Seed morphometry of four species of *Dactylorhiza* Neck. ex Nevski (Orchidaceae) from Kashmir Himalaya, India

Gowhar A Shapoo^{1,3}, Zahoor A Kaloo¹ and Aijaz Hassan Ganie²

¹Plant Tissue Culture Research Laboratory, Department of Botany, University of Kashmir, Hazratbal, Srinagar- 190 006, J & K, India

²Department of Botany, University of Kashmir, Kargil Campus, 194 103, Ladakh, India

³Corresponding author: gashapoo@gmail.com

[Received 12.09.2020; Revised 26.12.2020; Accepted 27.12.2020; Published 31.12.2020]

Abstract

Seed morphometrical study has been carried out for 4 species of *Dactylorhiza* Neck. ex Nevski (Orchidaceae) viz. *D. batagirea* (D.Don) Soo, *D. kafriana* Renz, *D. umbrosa* (Kar. & Kir.) Nevski and *D. viridis* (L.) R.M. Bateman, Pridgeon & M. W. Chase from Kashmir Himalaya. It has been observed that the seeds are spatulate with ovoid and rectangular testa cells in *D. batagirea* and *D. viridis* where as in *D. kafriana* the seeds are crescent with rectangular testa cells and in *D. umbrosa* they are elliptic with pentagonal testa cells. All the results of investigation are presented in a table and discussed in detail with the help of photographs.

Key words: *Dactylorhiza*, Seed morphology, Testa-cells, Kashmir Himalaya

INTRODUCTION

The macro and micro characters of seeds studied under light and scanning electron microscopes are predominant nowadays to delimit different species (Kaya *et al.* 2011). Orchid seeds are the smallest in the plant kingdom. They are dust like, non-endospermic and comprise an undifferentiated embryo suspended within a membranous and often transparent, but at times pigmented seed coat (Verma *et al.* 2013). A single fruit of *Cynoches ventricosum* var. *chlorochilon* encloses about 4 million seeds. Because of their small size they are extremely light-weighted and disperse through air. Maximum seed weight, 14 – 17 µg, has been reported in *Galeola* sp. whereas the lowest, 0.3 – 0.4 µg is recorded in *Anguloa* spp. (Arditti & Ghani 2000). The seed characteristics are more conserved in orchids than vegetative and floral characters (Chase & Phippen 1988), and therefore they are very important for taxonomic, phylogenetic, and phytogeographic studies in orchids (Gamarra *et al.* 2008; Chemisquy *et al.* 2009). The seed morphology can be utilized to delimit different species within a genus (Augustine *et al.* 2001; Swamy *et al.* 2004; Akin *et al.* 2010; Gammara *et al.* 2010). Barthlott and Ziegler (1981) reported 20 different types of seeds in orchids based on various morphological characters of seeds. Vij *et al.* (1992) recognized taxonomic significance of seed characters in 53 orchid species. The morphological variation in shape, size, colour, nature of seed coat, texture of seed coat, the number of testa cells and their size and number greatly vary among different orchid species (Arditi *et al.* 1980; Augustine *et al.* 2001). Verma *et al.* (2012) conducted a study on seed morphometry of seven threatened Himalayan orchids belonging to three different subfamilies, and exhibiting terrestrial (*Arundina graminifolia*, *Cypripedium cordigerum*, *Habenaria marginata*, *Herminium monorchis*), mycoheterotrophic (*Gastrodia falconeri*) or epiphytic (*Aerides multiflora*, *Pholidota articulata*) life modes. It was found that seeds were fusiform, filiform or ovoid in shape and their size showed direct correlation with plant habit. Seeds were smaller in epiphytes as compared to terrestrial or mycoheterotrophic orchid species. Seeds were truncated except in *Arundina graminifolia*. Scanning Electron Microscopy (SEM) studies revealed that the

