1600 mm, although it varies from a minimum of 350 mm at Lahaul Spiti to a maximum of 4400 mm at Dharamshala. The temperature of the state varies according to elevation. From end of February, mercury rises gradually till June, which is generally the hottest month in this region. With the onset of monsoons, there is a gradual fall in temperature. When the monsoon ends by the middle of September, temperature falls gradually at first and fairly rapidly after November (Balokhra, 2020).

For light microscopic studies, pollen slides were made according to the acetolysis method suggested by Erdtman (1952). For the scanning electron microscopic studies, the pollen grains/anthers of identified important pollen taxa were collected in glass vials and preserved at sub-zero temperature. Pollen samples were obtained from the mature specimens. While doing the scanning, an adhesive (a plastic dissolved in a volatile solvent) is applied to the smooth metal surface of the microscope stage, and a small quantity of pollen residue obtained above or of pollen collected directly from the plant is placed on the adhesive and teased thoroughly in alcohol. The pollen grains were then air dried in vacuum evaporator and coated first with carbon and then with gold with the help of fine coated ion Sputter J.F.C-1100 (Donmez, 1999). The pollen grains were scanned at accelerating voltage of 15 to 20 KV in a Scanning Electron Microscope,"JSM 6100" at Regional Sophisticated Instrumentation Centre, Panjab University, Chandigarh. Pollen grains are then ready for observation (Laere *et al.*, 1969). Thus, shape and size of pollen, type and number of aperture, surface pattern and ornamentation of exine were investigated from this palynomorphological study. The descriptive terminology is followed as by Sawyer (1981) and Vorwohl (1990).

RESULTS AND DISCUSSION

1. *Albizia lebbeck* Benth. : Compound grains (polyad), circular-ovoid and prolate-spheroidal, large grains, size ranges from 57µm x 49 µm, tricolporate, isopolar, radially symmetric. Exine was with granulate margins showing peculiar 16 celled (8 forming ring encircling 8 central block of two 4's above the other) which is the characteristic feature of Albizia pollens. Tectum was psilate/faveolate, sexine slightly thicker than nexine.

Figure. 1: Showing map of Himachal Pradesh

Table 1: Showing major, medium and minor honey plants

Plant species	Common name	Honey potentiality	Flowering period	Distribution	Nature/Economic importance
1	2	3	4	5	6
Albizia lebbek Benth.	Siris or East Indian Walnut	N3P3	APR-MAY	Valley, low and mid hills	Tree, Fodder, Medicinal, Timber, Fuel (W/C)
Bauhinia variegata L.	Kachna or Kanchan	N2P3	FEB-APR	Throughout	Tree, Fodder, Fuel, Ornamental, Vegetable, Pickle (W)
Caesalpinia decapetala (Roth.) Alston	Mauritius or Mysore thorn	N3P3	MAR- APR	Valley, low and mid hills	Shrub (Climber), Ornamental, Medicinal (W)
Cassia fistula L.	Indian laburnum	N2P2	APR-JULY	Valley, low and mid hills	Avenue tree, Ornamental, Timber, Medicinal (W/C)
Dalbergia sissoo Roxb.	Sissoo or Sissum	N1P1	MAR-MAY	Valley, low and mid hills	Tree, Timber, Fuel, Fodder, Shade, Soil erosion control, Medicinal (W)
Robinia pseudoacacia L.	Black locust	N1P2	APR-JUNE	Throughout	Tree, Ornamental, Fuel, Timber (W/C)

Table 2: Showing pollen morphology of honey plants

Plant Name and Sub Family Name	Pollen unit	Shape	Shape Class (100 P/E)	Size (Length x Breadth)	Aperture	Polarity	Symmetry	Ornamentation
Albizia lebbeck Benth. (Mimosoideae)	Polyad	Circular-ovoid	Prolate-spheroidal	Large grains 57µm x 49 µm	3-Colporate	Isopolar	Radial	Psilate/Faveolate
Bauhinia variegata Linn. (Caesalpinioideae)	Monad	Triangular	Prolate- spheroidal	Large grains 51.1µm x 50 µm	3-Colporate	Isopolar	Radial	Straite –reticulate
Caesalpinia decapetala (Roth.) Alston. (Caesalpinioideae)	Monad	Oval	Sub oblate to prolate- spheroidal	Large grains 51µm x 45.3µm	3-Colporate	Isopolar	Radial	Reticulate-rugulate
Cassia fistula Linn. (Caesalpinioideae)	Monad	Oval / Round	Prolate spheroidal to prolate	Medium sized grains 28.5µm x 16.5µm	3-Colporate	Isopolar	Radial	Reticulate-rugulate
Dalbergia sissoo Roxb. ex. DC. (Faboideae)	Monad	Triangular/ Round	Oblate- spheroidal	Small grains 19.2µm x17.6 µm	3-Colpate and 3-porate	Isopolar	Radial	Psilate
Robinia pseudoacacia Linn. (Faboideae)	Monad	Triangular/ elongated oval	Oblate- spheroidal	Medium sized grains 26.9µm x 22.3µm	3-Colporate	Isopolar	Radial	Psilate

