

## Study on seed morphometry of some threatened Western Himalayan orchids

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**Abstract:** Physical seed characteristics of 32 threatened Western Himalayan orchids belonging to 23 genera of 3 subfamilies were studied using light and scanning electron microscopy. The species showed remarkable seed shape variations (fusiform, spatulate, ovoid, filiform) irrespective of their taxonomic position. Epiphytes produced relatively small-sized seeds compared to their terrestrial, lithophytic, or mycoheterotrophic counterparts. Seeds were truncated (length/width < 6.0) in 27 species, and were mostly elongated (length/width =  $19.957 \pm 1.459$ ) in the orchidoid species *Goodyera biflora* (Lindl.) Hook.f. The number or size of testa cells was found fairly consistent at the genus level, and their walls were straight, sinuous, or undulate. Periclinal walls possessed vertical or oblique ornamentation in 6 species, which is of taxonomic implication. Embryos were rather tiny and most of the seed space (even >90% in 7 species) was occupied by air. The highest seed to embryo volume ratio was recorded in terrestrial *Liparis odorata* (Willd.) Lindl. and the lowest in epiphytic *Rhynchostylis retusa* (L.) Blume, both of which are members of the advanced Epidendroideae. Seed characteristics are important in elucidating taxonomic, phylogenetic, and phytogeographic relationships among different orchid taxa.

**Key words:** Himalaya, orchid, seed shape, embryo, micromorphology, percent air space

### 1. Introduction

Orchid seeds are the smallest in the plant kingdom. They are produced in very large numbers; a single fruit of *Cycnoches ventricosum* Bateman var. *chlorochilon* (Klotzsch) P.H.Allen can house up to 4 million seeds (Arditti and Ghani, 2000). If all of the orchid seeds managed to germinate, the jungles on earth would be converted into thick orchid mats with striking color combinations. Generally, such a huge number of seeds are produced by those plants that have some very specific requirement(s) for their germination (Rauh et al., 1975; Rasmussen, 1995), and the Orchidaceae are no exception to this. Despite the large numbers in which seeds are produced, seed germination is exceptionally low (less than 1%) in orchids. Their embryos lack access to nutrient reserves, and the successful germination of seeds in vivo is obligatory to a fungal stimulus (mycotrophy). Because of their minute size, the seeds are extremely light-weight; the heaviest seeds weigh just 14–17 µg in species of *Galeola* Lour., while they are the lightest (0.3–0.4 µg) in *Anguloa* Ruiz & Pav. spp. (Arditti and Ghani, 2000). Cellular organization of seeds is also very simple. They are just an undifferentiated mass of embryonal cells that is enclosed

within a more or less transparent seed coat (Arditti et al., 1979). However, despite their dust-like nature and simple organization, a great deal of diversity is met in the size, shape, and structure of orchid seeds. Barthlott and Ziegler (1981) recognized 20 different seed types in orchids, based primarily upon their shape, relative elongation of seed coat (testa) cells, cell wall sculpturing, and presence of intercellular gaps and beadings. Seed characteristics are quite conservative compared to vegetative and floral ones (Chase and Pippen, 1988), and therefore they are of good taxonomic, phylogenetic, and phytogeographic importance in orchids (Clifford and Smith, 1969; Barthlott, 1976; Healey et al., 1980; Molvray and Kores, 1995; Arditti and Ghani, 2000; Aybeke, 2007; Gamarra et al., 2008, 2010, 2012; Chemisquy et al., 2009; Akcin et al., 2010) as well as other plant groups (Abdel Khalik, 2013; Bona, 2013; Mostafavi et al., 2013). Furthermore, as seeds are directly responsible for the regeneration and distribution of species, they play an important role in orchid conservation.

The Himalayan range is one of the most important mountain ecosystems of the world. It stretches nearly 2400 km in length and arises from low-lying plains to over 8000 m a.s.l. The Himalayan range provides a

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home to some of the highest peaks on the planet, including the highest, Mount Everest. There are 3 important geographical divisions of the Himalayan range: 1- Western Himalaya, comprising the northern part of Afghanistan and Pakistan and India (Jammu and Kashmir, Himachal Pradesh, Uttarakhand) up to the western border of Nepal; 2- Central Himalaya, which falls in Nepal; and 3- Eastern Himalaya, extending from the North Bengal hills to Sikkim, Bhutan, and Arunachal Pradesh. Such a geographical and, therefore, climatic diversity is marvelously reflected in the rich variety of flora and fauna supported by this complex system of mountain chains. The Indian Himalayan Region alone houses nearly 50% of the total flowering plants of India (Singh, 2006) and is also an important habitat for more than 850 orchid species (Singh, 2001).

The present study was conducted on Western Himalayan orchids. The majority of these species are widely distributed across this Himalayan segment (Table 1), and seeds were collected from populations growing in the state of Himachal Pradesh, India. *Eria tomentosa* (J.Koenig) Hook.f. is an Eastern Himalayan taxon and does not grow naturally in Western Himalaya. A plant of this species was procured from the National Research Centre for Orchids (NRCO), Pakyong, Sikkim, India, and was kept in a polyhouse at Solan (Himachal Pradesh). Its flowers were hand-pollinated for obtaining fruits and seeds. The species has been grown ex situ for multiplication in vitro. Seedlings of this Eastern Himalayan species will be introduced to some natural habitats in Himachal Pradesh. Seeds of this species were also investigated in the present study. Himachal Pradesh (30°22'N–33°12'N, 75°47'E–79°04'E) comprises a series of several more or less parallel converging mountain ranges with a wider range of altitudes (350–7000 m). There are 5 physiographic zones in the state: a wet subtemperate zone, humid subtemperate zone, dry temperate zone, humid subtropical zone, and subhumid tropical zone. The climate is as varied as the physical configuration and is markedly influenced by the aspect and altitude of the mountains. It varies from hot and humid in the lower hills to warm-temperate, alpine, and glacial in the higher mountains. Spiti Valley experiences drier conditions as it is almost cut off by the high mountain ranges, while Kangra experiences maximum rainfall. Altitudes above 2000 m experience snowfall during winters. The soils are also quite variable and determine the nature and type of vegetation they support. At altitudes below 1000 m, these are mostly sandy loam, light gray to brown, superficial, and bound in pebbles, stones, and boulders. Between 1000 and 2200 m, they are deep, loam to silty loam, gray to dark brown, and with rich potassium and poor gravel contents; they are prone to drought due to quick internal drainage. At

higher elevations (2200–3200 m), the soils are shallower and range from silty loam to loamy, well-drained and dark-brown. The epiphytic species of Himachal Pradesh are confined to altitudes below 1400 m, where they prefer rough-barked hosts for their germination, growth, and development.

Depending on the dispersal capacity of seeds and nutritional and microclimatic requirements for their germination, orchids experience different distributional patterns across the Himalayan range. The Western Himalaya supports more ground-growing species, whereas epiphytes dominate in Eastern Himalaya. Despite their rich diversity in the Himalaya, only a few orchids have so far been studied for their detailed seed morphometric characteristics (Garg et al., 1992; Vij et al., 1992; Rani et al., 1993; Sharma et al., 2004; Verma et al., 2012). Furthermore, these investigations (except Sharma et al., 2004) lacked statistical analyses of the data. Therefore, a study was conducted on various seed characteristics (shape; length, width, number, and size of testa cells; seed and embryo volume; percent air space) of 32 Himalayan orchid species. They belong to 23 genera (11 tribes) of 3 subfamilies, Cypridiaceae, Orchidoideae, and Epidendroideae (Cameron et al., 1999), and they exhibit varied life modes (terrestrial, lithophytic, mycoheterotrophic, epiphytic). Owing to habitat loss and collection pressures, all of these species are facing varied degrees of threat to their natural populations. While all of them are mentioned in Appendix II of CITES, *Cypripedium cordigerum* D.Don is also listed in the Red Data Book of Indian plants (Nayar and Sastry, 1988). The aim of the present study was to assess the taxonomic, phylogenetic, and phytogeographical importance of seed morphometric characteristics in these orchids. The results were analyzed statistically and photographs (light microscope, scanning electron microscope) are provided uniformly for each species.

## 2. Materials and methods

Field trips were organized (2009–2012) in Western Himalaya to locate different orchid species. These species were identified following standard flora (King and Pantling, 1898; Deva and Naithani, 1986) using both vegetative and floral characteristics. In the field, 2 or 3 flowering individuals of each species were marked using paraffin wax-dipped paper tags and the seeds were collected later (after 30–40 days) from their mature/dehiscing fruits. Table 1 summarizes the taxonomic position, life modes, and collection and distributional details of the presently studied orchid species. For each species, a voucher specimen was deposited at the Herbarium of the Botany Department, Panjab University, Chandigarh, India (PAN). The seeds were directly fixed in FAA (1:1:18 of formalin,

**Table 1.** Taxonomic position and collection and distributional details of presently studied Western Himalayan orchids.

S. no.	Taxa	Collection details			Life mode*	Distribution	
		Locality, district (altitude)	Habitat	Voucher no.		India and neighboring countries**	Range (m)
Subfamily: Cypripedioideae							
Tribe: Cypripedieae							
1	<i>Cypripedium cordigerum</i> D.Don	Charabra, Shimla (2510 m)	Shady forest floor	Vij & Verma (281)	TER	CH, JK, HP, UK	2500–3000
Subfamily: Orchidoideae							
Tribe: Cranichideae							
2	<i>Goodyera biflora</i> (Lindl.) Hook.f.	Karol, Solan (1600 m)	Shady forest floor	Kusum (508)	TER	CH, HP, NP, UK	1500–2500
3	<i>Spiranthes sinensis</i> (Pers.) Ames	Karol, Solan (1600 m)	Open grassland	Kusum (507)	TER	BD, CH, EH, HP, JK, NP, PI, PK, SL, UK	500–2500
Tribe: Orchideae							
4	<i>Androcorys monophylla</i> (D.Don) Agrawala & H.J.Chowdhery	Khanog, Solan (1580 m)	Shady forest floor	Kusum (515)	TER	HP, NP, UK	800–2000
5	<i>Brachycorythis obcordata</i> (Buch.-Ham. ex D.Don) Summerh.	Kasauli, Solan (1827 m)	Shady forest floor	Kusum (503)	TER	BD, BH, EH, HP, UK	1500–2000
6	<i>Dactylorhiza hatagirea</i> (D.Don) Soo	Sissu, Lahul and Spiti (3050 m)	Open grassland	Kusum (513)	TER	BD, CH, EH, HP, JK, NP, PK, UK	2800–4000
7	<i>Dithrix griffithii</i> (Hook. f.) Ormerod & Gandhi	Dedhgharat, Solan (1420 m)	Shady forest floor	Kusum (515a)	TER	EH, HP, JK, PK, UK	1600–3000
8	<i>Habenaria aitchisonii</i> Rchb.f.	Khanog, Solan (1580 m)	Shady forest floor	Kusum (528)	TER	BD, EH, HP, JK, NP, PK, UK	2000–4000
9	<i>Habenaria intermedia</i> D.Don	Forest road, Solan (1460 m)	Shady forest floor	Vij & Verma (249)	TER	CH, EH, HP, JK, NP, UK	1500–2800
10	<i>Habenaria pectinata</i> (J.E.Sm.) D.Don	Garhkhal, Solan (1760 m)	Shady forest floor	Kusum (529)	TER	BD, CH, EH, HP, JK, NP, PK, UK	1500–3500
11	<i>Habenaria plantaginea</i> Lindl.	Jwalaji, Kangra (820 m)	Shady forest floor	Vij & Verma (308)	TER	EH, HP, JK, NP, PI, SL, UK	800–1000
12	<i>Habenaria pubescens</i> Lindl.	Sulmana, Solan (1450 m)	Shady forest floor	Kusum (504)	TER	EH, HP, NP, UK	600–1500
13	<i>Herminium lanceum</i> (Thunb. ex Sw.) Vujik	Dharampur, Solan (1350 m)	Shady forest floor	Kusum (517)	TER	BD, CH, EH, HP, JK, NP, PK, UK	1250–3000
14	<i>Pecteilis gigantea</i> (J.E.Sm.) Rafin.	Tihra, Mandi (1020 m)	Open grassland	Verma (509)	TER	EH, HP, JK, PI, PK, UK	900–2000
15	<i>Peristylus affinis</i> (D.Don) Seidenf.	Sadhupul, Solan (1250 m)	Shady forest floor	Vij & Verma (309)	TER	CH, EH, HP, NP, UK	1400–2200
16	<i>Platanthera clavigera</i> Lindl.	Karsog, Mandi (1560)	Open grassland	Kusum (514)	TER	CH, EH, HP, NP, UK	1550–4000
17	<i>Platanthera edgeworthii</i> (Hook.f. ex Collett) R.K.Gupta	Nauradhar, Sirmaur (2500 m)	Open grassland	Vij & Verma (220)	TER	EH, HP, JK, NP, PK, UK	1500–3000
18	<i>Platanthera latilabris</i> Lindl.	Taradevi, Shimla (1820 m)	Open grassland	Kusum (510)	TER	CH, EH, HP, JK, NP, UK	1500–3000
Tribe: Diseae							

Table 1. (Continued).

