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-spheroideal 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 microechmate to microverrucate and distinct perforations, (2) Viola pagonantha Type, prolate-spheroideal to prolate pollen, circular in outline in polar view, microverrucate uniform, not forming clusters, without distinct perforations, (3) Viola pilosa Type, prolate-spheroideal to prolate, sexine microverrucate, in clusters, perforations mostly inconspicuous, and (4) Viola tricolor Type, pollen grains large (38-51 mm), oblate, stelphano (4-5)-colporate, microverrucate 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, verrucate

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. novaes-anglicae, 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 Nayak (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 acetyolysis 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 acetyolysed 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 oculometer. 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 aluminum 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 pm X 14-43 μm, usually 3-colporate, rarely 4-5-colporate (V odorata, V tricolor), exine very thin and extremely delicate, sexine microechmate to microvermiculate, microvermiculae 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 microvermiculate, with distinct perforations. Microvermiculae 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, microvermiculae uniform, not in clusters, without distinct perforations.

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

Viola tricolor Type (V tricolor), pollen grains oblate-spheroidal, large (38-51 mm), stephano (4-5) colporate, microvermiculae 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 mm, Equatorial axis (E) = 17-21 mm, P/E ratio = 1.46, prolate. Sexine microvermiculate, verruculae 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 microvermiculate, verruculae 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

*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. 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
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
**Fig. 18.** _V. tricolor_, detail of the surface. SEM x 20,000

Intermediate Type

_V. kunawarensis_ Royle. Pollen grains 3-colporate, _P_ = 23-28, _E_ = 18-20, _P/E_ = 1.50, prolate. Sexine microverrucate, microverucae nearly clustered due to fissures, perforations inconspicuous (Fig. 19-20).

**Fig. 19.** _V. kunawarensis_, polar/oblique-view, SEM x 2,700

_V. mandshurica_ W. Becker. Pollen grains 3-colporate, _P_ = 22-29, _E_ = 21-28, _P/E_ = 1.02, prolate-spherical. Sexine microverrucate, microverucae nearly clustered due to fissures, perforations inconspicuous (Fig. 21).

**Fig. 20.** _V. kunawarensis_, detail of the surface, SEM x 20,000

_V. odorata_ L. Pollen grains stephano (4)-5 colporate, _P_ = 43-51, _E_ = 39-43, _P/E_ = 1.9, prolate-spherical. Sexine microverrucate, microverucae comparatively large, nonuniform, not in clusters, perforations inconspicuous (Fig. 22).

**Fig. 21.** _V. mandshurica_, detail of the surface, SEM x 20,000

**Fig. 22.** _V. odorata_, detail of the surface, SEM x 20,000.

_V. sikkimensis_ W. Becker. Pollen grains 3-colporate, _P_ = 20-32, _E_ = 16-18, _P/E_ = 1.84, prolate. Sexine microverrucate, microverucae nearly clustered due to fissure, slightly undulated, perforations inconspicuous (Fig. 23).

**Fig. 23.** _V. sikkimensis_, detail of the surface, SEM x 20,000

_V. thomsonii_ Oudem. Pollen grains 3-colporate, _P_ = 21-32, _E_ = 19-25, _P/E_ = 1.68, prolate. Sexine surface microverrucate, microverucae nearly clustered due to fissure, perforations inconspicuous (Fig. 24).

**Fig. 24.** _V. thomsonii_, detail of the surface, SEM x 20000

**Discussion**

The pollen grains of _Viola_ are usually delicate and with a very thin wall. During acetolysis the pollen of most
Viola species collapse due to its weak exine. This may be one of the reasons why very limited work has been done so far on the pollen morphology of Viola. Without acetylation the cell content is not destroyed, making light microscopic photography impossible. Only the measurements of pollen were made under LM.

To our knowledge, a SEM study of the pollen of Himalayan Viola has not been carried out so far. Thus the pollen morphological description of 13 species and three subspecies of Viola, with the exception of two introduced garden species, are given here for the first time.

To some extent, pollen morphology tends to support the classification of Viola, based on gross morphology. For example, placing Viola tricolor in Subgen. Melanium is justified due to its large-sized penta-colporate pollen, and sexine with comparatively large verrucae. Based on morphological grounds, Viola hookeri and V. wallichiana are referred to Subgen. Dischidium, and the pollen morphological characters also support this view. Similarly, the majority of species belonging to Section Viola have fairly uniform pollen grains. However, a few species such as V. kunawarensis, V. mandshurica, V. sikkimensis and V. thomsonii, belonging to Section Viola have different sexine ornamentation, suggesting that these species should be referred to a different group.

Based on the present findings, it is proposed that the existing classification of Viola should be revised. Along with morphological and palynological data, this revision should include data from cytology and molecular systematics.

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Specimens Examined

Viola L.

Subgen. Viola

V. betonicifolia Sm. var. betonicifolia. WEST NEPAL: Polunin, Sykes & Williams 1819, Mar 1952, BM; Dani 210 (TUCH).

V. betonicifolia var. cordifolia H. Hara. EAST NEPAL: Dobremez 2248, May 1973 (BM).

V. betonicifolia var. jaunsarensis (W. Becker) H. Hara. WEST NEPAL: Stainton 4218, May 1963 (BM).

V. bulbosa Maxim. WEST NEPAL: Polunin, Sykes & Williams 4038, May 1952 (BM).

V. canescens Wall. CENTRAL NEPAL: Dani 138, Apr 2000 (TUCH); Dani 203, Feb 2000 (TUCH); Bhattarai 70, Nov 2000 (TUCH).

V. hamiltoniana D. Don. CENTRAL NEPAL: Dani 208, Mar 2000, TUCH; Sunita s.n. (TUCH).

V. hookeri Hook.f. and Thoms. CENTRAL NEPAL: Stainton, Sykes & Williams 4881, Apr 1954 (BM).

V. kunawarensis Royle. WEST NEPAL: Polunin, Sykes & Williams 1078; Jun 1952 (BM).

V. mandshurica W. Becker. CENTRAL NEPAL, Dani 202, Feb 2000 (TUCH).

V. odorata L. CENTRAL NEPAL: Dani 205, Feb 2001 (TUCH).

V. pilosa Blume. CENTRAL NEPAL: Dani 136, Apr 2000, TUCH; Dani 139, Apr 2000 (TUCH).

V. pogonantha W.W. Sm. CENTRAL NEPAL: Dani 140, Apr 2000 (TUCH).

V. sikkimensis W. Becker, EAST INDIA (Darjeeling), Kanai et al. 2935, May 1960 (BM).

V. thomsonii Oudem. CENTRAL NEPAL: Dani 137, Apr 2000, TUCH; Pokhrel 9, Jun 2001 (TUCH).

Subgen. Dischidium (Ging.) Peterm.

V. biflora L. CENTRAL NEPAL: Dani 144, Jul 2000 (TUCH); Dani 147, Jul 2000 (TUCH); Shrestha 2 (TUCH).


Subgen. Melanium (Ging.) Peterm.

Viola tricolor L. CENTRAL NEPAL: Dani 206, Feb 2001 (TUCH).

References