2. *Bauhinia variegata* Linn.: Monad, triangular and prolate- spheroidal, large grains, size ranges from 51.1µm x 50 µm, , tricolporate, isopolar, radially symmetric. Tectum was straite –reticulate, sexine as thick as nexine.
3. *Caesalpinia decapetala* (Roth.) Alston: Monad, oval and sub oblate to prolate- spheroidal, large sized grains, size ranges from 51µm x 45.3µm, tricolporate, isopolar, radially symmetric. Tectum was reticulate-rugulate, sexine thicker than nexine.
4. *Cassia fistula* Linn.: Monad, oval/round and prolate spheroidal-prolate, medium sized grains, size ranges from 28.5µm x 16.5µm, tricolporate, isopolar, radially symmetric. Tectum was reticulate-rugulate, sexine thicker then nexine.
5. *Dalbergia sissoo* Roxb. ex. DC. : Monad, triangular/ round and oblate- spheroidal, small grains, size ranges from 19.2µm x17.6 µm, tricolpate and triporate, isopolar, radially symmetric. Tectum was psilate, sexine thicker than the nexine.
6. *Robinia pseudoacacia* Linn. : Monad, triangular/ elongated oval and oblate-spheroidal, medium sized grains, size ranges from 26.9µm x 22.3µm, tricolporate, isopolar, radially symmetric. Tectum was psilate, sexine thicker than nexine.

Pollen grains of six members of family fabaceae were investigated through light and scanning electron microscopy (Table 1&2, Fig. 2 &3). The pollen morphology varies among different members of same family; occur in varying shapes and forms. They also show variation in exine structure and sculpture.

Pollen characters used in this study included pollen unit, pollen shape, shape class (100 P/E)) size, aperture, polarity, symmetry, surface pattern and exine ornamentation. Out of six species, all had solitary grains (Monads), except *Albizzia lebbeck* having compound grains (Polyad). Diffferent pollen shapes were recorded in the study. The shape of pollen grains varies from circular-ovoid in *Albizia lebbeck*, triangular in *Bauhinia variegata,* oval / round in *Cassia fistula*, oval in *Caesalpinia decapetala* and triangular/ round *Robinia pseudoacacia.* The shape class of pollens was arrived at using ratio of the polar axis and equatorial diameter measurements. The pollens observed were either prolate-spheroidal (100-114) or oblate spheroidal (88-100). The size of pollen grains studied ranged from 19.2µm x17.6 µm to 57µm x 49 µm among members of family fabaceae. Two type of aperture types were recorded in the study. Most of pollen are tricolporate i.e having having both colpi and pori in same aperture except *Dalbergia sissoo* grains are tricolpate (with three colpa) and triporate (with three pores). Exine ornamentation include psilate/faveolate (exine is smooth and with little pits) in *Albizzia lebbeck*, smooth with pits <1 µm (psilate) in *Dalbergia sissoo* and *Robinia pseudoacacia*, straite –reticulate (radial projections are either parallel or form reticular pattern) in *Bauhinia variegata*, irregularly

