

ONTOGENY AND ORGANISATION OF FEMALE GAMETOPHYTE IN TRIANDROUS ORCHID, *NEUWIEDIA VERATRIFOLIA* BLUME (ORCHIDACEAE)– A RE-INVESTIGATION

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Abstract

The ovary in *Neuwiedia veratrifolia* Blume was observed as inferior, tricarpeal, syncarpous, and trilobular due to fusion of the placenta. Numerous anatropous, bitegmic, and tenuinucellate ovules were borne on the placental humps. Funicle lacks conducting tissue. Integuments developed from the epidermis of the ovular primordium, 2-cell layered thick; micropyle was formed from the inner integument alone. The development of embryo sac confirmed to monosporic C-1b type (Abe, 1972b). Chalazal megaspore in the triad configuration developed into 8-nucleate embryo sac. The mature embryo sac contained an egg apparatus, secondary nucleus, and 3 antipodal nuclei. Double fertilization occurred in a normal manner.

Introduction

THE GENUS *Neuwiedia* Blume (Tribe: Apostasioideae; sub-family: Apostasioideae; family: Orchidaceae of Dressler and Dodson, 1960) has 8 species, distributed in Malayan Peninsula, South Eastern China, Vietnam, Thailand, Indonesia, Philippines, and New Guinea (Burns-Balogh and Funk, 1986; Vogel, 1969; WCSP, 2019). Clements (1995, 1999) reported bisporic and G-2 type of embryo sac development in *Neuwiedia veratrifolia*. A re-investigation of this aspect of the same taxon collected from Singapore has revealed some interesting deviations which are presented here.

Material and Methods

Neuwiedia veratrifolia Blume is an erect terrestrial herb, 70-150 m in height. Leaves were lanceolate to linear, acuminate with prominent nerves. Inflorescence was terminal, 20-40 cm long, surpassing the leaves in length. Flowers were yellow, and subtended by long bracts (Figs. 1, 2). Sepals and petals 3 + 3, lateral ones obovate-lanceolate, median ones lanceolate. Stamens 3, basal part of the filament fused with style and form gynostegium. Ovary inferior, triangular, ellipsoid; style shorter than the stamens; stigma rounded to obscurely three lobed (Fig. 3). The specimen was procured from Singapore in the year 1982 by Prof Arekal D Govindappa, Head of the Department of Botany, Mysore University, Mysuru and comprised of few flower buds. The specimens were later misplaced and remain unnoticed for several years. Recently (2018), some flower buds were recovered from partially dried specimens and were used for the present study. Conventional micro techniques were followed. Serial transverse sections of 10-12 μ m thickness were stained with Heidenhain's iron-alum and Haematoxylin. Erythrosin in clove oil was used as a counter stain. Drawings were

made by using the specimen under camera lucida and Meopta microscope. Photomicrographs were taken by an Olympus-CH 20i microscope with built in analogue camera



Figs. 1-3. *Neuwiedia veratrifolia*: 1, Habit of the flowering plant (scale bar= 20 cm); 2, Terminal portion of inflorescence, note the bracts are longer than flowers (scale bar= 8 mm); 3, Flower with and without sepals and petals, note the fusion of filaments with style (scale bar= 8 mm).

