

PALYNO-ANATOMICAL STUDIES OF *CLEMATIS* L. (RANUNCULACEAE) FROM POONCH VALLEY AJK, PAKISTAN

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ABSTRACT

Palynological and leaf epidermal anatomical studies were performed on three *Clematis* L. species i.e. *C. connata* DC, *C. grata* Wall. and *C. montana* Buch. Ham. in DC using light and scanning electron microscopy. *Clematis* species usually possess tricolporate pollen but differ in details of polar and equatorial diameter, colpi length, colpi width, exine thickness and exine sculpturing. Anomocytic stomata are present in these species but they differ in details of size of stomatal complex, stomatal aperture, abaxial and adaxial cell length and breadth. Trichomes are present in *C. connata* and *C. grata* but absent in *C. montana*. It is concluded that these three species of genus *Clematis* are differentiated on the basis of palyno-anatomical characters which are important for correct taxonomic identification of these species along with morphological characters.

Key Words: Clematis, Palynology, anatomy, systematic significance.

INTRODUCTION

Among parameters of taxonomy morphological, palynological and leaf epidermal anatomical characters are the important ones. Palynology is the science of pollen and spore morphology applied in plant taxonomy, plant geography, climatology, aeropalynology, criminology, allergy, stratigraphic correlation of oil bearing rocks and coal field, pharmacopalynology. The variations within a species, genus or family is usually reflected in anatomical features as well thus the role of anatomy in taxonomy has long been recognized. Leaf epidermal anatomical features (stomata, trichomes and other characters) are useful anatomical tools. (Stace, 1980).

Ranunculaceae is a large family of 50 genera and 2000 species widely distributed throughout the northern hemisphere but also in southern temperate regions (Mabberley, 1987). In Pakistan, it is represented by 22 genera and 114 species, of which genera like *Nigella*, *Anemone*, *Aquilegia* and *Clematis* include plants of ornamental value (Riedle and Nasir 1991).

The genus *Clematis* L. (Ranunculaceae) comprises about 250 species world wide. The species are well adapted to highland areas and are economically important for their chemical properties including the saponin glycosides available in their leaves and roots and also as ornamentals. *Clematis* is a vigorous climbing, with attractive flowers. Almost all species are found throughout the temperate regions of both hemispheres, and also in mountains in the tropics (Tamura, 1993).

The present study is the first attempt made on palynological and anatomical studies of genus *Clematis*

in Poonch Valley, considering palynological and leaf epidermal anatomical studied in three species i.e. *C. connata*, *C. grata* and *C. Montana*.

MATERIALS AND METHODS

Light microscopic (LM) study of pollen and leaf epidermal anatomy was conducted during 2009 - 10 in the Experimental Taxonomy Laboratory of Quaid-i-Azam University, Islamabad. The study was confined to description of 3 species of the family genus *Clematis*. The plant material was collected from Poonch Valley situated in Azad Jammu and Kashmir. It lies between 33⁰- 36⁰ North latitude and 73⁰ -75⁰ East longitude. The region has got an average height of 1750-2500 meter from sea level, sloping from south north to north east.

Glycerin jelly was used in pollen staining and prepared according to modified method of (Meo and Khan, 2005). Mature floral buds were utilized from the dried plant material for palynomorph study. Different parameters were studied under light microscope for pollen morphology as Qualitative characters type of pollen, shape in polar and equatorial view, presence of colpi and spines and sculpturing. Quantitative characters included polar and equatorial diameter, P/E ratio, No. of spines/pollen, No. of spines between colpi, No. of colpi, length/width of colpi, No. of pores, spine length, No. of spine and exine thickness.

Scanning Electron Microscope (SEM) analyses of pollen was carried out in Centralized Science Laboratory, University of Karachi, Pakistan during the January 2010. The pollen grains were prepared for scanning electron microscopy (SEM) by the standard

methods described by Erdtman (1952). The pollen grains were suspended in a drop of water and were directly transferred to a metallic stub using double sided cello tape and coated with gold in a sputtering chamber (Ion-sputter JFC-1100). The S.E.M examination was carried out on a Jeol microscope JSM-2. The measurements were based on 5-7 readings from each specimen.

For epidermal studies Shultze's method of maceration with improved technique was followed (Subrahmanyam, 1996). The prepared slides were studied under light microscope. The characters noted on both abaxial and adaxial surface were shape of the cells, cell width, cell length, type of stomata, shape of the stomata, length and width of the stomatal complex and aperture,

type of trichomes, length of trichomes, breadth of trichome base and presence or absence of glands. Microphotography of pollen and leaf epidermis was carried out with the help of Camera fitted Nikon light Microscope (LM).