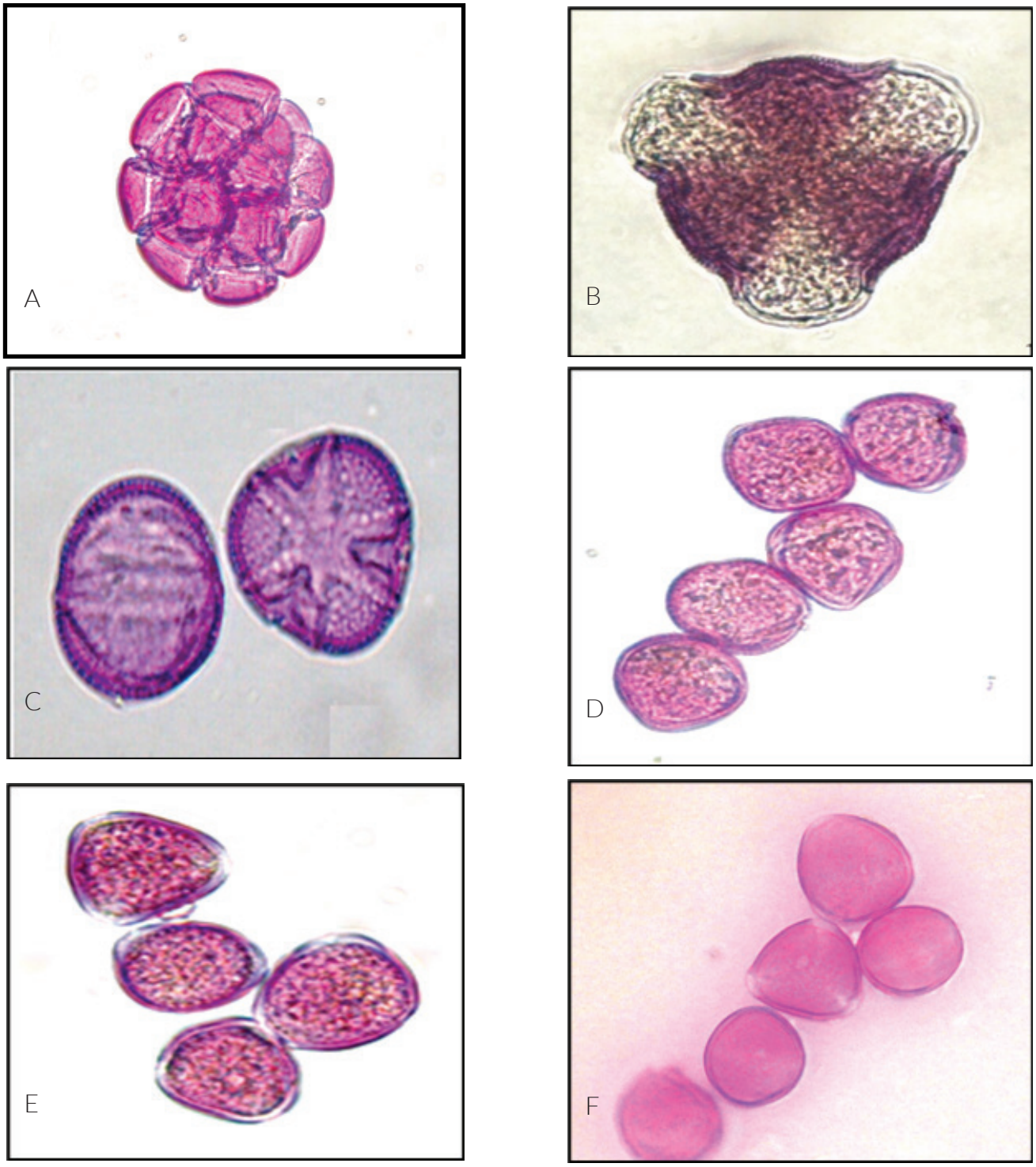


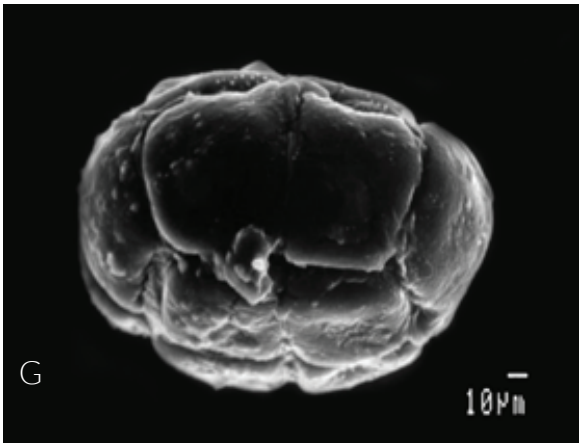
Figure 2: Showing photomicrographs of pollen grains through Light Microscopy .

