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**SCANNING ELECTRON MICROSCOPIC STUDIES ON POLLEN
MORPHOLOGY OF *BAUHINIA* (CAESALPINIACEAE)**

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ABSTRACT

The pollen morphology of four species of *Bauhinia* viz., *B. purpurea* L., *B. variegata* L., *B. acuminata* L. and *B. tomentosa* L. belonging to sub-family Caesalpiniaceae was studied by Light and Scanning Electron Microscopy. All the species showed distinct variations in terms of shape, size, aperture and exine ornamentation characters. Observations of present study were differing from earlier studies in certain characters. SEM was found to be vital instrument in studying pollen characters at high magnifying level.

Key Words: Pollen Morphology, *Bauhinia*, LM, SEM.

INTRODUCTION

The genus *Bauhinia*, one of the largest genera in the sub-family Caesalpiniaceae represents more than 300 species (Wunderlin *et.al.* 1987 and Sharma, 2007). *Bauhinia* has been extensively planted as a garden, park and roadside ornamental tree in many warm temperate and Sub-tropical region (Lau *et al.*, 2005). *Bauhinia* is also known as Mountain Ebony. Erdtman mentioned the Leguminosae as an euryapalynous group and studied pollen characters in two species of *Bauhinia* (Erdtman, 1971). Considering pollen structure, much variation was found in four species of *Bauhinia* which helps to differ *Bauhinia* at species level. The Scanning Electron Microscope offers a rapid means of observing pollen wall surfaces, as well as providing greater resolution than the Light Microscope which shows some of the possibilities of Scanning Electron Microscopy in extending the scope and precision of pollen analysis as well as pollen morphology (Pilcher, 1968). In earlier studies by Vishnu-Mittre and Sharma (1962), 19 species of *Bauhinia* from India were investigated. Whereas, a little detailed information was provided on pollen morphology of *Bauhinia* species. The portrayal of pollen surfaces has been greatly improved by the Scanning Electron Microscope (Ridgway and Skvarla, 1969). The present study was carried out to provide palynological information and variations in pollen morphology of four *Bauhinia* species by Scanning Electron Microscope.

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MATERIALS AND METHODS

Four species of *Bauhinia* were collected and Herbarium specimen of each species was prepared and kept at departmental herbarium, Department of Botany, Sant Gadge Baba Amravati University, Amravati. Anthers were collected from the mature flowers and stored in 70% alcohol. The collected material was crushed with a glass rod in plastic centrifuge tube. The crushed material was filtered through fine meshes to isolate pollen grains.

The pollen grains were prepared for light and scanning electron microscopy by the standard method described by Erdtman (1952) and Arora and Modi (2008). For light microscopy, the pollen grain were mounted in stained glycerine jelly and observations were made with Trinocular Fluorescence Microscope (Axiostar HBO 50/AC Carl zeiss). For SEM studies, pollen grain were suspended in a drop of ethanol and directly transpired with a fine pipette to a metallic stubs using double sided cello tape and coated with gold palladium in a sputtering chamber (POLARON SPUTTER COATER). The SEM examination was carried out on a LEO electron microscope (LEO 430). The measurements are based on 10 readings from each pollen type and the pollen grain size, colpi size, pore size was measured. The terminology used in accordance with Erdtman(1952), Fageri and Iverson (1964), Bhattacharya *et. al.* (2006) and Agashe (2006).

RESULTS

Description of pollen type:

1) *Bauhinia purpurea* L.: Pollen grains are 59.94-63.27×56.61-64.93µm(Table1), prolate-spheroidal, radially symmetrical, outline rounded triangular, tricolporate, colpi broad, narrow towards the ends, colpi length 49.95 µm, pori deep,12.6 in diameter, exine 2.8 µm in thickness, sculpturing striato-reticulate [Table 1, Fig. A (LM) and Fig. 1-2 (SEM)].

Table 1: Pollen grain characteristics of *Bauhinia* sps.

