

Pollen Morphology of Himalayan *Viola* (Violaceae)

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Abstract

Pollen of 15 species of *Viola* L. (Violaceae) from the Himalaya were investigated by light and scanning electron microscopy. The pollen of *Viola* are prolate-spheroidal to prolate, rarely oblate, 3 (4-5)-colporate, having a delicate exine with microverrucate ornamentation. Four types of pollen grains are recognized: (1) *Viola hookeri* Type, characterized by subprolate to prolate grains, sexine microechinate to microverrucae and distinct perforations, (2) *Viola pogonantha* Type, prolate-spheroidal to prolate pollen, circular in outline in polar view, microverrucae uniform, not forming clusters, without distinct perforations, (3) *Viola pilosa* Type, prolate-spheroidal to prolate, sexine microverrucate, in clusters, perforations mostly inconspicuous, and (4) *Viola tricolor* Type, pollen grains large (38-51 μ m), oblate, stephano (4-5) colporate, microverrucae comparatively large, nearly uniform, perforations inconspicuous (*V. tricolor*). The pollen morphological data suggest a rearrangement of species at the sectional level.

Key words: ornamentation, pollen grain, SEM, systematics, verrucae

Introduction

Viola is the largest genus of the family Violaceae, comprising ca. 400 species (Mabberley 1997). They are cosmopolitan, but mainly occur in the north temperate regions, being restricted in the tropics to mountainous areas. China comprises 125 species of *Viola*, 35 species in India, and 11 species in Bhutan. About 30 species of this genus are estimated to occur in the Himalayas, and 20 species of *Viola* have been reported from Nepal (Dani 2001).

The genus *Viola* has been classified into three subgenera namely, Subgen. *Viola* (most of the species), Subgen. *Dischidium* and Subgen. *Melanium* (Wang 1991). Based on his classification, the Himalayan species of *Viola* can be allocated to all three subgenera namely, *Viola betonicifolia*, *V. bulbosa*, *V. canescens*, *V. hamiltoniana*, *V. kunawarensis*, *V. mandshurica*, *V. odorata*, *V. pilosa*, *V. sikkimensis*, *V. thomsonii* (Subgen. *Viola*), *Viola biflora*, *V. wallichiana* (subgen. *Dischidium*), and *Viola tricolor* (Subgen. *Melanium*).

The pollen morphology of only a few species of *Viola* has been examined to date. Most of the work has been done on European species (*V. arvensis*, *V. calcarata*, *V. canina*, *V. lutea*, *V. odorata*, *V. riviniana*, *V. tricolor*) using LM and SEM (Moore *et al.* 1991, Halbritter & Buchner 2000). Kapp *et al.* (2000) have described two species (*V. novae-angliae*, *V. sagittata*) from the USA. Eastern Asiatic species were

investigated by Huang (1972) and Wang *et al.* (1995). So far only two Himalayan species (*V. betonicifolia*, *V. biflora*) have been described (Gupta & Sharma 1986). The pollen of *Hybanthus* (*H. enneaspermus*), a closely related genus of the family Violaceae, is described from Maharashtra, India by Nayar (1990). None of the above has been carried out using an electron microscope (Cooper 2002). Therefore, the present investigation on the pollen morphology of Himalayan *Viola* represents a pioneering work using both LM and SEM techniques.

Methodology

Pollen samples of all Himalayan species of *Viola* (Violaceae) were obtained from the Tribhuvan University Herbarium of Nepal (TUCH), and Natural History Museum London (BM).

For Light microscopic (LM) study, anthers from dried buds were transferred to a drop of acetolysis solution for 5 minutes to 24 hours, depending upon the strength of exine wall. As the pollen of *Viola* usually has a very delicate exine, the acetolysed pollen were directly mounted in glycerine on microscopic slides. The pollen samples were transferred using a specially adapted needle with a human nasal hair glued at the tip (Zetter 1989).

LM examination was carried out with a NIKON light microscope using x 85 objective, x 10 eyepiece.

Measurements of glycerine mounted pollen were made after slide preparation. The polar axis (P) and equatorial diameter (E) were measured using an oculo-micrometer. The mean value, the range for each parameter, and the ratio of polar to equatorial axis (P/E) were calculated from ten undistorted pollen per sample. Microphotography was made subsequently using an OLYMPUS OM-4 Ti camera.