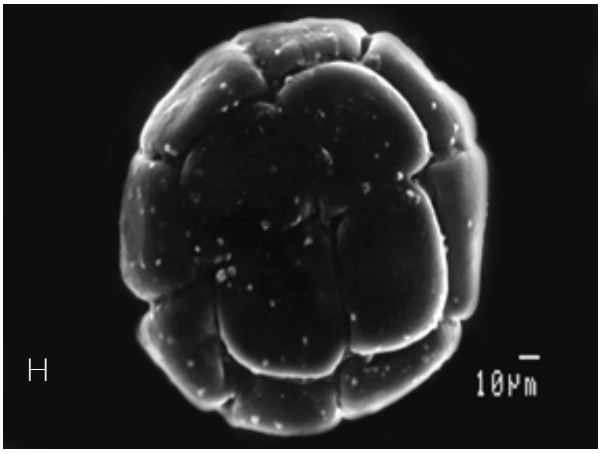
A. *Albizia lebbek* (OS $_{X\ 400}$), **B**. *Bauhinia variegata* (OS $_{X\ 400}$), **C**. *Caesalpinia decalpetala,* OS (EV&PV) W$_{X\ 400,}$ **D**. *Cassia fistula,* (OS $_{X\ 400}$), **E**. *Dalbergia sissoo* (OS $_{X\ 400}$), **F**. *Robinia pseudoacacia* (OS & SV $_{X\ 400}$)

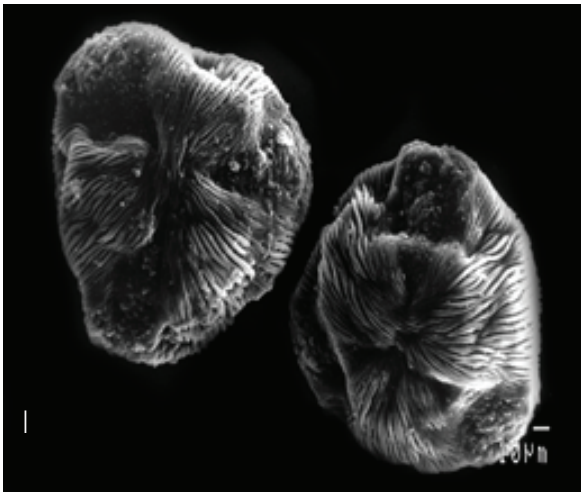
distributed radial projection or sculpturing elements forming reticular pattern (reticulate-rugulate) in *Cassia fistula* and *Caesalpinia decapetala*. The Fabaceae family is very heterogeneous based on pollen grains features. Thus, it is essential to examine a large number of pollen grains from one family in order to obtain a complete knowledge of different types within that family.

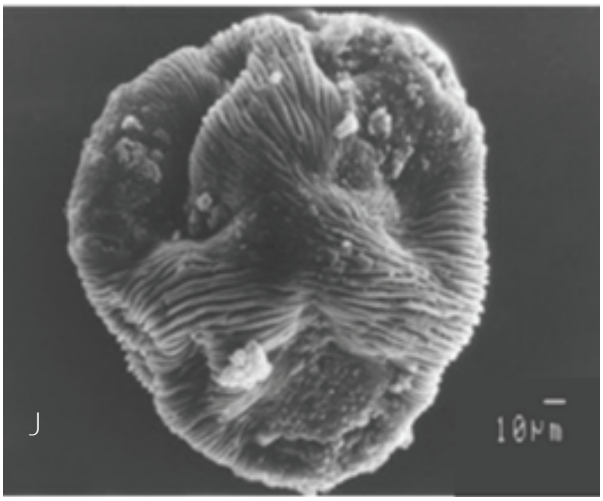
CONCLUSION

The present work will be very helpful for writing and identifying pollen flora a region. This work is important for researchers, palynologist, apiarist, horticulture and forest department for identification of plant on the basis of simply on pollen grains. Pollen morphology can be employed to determine botanical and geographical origin of honeys. These studies can be useful to detect adulteration in honeys and also very important for evolutionary studies and to detect adulterations in crude drugs of plant origin These palynological studies is can be utilized in forensic science to detect the crime on the basis of pollen grains & spores.