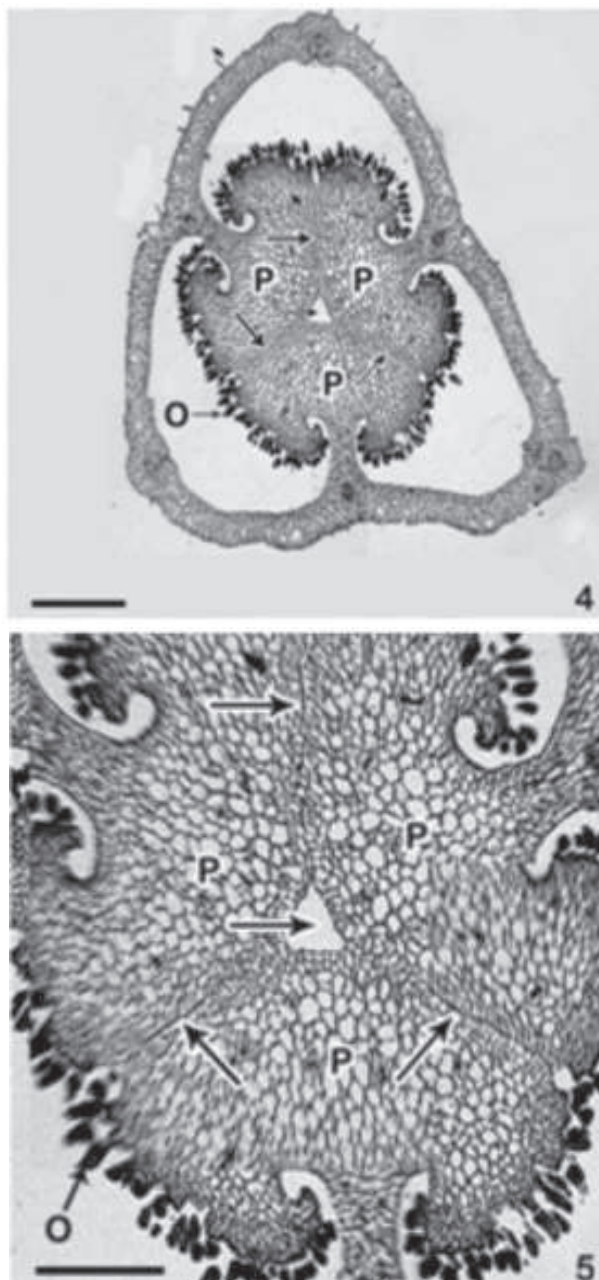
(CM OF 1.4 megapixel) and captured using Av-digitiser having grand VCD-200 captured guard.

Results

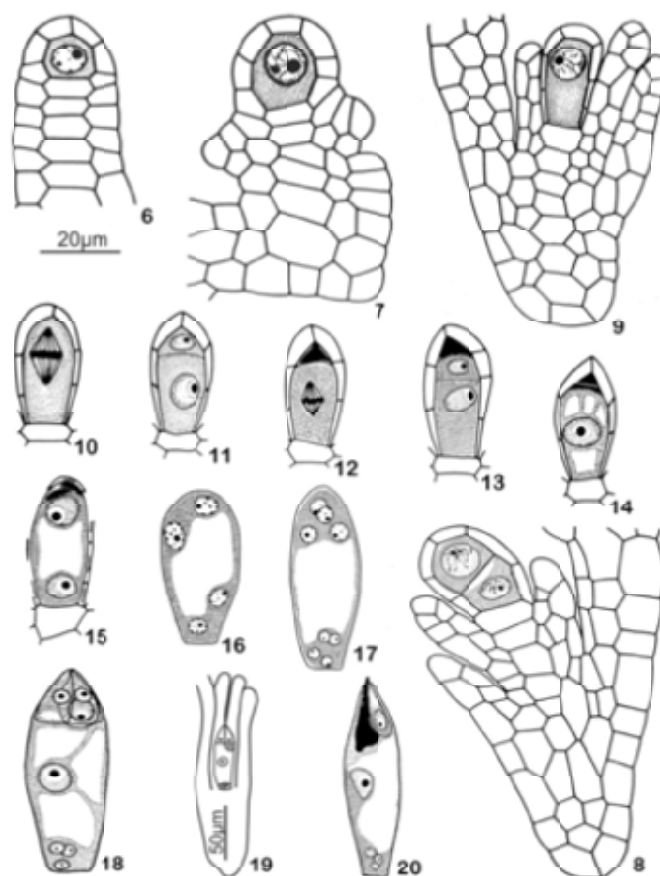
Megasporangium

The ovary was observed to be inferior, tricarpeillary, syncarpous, and appeared trilobular due to fusion of the placenta (Figs. 4, 5). Ovules were borne on placental

