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POLLEN FLORA OF PAKISTAN–LIII. VERBENACEAE

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Abstract

Pollen morphology of 13 species representing 9 genera of the family Verbenaceae from Pakistan has been examined by light and scanning electron microscope. Pollen grains are usually radially symmetrical, isopolar, tricolporate or tricolpate, mostly prolate-spheroidal to sub-prolate rarely oblate-spheroidal, sexine is much thicker or thinner than nexine. Tectum type varies from subpsilate to reticulate, rugulate-reticulate often spinulose-reticulate. On the basis of apertures and exine ornamentation eight distinct pollen types are recognized viz., *Caryopteris grata*-type, *Caryopteris odrata*-type, *Chascanum marrubifolium*-type, *Clerodendrum phlomoides* - type, *Lantana indica*–type, *Phyla nodiflora*–type *and Verbena officinalis*–type.

Introduction

Verbenaceae is a family of c. 100 genera and nearly 2600 species, mostly tropical and subtropical in distribution (Mebberely, 1987). In Pakistan it is represented by 17 genera and c. 35 species (Jafri & Ghafoor, 1974). The family is characterized mostly by woody habit (shrubs or trees, zygomorphic flowers, androecium didynamous, 2 carpels, ovary usually 4 loculed with terminal or subterminal style. Fruit usually drupe or nutlets, some chief genera of Verbenaceae are *Clerodendrum*, *Callicarpa*, *Vitex*, *Lantana* and *Verbena*. The family is important for teak timber i.e., *Tectona grandis* and some ornamental plants.

Pollen morphology of family has been examined by Erdtman (1952) Nair & Rehman (1962), Markgraf & D'Antoni (1978). Erdtman *et al*., (1963) examined pollen morphology of some Scandinavian species of family Verbenaceae taxa Scandinavian. Raj (1983) examined pollen morphology of some Verbenaceous taxa from Scandinavia. Punt & Langewis (1988) studied pollen morphology of the species *Verbena officinalis* for Northwest pollen Flora. There are no reports on pollen morphology of the family Verbenaceae from Pakistan. Present investigations are based on the pollen morphology of 13 species representing 9 genera of the family Verbenaceae by light and scanning electron microscope.

Materials and Methods

Pollen samples were obtained from Karachi University Herbarium (KUH) or collected from the field. The list of voucher specimens is deposited in KUH. The pollen grains were prepared for light (LM) and scanning microscopy (SEM) by the standard methods described by Erdtman (1952). For light microscopy, the pollen grains were mounted in unstained glycerin jelly and observations were made with a Nikon Type-2 microscope under (E40, 0.65) and oil immersion (E100, 1.25), using 10x eye piece. For SEM studies, pollen grains suspended in a drop of water were directly transferred with a fine pipette to a metallic stub using double sided cello tape and coated with gold in a sputtering chamber (Ion-sputter JFC-1100). Coating was restricted to 150 A. The S.E.M examination was carried out on a Jeol microscope JSM-2. The measurements are based

on 15-20 readings from each specimen. Pollen diameter, polar axis (P) and equatorial diameter (E), aperture size and exine thickness were measured.

The terminology used is in accordance with Erdtman (1952), Kremp (1965), Faegri & Iversen (1964) and Walker & Doyle (1975).

General pollen characters of the family Verbenaceae

Pollen grains usually radially symmetrical, isopolar. Mostly sub-prolate, prolate-spheroidal often oblate-spheroidal to sub-oblate. Tricolporate, rarely tricolpate, sexine thicker or thinner than nexine. Tectal surface various types ranges from subpsilate-spinulose or reticulate. On the basis of exine ornamentation eight distinct pollen types are recognized viz., *Callicarpa macrophylla*-type, *Caryopteris grata*-type, *Caryopteris odrata*-type, *Chascanum marrubifolium*-type, *Clerodendrum phlomides* - type, *Lantana indica*–type, *Phyla nodiflora*–type and *Verbena officinalis*–type.

Key to the pollen types

1. \+ Pollen grains colpate ……..……. *Clerodendrum phlomides*-type
   \- Pollen grains colporate ……..…… 2

2. \+ Tectum sub-psilate ..……..…….. *Phyla nodiflora*-type
   \- Tectum not as above ……... 3

3. \+ Tectum rugulate-fossulate ……..…….. *Lantana indica*-type
   \- Tectum not as above ……..……. 4

4. \+ Tectum scabrate ……..…… *Caryopteris odrata*-type
   \- Tectum reticulate or striate-rugulate or fossulate-striate ……..... 5

5. \+ Tectum fossulate-striate ……... *Chascanum marrubifolium* -type
   \- Tectum not as above ……... 6

6. \+ Tectum reticulate ……..…….. *Callicarpa macrophylla*-type
   \- Tectum not as above ……..…….. 7

