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Short Communication

Taxonomic Utility of Palynological Characters in the Genus *Fallopia*, Family Polygonaceae from Pakistan

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ABSTRACT

Pollen grains of two species of the genus *Fallopia* Adans from Pakistan were studied by light microscope (LM) and scanning electron microscope (SEM). Pollen grains usually radially symmetrical, isopolar, tricolporate, subprolate, sometimes prolate-spheroidal in equatorial view, while circular in polar outline. Based on exine ornamentation under SEM, two pollen types are distinguished i.e., Convolvulus pollen type with microechinate-punctate exine and Dumetorum type with reticulate exine.

Key Words: Pollen morphology; Isopolar; Circular; Microechinate; Punctate; Reticulate

INTRODUCTION

Fallopia Adans is an exceedingly variable but rather a small genus of family Polygonaceae consisting of 16 species (Kim *et al.*, 2000) and distributed in north temperate regions of Europe and Asia (Qaiser, 2001). Plants are mostly annual or perennial herbs, sometimes rhizomatous or twining vines. The distinguishing characters of the genus are their large, cordate or broadly ovate leaves, outer tepals keeled with single main vein, filaments dorsoventrally flattened and swollen at the base, styles 3, very short, fused at the base, fruits trigonous with prominent ribs, beak is absent or very short (Holub, 1970; Haraldson, 1978; Ronse Decraene & Akeroyd, 1988; Ronse Decraene *et al.*, 2000). This small genus is represented by 2-3 species in Pakistan. *F. dentato-alata* (F. Schmidt) Holub is a new species from Pakistan. It shows resemblance with *F. dumetorum* (L.) Holub but can be distinguished by its cuneate wings and large size nut (Qaiser, 2001). Unfortunately this species was unavailable for palynological studies, so in the current study palynological characters of potential taxonomic value of *F. convolvulus* (L.) A. Love and *F. dumetorum* (L.) Holub were investigated. Pollen morphological characters of the genus were previously examined by Nowicke and Skvarla (1979); Wang and Feng (1994) and Zhong and Zhou (1998). However, there are no reports on palynological studies of the genus from Pakistan. In the present studies an attempt has been made to provide complete information on pollen morphology of two species by light microscopy (LM) and scanning electron microscopy (SEM) and to evaluate the taxonomic value of micromorphological characters of pollen in order to distinguish *F. convolvulus* and *F. dumetorum* from each other.

MATERIALS AND METHODS

The flowers were extracted from herbarium specimens of Quaid-i-Azam University, Islamabad (Table I). The pollen grains were prepared for acetolysis by the modified procedure of Erdtmann (1952 & 1969). For light microscopy, acetolysed anthers were removed from filaments of stamen with the help of dissecting needles and then crushed to release pollen grains on a clean glass slide. Anther wall material was discarded. Then pollen grains were mounted in glycerin jelly stained with 1% safranine. The slide was placed on hot plate to melt glycerin jelly and to remove bubbles from the slide. Cover slip was placed on the prepared pollen-glycerin jelly mixture. When cooled, the glass slide was labeled and edges of the cover slip were sealed with transparent nail vanish. The prepared slides were studied under the light microscope. Pollen type, its shape and diameter in polar and equatorial view, P/E ratio, exine thickness and its sculpturing, intine thickness and length of colpi were examined. Details of pollen morphology were based on the measurements of 10-15 grains. The data were statistically analyzed i.e., range, mean and standard error (±) were calculated using MS excel sheet. Their photographs were taken with the Nikon FX-35 microscope equipped with Camera photomicrograph system.

For SEM studies, pollen grains suspended in a drop of 40% acetic acid were transferred to clean metallic stubs and coated with gold using a JEOL JFC 1100 E ion sputtering device. SEM observations were carried out on a JEOL microscope JSM5910. The work was carried out in the Centralized Resource Laboratory, University of Peshawar (Pakistan).

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The terminology used is in accordance to Erdtman (1952), Kremp (1965), Punt *et al*. (1994 & 2007).

RESULTS

Summarized results of pollen morphology of the genus *Fallopia* are presented in Table II. Pollen morphology of the genus is noted as follows.