For SEM, the pollen samples were acetolysed as in LM study, and then transferred to the glycerine. The treated pollen were then transferred to aluminium stubs, applying a drop of ethanol to the stub to dissolve the glycerine. Later, the stubs were coated with gold for 4-8 minutes, using a Sputter coater (Bio-Rad SC 500). Scanning microphotographs were made with a Jeol JSM – 6400 Scanning Electron Microscope.

The morphological terms and concepts used in the present text follow Faegri and Iversen (1989) and Punt *et al.* (1994).

Results

The pollen of *Viola* are prolate, sub-prolate to prolate-spheroidal, 18-51 μm X 14-43 μm , usually 3-colporate, rarely 4-5-colporate (*V. odorata*, *V. tricolor*), exine very thin and extremely delicate, sexine microechinate to microverrucate, microverrucae sometimes grouped in clusters, perforations conspicuous or inconspicuous. Four types of pollen grains are recognized based on exine ornamentation:

Viola hookeri Type (*V. bulbosa*, *V. hookeri*, *V. wallichiana*), characterized by subprolate to prolate grains, sexine microechinate to microverrucate, with distinct perforations. Microverrucae arranged in rosette pattern around perforations.

Viola pogonantha Type (*V. betonicifolia* var. *cordifolia*, *V. betonicifolia* var. *jaunsarensis*, *V. biflora*, *V. canescens*, *V. hamiltoniana*, *V. pogonantha*), pollen prolate-spheroidal to prolate, circular in outline in polar view, microverrucae uniform, not in clusters, without distinct perforations.

Viola pilosa Type (*V. betonicifolia* var. *betonicifolia*, *V. pilosa*), prolate-spheroidal to prolate, ornamentation verrucose, microverrucae in clusters, to form supra sculpture, perforations mostly inconspicuous.

Viola tricolor Type (*V. tricolor*), pollen grains oblate-spheroidal, large (38-51 μm), stephano (4-5) colporate, microverrucae comparatively large, nearly uniform, perforations inconspicuous.

The following species of *Viola* shows intermediate type of ornamentation between *Viola pogonantha* Type

and *Viola pilosa* Type: *V. kunawarensis*, *V. mandshurica*, *V. sikkimensis*, and *V. thomsonii*. Similarly, in shape and size *Viola odorata* seems to be closer to *V. tricolor* Type, because both species are introduced species in Nepal, but in terms of ornamentation, it is related to *Viola pogonantha* Type.

The size, shape and exine ornamentation of all species of Himalayan *Viola* examined here, is given below:

Type 1. *Viola hookeri* Type

Viola bulbosa Maxim. Pollen grains 3-colporate, Polar axis (P) = 20-25 μm , Equatorial axis (E) = 17-21 μm , P/E ratio = 1.46, prolate. Sexine microverrucate, verrucae uniform, with distinct perforations (Fig. 1-2).

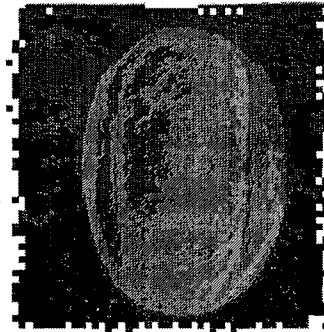


Fig. 1. *V. bulbosa*, equatorial view, SEM x 3,000

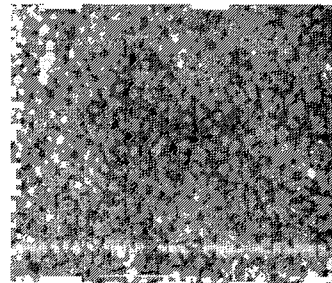


Fig. 2. *V. bulbosa*, detail of the surface, SEM x 20,000

V. hookeri Hook.f. & Thoms. Pollen grains 3-colporate, P = 20-32, E = 14-18, P/E = 2.2, prolate. Sexine microverrucate, verrucae very small, uniform, with distinct perforations (Fig. 3-4).