RESULTS AND DISCUSSION

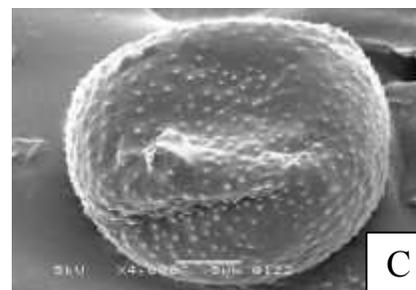
Table 1 presents the pollen characteristics of selected species of genus *Clematis* of Ranunculaceae. Table 2 presents the comparative summary of leaf epidermal anatomical features of three species of *Clematis*.

Table 1: Pollen characteristics of selected species of genus *Clematis* of Ranunculaceae

Botanical name	Polar Diameter (µm)	Equatorial Diameter (µm)	P/E ratio	Length of calpi (µm)	Width of calpi (µm)	Exine thickness (µm)	SEM of Pollen
<i>Clematis connata</i>	26 (25-27)	27.5 (25-30)	0.94	1.33 (1-1.7)	4.7 (3.7-5.7)	2.37 (2-2.7)	Scabrate
<i>Clematis grata</i>	20.4 (19-22)	17.1 (15.5-19)	1.19	1.8 (1.2-2.2)	3.2 (3-3.5)	2 (1.7-2.3)	Scabrate or granulate
<i>Clematis montana</i>	22.2 (18-25)	21.6 (20-23)	1.02	2.2 (2-2.5)	3.05(2.7-3.7)	2.3 (2-2.8)	Scabrate

Table 2: Comparative Summary of Leaf Epidermal Anatomical Features of Three Species of *Clematis*

Botanical name	Type of Stomata	Trichome structure on Adaxial surface	Trichome structure on Abaxial surface	Epidermal cells on Adaxial surface	Epidermal cells on Abaxial surface	Length x Width of epidermal cells on Adaxial surface (µm)	Length x Width of epidermal cells on Abaxial surface (µm)
<i>Clematis connata</i>	Anomocytic	Absent	Bicellular and tricellular hair-like trichomes	Undulating	Undulating	67.5 (52.5-82.5) × 38.7 (32.5-45)	61.2 (37.5-100) × 38.7 (20-75)
<i>Clematis grata</i>	Anomocytic	Absent	Unicellular hair-like trichomes	Undulating	Undulating	53.7 (45-62.5) × 25 (22.5-30)	35.8 (25-55) × 33.2 (22.5-45)
<i>Clematis montana</i>	Anomocytic	Absent	Absent	Polyhedral	Undulating	115.5 (75-150) × 43.3 (30-62.5)	66.6 (37.5-87.5) × 46.6 (35-57.5)