S. no.	Taxa	Collection details			Life mode*	Distribution	
		Locality, district (altitude)	Habitat	Voucher no.		India and neighboring countries**	Range (m)
19	<i>Satyrium nepalense</i> D.Don Subfamily: Epidendroideae Tribe: Neottieae	Taradevi, Shimla (1820 m)	Shady forest floor	Vij & Verma (101)	TER	BD, CH, EH, HP, JK, NP, PI, PK, SL, UK	1500–3000
20	<i>Epipactis gigantea</i> Dougl. ex Hook.f.	Taradevi, Shimla (1820 m)	Open grassland	Vij & Verma (216)	TER	CH, EH, HP, JK, NP, PK, UK	1800–4000
21	<i>Epipactis helleborine</i> (L.) Crantz Tribe: Gastrodieae	Taradevi, Shimla (1820 m)	Open grassland	Vij & Verma (217)	TER	CH, EH, HP, JK, NP, PK, UK	1500–4000
22	<i>Gastrodia falconeri</i> D.L.Jones & M.A.Clem. Tribe: Malaxideae	Kothi, Kullu (2586 m)	Shady forest floor	Vij & Verma (219)	MYC	EH, HP, JK, PK, UK	2500–3000
23	<i>Crepidium acuminatum</i> (D.Don) Szlach.	Kasauli, Solan (1820 m)	Shady forest floor	Kusum (521)	TER/ LIT	BD, CH, EH, HP, NP, PI, UK	1500–2300
24	<i>Liparis odorata</i> (Willd.) Lindl.	Kaithalighat, Solan (1750 m)	Open grassland	Vij & Verma (298)	TER	CH, EH, HP, NP, UK	1400–2400
25	<i>Liparis rostrata</i> Rchb.f. Tribe: Calypsoeae	Taradevi, Solan (1800 m)	Shady forest floor	Kusum (501)	TER	EH, HP, JK, NP, PK, UK	1500–2500
26	<i>Oreorchis micrantha</i> Lindl. Tribe: Cymbidieae	Kufri, Shimla (2480 m)	Shady forest floor	Vij & Verma (284)	TER	CH, EH, HP, JK, NP, PK, UK	2400–3300
27	<i>Cymbidium macrorhizon</i> Lindl.	Jatoli, Solan (1380 m)	Shady forest floor	Kusum (524)	MYC	CH, EH, HP, NP, PK, UK	1300–2500
28	<i>Eulophia herbacea</i> Lindl.	Solan (1500 m)	Open grassland	Kusum (520)	TER	BD, CH, EH, HP, NP, PI, UK	1200–1500
29	<i>Eulophia hormusjii</i> Duthie Tribe: Podochileae	Shaktinagar, Solan (1450 m)	Open grassland	Kusum (506)	TER	EH, HP, NP, PI, PK, UK	1400–1800
30	<i>Eria tomentosa</i> (J.Koenig) Hook.f.*** Tribe: Vandeeae	Solan (1450 m)	Kept ex situ at a polyhouse, otherwise bright forest	Kusum (521a)	EPI	BD, CH, EH	800–1500
31	<i>Gastrochilus calceolaris</i> (J.E.Sm.) D.Don	Nagrota, Kangra (860 m)	Bright forest	Vij & Verma (178)	EPI	AN, CH, EH, HP, JK, NP, UK	850–1600
32	<i>Rhynchostylis retusa</i> (L.) Blume	Tihra, Mandi (1020 m)	Bright forest	Vij & Verma (316)	EPI	AN, BD, CH, EH, HP, JK, NP, PI, SL, UK	600–1200

\*Life mode: EPI, epiphyte; LIT, lithophytic; MYC, mycoheterotrophic; TER, terrestrial. \*\*Distribution: AN, Andaman & Nicobar Islands; BD, Bangladesh; CH, China; EH, Eastern Himalaya & Northeast India; HP, Himachal Pradesh; JK, Jammu & Kashmir; NP, Nepal & Bhutan; PI, Peninsular India; PK, Pakistan; SL, Sri Lanka; UK, Utrakhand. \*\*\**Eria tomentosa* does not occur naturally in Western Himalaya. A plant of this species was procured from the National Research Centre for Orchids (Pakyong, Sikkim) and kept in a polyhouse at Solan. Its flowers were hand-pollinated to obtain fruits and seeds.

acetic acid, and 50% ethyl alcohol) at the time of collection in the field and were later mounted in 10% glycerin for light microscopic studies. Seed length and width were measured (at the longest and widest axis) using a light microscope and standardized ocular meter. The results are based on observations made in seed samples taken randomly from 1–2 fruit(s) in each species. Characteristics such as seed volume, embryo volume, and percent air space were calculated following Arditti et al. (1979). For calculating seed volume, the formula  $2[(W/2)^2 \times (L/2) \times (1.047)]$  was applied, where  $W$  = seed width,  $L$  = seed length, and  $1.047$  = value of  $(\pi/3)$ . As embryos were generally elliptical in cross-section, their volume was calculated using the formula  $4/3 \times \pi \times (L/2) \times (W/2)^2$ , where  $L$  = embryo length and  $W$  = embryo width. Seeds with length/width ( $L/W$ ) ratio of  $<6.0$  were designated as truncated and those with  $L/W$  of  $>6.0$  as elongated. The percent air space was calculated by subtracting embryo volume from the seed volume of the same seed. Detailed seed coat characteristics were observed by scanning electron microscopy following Vij et al. (1992). For this purpose, the seeds were gradually dehydrated through an ethyl alcohol series first, and finally dried to critical-point (for 10–15 min) using  $\text{CO}_2$  as a transition fluid. They were then mounted on aluminum stubs and stored in a calcium chloride desiccator until use. The samples were subsequently gold-coated and observed using a JEOL JSM-6100 scanning electron microscope at 10 kV. For seed shape, the terminology of Clifford and Smith (1969) and Vij et al. (1992) was used with a slight modification: for ‘filamentous’ seed shape, we have used the word ‘filiform’.

The data for each species were collected in 10 replicates and the values expressed in Table 2 are means of these. Results were analyzed using a completely random design. These were subjected to one-way analysis of variance and post hoc tests to detect the significant differences ( $P \leq 0.05$ ) in seed characteristics among different species using SPSS 17.0 (SPSS Inc., USA).

### 3. Results

The seeds of the presently studied Himalayan orchids exhibited diversity in their shape, size, and seed coat (testa cell number, size, ornamentation) and embryo characteristics. The salient results are summarized in Table 2 and in what follows they are presented in detail.

Seeds were fusiform in 16 species, spathulate in 11, and ovoid in 4 (Figures 1–5). Fusiform seeds were observed in all subfamilies while filiform were present only in Orchidoideae species *Goodyera biflora* (Lindl.) Hook.f. (Figure 1). Variations were observed in seed shape even at the tribe level; they were filiform and ovoid in Cranichideae; ovoid, spathulate, and fusiform in Orchideae; and spathulate, fusiform, and ovoid in Cymbidieae. In the

majority of cases, however, the seed shape was consistent at the genus level. Seeds were uniformly spathulate in the presently investigated species of *Platanthera* Rich. (Figure 3) and *Liparis* Rich. (Figure 4) and were fusiform in *Epipactis* Zinn (Figure 3) and *Habenaria* Willd. (Figure 2), except for *H. aitchisonii* Rchb.f., where they were spathulate (Figure 2). Seed shape, however, differed in 2 species of *Eulophia* R.Br. ex Lindl.; it was fusiform in *E. herbacea* Lindl. (Figure 4) but ovoid in *E. hormusjii* Duthie (Figure 5). Seed shape showed no definite relation with plant habit; while all epiphytes studied presently possessed fusiform seeds (Figure 5), this was not the situation in the case of terrestrials and mycoheterotrophs.

Presence of smallest seeds is one of the most distinctive features of the family Orchidaceae. However, despite their microscopic nature, the seeds showed a great diversity in their size (Table 2). The seed length ranged between  $0.113 \pm 0.005$  mm (*Eria tomentosa*) and  $1.796 \pm 0.037$  mm (*Goodyera biflora*), and width ranged from  $0.041 \pm 0.003$  mm [*Rhynchostylis retusa* (L.) Blume] to  $0.296 \pm 0.038$  mm [*Spiranthes sinensis* (Pers.) Ames]. Significant differences were noticed in seed length and width at tribe and/or genus level. The  $L/W$  ratio, which provides information on the degree of seed truncation, revealed that the seeds were truncated ( $L/W < 6.0$ ) in 27 species and elongated ( $L/W > 6.0$ ) in the remaining 5 (*Goodyera biflora*, *Habenaria pubescens* Lindl., *Peristylus affinis* (D. Don) Seidenf., *Oreorchis micrantha* Lindl., *Eulophia herbacea*). The most truncated seeds ( $L/W = 1.700 \pm 0.122$ ) were observed in *Androcorys monophylla* (D. Don) Agrawala & H.J. Chowdhery (Figure 1) and the most elongated ( $L/W = 19.957 \pm 1.459$ ) in *Goodyera biflora* (Figure 1), both of which are members of subfamily Orchidoideae. Seed volume showed significant variations both in orchidoid and epidendroid orchids; it was lowest in epiphytic *Eria tomentosa* [ $(0.096 \pm 0.010 \text{ mm}^3) \times 10^{-3}$ ] and highest in terrestrial *Epipactis gigantea* Dougl. ex Hook.f. [ $(14.849 \pm 0.742 \text{ mm}^3) \times 10^{-3}$ ].

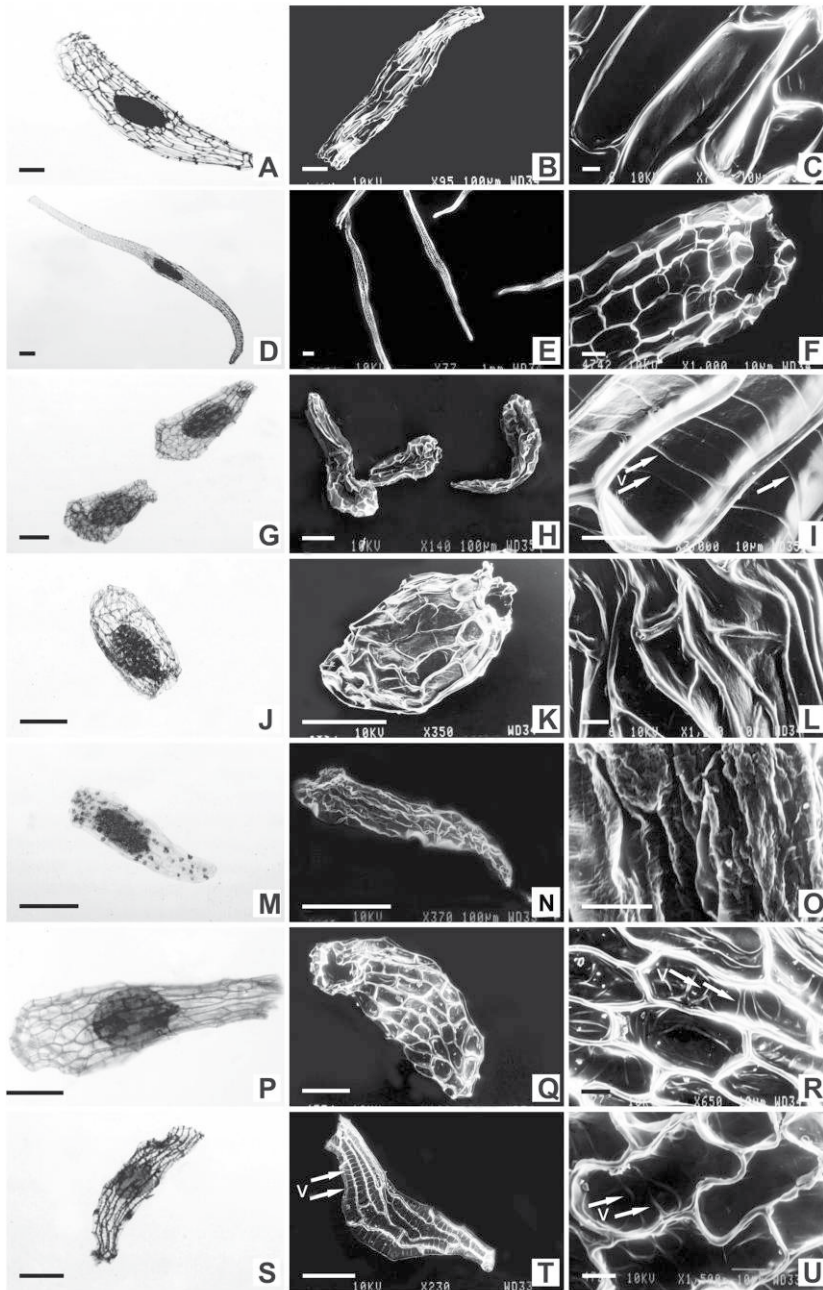
The micromorphology of seeds revealed that their testa cells were quadrilateral in shape. However, more or less pentagonal to hexagonal cells were observed in *Androcorys monophylla* (Figure 1), *Dactylorhiza hatagirea* (D. Don) Soo (Figure 1), and *Gastrodia falconeri* D.L. Jones & M.A. Clem. (Figure 4). In *Crepidium acuminatum* (D. Don) Szlach. (Figure 4) and *Liparis odorata* (Willd.) Lindl. (Figure 4), they were irregular in shape. The testa cells were oriented longitudinally and arranged straight except in *Eulophia herbacea* (Figure 4) and *Eria tomentosa* (Figure 5), where they were twisted. Cell walls were straight to sinuous in the majority of the species but moderately to highly undulate in *Spiranthes sinensis* (Figure 1), *Dithrix griffithii* (Hook.f.) Ormerod & Gandhi (Figure 1), *Crepidium acuminatum* (Figure 4), and *Liparis rostrata* Rchb.f. (Figure 4).