7. \+ Tectum rugulate ……..…….. *Caryopteris grata*-type
   \- Tectum striate-rugulate ……..……. *Verbena officinalis*-type

Pollen type: *Callicarpa macrophylla*-type.
Pollen class: Tricolporate
P/E ratio: 1.19
Shape: sub-prolate.
Apertures: Ectocolpus long narrow with acute ends.
Exine: Sexine thicker or thinner than nexine.
Ornamentation: reticulate
Measurements: Size: Length = (25.25-) 31.37 ± 0.2 (-37.5) μm and breadth (22.5) 21 ± 0.11 (27.5) μm, colpi (25.5–) 24.11 ± 0.42 (30.5) μm in long. Mesocolpium 22.5 (22.73 ± 0.25) 25.5 μm. Apocolpium 2.5 (2.6 ± 1.24) 2.75 μm. Exine c. 2.5 μm thick, sexine thicker than nexine. Tectum medium reticulate.
Species included: *Callicarpa macrophylla* Vahl.

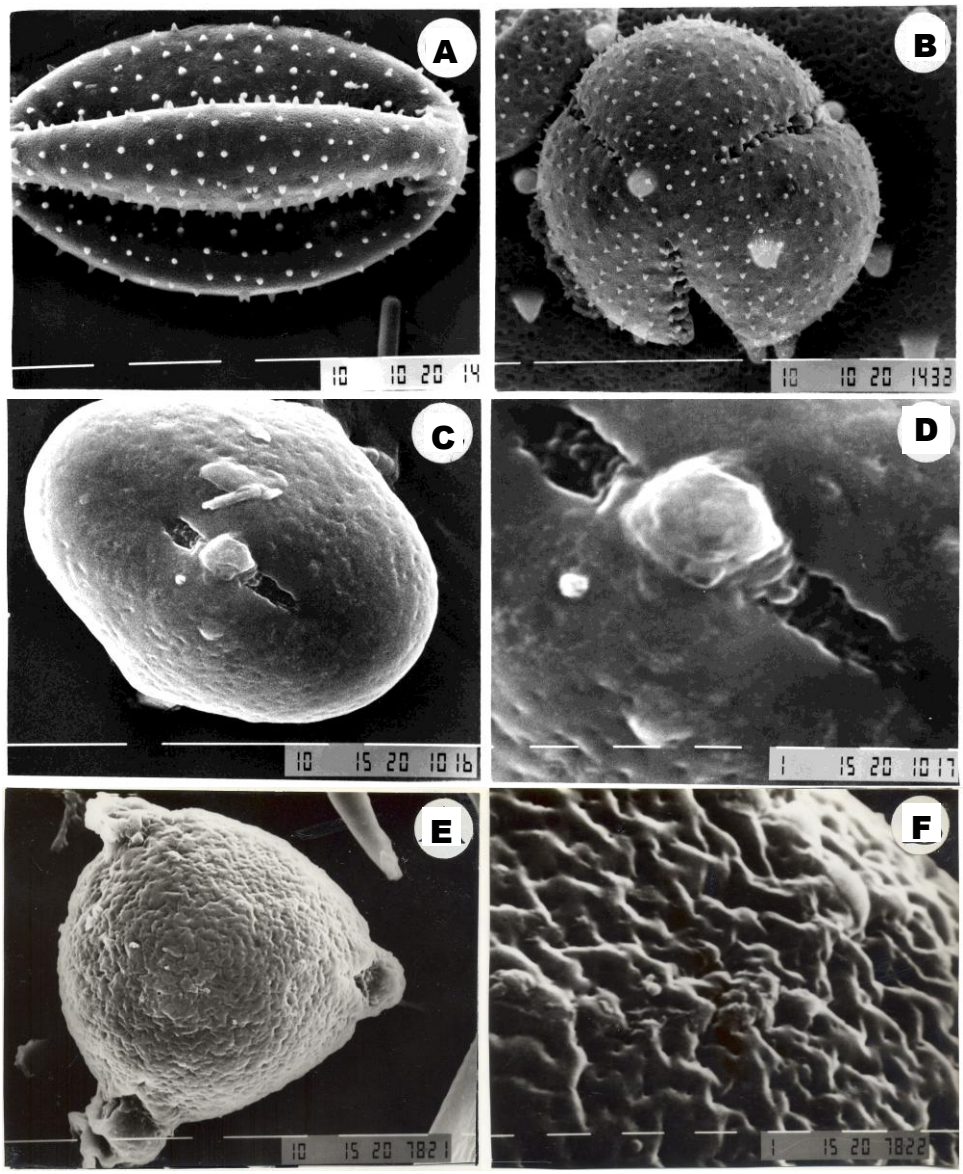

Fig. 1. Scanning Electron micrographs of pollen grains. *Clerodendrum phlomides*: A, Equatorial view, B, Polar view. *Phyla nodiflora*: C, Equatorial view, D, Exine pattern. *Priva cordifolia*: E, Polar view, F, Exine pattern.
Scale bar = A, B, C & E = 10 μm