Sr. No.	Species	Pollen shape	Pollen grain size (µm) P×E	Colpi/pori size (µm)	Amb	Exine ornamentation
1.	<i>B. purpurea</i> L.	Prolate spheroidal	59.94×64.93	49.95×12.6	Rounded triangular	Striato-reticulate
2.	<i>B. variegata</i> L.	Spheroidal-Prolate spheroidal	36.63-×46.62	36.39×4-6.	Triangular	Striate
3.	<i>B. acuminata</i> L.	Spheroidal-Prolate spheroidal	97.17×106.56	69.03×12.87	circular	Reticulate
4.	<i>B. tomentosa</i> L.	Suboblate-spheroidal	83.25×93.24	49.95×5.95	circular	Clavate

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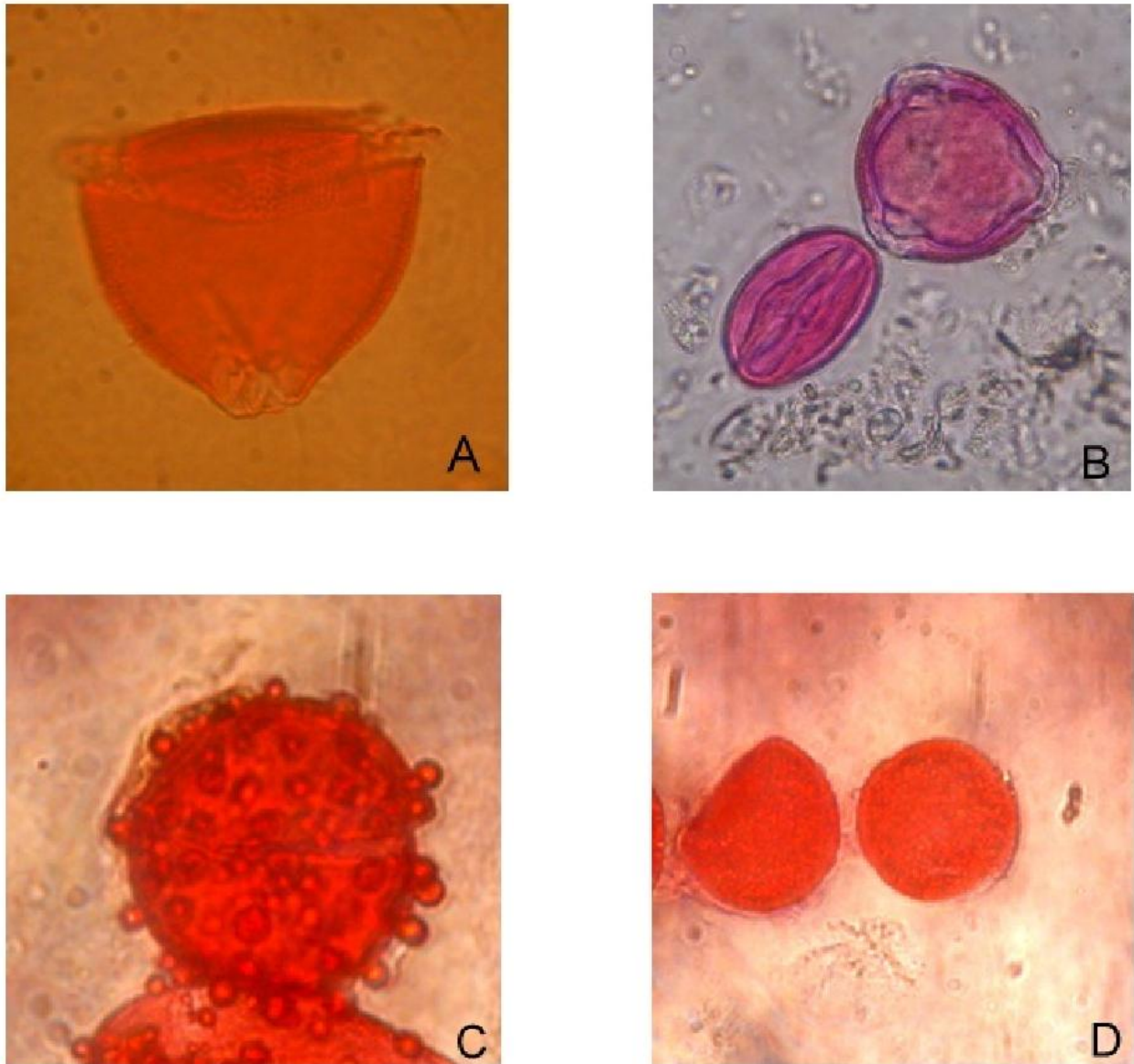


Fig. 1 A-D : Light micrographs of *Bauhinia* pollen. A *Bauhinia purpurea*, B *Bauhinia Variegata*, C *Bauhinia acuminata*, D *Bauhinia tomentosa*.