Table 2. Seed and embryo dimensions of presently studied Western Himalayan orchids.

S. no.	Taxa	Shape	Seeds				Testa cells				Embryo			Seed vol/embryo vol		Seed air space (%)
			L (mm)	W (mm)	L/W	Vol (mm <sup>3</sup> × 10 <sup>-3</sup> )	Walls	No. at long. axis	Length (µm)	L (mm)	W (mm)	L/W	Vol (mm <sup>3</sup> × 10 <sup>-3</sup> )	Seed vol/embryo vol		
A. Subfamily: Cypripedioideae																
Tribe: Cypripedieae																
1.	<i>Cypripedium cordigerum</i>	Fusiform	0.734 ± 0.732 <sup>n</sup>	0.149 ± 0.011 <sup>n</sup>	4.966 ± 0.515 <sup>gh</sup>	4.262 ± 0.552 <sup>k</sup>	Simuous	9.800 ± 0.788 <sup>i</sup>	75.557 ± 8.870 <sup>jk</sup>	0.147 ± 0.011 <sup>m</sup>	0.086 ± 0.004 <sup>oop</sup>	1.722 ± 0.136 <sup>deefg</sup>	0.565 ± 0.038 <sup>de</sup>	7.710 ± 1.710 <sup>abc</sup>	86.483 ± 2.782 <sup>gh</sup>	
B. Subfamily: Orchidoideae																
Tribe: Cranichideae																
2.	<i>Goodyera biflora</i>	Filiform	1.796 ± 0.037 <sup>q</sup>	0.090 ± 0.005 <sup>efgh</sup>	19.957 ± 1.459 <sup>m</sup>	3.849 ± 0.403 <sup>j</sup>	Straight/ simuous	27.600 ± 2.913 <sup>m</sup>	65.962 ± 9.110 <sup>hi</sup>	0.132 ± 0.004 <sup>gh</sup>	0.056 ± 0.002 <sup>lmn</sup>	2.326 ± 0.095 <sup>ij</sup>	0.222 ± 0.025 <sup>bc</sup>	17.824 ± 1.901 <sup>def</sup>	91.117 ± 0.885 <sup>gh</sup>	
3.	<i>Spiranthes sinensis</i>	Ovoid	0.287 ± 0.010 <sup>gh</sup>	0.296 ± 0.038 <sup>kl</sup>	2.728 ± 0.386 <sup>bc</sup>	0.882 ± 0.261 <sup>def</sup>	Simuous/ undulate	9.500 ± 0.527 <sup>hi</sup>	30.337 ± 2.336 <sup>bc</sup>	0.094 ± 0.016 <sup>fgh</sup>	0.074 ± 0.005 <sup>ijklm</sup>	1.253 ± 0.084 <sup>ab</sup>	0.277 ± 0.084 <sup>cd</sup>	3.311 ± 1.167 <sup>ab</sup>	67.768 ± 6.625 <sup>de</sup>	
Tribe: Orchideae																
4.	<i>Androcorys monophylla</i>	Ovoid	0.180 ± 0.008 <sup>bed</sup>	0.112 ± 0.008 <sup>ghl</sup>	1.700 ± 0.122 <sup>a</sup>	0.627 ± 0.105 <sup>bode</sup>	Simuous	5.100 ± 0.875 <sup>de</sup>	38.093 ± 6.115 <sup>bcedf</sup>	0.118 ± 0.011 <sup>kl</sup>	0.084 ± 0.011 <sup>mmo</sup>	1.407 ± 0.067 <sup>bcd</sup>	0.451 ± 0.148 <sup>gh</sup>	1.553 ± 0.610 <sup>a</sup>	28.228 ± 2.539 <sup>b</sup>	
5.	<i>Brachycorythis obcordata</i>	Spathulate	0.247 ± 0.032 <sup>efg</sup>	0.048 ± 0.004 <sup>ab</sup>	5.079 ± 0.585 <sup>gh</sup>	0.156 ± 0.044 <sup>ab</sup>	Undulate	8.100 ± 0.994 <sup>gh</sup>	31.177 ± 7.451 <sup>bcd</sup>	0.081 ± 0.003 <sup>acdef</sup>	0.038 ± 0.004 <sup>bc</sup>	2.170 ± 0.236 <sup>hi</sup>	0.060 ± 0.012 <sup>a</sup>	2.637 ± 0.620 <sup>ab</sup>	60.182 ± 9.129 <sup>d</sup>	
6.	<i>Dactylothis hatagirea</i>	Spathulate	0.321 ± 0.328 <sup>ij</sup>	0.133 ± 0.009 <sup>m</sup>	2.399 ± 0.505 <sup>abc</sup>	1.519 ± 0.533 <sup>hi</sup>	Simuous	9.200 ± 0.788 <sup>hi</sup>	34.953 ± 8.420 <sup>bode</sup>	0.132 ± 0.011 <sup>lmn</sup>	0.089 ± 0.003 <sup>op</sup>	1.486 ± 0.109 <sup>bode</sup>	0.545 ± 0.075 <sup>hi</sup>	2.821 ± 1.026 <sup>ab</sup>	59.835 ± 14.686 <sup>d</sup>	
7.	<i>Dithrix griffithii</i>	Fusiform	0.351 ± 0.347 <sup>jk</sup>	0.081 ± 0.005 <sup>de</sup>	4.312 ± 0.883 <sup>ef</sup>	0.615 ± 0.144 <sup>bode</sup>	Undulate	4.700 ± 0.483 <sup>cd</sup>	75.320 ± 16.921 <sup>ijk</sup>	0.074 ± 0.011 <sup>ate</sup>	0.052 ± 0.004 <sup>fg</sup>	1.424 ± 0.109 <sup>bcd</sup>	0.108 ± 0.033 <sup>a</sup>	6.163 ± 2.484 <sup>abc</sup>	81.708 ± 5.827 <sup>efgh</sup>	
8.	<i>Habenaria aitchisonii</i>	Spathulate	0.240 ± 0.233 <sup>defg</sup>	0.087 ± 0.010 <sup>efg</sup>	2.793 ± 0.506 <sup>bc</sup>	0.482 ± 0.109 <sup>abcd</sup>	Straight/ simuous	4.600 ± 0.516 <sup>bcd</sup>	53.080 ± 10.879 <sup>gh</sup>	0.102 ± 0.010 <sup>ghij</sup>	0.074 ± 0.005 <sup>ijkl</sup>	1.400 ± 0.191 <sup>bed</sup>	0.289 ± 0.038 <sup>de</sup>	1.658 ± 0.297 <sup>a</sup>	37.492 ± 14.005 <sup>bc</sup>	
9.	<i>Habenaria intermedia</i>	Fusiform	0.523 ± 0.516 <sup>i</sup>	0.091 ± 0.008 <sup>efgh</sup>	5.773 ± 0.836 <sup>hi</sup>	1.144 ± 0.219 <sup>gh</sup>	Straight/ simuous	4.500 ± 0.527 <sup>bcd</sup>	118.000 ± 21.881 <sup>i</sup>	0.139 ± 0.008 <sup>mm</sup>	0.070 ± 0.003 <sup>ijk</sup>	1.978 ± 0.070 <sup>fgh</sup>	0.273 ± 0.048 <sup>def</sup>	3.142 ± 0.274 <sup>ab</sup>	67.967 ± 2.642 <sup>de</sup>	
10.	<i>Habenaria pectinata</i>	Fusiform	0.379 ± 0.008 <sup>h</sup>	0.101 ± 0.009 <sup>ghij</sup>	3.793 ± 0.381 <sup>de</sup>	1.016 ± 0.184 <sup>efg</sup>	Straight/ simuous	4.800 ± 0.421 <sup>cd</sup>	79.600 ± 7.795 <sup>ijk</sup>	0.090 ± 0.007 <sup>efg</sup>	0.076 ± 0.004 <sup>klmm</sup>	1.192 ± 0.112 <sup>ab</sup>	0.273 ± 0.040 <sup>cd</sup>	3.764 ± 0.781 <sup>ab</sup>	72.362 ± 5.848 <sup>def</sup>	
11.	<i>Habenaria plantaginea</i>	Fusiform	0.401 ± 0.140 <sup>b</sup>	0.082 ± 0.005 <sup>def</sup>	4.892 ± 0.358 <sup>gh</sup>	0.716 ± 0.100 <sup>def</sup>	Straight/ simuous	4.500 ± 0.527 <sup>bcd</sup>	90.240 ± 9.906 <sup>k</sup>	0.090 ± 0.005 <sup>efg</sup>	0.046 ± 0.007 <sup>def</sup>	1.983 ± 0.283 <sup>ghi</sup>	0.104 ± 0.039 <sup>a</sup>	7.426 ± 2.152 <sup>abc</sup>	85.456 ± 4.415 <sup>gh</sup>	
12.	<i>Habenaria pubescens</i>	Fusiform	0.578 ± 0.009 <sup>m</sup>	0.094 ± 0.011 <sup>efgh</sup>	6.248 ± 0.669 <sup>i</sup>	1.343 ± 0.312 <sup>gh</sup>	Straight/ simuous	4.600 ± 0.516 <sup>bcd</sup>	127.280 ± 15.376 <sup>i</sup>	0.072 ± 0.021 <sup>cd</sup>	0.051 ± 0.018 <sup>efg</sup>	1.492 ± 0.534 <sup>bode</sup>	0.124 ± 0.022 <sup>ab</sup>	24.229 ± 5.814 <sup>fg</sup>	90.204 ± 8.797 <sup>efgh</sup>	
13.	<i>Herminium lancum</i>	Spathulate	0.264 ± 0.006 <sup>efgh</sup>	0.095 ± 0.005 <sup>efgh</sup>	2.778 ± 0.151 <sup>bc</sup>	0.627 ± 0.061 <sup>bode</sup>	Simuous	5.300 ± 0.674 <sup>de</sup>	50.560 ± 6.635 <sup>efgh</sup>	0.112 ± 0.010 <sup>ijk</sup>	0.082 ± 0.007 <sup>lmno</sup>	1.358 ± 0.030 <sup>bc</sup>	0.404 ± 0.094 <sup>efg</sup>	1.615 ± 0.309 <sup>a</sup>	36.077 ± 11.775 <sup>bc</sup>	
14.	<i>Pecteilis gigantea</i>	Fusiform	0.314 ± 0.013 <sup>hi</sup>	0.062 ± 0.014 <sup>bc</sup>	5.310 ± 1.079 <sup>gh</sup>	0.327 ± 0.151 <sup>abc</sup>	Simuous	9.300 ± 0.823 <sup>hi</sup>	33.971 ± 2.297 <sup>bcd</sup>	0.074 ± 0.011 <sup>ate</sup>	0.037 ± 0.004 <sup>bc</sup>	2.000 ± 0.236 <sup>efgh</sup>	0.054 ± 0.017 <sup>a</sup>	6.087 ± 1.826 <sup>abc</sup>	82.188 ± 5.931 <sup>efgh</sup>	
15.	<i>Peristylus affinis</i>	Fusiform	0.499 ± 0.033 <sup>i</sup>	0.064 ± 0.011 <sup>c</sup>	7.959 ± 1.092 <sup>i</sup>	0.557 ± 0.234 <sup>abcde</sup>	Simuous	7.000 ± 0.942 <sup>fg</sup>	72.252 ± 8.760 <sup>ij</sup>	0.078 ± 0.014 <sup>def</sup>	0.026 ± 0.003 <sup>a</sup>	3.067 ± 0.439 <sup>i</sup>	0.028 ± 0.013 <sup>a</sup>	19.865 ± 8.142 <sup>efg</sup>	91.253 ± 1.270 <sup>gh</sup>	
16.	<i>Platanthera clavigera</i>	Spatulate	0.250 ± 0.010 <sup>efg</sup>	0.119 ± 0.013 <sup>i</sup>	2.119 ± 0.185 <sup>ab</sup>	0.946 ± 0.232 <sup>defg</sup>	Simuous	4.400 ± 0.516 <sup>bcd</sup>	57.600 ± 6.948 <sup>gh</sup>	0.108 ± 0.005 <sup>ijkl</sup>	0.094 ± 0.008 <sup>pq</sup>	1.150 ± 0.092 <sup>ab</sup>	0.507 ± 0.103 <sup>gh</sup>	1.863 ± 0.468 <sup>a</sup>	44.388 ± 13.582 <sup>c</sup>	