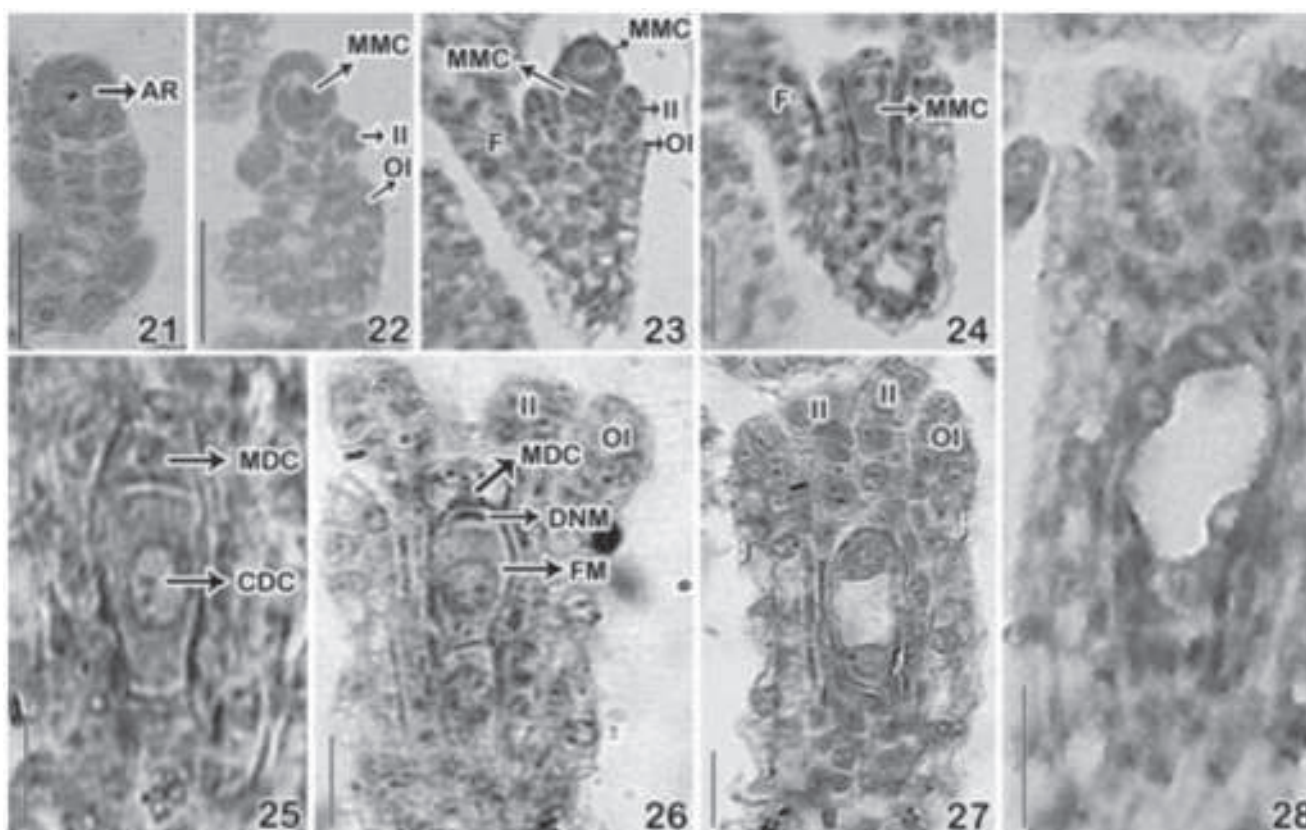
mass. Before pollination, numerous ovular primordia arose from the placental mass. Each ovular primordium was composed of an axial row of 7-8 cells surrounded by an epidermis. The terminal cell of the axial row forms a large, densely cytoplasmic archesporial cell (Figs. 6, 21). Further, it enlarged and functioned directly as megaspore mother cell. The integumentary primordia arose from the epidermis of the ovular primordium immediately below the megaspore mother cell (Figs. 7, 22). Although the inner layer developed faster during early stages, the outer layer soon matched that of the inner layer while developing the two integuments. Both the integuments were two layered throughout the developmental stages of the tenuinucellate anatropous ovule. The funicle was found to be devoid of vasculature.



Figs. 4-5. *Neuwiedia veratrifolia*, T. S. of ovary: 4, Ovary in T. S. (scale bar= 1000 µm); 5, Central portion of the ovary enlarged, note triangular opening in the centre and post-genitally united septa (indicated by the arrow), (scale bar= 500 µm). (O, Ovule; P, Placenta.)