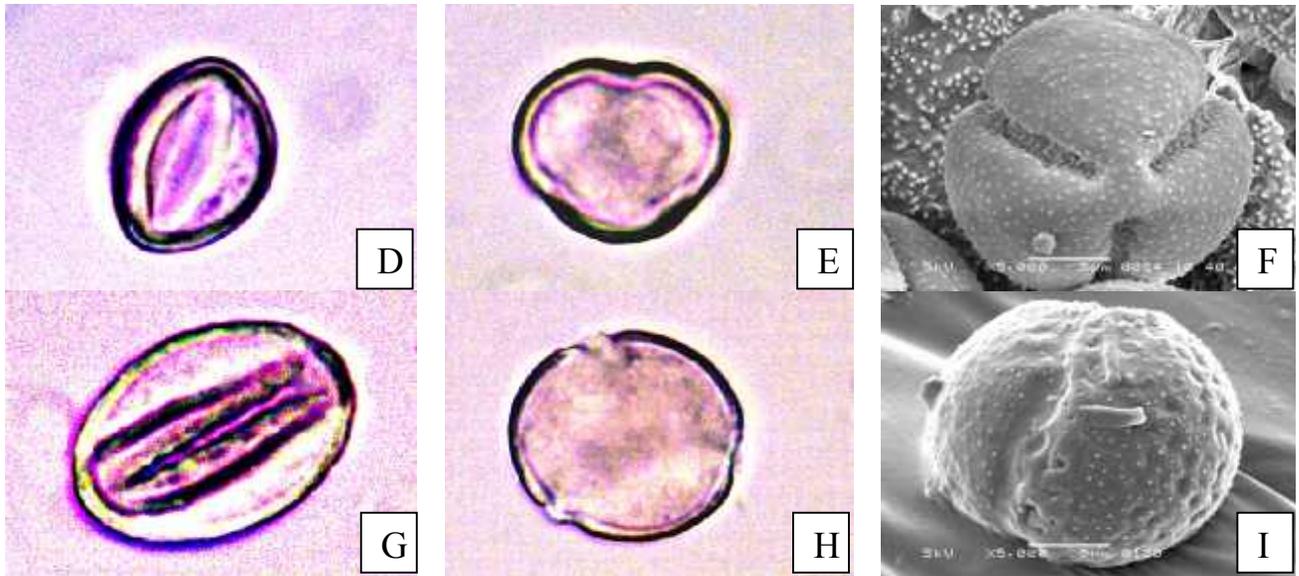
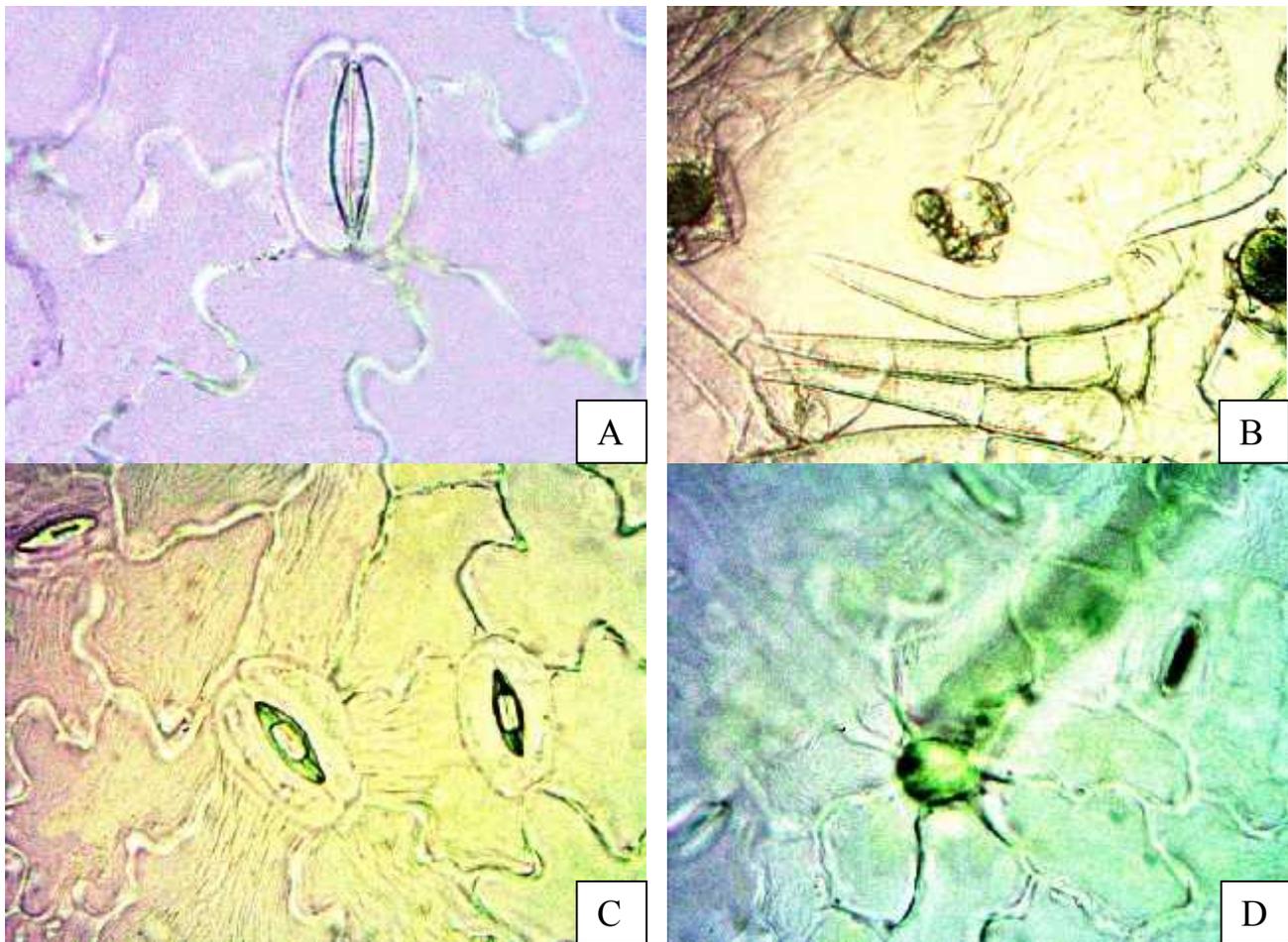


Plate 1 A-C *Clematis connate*: Equatorial View of Pollen, Polar View of Pollen, SEM of Pollen. D-F *Clematis grata*: Equatorial View of Pollen, Polar View of Pollen, SEM of Pollen. G-I *Clematis montana*: Equatorial View of Pollen, Polar View of Pollen, SEM of Pollen.