17.	<i>Platanthera edgeworthii</i>	Spathulate	0.248 ± 0.009 <sup>efg</sup>	0.115 ± 0.004 <sup>hi</sup>	2.154 ± 0.072 <sup>ab</sup>	0.863 ± 0.086 <sup>def</sup>	Simuous	5.400 ± 0.843 <sup>de</sup>	47.093 ± 8.377 <sup>defg</sup>	0.116 ± 0.015 <sup>hkl</sup>	0.099 ± 0.010 <sup>q</sup>	1.174 ± 0.548 <sup>ab</sup>	0.618 ± 0.182 <sup>ij</sup>	1.395 ± 0.520 <sup>a</sup>	28.924 ± 8.472 <sup>b</sup>
18.	<i>Platanthera latilabris</i>	Spathulate	0.199 ± 0.024 <sup>bcde</sup>	0.092 ± 0.009 <sup>efgh</sup>	2.189 ± 0.360 <sup>ab</sup>	0.441 ± 0.088 <sup>abcd</sup>	Simuous/ undulate	5.600 ± 0.516 <sup>de</sup>	35.866 ± 5.386 <sup>bcde</sup>	0.092 ± 0.005 <sup>ghlmn</sup>	0.076 ± 0.005 <sup>klmn</sup>	1.223 ± 0.063 <sup>ab</sup>	0.282 ± 0.056 <sup>cd</sup>	1.607 ± 0.394 <sup>a</sup>	34.052 ± 7.158 <sup>bc</sup>
Tribe: Diaseae															
19.	<i>Satyrium nepalense</i>	Ovoid	0.253 ± 0.005 <sup>efg</sup>	0.102 ± 0.015 <sup>hij</sup>	2.542 ± 0.349 <sup>bc</sup>	0.698 ± 0.215 <sup>cdef</sup>	Simuous	3.200 ± 0.918 <sup>ab</sup>	86.533 ± 28.711 <sup>jk</sup>	0.122 ± 0.003 <sup>kl</sup>	0.078 ± 0.007 <sup>klmn</sup>	1.578 ± 0.062 <sup>bcdef</sup>	0.384 ± 0.062 <sup>def</sup>	1.817 ± 0.474 <sup>a</sup>	42.305 ± 11.496 <sup>bc</sup>
C. Subfamily: Epidendroideae															
Tribe: Neottieae															
20.	<i>Epipactis gigantea</i>	Fusifiform	0.780 ± 0.014 <sup>n</sup>	0.269 ± 0.005 <sup>o</sup>	2.893 ± 0.060 <sup>bcd</sup>	14.849 ± 0.742 <sup>n</sup>	Simuous	16.900 ± 1.286 <sup>i</sup>	46.385 ± 3.472 <sup>cdefg</sup>	0.218 ± 0.012 <sup>p</sup>	0.120 ± 0.007 <sup>r</sup>	1.827 ± 0.170 <sup>defgh</sup>	1.646 ± 0.217 <sup>h</sup>	8.844 ± 0.953 <sup>abc</sup>	88.923 ± 1.285 <sup>gh</sup>
21.	<i>Epipactis helleborine</i>	Fusifiform	0.840 ± 0.097 <sup>o</sup>	0.181 ± 0.006 <sup>o</sup>	4.632 ± 0.579 <sup>efg</sup>	7.255 ± 1.017 <sup>i</sup>	Simuous	14.800 ± 1.032 <sup>k</sup>	56.995 ± 8.030 <sup>gh</sup>	0.122 ± 0.011 <sup>q</sup>	0.101 ± 0.006 <sup>q</sup>	1.209 ± 0.131 <sup>ab</sup>	0.661 ± 0.109 <sup>i</sup>	11.138 ± 2.734 <sup>abcd</sup>	90.740 ± 1.914 <sup>gh</sup>
Tribe: Gastrodieae															
22.	<i>Gastrodia falconeri</i>	Fusifiform	0.494 ± 0.078 <sup>i</sup>	0.103 ± 0.006 <sup>hik</sup>	4.840 ± 0.954 <sup>efg</sup>	1.367 ± 0.212 <sup>gh</sup>	Simuous	9.400 ± 1.264 <sup>hi</sup>	53.132 ± 10.116 <sup>gh</sup>	0.100 ± 0.014 <sup>gh</sup>	0.045 ± 0.007 <sup>cdef</sup>	2.219 ± 0.318 <sup>hi</sup>	0.114 ± 0.046 <sup>e</sup>	14.634 ± 8.072 <sup>cde</sup>	91.690 ± 3.198 <sup>gh</sup>
Tribe: Malaxideae															
23.	<i>Crepidium acuminatum</i>	Spathulate	0.177 ± 0.005 <sup>bc</sup>	0.076 ± 0.003 <sup>a</sup>	2.320 ± 0.163 <sup>ab</sup>	0.274 ± 0.028 <sup>abc</sup>	Highly undulate	15.000 ± 0.942 <sup>k</sup>	11.886 ± 0.918 <sup>a</sup>	0.049 ± 0.004 <sup>b</sup>	0.032 ± 0.003 <sup>ab</sup>	1.570 ± 0.232 <sup>bcdef</sup>	0.026 ± 0.007 <sup>a</sup>	11.106 ± 3.865 <sup>bcd</sup>	90.183 ± 2.406 <sup>gh</sup>
24.	<i>Liparis odorata</i>	Spathulate	0.277 ± 0.022 <sup>ghi</sup>	0.096 ± 0.010 <sup>ghi</sup>	2.923 ± 0.392 <sup>bcd</sup>	0.676 ± 0.160 <sup>cdef</sup>	Highly undulate	6.400 ± 0.516 <sup>ef</sup>	43.580 ± 4.564 <sup>bcdefg</sup>	0.045 ± 0.010 <sup>ab</sup>	0.034 ± 0.003 <sup>ab</sup>	1.320 ± 0.265 <sup>abc</sup>	0.029 ± 0.013 <sup>a</sup>	25.854 ± 8.457 <sup>b</sup>	91.821 ± 1.685 <sup>b</sup>
25.	<i>Liparis rostrata</i>	Spathulate	0.273 ± 0.006 <sup>efghi</sup>	0.095 ± 0.009 <sup>efghi</sup>	2.898 ± 0.267 <sup>bcd</sup>	0.655 ± 0.140 <sup>ade</sup>	Simuous	8.300 ± 0.823 <sup>gh</sup>	33.257 ± 3.381 <sup>bcd</sup>	0.086 ± 0.004 <sup>defg</sup>	0.049 ± 0.003 <sup>defg</sup>	1.745 ± 0.094 <sup>cdefg</sup>	0.111 ± 0.021 <sup>a</sup>	5.771 ± 1.449 <sup>ab</sup>	82.127 ± 5.527 <sup>efgh</sup>
Tribe: Calypsoeae															
26.	<i>Oreorchis micrantha</i>	Fusifiform	0.863 ± 0.010 <sup>o</sup>	0.091 ± 0.008 <sup>efgh</sup>	9.538 ± 0.833 <sup>k</sup>	1.895 ± 0.365 <sup>i</sup>	Simuous	15.900 ± 1.197 <sup>kl</sup>	54.592 ± 4.542 <sup>gh</sup>	0.167 ± 0.016 <sup>o</sup>	0.064 ± 0.005 <sup>hi</sup>	2.596 ± 0.318 <sup>kl</sup>	0.369 ± 0.077 <sup>def</sup>	5.364 ± 1.653 <sup>ab</sup>	79.444 ± 7.559 <sup>efg</sup>
Tribe: Cymbidieae															
27.	<i>Cymbidium macrorhizon</i>	Spathulate	0.161 ± 0.006 <sup>b</sup>	0.048 ± 0.004 <sup>ab</sup>	3.341 ± 0.373 <sup>cd</sup>	0.101 ± 0.018 <sup>a</sup>	Simuous/ undulate	11.400 ± 0.699 <sup>i</sup>	14.206 ± 0.786 <sup>e</sup>	0.031 ± 0.002 <sup>a</sup>	0.033 ± 0.003 <sup>ab</sup>	0.935 ± 0.100 <sup>a</sup>	0.018 ± 0.005 <sup>a</sup>	5.661 ± 1.452 <sup>ab</sup>	80.839 ± 6.437 <sup>efgh</sup>
28.	<i>Eulophia herbacea</i>	Fusifiform	0.964 ± 0.009 <sup>p</sup>	0.087 ± 0.002 <sup>defg</sup>	11.074 ± 0.388 <sup>i</sup>	1.921 ± 0.102 <sup>i</sup>	Straight/ simuous	11.200 ± 0.918 <sup>i</sup>	86.706 ± 7.688 <sup>ghk</sup>	0.122 ± 0.003 <sup>lmn</sup>	0.065 ± 0.003 <sup>hij</sup>	1.870 ± 0.115 <sup>efgh</sup>	0.275 ± 0.027 <sup>cd</sup>	7.100 ± 0.898 <sup>abc</sup>	85.743 ± 1.460 <sup>efgh</sup>
29.	<i>Eulophia hormusjii</i>	Ovoid	0.220 ± 0.037 <sup>cdef</sup>	0.104 ± 0.010 <sup>hik</sup>	2.163 ± 0.505 <sup>ab</sup>	0.622 ± 0.116 <sup>bcde</sup>	Straight/ simuous	2.600 ± 0.516 <sup>a</sup>	87.200 ± 18.305 <sup>kl</sup>	0.074 ± 0.011 <sup>ate</sup>	0.048 ± 0.008 <sup>defg</sup>	1.555 ± 0.101 <sup>bcde</sup>	0.096 ± 0.050 <sup>a</sup>	8.223 ± 3.997 <sup>abc</sup>	84.441 ± 8.282 <sup>efgh</sup>
Tribe: Podochileae															
30.	<i>Eria tomentosa</i>	Fusifiform	0.113 ± 0.005 <sup>a</sup>	0.056 ± 0.002 <sup>bc</sup>	2.003 ± 0.135 <sup>ab</sup>	0.096 ± 0.010 <sup>a</sup>	Simuous	3.500 ± 0.527 <sup>abc</sup>	33.266 ± 6.153 <sup>bcd</sup>	0.068 ± 0.005 <sup>c</sup>	0.040 ± 0.005 <sup>bcd</sup>	1.748 ± 0.261 <sup>cdefg</sup>	0.058 ± 0.016 <sup>a</sup>	1.673 ± 0.539 <sup>a</sup>	38.486 ± 9.007 <sup>bc</sup>
Tribe: Vandeeae															
31.	<i>Gastrochilus calceolaris</i>	Fusifiform	0.227 ± 0.024 <sup>cdef</sup>	0.049 ± 0.004 <sup>ab</sup>	4.650 ± 0.027 <sup>efg</sup>	0.146 ± 0.029 <sup>ab</sup>	Undulate	7.800 ± 0.918 <sup>f</sup>	29.574 ± 5.435 <sup>b</sup>	0.111 ± 0.006 <sup>ijk</sup>	0.041 ± 0.004 <sup>bcde</sup>	2.708 ± 0.370 <sup>k</sup>	0.102 ± 0.028 <sup>a</sup>	1.478 ± 0.468 <sup>a</sup>	30.776 ± 9.358 <sup>bc</sup>
32.	<i>Rhynchostylis retusa</i>	Fusifiform	0.231 ± 0.007 <sup>cdefg</sup>	0.041 ± 0.003 <sup>a</sup>	5.583 ± 0.377 <sup>ghi</sup>	0.105 ± 0.020 <sup>a</sup>	Straight/ simuous	7.100 ± 0.875 <sup>fg</sup>	33.009 ± 4.175 <sup>bcd</sup>	0.133 ± 0.021 <sup>lmn</sup>	0.036 ± 0.003 <sup>bc</sup>	3.705 ± 0.851 <sup>m</sup>	0.094 ± 0.021 <sup>a</sup>	1.182 ± 0.265 <sup>a</sup>	11.122 ± 8.742 <sup>a</sup>

Data are shown as mean ± standard deviation. Values in a column with the same superscripts are not significantly different at P ≤ 0.05. L, length; Vol, volume; W, width.