Caryopteris grata -type
Pollen class: Tri-colporate,
P/E ratio: 1.39
Shape: Prolate
Apertures: Ectocolpus long narrow with acute ends.
Exine: Sexine thicker than nexine.
Ornamentation: Densely rugulate with baccule.
Measurements: Size: Length = (47.5-) 31.51 ± 1.24 (-55.5) μm and breadth (33.75) 37.5 ± 0.90 (50.5) μm, colpi (37. 5 -) 44.69 ± 0.42 (-50.25) μm in long. Mesocolpium 27.5-37.5 μm. Apocolpium c. 2.5 μm. Exine 2.5 μm thick, sexine thicker than nexine. Tectum densely rugulate.
Species included: *Caryopteris grata* Benth.
Caryopteris odorata-type
P/E ratio: 1.32
Pollen class: Tricolporate
Shape: sub-prolate
Apertures: Ectocolpus long narrow with acute ends.
Exine: Sexine thinner than nexine.
Ornamentation: Scabrate.
Measurements: Size: Length = (42.5-) 50.51 ± 0.89 (-60.5) μm and breadth (30.75) 37.5 ± 0.48 (45.5) μm, colpi (37. 5-) 44.69 ± 0.42 (-50.25) μm long. Mesocolpium (22.5-) 28.69 ± 0.27 (-32.5) μm. Apocolpium (7.5-) 9.69 ± 0.42 (-15.25) μm. Exine 2.5 μm thick, sexine thinner than nexine. Tectum scabrate.
Species included: *Caryopteris odorata* (Ham.) Robinson

Chascanum marrubifolium-type
P/E ratio: 1
Pollen class: Tricolporate
Shape: sub-prolate
Apertures: Ectocolpus long narrow with acute ends.
Exine: Sexine thinner than nexine.
Ornamentation: fossulate-striate
Measurements: Size: Length = (60.5-) 50.51 ± 0.89 (-82.5) μm and breadth (47.75) 56.2 ± 0.48 (60.5) μm, colpi (40.5-) 56.69 ± 0.87 (-50.25) μm in long. Mesocolpium (30.5-) 36.25 ± 0.32 (-45.5) μm. Apocolpium (8.5-) 9.69 ± 0.42 (-12.5) μm. Exine 2.5-5 μm thick, sexine thinner than nexine. Tectum fossulate-striate
Species included: *Chascanum marrubifolium* Fenzl. ex Walp.

Clerodendrum phlomides –type (Fig.1 A & B).
P/E ratio: 1.32-1.54
Pollen class: Tricolporate
Shape: sub-prolate or prolate
Apertures: Ectocolpus long narrow with acute ends.
Exine: Sexine thinner than nexine.
Ornamentation: Tectum reticulate-rugulate or very finely reticulate with spinules widely distributed, 0.66-1.6
Measurements: Size: Length = (25.5-) 61.36 ± 1.37 (-75.39) μm and breadth (15.67) 51.5 ± 0.48 (45.5) μm, colpi (17.5-) 51.51 ± 1.73 (-57.44) μm in long. Mesocolpium (17.

08-) 8 .69 ± 0.27 (-46.5) µm. Apocolpium (7.5-) 9.69 ± 0.42 (-12.56) µm. Exine (1.87-) 2.25 ± 0. 27 (-2.5) µm thick, sexine as thick as nexine. Tectum reticulate-rugulate or finely reticulate with spinules widely distributed.
Species included: *Clerodendrum phlomides* L.f. , *Vitex trifolia* L.

Key to the species

1. \+ Tectum finely reticulate with spinules ……. *Clerodendrum phlomides*
   \- Tectum reticulate-rugulate ..…… *Vitex trifolia*

Pollen type: ***Lantana indica***
Pollen class: Tricolporate,
P/E ratio: 120-123
Shape: Prolate-spheroidal
Apertures: Ectocolpus long narrow with acute ends.
Exine: Sexine thicker than nexine.
Ornamentation: Rugulate-fossulate
Measurements: Size: Length = (30.5-) 40.51 ± 0.2 (-50.5) µm and breadth (27.75) 39.5 ± 0.11 (50.5) µm, colpi (20.0–) 28.15 ± 0.42 (36.25) µm long. Mesocolpium 12.5-17.5 µm. Apocolpium c. 1.25-8.5 µm. Exine 2.5 (2.6 ± 0.5) 2.75 µm thick, sexine thicker than nexine. Tectum rugulate-fossulate.
Species included: *Lantana indica* Roxb., *Lantana camara* L., and *Verbena tenuisecta* Briq.