testa cells were longitudinally oriented, arranged either straight or in a spiral, and their periclinal walls were comparatively irregularly placed in epiphytic species. Furthermore Verma *et al.* (2014) examined physical characteristics of seeds of 32 threatened Western Himalayan orchids belonging to 23 genera of 3 subfamilies using light and scanning electron microscopy. It was observed that these species showed remarkable variations in seed shape (fusiform, spathulate, ovoid, filiform) irrespective of their taxonomic position. The study further reported that epiphytes produced small-sized seeds compared to their terrestrial, lithophytic, or mycoheterotrophic counterparts. Seeds were truncated in 27 species, and were mostly elongated in other orchid species. Swamy *et al.* (2014) conducted SEM studies on seed morphology of 10 species of epiphytic orchids from Western Ghats of Karnataka. All the investigated taxa are different in their seed surface characters including size, shape, visibility of embryo, testa cells and structure, curvature and ridges. Seed colour ranged from pale yellow to yellow-brown and white. Variations in seed and embryo volume and percentage of air space could exist among the different taxa of orchids. Akbulut and Senel (2016) examined the seeds of 7 *Dactylorhiza* taxa including 2 endemics from Turkey (*D. nieschalkiorum*, *D. osmanica* var. *osmanica*, *D. euxina* var. *euxina*, *D. iberica*, *D. romana* subsp. *romana*, *D. saccifera*, *D. urvilleana*) in terms of morphological, micromorphological and morphometric properties. They analyzed the seeds using standard procedures of both light and scanning electron microscopy. The seeds collected from different localities were examined in terms of 22 characters, such as seed shape and size, length/width ratio, embryo volume, seed/embryo volume, percent airspace, position of the embryo and seed surface features were analyzed. Furthermore, the morphometric measurements showed that some diagnostic characters could be important for these taxa. As a result of the study, the dendograms were formed based on the numerical analysis and important differences were determined in terms of examined characters among taxa.

Though several reports on orchid seed morphometric studies by using light microscopy and scanning electron microscopy (SEM) techniques are reported from various parts of India, so far no report is published from Jammu and Kashmir Himalayas. Therefore, to fill the gap, for the first time, the present study has been undertaken to analyze seed morphometry of four imperative *Dactylorhiza* (Orchidaceae) species growing in Kashmir Himalaya-India which may prove useful in species delimitation and taxonomic studies.

MATERIALS AND METHODS

Study area

Kashmir Himalaya is situated in northern fringe of the Indian sub-continent between 33°22' and 34°50'N latitudes and 73°55' and 73°33'E longitudes covering an area of about 2, 22.797 sq Km. Extensive field surveys between the months of April to October (2010 – 2016) covering an altitudinal range of 2100 – 4300m amsl were undertaken in South, Central, North and Western areas of Kashmir. Voucher specimens for each species were collected, processed into mounted herbarium-sheets and deposited in Kashmir University Herbarium (KASH) of the Department of Botany, University of Kashmir, with Voucher Specimen Numbers from 1891 – 1894 (Ref. No. F/GS Herbarium-Specimen vouchers, KU/2016) for the four species of *Dactylorhiza*.

Collection of plant material and microscopic studies

The mature seeds of the species under study have been collected from natural populations (Table 1). The geo-coordinates of the selected sites were recorded by using GPS (Model: GPS map76CSx). The morphological characters of seeds were analyzed and photographed with the help of Trinocular Stereo-Zoom Microscope (Model: Carl Zeiss Discovery V8). For SEM studies, double sized conductive tap was fixed with stub, mature seeds were loaded on the stub

and then sputter coated with gold and finally observed under Scanning Electron Microscope (Model: Hitich-S-3000H). Seed dimensions were measured under light microscope with micrometer.

Table 1. Collection sites and fruiting period of selected species of *Dactylorhiza* Neck. ex Nevski of Kashmir Himalaya

S. No.	Species	Collection site (Geo-coordinates), altitude	Fruiting period (months)
1	<i>D. hatageria</i>	Daksum – Anantnag 34°22.321' N & 74°03.321' E 3549m amsl	August – September
2	<i>D. kafiriana</i>	Tulail – Bandipora 34°31.334' N & 75°10.757' E 3212m amsl	July – August
3	<i>D. umbrosa</i>	Vishansar – Ganderbal 34°24.109' N & 75°08.122' E 3645m amsl	August – September
4	<i>D. viridis</i>	Vishansar – Ganderbal 34°24.210' N & 75°08.101' E 3704m amsl	August – September