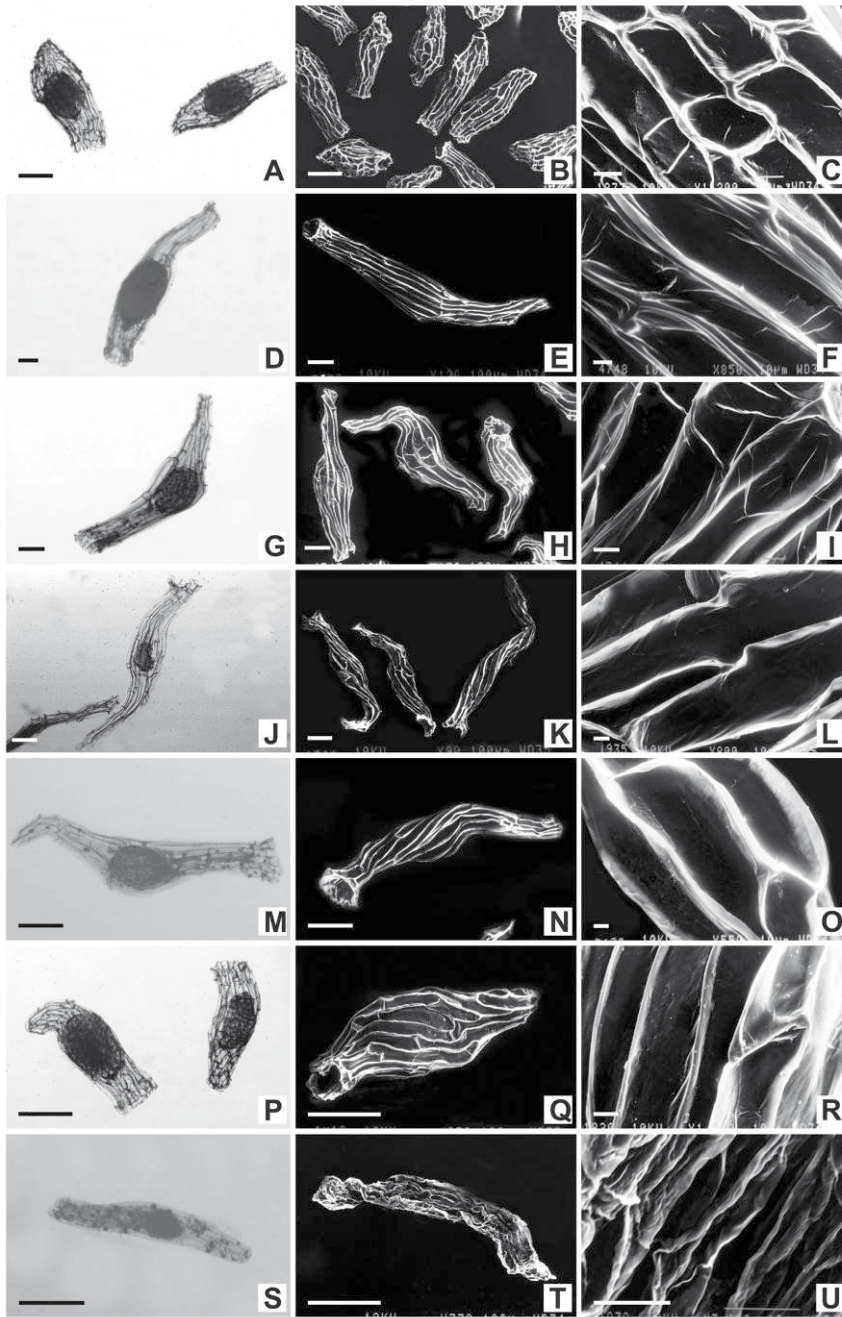


**Figure 1.** Seed and embryo structure of Western Himalayan orchids. A–C = *Cypripedium cordigerum*, D–F = *Goodyera biflora*, G–I = *Spiranthes sinensis*, J–L = *Androcorys monophylla*, M–O = *Brachycorythis obcordata*, P–R = *Dactylorhiza hatagirea*, S–U = *Dithrix griffithii*. Scale bars: A, B, D, E, G, H, J, K, M, N, P, Q, S, T = 100 µm; C, F, I, L, O, R, U = 10 µm. v = vertical ornamentation in periclinal walls of testa cells.

Intercellular spaces were observed in both of the members of tribe Cranichideae (*Goodyera biflora* and *Spiranthes sinensis*), and these were prominent at cell corners (Figure 1). The periclinal walls possessed external ornamentation (ridges) in a few species. These were thick and vertical in orchidoids [*Spiranthes sinensis* (Figure 1), *Dactylorhiza*

*hatagirea* (Figure 1), *Dithrix griffithii* (Figure 1)] and thin and oblique in epidendroid orchids [*Oreorchis micrantha* (Figure 4), *Eulophia herbacea* (Figure 4), *E. hormusjii* (Figure 5)]. The cell walls in all epiphytes possessed very thick (cord-like) depositions (Figure 5). Number of testa cells (at longest seed axis) ranged between  $2.600 \pm$



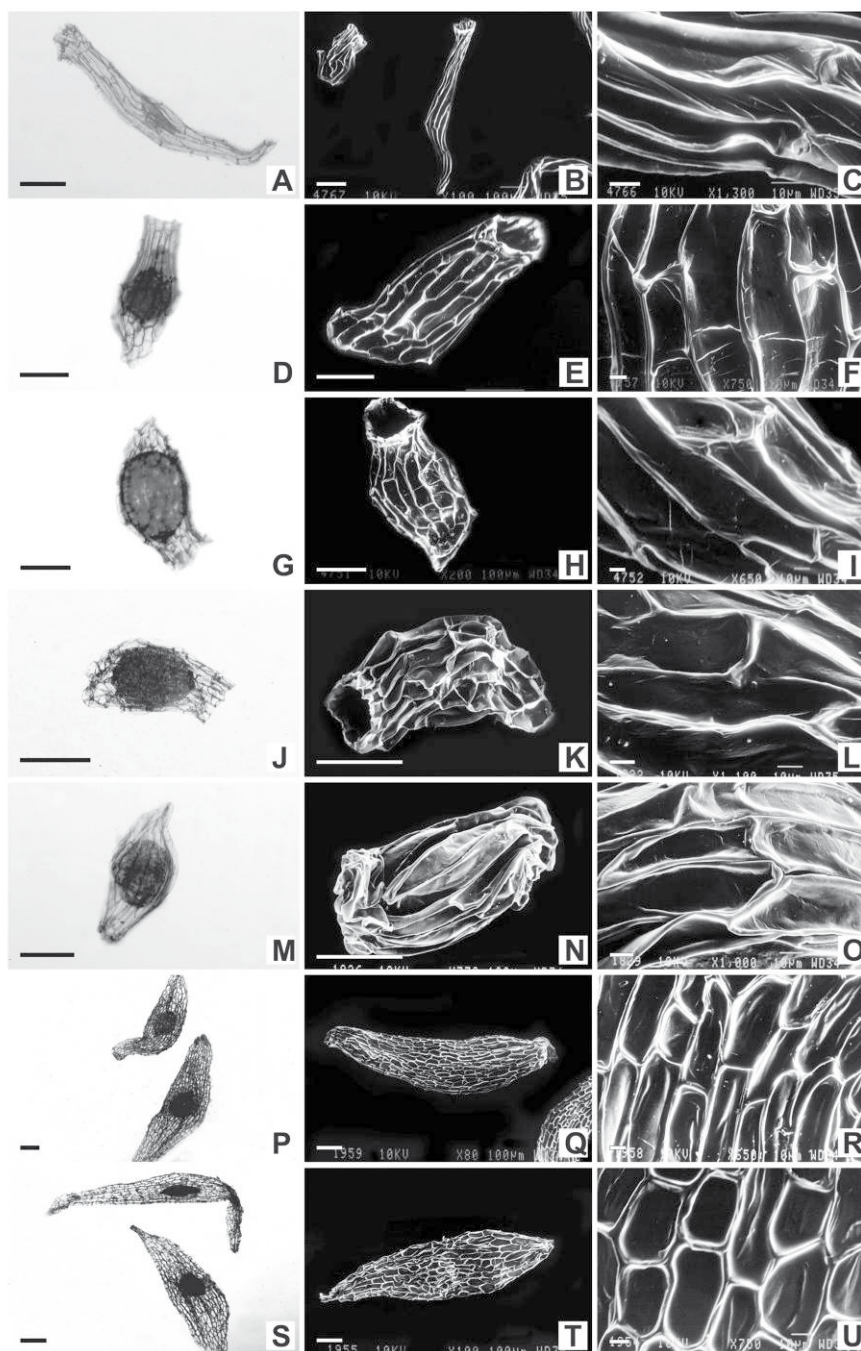


**Figure 2.** Seed and embryo structure of Western Himalayan orchids. A–C = *Habenaria aitchisonii*, D–F = *Habenaria intermedia*, G–I = *Habenaria pectinata*, J–L = *Habenaria plantaginea*, M–O = *Habenaria pubescens*, P–R = *Herminium lanceum*, S–U = *Pecteilis gigantea*. Scale bars: A, B, D, E, G, H, J, K, M, N, P, Q, S, T = 100  $\mu$ m; C, F, I, L, O, R, U = 10  $\mu$ m.

0.516 (*Eulophia hormusjii*) and  $27.600 \pm 2.913$  (*Goodyera biflora*). Such a variation in number may be significantly (*Epipactis*, *Liparis*, *Eulophia*) or nonsignificantly (*Habenaria*, *Platanthera*) different at genus level. These cells were longest ( $127.280 \pm 15.376$   $\mu$ m) in *Habenaria pubescens* (Orchidoideae) and shortest ( $11.886 \pm 0.918$

$\mu$ m) in *Crepidium acuminatum* (Epidendroideae). It is worth mentioning that not all of the testa cells along the longitudinal seed axis were of the same length; they were comparatively shorter both at apical and chalazal ends.

The small-sized and ellipsoidal embryos filled the central area (Figures 1–5) conforming to the widest zone

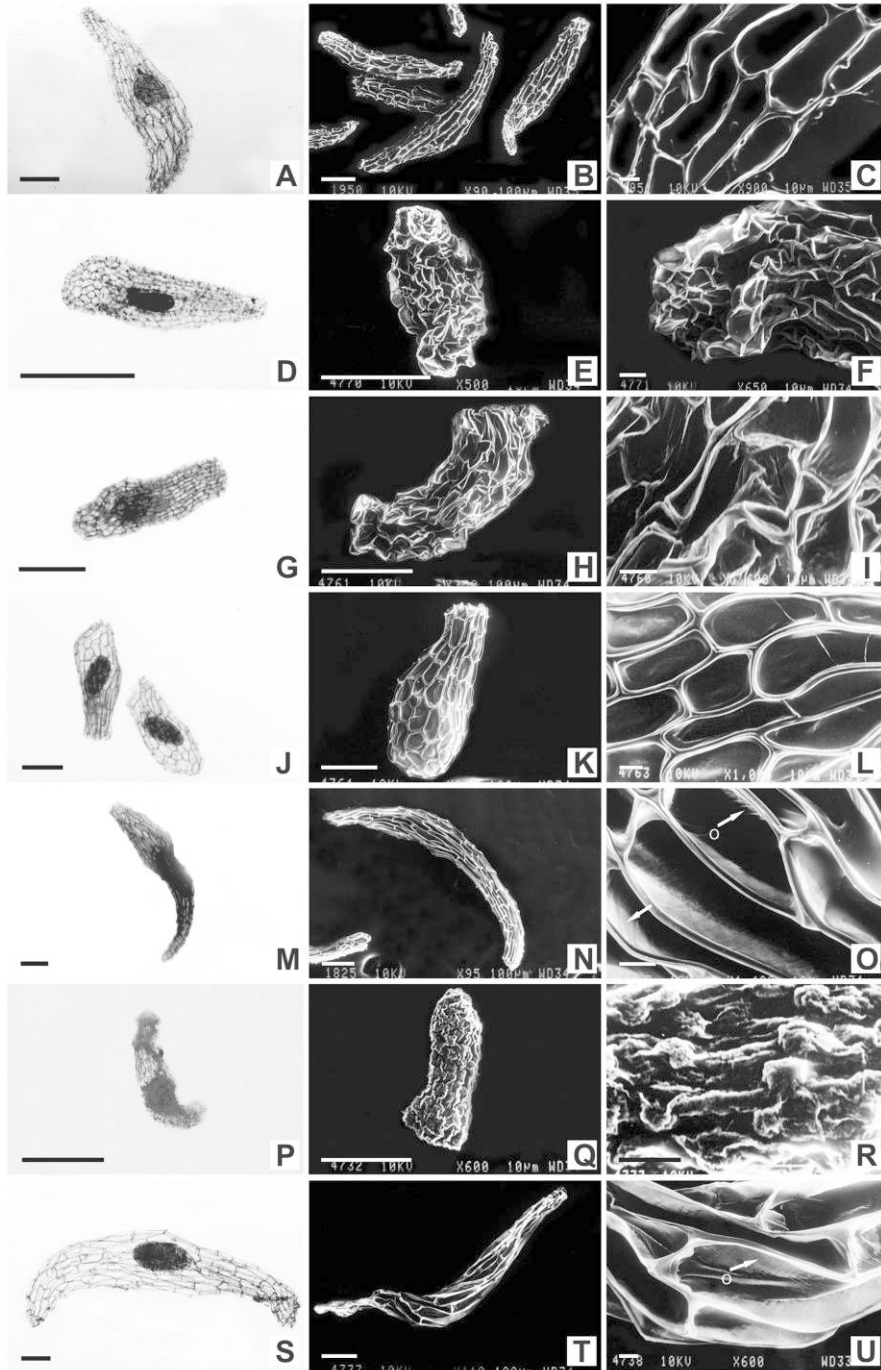


**Figure 3.** Seed and embryo structure of Western Himalayan orchids. A–C = *Peristylus affinis*, D–F = *Platanthera clavigera*, G–I = *Platanthera edgeworthii*, J–L = *Platanthera latilabris*, M–O = *Satyrium nepalense*, P–R = *Epipactis gigantea*, S–U = *Epipactis helleborine*. Scale bars: A, B, D, E, G, H, J, K, M, N, P, Q, S, T = 100 µm; C, F, I, L, O, R, U = 10 µm.