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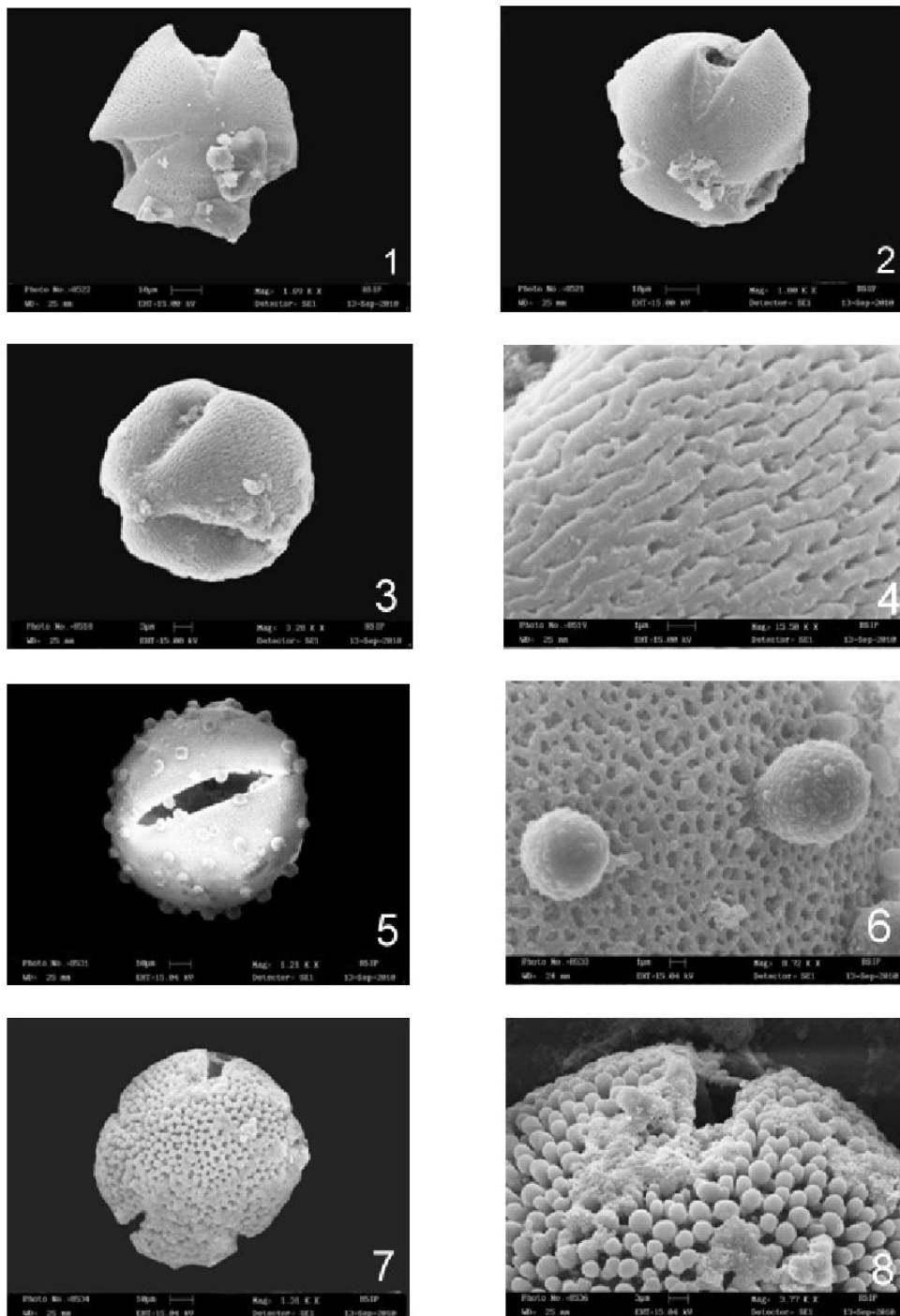


Fig. 2 1-8 : SEM micrographs showing structure and exine sculpture of pollen grains, 1 -2 *Bauhinia purpurea*, 2 -3 *Bauhinia variegata*, 4-5 *Bauhinia acuminata*, 7-8 *Bauhinia tomentosa*.