G
10µm


H
10µm


I


J
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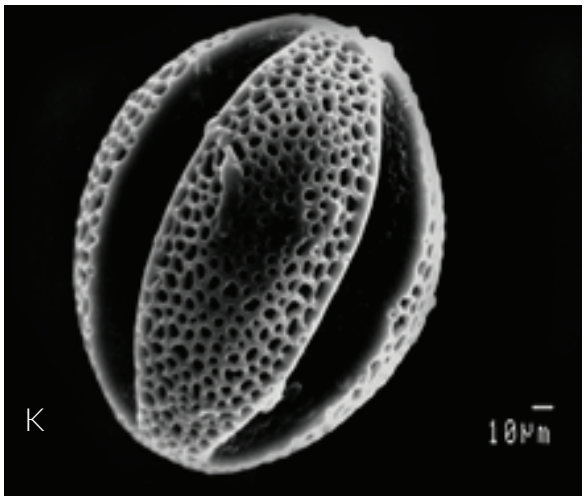
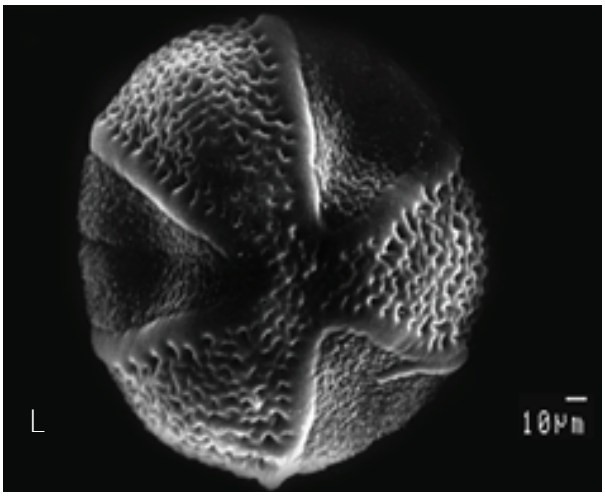
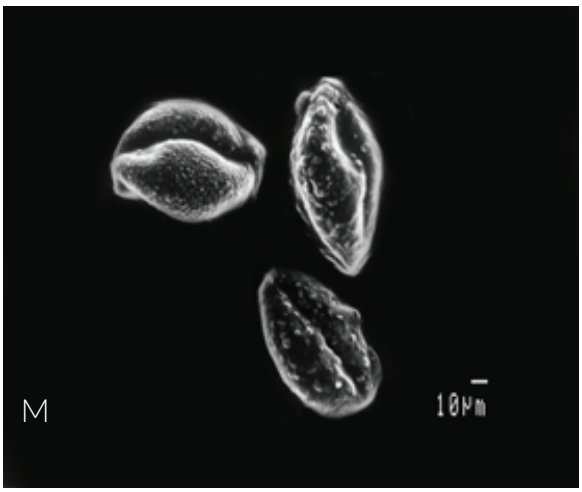
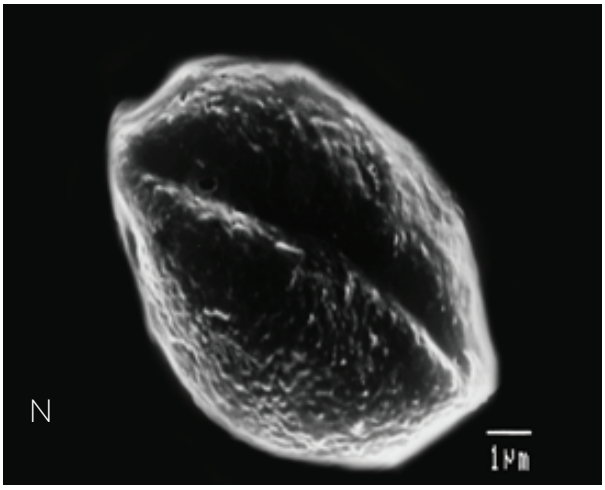