of the seeds. Embryo length ranged between  $0.031 \pm 0.002$  mm (*Cymbidium macrorhizon* Lindl.) and  $0.218 \pm 0.012$  mm (*Epipactis gigantea*) and width between  $0.026 \pm 0.003$  mm (*Peristylus affinis*) and  $0.120 \pm 0.007$  mm (*Epipactis gigantea*). On the other hand, embryo L/W ratio was

found lowest in *Cymbidium macrorhizon* ( $0.935 \pm 0.100$ ) and highest in *Rhynchostylis retusa* ( $3.705 \pm 0.851$ ), both of which belong to subfamily Epidendroideae.

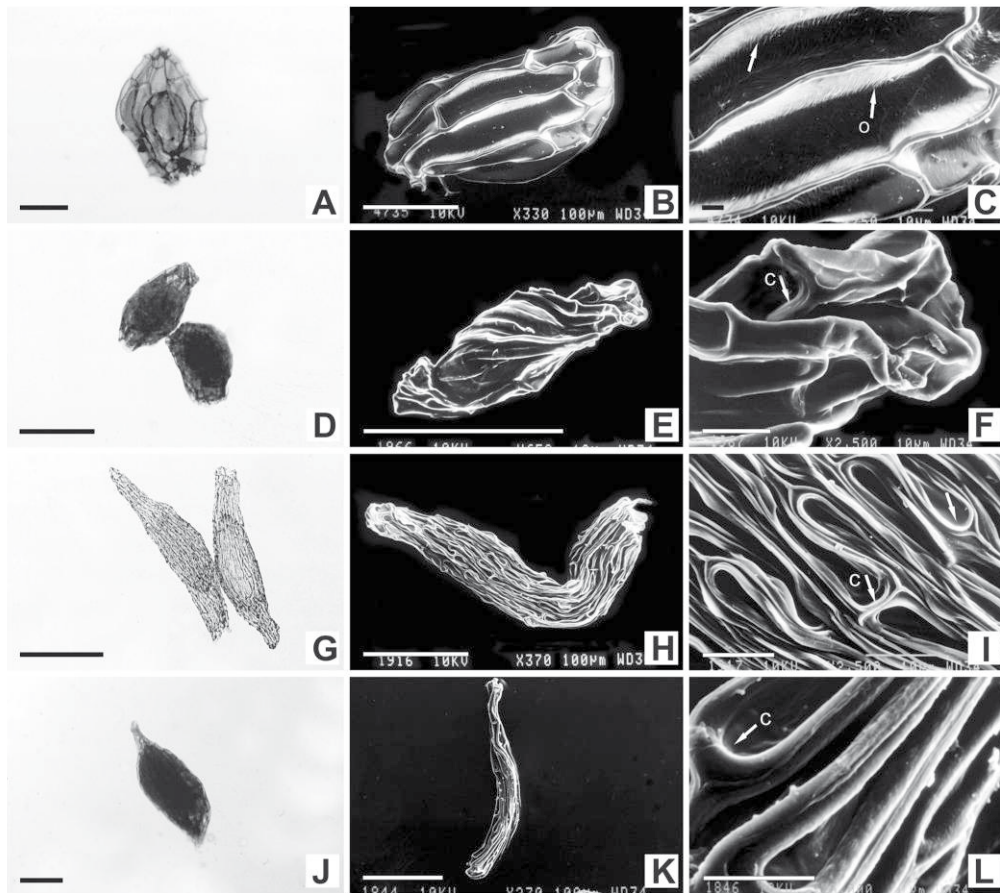
Embryo volume is an important attribute as it directly affects the percentage of available air space inside seed.



**Figure 4.** Seed and embryo structure of Western Himalayan orchids. A-C = *Gastrodia falconeri*, D-F = *Crepidium acuminatum*, G-I = *Liparis odorata*, J-L = *Liparis rostrata*, M-O = *Oreorchis micrantha*, P-R = *Cymbidium macrorhizon*, S-U = *Eulophia herbacea*. Scale bars: A, B, D, E, G, H, J, K, M, N, P, Q, S, T = 100  $\mu$ m; C, F, I, L, O, R, U = 10  $\mu$ m. o = oblique ornamentation in periclinal walls of testa cells.

Like their L/W ratios, variable embryo volumes were recorded in various epidendroid species; it was lowest in *Cymbidium macrorhizon* [(0.018  $\pm$  0.005 mm<sup>3</sup>)  $\times$  10<sup>-3</sup>] and highest in *Epipactis gigantea* [(1.646  $\pm$  0.217 mm<sup>3</sup>)  $\times$  10<sup>-3</sup>].

In Orchidoideae, embryo volume ranged between (0.028  $\pm$  0.013 mm<sup>3</sup>)  $\times$  10<sup>-3</sup> (*Peristylus affinis*) and (0.618  $\pm$  0.182 mm<sup>3</sup>)  $\times$  10<sup>-3</sup> [*Platanthera edgeworthii* (Hook.f. ex Collett) R.K.Gupta]. Because of the tiny nature of the embryos



**Figure 5.** Seed and embryo structure of Western Himalayan orchids. A–C = *Eulophia hormusjii*, D–F = *Eria tomentosa*, G–I = *Gastrochilus calceolaris*, J–L = *Rhynchosstylis retusa*. Scale bars: A, B, D, E, G, H, J, K = 100  $\mu$ m; C, F, I, L = 10  $\mu$ m. c = cord-like thickenings in cell walls; o = oblique ornamentation in periclinal walls of testa cell.

in the majority of species, most of the seed space was occupied by air. The percent air space varied remarkably in epidendroid orchids as the species with the lowest ( $11.122 \pm 8.742\%$  in *Rhynchosstylis retusa*) and the highest ( $91.821 \pm 1.685\%$  in *Liparis odorata*) air spaces belonged to this subfamily. We observed that the epiphytic species possess comparatively lower percentages of air space in their seeds as compared to their terrestrial or mycoheterotrophic counterparts. Significant differences in percent air space were observed between taxa belonging to same tribe and/or genus. The lowest ( $1.182 \pm 0.265$ ) seed to embryo (s/e) volume ratio was also observed in *Rhynchosstylis retusa*, and it was highest ( $25.854 \pm 8.457$ ) in *Liparis odorata*. The studied orchid species were found to dwell in a variety of habitats (shady forest floors, bushy grasslands, bright forests), but no possible correlation was seen between species habitat and their seed characteristics.

During our field visits for seed collection, we found that orchid habitats in a majority of the localities were under different anthropogenic pressures. Overgrazing,

fodder and fuel wood collection, forest fires, landslides, and large-scale tourism have resulted in soil erosion and degradation of natural habitats. Construction of houses, hydroelectric projects, and roads to meet the needs of the expanding human population have added to the woes by contributing either in shrinkage of natural habitats or their fragmentation. Another important reason for decline in orchid populations is a slow and gradual replacement of native species (including orchids) by some exotic weeds such as *Ageratum conyzoides* L., *Lantana camara* L., and *Parthenium hysterophorus* L. All of the above-mentioned factors directly or indirectly affect the fruit and seed set in orchids and therefore their conservation.

#### 4. Discussion

Seeds represent an integral part of the plant life cycle. The present investigation of seed characteristics of 32 Himalayan orchids yielded interesting results. We observed that as many as 50% of the presently studied species spread over 3 different subfamilies produced fusiform seeds, and

there was no uniformity in seed shape even at the tribe level. Seed shape was found uniform at the generic level in the case of *Epipactis*, *Liparis*, and *Platanthera*. Chase and Phippen (1988) observed a remarkably consistent seed shape over a large and variable set of orchidoid genera. Tsutsumi et al. (2007) also reported a uniform occurrence of fusiform seeds in some *Liparis* species irrespective of their life mode (terrestrial/epiphytic). Aybeke (2007) studied seed morphometry of 8 *Ophrys* L. species and found uniformly fusiform seeds in all of them. However, the differences in seed shapes of the presently studied species of *Habenaria* (fusiform, spatulate) and *Eulophia* (fusiform, ovoid) reflect that this character could not be employed for delimitation of different genera with certainty. Seed characteristics support the transfer of 3 *Habenaria* species [*H. clavigera* (Lindl.) Dandy, *H. edgeworthii* Hook.f. ex Collett, *H. latilabris* (Lindl.) Hook.f.] to genus *Platanthera* (Govaerts et al. 2013). Their seeds were uniformly spatulate (Figure 3) and smaller (Table 2) as compared to the presently studied species of *Habenaria*. Only *H. aitchisonii* possessed spatulate seeds (Figure 2), which depicts closer affinities between these 2 genera (*Habenaria*, *Platanthera*). The shape of the seeds is also thought to have phylogenetic significance. Fusiform seeds that are found in all of the subfamilies (Arditti et al., 1979, 1980; Healey et al., 1980; Rasmussen, 1995; Verma et al., 2012; present study) appear to be basic in orchids, from which all other seed shapes might have evolved. Arditti et al. (1979) and Vij et al. (1992) demonstrated that seeds show the least shape variability and are usually fusiform in primitive orchids (Cypripedioideae) but exhibit great variations (fusiform, ovoid, elliptical, filamentous, cylindrical) in advanced epidendroid orchids. The present results are in line with these findings. Such observations also suggest a possible correlation between the number of seed shapes and the extent of species diversification in different orchid subfamilies. Different seed shapes in Epidendroideae might also have evolved as an adaptive strategy of its members in response to much varied life modes (terrestrial, mycoheterotrophic, lithophytic, epiphytic).

The 'dust-like' seeds of orchids are very well suited for long-range dispersal by wind. They show a significant variation in their length (0.05–6.0 mm) as well as width (0.01–0.9 mm), and such differences are of good taxonomic importance at genus and/or species level (Arditti and Ghani, 2000). Variable seed sizes were observed in Epidendroideae in the present study. Vij et al. (1992) also observed such variations and demonstrated that the seeds are generally long and intermediately sized in primitive cypripedoid orchids, short and intermediate in orchidoid orchids, and short, intermediate, and long in advanced epidendroid orchids. Seed length is very important in

calculating the degree of seed truncation. Presently, 27 species were found to possess truncated seeds where  $L/W < 6.0$ . Elongated ( $L/W > 6.0$ ) seeds were observed only in 5 species irrespective of their taxonomic position. The lowest  $L/W$  value was recorded in ovoid seeds of *Androcorys monophylla* (Figure 1). In a study on some Himalayan orchids, Verma et al. (2012) also recorded the lowest  $L/W$  ratio in its closely allied species, *Herminium monorchis* (L.) R.Br. According to Arditti et al. (1979), the relative degree of truncation of orchid seeds is directly correlated with an increase in their length rather than their width. Chase and Phippen (1988) demonstrated that during the seed drying process there is no significant change in seed length, but the width may decrease by 25%–40%. Such findings suggest that the  $L/W$  ratio is not of much conservative value. One may more or less expect differences in  $L/W$  ratios of seeds belonging to the same or different individuals of the same species growing in the same locality but either harvested at different time periods after pollination or dehydrated in different ways (e.g., in one case directly collected in FAA and in another case collected in a polythene packet and transferred to FAA a few days after arriving at the laboratory). Arditti et al. (1979) suggested that instead of length and width, volume should be considered as a better reflection of seed size in orchids. On average, seed volume was recorded as high in the presently studied epidendroid orchids. When compared with ground-growing species, the seed volume ( $\text{mm}^3 \times 10^{-3}$ ) was found comparatively lower in the epiphytic ones [ $0.096 \pm 0.010$  (the lowest) in *Eria tomentosa*,  $0.105 \pm 0.020$  in *Rhynchostylis retusa*,  $0.146 \pm 0.029$  in *Gastrochilus calceolaris* (J.E.Sm.) D.Don]. Kiyohara et al. (2012), in a study on 68 Japanese orchids, also observed the lowest seed volume in a species of *Eria* Lindl. [*E. reptans* (Kuntze) Makino]. According to Clifford and Smith (1969), Vij et al. (1992), Rasmussen (1995), Swamy et al. (2004), and Verma et al. (2012), the seed sizes show a direct correlation with plant habit; epiphytic orchids generally possess smaller seeds as compared to the terrestrials. Yoder et al. (2010) also explained that the seeds of epiphytic orchids are smaller, lighter, and more porous than the ground-growing ones. However, the data presented by Arditti and Ghani (2000) on numerical and physical characteristics of orchid seeds suggest that this is not a rule.