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2) *Bauhinia variegata* L.: Pollen grains are $36.63\text{--}49.95\text{ }\mu\text{m}\times 39.96\text{--}46.62$, spheroidal-prolate spheroidal, radially symmetrical, outline triangular, equatorial outline elliptic, tricolporoidate colpi $36.39\times 4\text{--}6\text{ }\mu\text{m}$, colpi long narrow toward the ends, exine thick $3.33\text{--}4.99\text{ }\mu\text{m}$, sculpturing striate [Table 1, Fig. B (LM) and Fig. 3-4 (SEM)].

3) *Bauhinia acuminata* L.: Pollen grains are $97.17\text{--}98.28\times 95.94\text{--}106.56\text{ }\mu\text{m}$, spheroidal to prolate-spheroidal, outline circular, radially symmetrical, Colpate, colpi elliptic, $69.03\times 12.87\text{ }\mu\text{m}$, exine thick, consisting rod like supratectal process, processes usually rounded varying in size $7.35\text{ }\mu\text{m}$ high $\times 5.7\text{ }\mu\text{m}$ in diameter, heterobrochate, sculpturing reticulate [Table 1, Fig. C (LM) and Fig. 5-6 (SEM)].

4) *Bauhinia tomentosa* L.: Pollen grains are $65.75\text{--}83.25\text{ }\mu\text{m}\times 66.6\text{--}93.24\text{ }\mu\text{m}$, sub-oblate to spheroidal, outline circular, 5-colpate, rarely 4-colpate, colpi length $49.95\times 5.95\text{ }\mu\text{m}$, colpi narrow, Exine thick $6.65\text{--}7\text{ }\mu\text{m}$, exine thicker than nexine, clavate, clavae $3\text{--}4.5\text{ }\mu\text{m}$ high $\times 2\text{--}3\text{ }\mu\text{m}$ in diameter, angular to rounded in outline [Table 1, Fig. D (LM) and Fig. 7-8 (SEM)].

DISCUSSION

A considerable variation in pollen morphology was observed among the investigated *Bauhinia* species, particularly exine ornamentation and characters of the aperture. All the four species of *Bauhinia* showed distinct variations in their pollen structure and were differ from earlier studies. Larsen (1975) reported inaperturate pollen in *Bauhinia acuminata* whereas, In present study a well defined aperture is noted. Larsen (1975) also mentioned colpoidate pollen in *B. purpurea* whereas present study reveals colpi with large pore. The reports of Perveen (1998) on striate sculpturing in *B. variegata* pollen is much similar to present findings.

The pollen size of all studied species is comparatively larger and pollen surface was found to be striate-reticulate-clavate which is rough in nature. Present findings were strongly supported by earlier studies of Ferguson and Pearce (1986) and Stroo (2000) mentioning large size and rough surface of *Bauhinia* pollen is a key factor for Bat mediated pollen transfer. Based on pollen morphological similarities it can be estimated that, the present investigated species of *Bauhinia* may also be pollinated by Bat.

B. purpurea, *B. variegata* and *B. acuminata* was found to be tricolporate, spheroidal- prolate spheroidal pollen which is in accordance with Lau et. al. (2006) observations; whereas *B. tomentosa* stood aside from above three species by showing five colpi. SEM studies on *Bauhinia* shows the importance of pollen character in taxonomic discrimination of species.

A scanning electron microscope was found to be an invaluable asset in the high tech world of research. The SEM offer many advantages over their counterparts. Its high magnification capacity is

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used to study minute biological material like pollen grains which can be an additional tool in taxonomic discrimination and classification of critical plant species. The pollen morphological characters observed from SEM images were stored in an online information retrieval tool indigenously designed and developed for pollen morphological studies.

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