K
10µm


L
10µm


M
10µm


N
1µm

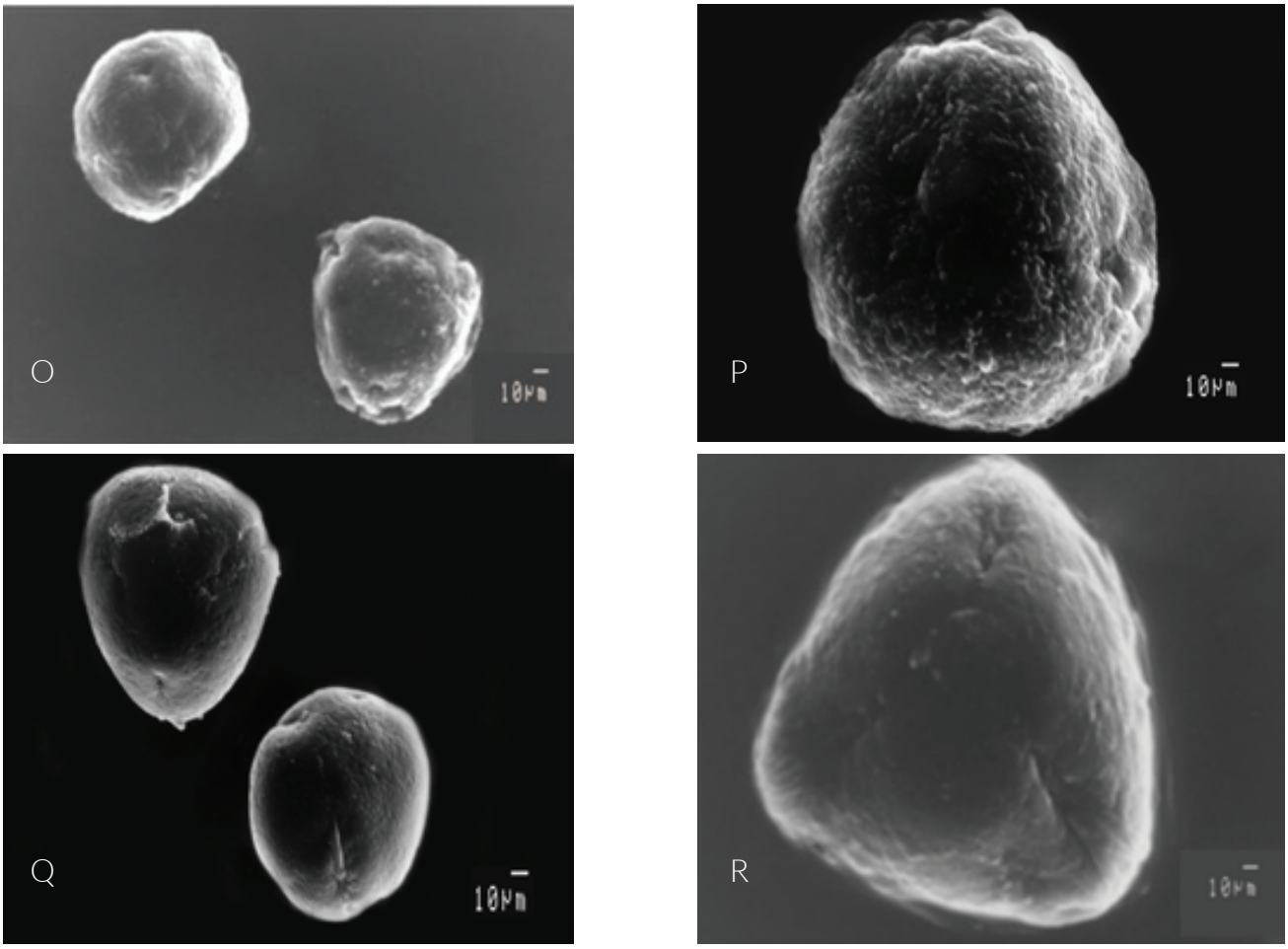


Figure 3: Showing morphological details of pollen grain through Scanning Electron Microscopy (SEM).

G. *Albizia lebbeck,* EV ($X_{1,200}$), **H.** *Albizzia lebbeck,* PV ($X_{1,200}$), **I**. *Bauhinia variegata,* (X_{850}), **J**. *Bauhinia variegata* ($X_{1,300}$), **K**. *Caesalpinia decalpetala,* EV ($X_{1,500}$), **L**. *Caesalpinia decalpetala,* PV ($X_{2,000}$), **M**. *Cassia fistula,* EV (X_{900}), **N**. *Cassia fistula,* EV ($X_{2,500}$), **O**. *Dalbergia sissoo,* ($X_{1,600}$), **P**. *Dalbergia sissoo,* ($X_{3,300}$), **Q**. *Robinia pseudoacacia,* ($X_{1,500}$), **R**. *Robinia pseudoacacia,* ($X_{2,200}$) 10um

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