Seed coat in orchids is generally represented by a single layer of elongated and transparent testa cells derived from the outer integument (Vij, 2006). The testa cells were quadrilateral in shape in the majority of the presently studied species. More or less pentagonal to hexagonal cells were, however, seen in *Androcorys monophylla*, *Dactylorhiza hatagirea*, and *Gastrodia falconeri*. Testa cells were irregularly shaped in closely related *Crepidium acuminatum* and *Liparis odorata*. Swamy et al. (2004)

also reported the presence of longitudinally oriented and irregularly shaped testa cells in another species of *Liparis* (*L. elliptica* Wight). Earlier, Clifford and Smith (1969), Vij et al. (1992), and Verma et al. (2012) suggested that quadrilateral testa cells are found commonly in ground-growing species, and they are generally fusiform in epiphytes. However, such differences were not seen presently. Testa cells that are straightly arranged along the longitudinal seed axis in a majority of species were arranged in a spiral (twisted) in *Eulophia herbacea* (Figure 4) and *Eria tomentosa* (Figure 5). Cell walls were straight to sinuous in the majority of the species. They were moderately to highly undulate in *Spiranthes sinensis*, *Dithrix griffithii*, *Crepidium acuminatum*, and *Liparis rostrata* and they depict no taxonomic or evolutionary significance. Some orchids possess uneven deposition of thickening materials on their periclinal or anticlinal walls, and such ornamentation(s) is of taxonomic importance. Clifford and Smith (1969) distinguished continuous, discontinuous, and beaded patterns of wall thickenings in testa cells of orchid seeds. Kurzweil (1993) and Molvray and Kores (1995) suggested that the seed coat in orchids may be made of cells that differ in shape and ornamentation. The surface walls were bereft of any cross-band thickenings in most of the presently studied taxa. However, such structures were present in 6 species. Thick and vertical reticulations were observed in 3 orchidoid species (*Dactylorhiza hatagirea*, *Dithrix griffithii*, *Spiranthes sinensis*), and these were thin and oblique in epidendroid *Oreorchis micrantha* and 2 *Eulophia* spp. (*E. herbacea*, *E. hormusjii*). Such wall thickenings were earlier reported in *Spiranthes sinensis* (Clifford and Smith, 1969; Vij et al., 1992); *Spiranthes vernalis* Engelm. & A.Gray (Molvray and Kores, 1995); *Dendrobium longicornu* Lindl., *Orchis habenarioides* King & Pantl. (= *Gymnadenia orchidis* Lindl.), and *Goodyera repens* (L.) R.Br. (Vij et al., 1992); *Spiranthes romanzoffiana* Cham. (Healey et al., 1980); *Eulophia guineensis* Lindl. (Barthlott, 1976); and species of *Calypso* Salisb. (Arditti et al., 1980). Aybeke (2007) and Gamarra et al. (2010, 2012) reported different types of ornamentations in cell walls of some *Ophrys* species and orchidoid orchids, respectively, and suggested their taxonomic importance. Ortunez et al. (2006) observed that the periclinal walls of *Cypripedium calceolus* L. were generally unsculptured, but some cells at the chalazal pole possessed transverse ridges. Vij et al. (1992) reported the occurrence of wall thickenings in all 4 Himalayan *Cymbidium* Sw. spp. studied by them (*Cymbidium eburneum* Lindl., *C. lancifolium* Hook., *C. aloifolium* (L.) Sw., *C. macrorhizon*) irrespective of their habit (epiphytic/mycoheterotrophic). However, we have not observed such structures presently in *Cymbidium macrorhizon*. As our material was juvenile (Figure 4), the testa cell walls had probably not developed such reticulations up to the time

of seed collection. The cell walls in all epiphytic species possessed very thick depositions (Figure 5). Such a profuse development of wall thickenings is of common occurrence in epiphytes and is thought to have an adaptive significance. They provide rigidity to the seed coat and thus protect the embryo (Swamy et al., 2004). Their hygroscopic nature is also thought to aid initiation of metabolic activities during seed germination (Vij et al., 1992). Shimizu (2012), on the other hand, demonstrated that seed coat patterns are independent of plant habit and these only make seeds to ride upon the winds to help in wider species distribution. The number of testa cells and their size showed variations in different species (Table 2). They were generally short-sized, both at apical and chalazal ends. Ortunez et al. (2006) and Gamarra et al. (2007), while investigating seed micromorphology of *Cypripedium calceolus* and genus *Neotinea* Rchb.f., respectively, also demonstrated the same pattern of testa cell lengths (short apical and chalazal cells, elongated medial cells) in these taxa. According to Arditti et al. (1979), for any given orchid genus one might expect either testa cell number or cell length to be somewhat consistent. Present observations agree with this generalization. The number of testa cells in different *Habenaria* spp. and *Platanthera* spp. was found to be similar (Table 2). On the other hand, 2 species of *Eulophia* showed significant difference in number of testa cells ( $11.200 \pm 0.918$  in *E. herbacea*,  $2.600 \pm 0.516$  in *E. hormusjii*) but exhibited uniformity in their size. Intercellular spaces were observed in both of the members of tribe Cranichideae (*Goodyera biflora* and *Spiranthes sinensis*) and these were prominent at cell corners. These spaces might have originated through restricted dissolution of the middle lamella along the cell corners. Such observations were also made earlier in some species of *Goodyera*, *Spiranthes*, and *Zeuxine* Lindl. (Healey et al., 1980; Tohda, 1985; Vij et al., 1992; Molvray and Kores, 1995), all of which belong to tribe Cranichideae.

Like seeds, the embryos are also minute in Orchidaceae. Orchid embryos are generally spherical or oval in shape (Arditti and Ghani, 2000) and the present species were no exception. A wide range of embryo volumes was seen in advanced Epidendroideae. Embryo volume directly affects the percentage of air inside seed, and therefore it has an important role in seed dispersal and species distribution. Fahn and Werker (1972) distinguished wind-dispersed (anomochores) seeds as flyers (meteoranomochores) and rollers (chamaeochores), and orchid seeds, because of their small size and light weight, belong to the former category (flyers). The tiny nature of embryos makes them exceedingly air-filled, therefore helping them to float (fly) across longer distances in air for a wider dispersal (Arditti, 1992; Yam et al., 2002). Burgeff (1936) experimentally demonstrated the relationship between s/e volume ratio

and seed buoyancy; seeds with a high value of  $s/e$  ratio are found more buoyant as they possess more air space. In the presently investigated species, the highest  $s/e$  volume ratio ( $25.854 \pm 8.457$ ) was obtained in *Liparis odorata* and the lowest ( $1.182 \pm 0.265$ ) in *Rhynchostylis retusa*, both of which are members of subfamily Epidendroideae. The seeds of epiphytic species were found to possess comparatively low  $s/e$  volume ratios and lower percentages of air space than the terrestrials. According to Tsutsumi et al. (2007), the comparative  $s/e$  volume ratio has evolutionary implication; seeds of epiphytic (advanced) orchids are generally shorter but possess large-sized embryos. These authors suggested that the larger embryos are likely to be developmentally advanced and have potential to germinate earlier than the smaller ones. Therefore, large embryos are thought to play an important role in early and better establishment of seedlings in the epiphytic life style. Moreover, in epiphytes, as the seeds are released from greater heights, they remain in the air for more time and consequently could disperse longer distances despite being less buoyant. Earlier, Garg et al. (1992) also mentioned that the seeds of terrestrial orchids are voluminous, with a larger air space (smaller embryos) than those of epiphytes. All of the presently studied epidendroid orchids are widely distributed, except for *Gastrodia falconeri* (Table 1). This species produces highly floating (>90% air space) seeds but exhibits a restricted distribution in the Himalayan range. Similar is the case of the presently studied lady slipper (*Cypripedium cordigerum*), which, despite its very buoyant seeds (air space > 85%), is a Himalayan endemic and exhibits patchy distribution along mountain ranges. Both of these orchids seem to be highly habitat-specific. According to Vij et al. (1998), some orchid species exhibit higher habitat specificity (narrow preferences towards exposure and shade, moisture, soil pH, mineral elements in soil, etc.), and this phenomenon is much pronounced in the mycoheterotrophs. The buoyant seeds that can successfully disperse long distances in such species might not be able to germinate and/or establish themselves in the absence of suitable biotic (mycorrhizal) and abiotic (desired habitat and substrates) factors. According to Benzing (1981, 1987), both the large number and the physical characteristics (size, air space) of orchid seeds help in the wider distribution of Orchidaceae by effective dispersion and successful colonization by at least a few of them in new substrates at newer localities. Such findings explain the discontinuous (patchy) distribution of many orchid species, including the presently studied *Cypripedium cordigerum*, across large ecosystems like the Himalayan range.

The Himalayan range is geodynamically young, and the top soil layer here that actually sustains the flora and fauna is thin and fragile. The majority of

natural habitats across the Himalaya are under a variety of anthropogenic pressures. Orchids are highly habitat-specific and even small fluctuations in the microclimatic factors affect their germination, growth, and development. Different anthropogenic activities have resulted in excessive soil erosion and the shrinkage, degradation, and fragmentation of habitats. Intact and healthy (least disturbed) habitats are necessary not only for the germination, growth, and reproduction of orchids, but also for those of the other 2 specific biotic elements (mycorrhiza, pollinators) that are obligatory for undergoing all processes from seed germination to seed formation. Exotic weeds (especially *Lantana camara*) pose a threat to many native species, including orchids. A wider and successful distribution of orchids, like other flowering plants, depends upon the success of seed (fruit) production and their effective dispersal thereafter. Moreover, the production and germination of seeds in any species in a given area directly depends upon its population structure (more individuals, chances of more fruit and seed production) and the habitat characteristics (which decide seed germination). The fruit set was not found to be similar in all of the presently studied species and it ranged between 60% and 90% in the majority of cases (unpublished data). The lowest (26.45%) fruit set was seen in *Crepidium acuminatum* and the highest (96.26%) in *Rhynchostylis retusa*. It was observed that the species having higher (>90%) fruit set (*Rhynchostylis retusa*, *Gastrochilus calceolaris*, *Platanthera edgeworthii*, *P. latilabris* Lindl.) generally produced small-sized seeds with smaller air space (11.122%–34.052%) inside them. On the other hand, orchids with low percentages (25%–50%) of fruit set (*Crepidium acuminatum*, *Liparis odorata*, *L. rostrata*, *Cypripedium cordigerum*) produced comparatively larger seeds with larger air space (82.127%–95.650%). The epiphytes produced more fruits (per inflorescence) due to easy access (less competition) to pollinators as well as lesser damage to their plants from herbivores. Such an observation suggests that despite less fruit set in ground-growing taxa, the seeds are well adapted (high air space) for effective dispersal to longer distances and therefore newer localities. Inflorescences of *Crepidium acuminatum* possess many (10–25) flowers but we could hardly find 1–2 fruit(s) in different populations comprising 15–20 individuals. According to Calvo (1990), the larger inflorescence size in orchids did not necessarily result in increased fruit set percentage but tended to increase the chances to produce at least one fruit. This appears true in the case of closely allied *Crepidium acuminatum*, *Liparis odorata*, and *L. rostrata*. In terrestrial orchids, grazing animals eat away the young inflorescences and/or fruits, affecting fruit/seed production, and also uproot the underground perennating plant parts (roots, rhizomes,

pseudobulbs, tubers). This affects the natural increment in orchid populations both through sexual and asexual means. Therefore, grazing should be regulated. The same area should not be used for this purpose year after year so that roots have enough time to establish themselves better. The overgrazed areas are also more prone to soil erosions. Littering with nonbiodegradable objects (polythene bags, food packaging, plastic glasses/bottles) was seen only in those habitats that fall in and around some popular tourist destinations (Kasauli, Kothi, Kufri, Taradevi). It was observed that this nonbiodegradable matter goes on accumulating year after year in the forest/grassland/riverbed areas and ultimately gets buried under soil-forming layers. Such soils become plastic-contaminated and are not suitable for the growth and development of plant species. Habitat fragmentation, on the other hand, affects seed set by altering pollinator population and behavior. Orchids have a long vegetative phase in their life cycle, and during fodder collection activities each year, many seedlings are cut immature before reaching their flowering and fruiting stage.