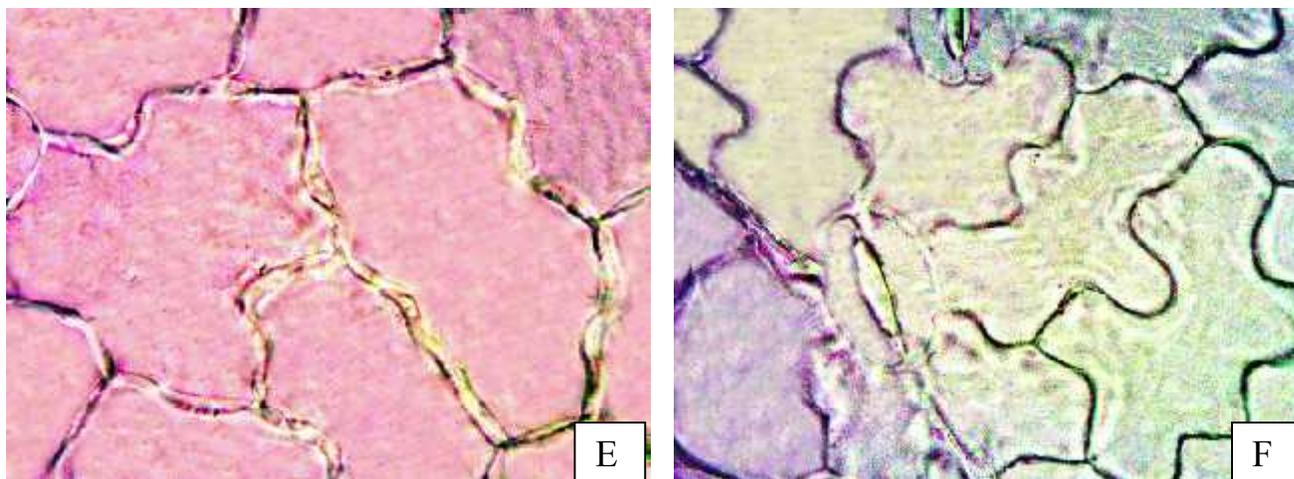


Plate 2 A-B *Clematis connata*, A: Stomata on Adaxial surface, B: Trichomes and glands on Abaxial surface (40x). **C-D** *Clematis grata*, C: Trichome on Adaxial surface, D: Stomata on Abaxial surface (40x). **E-F** *Clematis Montana*, E: Adaxial surface, F: Stomata on Abaxial surface (40x).

In this study the pollen of three *Clematis* species observed are monad and tricolporate, generally circular to semi-angular in polar view but circular to circular-lobate in *Clematis grata*. Minimum polar diameter i.e. 20.4 μm and equatorial diameter i.e. 17.1 μm is found in *Clematis grata* whereas maximum polar and equatorial diameter is observed in *Clematis connata* i.e. 26 μm and 27.5 μm respectively (Table 1). Highest P/E ratio is 1.19 μm found in *Clematis grata*. Maximum exine thickness is observed in *Clematis connata* i.e. 2.3 μm and minimum exine thickness is 2 μm in *Clematis grata*. Sculpturing is scabrate or granulate in these species. Sheidai *et al.*, (2009) also observed granulate sculpturing with microechinate projections in five species of *Clematis*. Anomocytic stomata are found in all species. Cells are undulating in *Clematis* species. Trichomes are absent in *C. montana* but present in other two species on the abaxial surface only. The trichomes are uni or bicellular hair-type. Variation in length and breadth of both surfaces is shown in table 2.

As no previous record exists on palynology and anatomy of most of the medicinal species of the study area, so the present study proved that in addition to morphology, palynology and leaf epidermal anatomy are the multiple approaches by which we can not only differentiate the different plants but can also place them at their correct taxonomic positions and the taxonomic problems can be solved among problematic taxa.

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