Key to the species

1. \+ Equatorial diameter of pollen grains 27-32 µm ……... *Lantana indica*
   \- Equatorial diameter of pollen grains 35-50 µm …….. 2

2. \+ Equatorial diameter of pollen grains 35-40 µm ……..… *Lantana camara*
   \- Equatorial diameter of pollen grains 42-50 µm ……. *Verbena tenuisecta*

Pollen type: *Phyla nodiflora***-** type - (Fig. 1C-F).
Pollen class: Tricolporate
P/E ratio: 80-103
Shape: Spheroidal, oblate-spheroidal, often sub-oblate
Apertures: Ectocolpus long narrow with acute ends.
Exine: Sexine thicker or thinner than nexine.
Ornamentation: sub-psilate-punctate
Measurements: Size: Length = (25.5-) 39.0 ± 0.2 (-52.5) µm and breadth (23.30) 41.5 ± 0.11 (60.00) µm, colpi (14.0–) 28.25 ± 0.42 (42.5) µm in long. Mesocolpium 12.5-17.5 µm. Apocolpium c. 1.25-8.5 µm. Exine 1.87- 3.6 µm thick, sexine thicker than nexine. Tectum sub-psilate-punctate
Species included: *Phyla nodiflora* (L.) Greene, *Priva cordifolia* (L.f.) Durce and *Verbena bonariensis* L.

Key to the species

1. + Pollen grains Prolate-spheroidal …….. *Phyla nodiflora*
   - Pollen grains sub-oblate to oblate-spheroidal ……. 2

2. + Pollen grains oblate-spheroidal ……..… *Priva cordifolia*
   Pollen grains sub-oblate ……..…… *Verbena bonariensis*

Pollen type: ***Verbena officinalis*****–**type
P/E ratio: 0.85
Shape: Oblate-spheroidal
Apertures: Ectocolpus long narrow with acute ends.
Exine: Sexine thinner than nexine.
Ornamentation: Tectum striate-rugulate
Measurements: Size: Length = (25.5-) 25.79 ± 0.37 (-27.5) μm and breadth (30.67) 31.13 ± 0.48 (32.5) μm, colpi (17.5-) 19.40 ± 0.41 (-22.44) μm in long. Mesocolpium (25.08-) 27.30 ± 0.27 (-30.5) μm. Apocolpium (2.5-) 5.52 ± 0.42 (-7.5) μm. Exine 2.5 μm thick, sexine thicker than nexine. Tectum striate-rugulate.
Species included: *Verbena officinalis* L.

Discussion

Pollen morphology of family Verbenaceae is quite heterogeneous, considerable variation is observed in apertural types and exine ornamentation. Almost all types of tectal surface are found within the family. However, pollen grains are generally isopolar, radially symmetrical, tricolporate rarely tricolpate, sub-psilate-reticulate, or striate tectum.

The family Verbenaceae has long been recognized as the closest ally of Labiatae as there is not a single character which satisfactorily separates the two families. There are number of taxa in both the families which have intermediate morphology (Crouquist, 1981). The boundary between the two subfamilies in somewhat arbitrary.

The eight pollen types recognized in the present study clearly indicates heterogeneous assemblage of various taxa in the family Verbenaceae. This has also been depicted in the molecular and phylogenetic studies. Cantino (1992) has also demonstrated Verbenaceae is a polyphyletic family and some of the subfamilies to be merged with Labiatae. Cantino *et al*., (1992) proposed a classification of Labiatae which was substantially different from that of Bentham (1876) and Briquet (1895-1897). The classification was supported by Thorne (1992) and considered Labiatae (s.l.) having subfamilies of Verbenaceae Caryoptenidoideae, Chloanoideae, Viticoidae and tribe Monochileae of subfamily Verbenoidae. He also treated narrowly circumscribed Verbenaceae (s.str.) as a monophyletic taxon. Wegstaff & Olmstead (1997) also demonstrated the polyphyletic nature of Verbenaceae (s.l.) on the basis of rbcL sequences and supported the classification of Cantino *et al*., (1992) and accepted Verbenaceae (s.str.) excluding the tribe of Monochileae. The present palynological studies include the representative from various subfamilies of Verbenaceae (s.l.) including that of Viticidae, Verbenoidae, Caryopteiodeae. The eurypalynous nature of the family also support the polyplyletic nature of the family Verbenaceae and within a single family all type of tectal surface in found. However, pollen morphology of Labiatae in quite distinct and in the family Labiatae mostly pantocolpate pollen are found with reticulate tectum (Perveen & Qaiser, 2003).

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