RESULTS AND DISCUSSION

The present investigation revealed that the selected orchid species exhibit diversity in the seed shape and size, and also shape and size of testa cells (Table 2 and Plates I – IV). It has been observed that the seeds are spatulate with ovoid and rectangular testa cells in *D. hatageria* and *D. viridis* where as in *D. kafiriana* the seeds are crescent with rectangular testa cells and in *D. umbrosa* they are elliptic with pentagonal testa cells. The seeds are longer in *D. kafiriana* followed by *D. umbrosa* and shorter in *D. viridis* followed by *D. hatageria*. The seed width is broader in *D. umbrosa* followed by *D. viridis* and narrower in *D. kafiriana*. Longer testa cells are seen in *D. kafiriana* and shorter in *D. umbrosa*. Similarly testa cells are observed wider in *D. hatageria* and narrower in *D. umbrosa*. The seed coat is transparent-light brown and embryo is dark brown in *D. hatageria*. In case of *D. kafiriana* the seed coat is transparent and embryo is light brown. In *D. viridis* the seed coat is transparent-light brown and embryo is dark. In *D. umbrosa*, the seed coat is transparent and embryo is brown in colour. Different morphological characters observed in different species are summarized in Table 2.

The different seed shapes like fusiform, spatulate and elliptic are characteristic feature of orchids (Arditi *et al.* 1979; Rasmussen 1995; Verma 2014), of which fusiform shape is considered as primitive (Arditi *et al.* 1979; Verma *et al.* 2014) and all other shapes are evolved (Verma *et al.* 2014). With this concept, the species under present study with spatulate, crescent and elliptic shape seeds may be considered as advanced. Further, as per the statement that the longer seeds are considered as primitive over the shorter ones (Vij *et al.* 1992; Verma *et al.* 2014), among the present studied species, *D. kafiriana* may be considered as the most primitive species than the rest three.

From the literature, it is noted that the variation in shape and size of testa cells is also one of the characteristic features of orchid seeds (Vij *et al.* 1992; Swamy *et al.* 2004 Verma *et al.*

PLATE I. Seed morphometry of *Dactylorhiza hatagirea* : **A & B.** Seeds and single seed in closer view under Stereo-zoom microscope; **C – E.** Seed shape, coat and testa cells under Scanning Electron Microscope

PLATE II. Seed morphometry of *Dactylorhiza kafiriana* : **A & B.** Seeds and single seed in closer view under Stereo-zoom microscope; **C – E.** Seed shape, coat and testa cells under Scanning Electron Microscope

PLATE III. Seed morphometry of *Dactylorhiza umbrosa* : **A & B.** Seeds and single seed in closer view under Stereo-zoom microscope; **C – E.** Seed shape, coat and testa cells under Scanning Electron Microscope

PLATE IV. Seed morphometry of *Dactylorhiza viridis* : **A & B.** Seeds and single seed in closer view under Stereo-zoom microscope; **C – E.** Seed shape, coat and testa cells under Scanning Electron Microscope

Table 2. Seed morphometry of four *Dactylorhiza* species of Kashmir Himalaya, India

Species	Seeds			Testa cells		
	Shape	Length (mm)	Width (mm)	Shape	Length (µm)	Width (µm)
<i>D. hatageria</i>	Spathulate	0.36±0.05	0.16±0.001	Ovoid and rectangular	345±240	230±170
<i>D. kafiriana</i>	Crescent	0.85±0.01	0.10±0.003	Rectangular	560±340	90±65
<i>D. umbrosa</i>	Elliptical	0.67±0.02	0.27±0.003	Pentagonal	180±130	30±25
<i>D. viridis</i>	Spathulate	0.25±0.02	0.17±0.004	Ovoid and rectangular	320±290	180±110

2014) and the shape, size and wall thickening pattern have great utility in taxonomic delimitation (Healey *et al.* 1980). The authors are of the opinion that the findings from the present study may be effectively used for taxonomic delimitation of the species in future revisionary studies of the genus *Dactylorhiza*.

Acknowledgments

Authors are highly thankful to the Head, Department of Botany, University of Kashmir-Srinagar, for providing necessary facilities to carry out this work. They also acknowledge the kind help of Mr. Tariq Ahmad of USIC, University of Kashmir in SEM studies. Authors are also highly thankful to Late. Prof. Suraj Prakash Vij and Prof. Promila Pathak of Department of Botany, Punjab University Chandigarh, India for their valuable suggestions and for providing relevant literature.