Key to the species of *Fallopia*

1a: Exine microechinate – punctate. 2. *F. convolvulus.*

1b: Reticulate exine with regularly distributed lumina and muri. 1. *F. dumetorum.*

Pollen class: Tricolporate

Size. The size of pollen grains (polar axis × equatorial diameter) in *F. convolvulus* is 21×19.5 µm and 21×21 µm in *F. dumetorum* (Table II).

Symmetry and shape. The pollen grains are radially symmetrical and isopolar. Outline is circular in polar view and sub-prolate, sometimes prolate-spheroidal in equatorial view (Fig. 1a-d). The P/E (polar axis/equatorial diameter) ratio varies from 1.00 in *F. dumetorum* to 1.07 in *F. convolvulus* with very small size variation among the taxa studied (Table II). Columella is well developed and regularly distributed.

Aperture. Non-lacunate types of pollen are observed in *Fallopia* species. Pores are elongated and narrow. Length of colpi in *F. convolvulus* is 12.8 µm, while 12.0 µm in *F. dumetorum* (Table II).

Exine and intine. Thickness of exine in *F. convolvulus* is 3.8 µm, while it is 2.00 µm in *F. dumetorum*. Intine is indistinct in *F. dumetorum* (Table II).

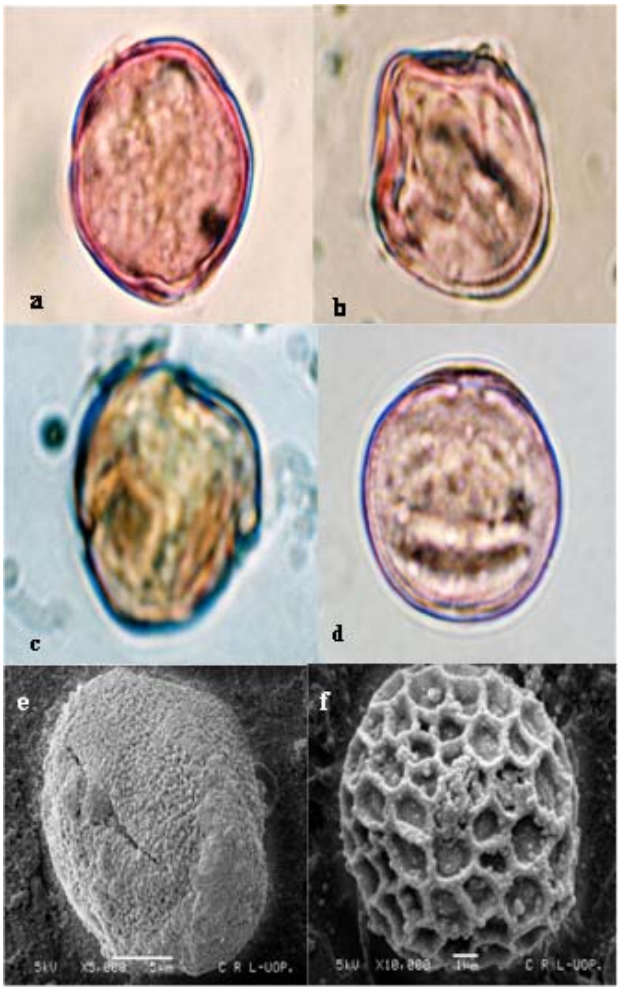
Exine sculpturing. When observations are made under LM, areolate pattern is visible in polar view of *F. convolvulus* but its equatorial view and both views of *F. dumetorum* do not show any clear pattern. Under SEM, exine appears as microechinate-punctate type in *F. convolvulus*, while it is reticulate in *F. dumetorum* with regularly distributed lumina and muri. Size of lumina varies from 0.8-1.2 µm and slight granulation is also seen in lamina (Table II).

DISCUSSION

Not only general morphology of plant but also pollen morphology is of great taxonomic significance (Stix, 1960). Pollen morphological characters have been used for the identification of taxa (Erdtmann, 1966). Palynological data is of significance in the distinction of species and also related with morphological characters (Qureshi *et al*., 2002). Pollen morphology is also helpful to justify taxonomic position of the species within its family (Shakira *et al*., 2007). The discovery of electron microscope has revolutionized the study of surface structures in depth, which was not ever plausible with light microscope.