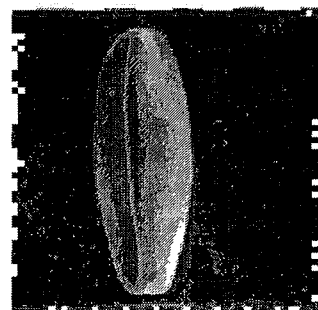


Fig. 3. *Viola hookeri*, equatorial view, SEM x 2,000

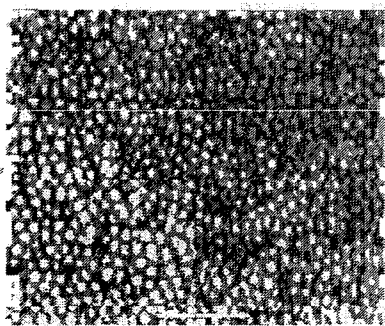


Fig. 4. *V. hookeri*, detail of the surface, SEM x 20,000

V. wallichiana Ging. ex DC. Pollen grains 3-colporate, P = 20-22, E = 19-21, P/E = 1.07, prolate spheroidal. Sexine surface microverrucate, microverrucae, perforations distinct (Fig. 5).



Fig. 5. *V. wallichiana*, detail of the surface, SEM x 20,000

Type 2. *Viola pogonantha* Type

V. betonicifolia var. *cordifolia* H. Hara. Pollen grains 3-colporate, P = 22-23, E = 19-20, P/E = 1.16, subprolate. Sexine microverrucate, microverrucae not clustered, perforations inconspicuous (Fig. 6).

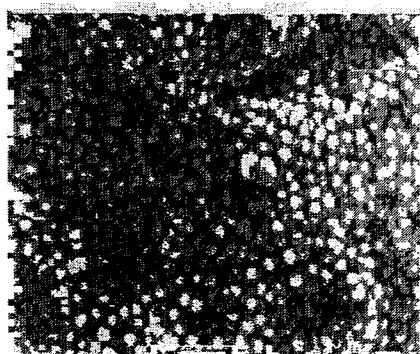


Fig. 6. *V. betonicifolia* var. *cordifolia*, detail of the surface, SEM x 20,000

V. betonicifolia var. *jaunsarensis* (W. Becker) H. Hara. Pollen grains 3-colporate, P = 21-23, E = 21-25, P/E = 1.10, prolate-spheroidal. Sexine microverrucate, perforations inconspicuous (Fig. 7).

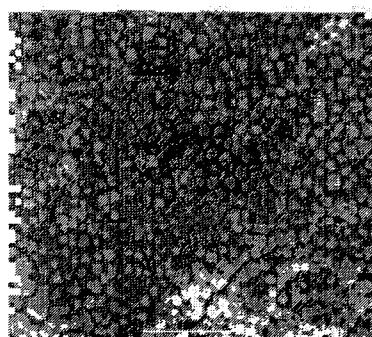


Fig. 7. *V. betonicifolia* var. *jaunsarensis*, detail of the surface, SEM x 20,000

V. biflora L. Pollen grains 3-colporate, P = 19-25, E = 17-25, P/E = 1.14, subprolate. Sexine microverrucate, perforations inconspicuous (Fig. 8-9).

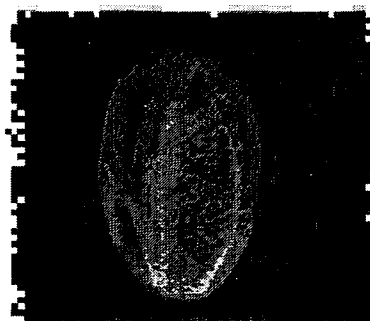


Fig. 8. *V. biflora*, equatorial view, SEM x 3,000



Fig. 9. *V. biflora*, detail of the surface, SEM x 20,000

V. canescens Wall. Pollen grains 3-colporate, P = 18-26, E = 17-20, P/E = 1.47, prolate. Sexine microverrucate, microverrucae nearly clustered, without perforations (Fig. 10-11).

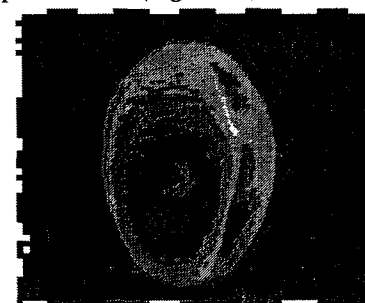


Fig. 10. *V. canescens*, equatorial view, SEM x 2,700

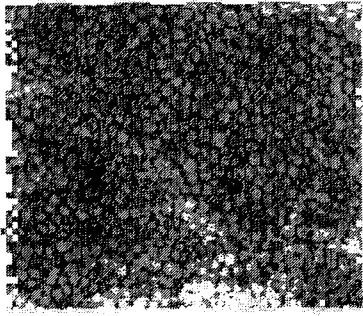


Fig. 11. *V. canescens*, detail of the surface, SEM x 20,000

V. hamiltoniana D. Don. Pollen grains 3-colporate, $P = 19-33$, $E = 17-20$, $P/E = 1.57$, prolate. Sexine microverrucate, microverrucae fairly large and somewhat clustered, without perforations (Fig. 12).