Orchids are at the front-line of extinction, with more species under threat globally than in any other plant family (Kull et al., 2006; Swarts and Dixon, 2009). According to Salazar (1996), destruction, modification, and fragmentation of natural forests, as well as illegal extraction of orchids, hasten their local extinction. Thus, to conserve the species, initiatives must be taken at the local level where it actually grows (in situ). Species can be

better conserved in protected areas (national parks, wild life sanctuaries, biosphere reserves), where anthropogenic interference is very low. Moreover, in such places, conservation is done on a community basis rather than by targeting a single specific species, and therefore the ecological equilibrium is well maintained. It is, however, now felt that conservation through protected reserves alone is unlikely to achieve protection of plant species. The species that are more sensitive to environmental fluctuations, such as orchids, need artificial assistance to migrate from hostile environments to new climatically buffered sites. Orchid seed banking is a viable option for seed storage for longer time periods (Seaton and Pritchard, 2000) that can later be utilized for mass propagation of species in vitro. The micropropagated seedlings (raised ex situ), after better acclimatization, could successfully be rehabilitated back into their natural or natural-like artificial abodes with desired substratum and microclimatic characteristics. Such practices can help in restoring the otherwise depleting orchid populations in and around the Himalayan range.

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#### References

- Abdel Khalik K (2013). Systematic implications of seed coat diversity in some representatives of the genus *Ipomoea* (Convolvulaceae). *Turk J Bot* 37: 811–824.
- Akcin TA, Ozdener Y, Akcin A (2010). Taxonomic value of seed characters in orchids from Turkey. *Belgian J Bot* 142: 124–139.
- Arditti J (1992). *Fundamentals of Orchid Biology*. New York, NY, USA: John Wiley and Sons.
- Arditti J, Ghani AKA (2000). Numerical and physical properties of orchid seeds and their biological implications. *New Phytologist* 145: 367–421.
- Arditti J, Michaud JD, Healey PL (1979). Morphometry of orchid seeds. I. *Paphiopedilum* and native California and related species of *Cypripedium*. *Amer J Bot* 66: 1128–1137.
- Arditti J, Michaud JD, Healey PL (1980). Morphometry of orchid seeds. II. Native California and related species of *Calypso*, *Cephalanthera*, *Corallorhiza* and *Epipactis*. *Amer J Bot* 67: 347–365.
- Aybeke M (2007). Pollen and seed morphology of some *Ophrys* L. (Orchidaceae) taxa. *J Plant Biol* 50: 387–395.
- Barthlott W (1976). Morphologie der Samen von Orchideen im Hinblick auf taxonomische und funktionelle Aspekte. In: Senghas K, editor. *Proceedings of the 8th World Orchid Conference*. Frankfurt, Germany: Deutsche Orchideen Gesellschaft, pp. 444–455 (in German).
- Barthlott W, Ziegler B (1981). Mikromorphologie der Samenschalen als systematisches Merkmal bei Orchideen. *Berichte der Deutsche Botanische Gesellschaft* 94: 267–273 (in German).
- Benzing DH (1981). Why is Orchidaceae so large, its seeds so small, and its seedlings mycotrophic? *Selbyana* 5: 241–242.
- Benzing DH (1987). Major patterns and processes in orchid evolution: a critical synthesis. In: Arditti J, editor. *Orchid Biology: Reviews and Perspectives*, Vol. IV. New York, NY, USA: Cornell University Press, pp. 33–77.
- Bona M (2013). Seed-coat microsculpturing of Turkish *Lepidium* (Brassicaceae) and its systematic application. *Turk J Bot* 37: 662–668.



- Burgett H (1936). Samenkeimung der Orchideen und Entwicklung ihrer Keimpflanzen. Jena, Germany: Verlag von Gustav Fischer (in German).
- Calvo RN (1990). Inflorescence size and fruit distribution among individuals in three orchid species. *Amer J Bot* 77: 1378–1381.
- Cameron KM, Chase MW, Whitten WM, Kores PJ, Jarrell DC, Albert VA, Yukawa T, Hills HG, Goldman DH (1999). A phylogenetic analysis of the Orchidaceae: evidence from rbcL nucleotide sequences. *Amer J Bot* 86: 208–224.
- Chase MW, Pippen JS (1988). Seed morphology in the Oncidiinae and related subtribes (Orchidaceae). *Systematic Bot* 13: 313–323.
- Chemisquy MA, Prevosti FJ, Morrone O (2009). Seed morphology in the tribe Chloraeae (Orchidaceae): combining traditional and geometric morphometrics. *Bot J Linn Soc* 160: 171–183.
- Clifford HT, Smith WK (1969). Seed morphology and classification of Orchidaceae. *Phytomorphology* 19: 133–139.
- Deva S, Naithani HB (1986). The Orchid Flora of North West Himalaya. New Delhi, India: Print and Media Associates.
- Fahn A, Werker E (1972). Anatomical mechanisms of seed dispersal. In: Kozłowski TT, editor. *Seed Biology*. New York, NY, USA: Academic Press, pp. 151–221.
- Gamarra R, Dorda E, Scrugli A, Galan P, Ortunez E (2007). Seed micromorphology in the genus *Neotinea* Rchb.f. (Orchidaceae, Orchidinae). *Bot J Linn Soc* 153: 133–140.
- Gamarra R, Galan P, Herrera I, Ortunez E (2008). Seed micromorphology supports the splitting of *Limnorchis* from *Platanthera* (Orchidaceae). *Nordic J Bot* 26: 61–65.
- Gamarra R, Ortunez E, Cela PG, Guadano V (2012). *Anacamptis* versus *Orchis* (Orchidaceae): seed micromorphology and its taxonomic significance. *Plant Syst Evol* 298: 597–607.
- Gamarra R, Ortunez E, Sanz E, Galan P (2010). Seeds in subtribe Orchidinae (Orchidaceae): the best morphological tool to support molecular analyses. In: Nimis PL, Vignes LR, editors. *Tools for Identifying Biodiversity: Progress and Problems*. Trieste, Italy: EUT, pp. 323–326.
- Garg V, Gupta S, Singh SG, Rani U (1992). Morphometry of some orchid seeds from west Himalaya. *J Orchid Soc India* 6: 85–90.
- Govaerts R, Bernet P, Kratochvil K, Gerlach G, Carr G, Alrich P, Pridgeon AM, Pfahl J, Campacci MA, Baptista DH et al. (2013). World Checklist of Orchidaceae. Kew, UK: The Royal Botanic Gardens (<http://apps.kew.org/wcsp/>).
- Healey PL, Michaud JD, Arditti J (1980). Morphometry of orchid seeds. III. Native California and related species of *Goodyera*, *Piperia*, *Platanthera* and *Spiranthes*. *Amer J Bot* 67: 508–518.
- King G, Pantling R (1898). The orchids of Sikkim Himalaya. *Ann Roy Bot Gard Calcutta* 8: 1–342.
- Kiyohara S, Fukunaga H, Sawa S (2012). Characteristics of the falling speed of Japanese orchids. *International J Biol* 4: 10–12.
- Kull T, Kindlmann P, Hutchings MJ, Primack RB (2006). Conservation biology of orchids: introduction to the special issue. *Biological Conservation* 129: 1–3.
- Kurzweil H (1993). Seeds morphology in Southern African Orchidoideae (Orchidaceae). *Plant Syst Evol* 185: 229–247.
- Molvray M, Kores PJ (1995). Character analysis of the seed coat in Spiranthoideae and Orchidoideae, with special reference to the Diurideae (Orchidaceae). *Amer J Bot* 82: 1443–1454.
- Mostafavi G, Assadi M, Nejadstattari T, Sharifnia F, Mehregan I (2013). Seed micromorphological survey of the *Minuartia* species (Caryophyllaceae) in Iran. *Turk J Bot*. 37: 446–454.
- Nayar MP, Sastry ARK (1988). Red Data Book of Indian Plants, Vol. 2. Calcutta, India: Botanical Survey of India.
- Ortunez E, Dorda E, Cela PG, Gamarra R (2006). Seed micromorphology in the Iberian Orchidaceae. I. Subfamily Cyripedioideae. *Bocconea* 19: 271–274.
- Rani U, Singh SG, Gupta S, Garg V (1993). Morphometry of orchid seeds in Epidendroidae as revealed by SEM. *Advances Plant Sci* 6: 128–133.
- Rasmussen HN (1995). *Terrestrial Orchids from Seed to Mycotrophic Plant*. Cambridge, UK: Cambridge University Press.
- Rauh W, Barthlott W, Ehler N (1975). Morphologie und Funktion der Testa staubformiger Flugsamen. *Botanische Jahrbucher fur Systematik, Pflanzengeschichte und Pflanzengeographie* 96: 353–374 (in German).
- Salazar GA (1996). Conservation threats. In: Hagsater E, Dumont V, editors. *Orchids-Status Survey and Conservation Action Plan*. Gland, Switzerland and Cambridge, UK: IUCN, pp. 6–10.
- Seaton PT, Pritchard HW (2000). Orchid seed banking: a viable option. *Orchid Conservation News* 3: 6–8.
- Sharma J, Kumar Y, Joy KA (2004). SEM characterization of Indian *Paphiopedilum* Pfitz. (Orchidaceae) based on seed morphometry. In: Manilal KS, Sathish Kumar C, editors. *Orchid Memories-A Tribute to Gunnar Seidenfaden*. Calicut, India: Mentor Books, pp. 49–56.
- Shimizu N (2012). Adaptation and evolution of seed shape on bleeding area in Japanese orchids. *International J Biol* 4: 47–53.
- Singh DK (2001). Orchid diversity in India. In: Pathak P, Sehgal RN, Shekhar N, Sharma M, Sood A, editors. *Orchids Science and Commerce*. Dehradun, India: Bishen Singh Mahendra Pal Singh, pp. 35–65.
- Singh JS (2006). Sustainable development of the Indian Himalayan Region: linking ecological and economic concerns. *Curr Sci* 90: 784–788.
- Swamy KK, Krishna Kumar HN, Ramakrishna TM, Ramaswamy SN (2004). Studies on seed morphometry of epiphytic orchids from Western Ghats of Karnataka. *Taiwania* 49: 124–140.
- Swartz ND, Dixon KW (2009). Terrestrial orchid conservation in the age of extinction. *Ann Bot* 104: 543–556.

- Tohda H (1985). Seed morphology in Orchidaceae II. Tribe Cranichideae. Science Report Tohoku University (4th Ser.) 39: 21–43.
- Tsutsumi C, Yukawa T, Lee NS, Lee CS, Kato M (2007). Phylogeny and comparative seed morphology of epiphytic and terrestrial species of *Liparis* (Orchidaceae) in Japan. J Plant Res 120: 405–412.
- Verma J, Kusum, Thakur K, Sembi JK, Vij SP (2012). Study on seed morphometry of seven threatened Himalayan orchids exhibiting varied life modes. Acta Bot Gallica 159: 443–449.
- Vij SP (2006). Orchids: Ingenuity at its best. In: Proceedings of the 93rd Indian Science Congress, Hyderabad. Kolkata, India: The Indian Science Congress association, pp. 1–26.
- Vij SP, Jalota RK, Gupta A (1998). Distribution pattern and substratum analysis of Shimla hill orchids. J Orchid Soc India 12: 15–28.
- Vij SP, Kaur P, Kaur S, Kaushal PS (1992). The orchid seeds: taxonomic, evolutionary and functional aspects. J Orchid Soc India 6: 91–107.
- Yam TW, Yeung EC, Ye XL, Zee SY, Arditti J (2002). Orchid embryos. In: Kull T, Arditti J, editors. Orchid Biology: Reviews and Perspectives VIII. Dordrecht, the Netherlands: Kluwer, pp. 287–385.
- Yoder JA, Imfeld SM, Heydinger DJ, Hart CE, Collier MH, Gribbins KM, Zettler LW (2010). Comparative water balance profiles of Orchidaceae seeds for epiphytic and terrestrial taxa endemic to North America. Plant Ecol 211: 7–17.