Present study revealed the utility of both qualitative and quantitative characters in taxonomic studies. This study particularly showed that distinct variation in exine ornamentation could be seen only by using scanning electron microscope (SEM). On the basis of exine ultrastructure (in SEM), two pollen types could be recognized in the species examined.

Fig. 1a-f. Light micrographs of the pollen grains of genus *Fallopia* (1000 X)
F. convolvulus: **a**. Polar view, **b.** Equatorial view. ***F. dumetorum***: **c.** Polar view, **d**. Equatorial view. ***F. convolvulus***: **e.** SEM micrograph showing equatorial view. ***F. dumetorum*: f.** SEM micrograph, overall view showing lumina and muri



Key to different pollen types:

1a: Pollen surface microechinate-scabrate. Convolvulus type. (*F. convolvulus*).
1b: Pollen surface reticulate with hexagonal lumina. Dumetorum type (*F. dumetorum*).

Convolvulus type pollen. This pollen type is represented in *F. convolvulus* corresponding to Convolvulus type (Nowicke & Skvarla, 1979; Wang & Feng, 1994; Zhong & Zhou, 1998). Pollen grains were tricolporate, prolate-spheroidal to subprolate, tricolporate with nearly equal polar

Table I. List of species investigated with locality, district, collector name and accession numbers

Species	Locality	District	Collector name	Acc. No.
Fallopia convolvulus	Nar Shikwa (stony soil)	Chitral	Muqarrab Shah and Dilawar	63867
	Dhilkot (stony soil)	Poonch	Bashir Ahmad and Javed	109452
F.dumetorum	Kulandi	Dir	Muqarrab Shah and Dilawar	63919
	Gumula	Dera Ismail Khan	Hafizullah and Ayaz	61273

Table II. Summary of Pollen measurements, shape and sculpturing features in *Fallopia* Adans species, (All measurements are in µm)

Species	Pollen class	Aperture Type	Shape in Equatorial view	Shape in Polar view	Equatorial diameter µm	Polar diameter µm	P/E ratio	Length of colpi µm	Exine thickness µm	Intine thickness µm	Sculpturing of exine	
											Under LM	UnderSEM
F. convolvulus	Tricolporate	Non-lacunate	Prolate-spheroidal to subprolate	Circular	19.5±0.93 (17.5-22.5)	21±1.00 (20-25)	1.07	12.8±0.2 (12-13.5)	3.8	2	Areolate in polar view but indistinct in equatorial viewx	Microechinate-punctate
F. dumetorum	Tricolporate	Non-lacunate	Subprolate	Circular	*21±0.79 (20-23.5)	21±0.85 (22.5-27)	1.00	12±0.7 (10-15)	2	Indistinct	Not clear	Reticulate, slightly granulate on lumina and muri

*Mean values followed by min-max in parentheses. P=Polar, E=Equatorial, ± Standard error

and equatorial diameter (Fig. 1a-b, Table II). The prolate pollen grains were 28.2 (25.6-28.2) × 20.1 (20.1-23.0) µm, possessed somewhat longer colpi (Wang & Feng, 1994). Zhang and Zhou (1998) described the grains of *F. convolvulus* as tricolporate prolate with 24.7 (23.0-26.0) × 20 (19.0-22.5) µm dimensions. Areolate pattern of exine sculpturing was noted in polar view of *F. convolvulus* when observations were made with LM (Fig. 1a-b). A clearer picture of the exine ornamentation was obtained through SEM studies, which exposed echinate-punctate ornamentation (Fig. 1e). Previously, ultra structure studies of *F. convolvulus* revealed two types of tectum i-e., echinate around ectocolpi and smooth around mesocolpium (Nowicke & Skvarla, 1979; Wang & Feng, 1994). Under LM, Zhang and Zhou (1998) did not observe any distinct type of ornamentation but in SEM they observed dimorphic exine.

Dumetorum type pollen. This pollen type is related to Zhang and Zhou's (1998) Tiniaria type pollen, characterized by reticulate exine (Fig 1f, Table II). *F. dumetorum* comprised this type with same polar and equatorial diameter (21 × 21 µm). Zhang and Zhou (1998) gave 23.8 × 20.4 µm size for subprolate or subspheroidal grains of *F. dumetorum*.

In conclusion pollen micromorphological features proved to be a good taxonomic marker at the specific level in the genus.

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