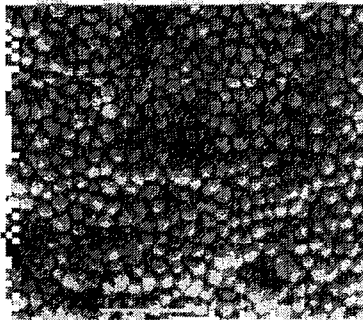


Fig. 12. *V. hamiltoniana*, detail of the surface, SEM x 20,000

V. pogonantha W.W. Sm. Pollen grains 3-colporate, $P = 18-23$, $E = 17-20$, $P/E = 1.17$, subprolate. Sexine microverrucate, verrucae fairly large and nearly clustered, perforations inconspicuous (Fig. 13-14).

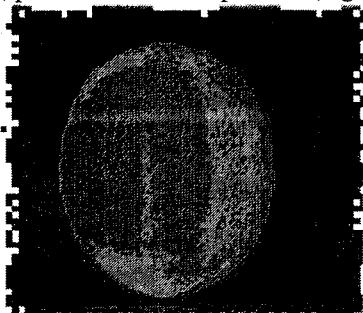


Fig. 13. *V. pogonantha*, equatorial view, SEM x 3,300

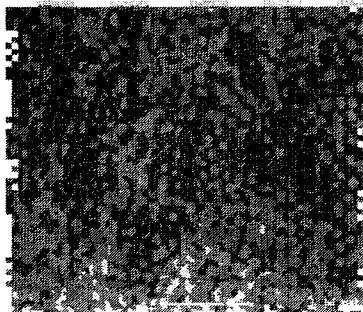


Fig. 14. *V. pogonantha*, detail of the surface, SEM x 20,000

Type 3. *Viola pilosa* Type

V. betonicifolia Sm. var. *betonicifolia*. Pollen grains 3-colporate, $P = 22-26$, $E = 21-24$, $P/E = 1.05$, prolate-spheroidal. Sexine microverrucate, microverrucae clustered, perforations distinct (Fig. 15).

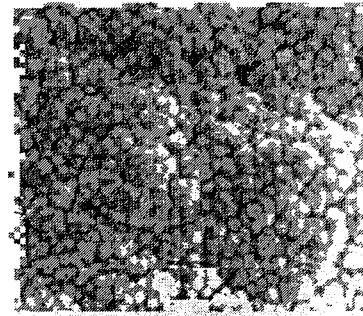


Fig. 15. *V. betonicifolia* var. *betonicifolia*, detail of the surface, SEM x 20,000

V. pilosa Blume. Pollen grains 3-colporate, $P = 23-26$, $E = 20-21$, $P/E = 1.25$, subprolate. Sexine microverrucate, microverrucae uniform and clustered, with perforations (Fig. 16).

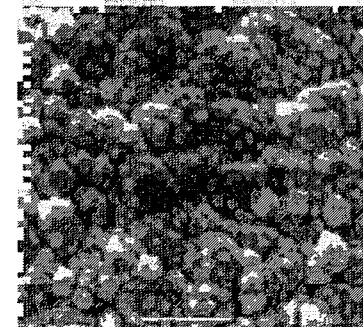


Fig. 16. *V. pilosa*, detail of the surface, SEM x 20,000

Type 4. *Viola tricolor* Type

V. tricolor L. Pollen grains 4-5 colporate, $P = 38-45$, $E = 35-41$, $P/E = 0.74$, oblate. Sexine microverrucate, verrucae undulated, perforations inconspicuous (Fig. 17-18).

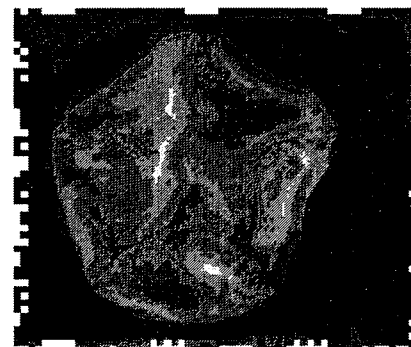


Fig. 17. *V. tricolor*, polar view, SEM x 1,300