LITERATURE CITED

- Akbulut, M. K. & Aneel, G. 2016. The seeds micromorphology and morphometry of certain *Dactylorhiza* (Orchidaceae) species distributed in Turkey. *Rendiconti Lincei* 27(4): 679 – 686.
- Akçin, T.A.; Ozdener, Y. & Akçin, A. 2010. Taxonomic value of seed characters in orchids from Turkey. *Belgian Journal of Botany* 142(1): 124 – 139.
- Arditti, J. & Ghani, A.K.A. 2000. Tansley Review No. 110. Numerical and physical properties of orchid seeds and their biological implications. *The New Phytologist* 145(3): 367 – 421.
- Arditti, J.; Michaud, J.D. & Healey, P.L. 1980. Morphometry of orchid seeds. II. Native California and related species of *Calypso*, *Cephalanthera*, *Corallorhiza* and *Epipactis*. *American Journal of Botany* 67: 347 – 365.
- Arditti, J.; Michaud, J.D. & Healey, P.L., 1979. Morphometry of Orchid Seeds. I. *Paphiopedilum* and native California and related species of *Cypripedium*. *American Journal of Botany* 66 (10): 1128 – 1137.
- Augustine, J.; Kumar, Y. & Sharma, J. 2001. Orchids of India. II. *Biodiversity and status of Bulbophyllum Thou.* Daya, New Delhi.
- Barthlott, W. & Ziegler, B. 1981. Morphologie der Samenschalen als systematische Merkmal bei Orchideen. *Ber. Dtsch. Bot. Ges.* 94: 267 – 273.

- Chase, M.W. & Pippen, J.S. 1988. Seed morphology in the *Oncidiinae* and related subtribes (Orchidaceae). *Systematic Botany* 13(2): 313 – 323.
- Chemisquy, M.A.; Prevosti, F.J. & Morrone, O. 2009. Seed morphology in the tribe Chloraeae (Orchidaceae): combining traditional and geometric morphometrics. *Botanical Journal of the Linnean Society* 160(2): 171 – 183.
- Gamarra, R.; Galan, P.; Herrera, I. & Ortunez, E. 2008. Seed micromorphology supports the splitting of *Limnorchis* from *Platanthera* (Orchidaceae). *Nordic Journal of Botany* 26(1 2): 61 – 65.
- Gamarra, R.; Ortunez, E.; Sanz, E.; Esparza, I. & Galan, P. 2010. *Seeds in subtribe Orchidinae (Orchidaceae): the best morphological tool to support molecular analyses*. EUT Edizioni Universita di Trieste
- Healey, P.L.; Michaud, J.D. and Arditti, J. 1980. Morphometry of orchid seeds. III Native California and related species of *Goodyera*, *Piperia*, *Platanthera* and *Spiranthes*. *American Journal of Botany* 67: 508 – 518.
- Kaya, A.; Unal, M.; Ozgokce, F.; Dogan, B., & Martin, E. 2011. Fruit and seed morphology of six species previously placed in *Malcolmia* (Brassicaceae) in Turkey and their taxonomic value. *Turkish Journal of Botany* 35(6): 653 – 662.
- Rasmussen, H.N. 1995. *Terrestrial orchids: from Seed to Mycotrophic Plant*. Cambridge University Press, Cambridge, UK.
- Swamy, K.K.; Kumar, H.K.; Ramakrishna, T.M. & Ramaswamy, S.N. 2004. Studies on seed morphometry of epiphytic orchids from Western Ghats of Karnataka. *Taiwania* 49(2): 124 – 140.
- Swamy, M.K.; Mohanty, S.K. & Anuradha, M. 2014. The effect of plant growth regulators and natural supplements on *in vitro* propagation of *Pogostem oncablin* Benth. *Journal of Crop Science and Biotechnology* 17(2): 71 – 78.
- Verma, J.; Thakur, K.; Sembi, J.K. & Vij, S.P. 2012. Study on seed morphometry of seven threatened Himalayan orchids exhibiting varied life modes. *Acta Botanica Gallica* 159(4): 443 – 449.
- Verma, J.; Sharma, K.; Thakur, K.; Sembi, J.K. & Vij, S.P. 2014. Study on seed morphometry of some threatened Western Himalayan orchids. *Turkish Journal of Botany* 38(2): 234 – 251.
- Verma, J.; Thakur, K. & Vij, S. P. 2013. On the occurrence of an interesting leafless orchid *Neottia listeroides* Lindl. In Himachal Pradesh, northwestern Himalaya, India. *Journal of Threatened Taxa* 5(11): 4601 – 4603.
- Vij, S. P.; Kaur, P.; Kaur, S. & Kaushal, P.S. 1992. The orchid seeds: taxonomic, evolutionary and functional aspects. *Journal of Orchid Society of India* 6: 91 – 107.