Figs. 6-20. *Neuwiedia veratrifolia*, ontogeny and organization of female gametophyte: 6, Ovular primordium showing hypodermal archesporial; 7, Ovular primordium with megaspore mother cell, note the initiation of integuments; 8, Ovule with double superposed megaspore mother cell; 9, Ovule with megaspore mother cell, note funicle without vasculature; 10, Megaspore mother cell at meiosis-I; 11, Unequal dyad cells; 12, Shows degenerating micropylar dyad cell and chalazal dyad cell at meiosis-II; 13, A triad; 14, Functional megaspore; 15, 2-nucleate embryo sac; 16, 4-nucleate embryo sac; 17, 8-nucleate embryo sac; 18-19, Organized embryo sac, note inner integument alone forms the micropyle; 20, Show the stages of double fertilization.



Figs. 21-28. *Neuwiedia veratrifolia*, photomicrograph showing different stages during embryo sac development: 21, Ovular primordium showing hypodermal archesporial cell (scale bar= 10 μ m); 22, Ovular primordium with megaspore mother cell; note the initiation of inner and outer integuments (scale bar= 10 μ m); 23, Ovule with double superposed megaspore mother cells (scale bar= 10 μ m); 24, Anapous ovule with megaspore mother cell, note funicle without vasculature (scale bar= 10 μ m); 25, Ovule with dyad cells, note the smaller micropylar dyad cell (scale bar= 10 μ m); 26, A triad; note degenerating micropylar dyad cell; degenerating non-functional megaspore and chalazal larger functional megaspore (scale bar= 10 μ m); 27, Two-nucleate embryo sac (scale bar= 10 μ m); 28, Four-nucleate embryo sac (scale bar= 15 μ m). (AR, Archesporial cell; CDC, Chalazal dyad cell; DNM, Degenerating non-functional megaspore; F, Funicle; FM, Functional megaspore; II, Inner integument; MDC, Micropylar dyad cell; MMC, Megaspore mother cell; OI, Outer integument).

The micropyle was formed by the inner integument alone (Figs. 19, 27). Usually a single megaspore mother cell (MMC) was formed in a developing ovule. Very rarely two MMCs were observed within a common nucellus (Figs. 8, 23). Further development of the second megaspore mother cells could not be recorded.

Megasporogenesis and Development of Female Gametophyte

The megaspore mother cell underwent meiosis-I and produced two superposed dyad cells (Figs. 10, 11, 25). Usually, the smaller micropylar dyad cell degenerated. The larger chalazal dyad, after meiosis-II, gave rise to two unequal superposed megaspores. Thus, a triad was formed which consisted of a degenerating micropylar dyad cell, a small degenerating non-functional megaspore and a larger chalazal functional megaspore (Figs. 12, 13, 14, 26). A free nuclear division occurred in the functional megaspore. The resulting daughter nuclei were pushed towards the opposite poles and a central vacuole was formed in the cytoplasm. Thus, a

2-nucleate embryo sac was organised (Figs. 15, 27). The nuclei of the 2-nucleate embryo sac further divided simultaneously and gave rise to two pairs of nuclei, (one pair being located at each pole). A 4-nucleate embryo sac was therefore organized (Figs. 16, 28). The four nuclei of the sac divided synchronously forming eight nuclei, disposing a micropylar and chalazal quartet. The nuclei of the chalazal quartet are smaller than those of the micropylar quartet (Fig. 17). Three of the micropylar quartet of nuclei organized into the egg apparatus and the other three at the chalazal end from the antipodal nuclei. The remaining two, one from each quartet fused together to form the secondary nucleus prior to fertilization (Figs. 18, 19). Double fertilization occurred normally (Fig. 20).

Discussion

Female Gametophyte

The ovary was inferior, tricarpeal, syncarpous, and appeared trilobular due to fusion of the placenta. This

conclusion was derived due to the presence of a central triangular opening at the centre of ovary and the post genital septa. The present observation has also been supported by Kocyan and Endress (2001), who studied the floral structure and development of *Neuwiedia veratrifolia*. They have noticed that the inferior ovary was first unilocular and later became trilocular by fusion of the placenta. The ovary of *N. veratrifolia* must be a transitional stage between true axile placentation and parietal placentation, as has been recorded in majority of the orchids.

The initiation of the placenta was found to be triggered before pollination. Similar observations have been made in *Epipogium aphyllum* (Afzelius, 1954), *Epipactis papillosa* (Sato, 1974), and *Epipogium roseum* (Arekal and Karanth, 1981). However, in majority of orchids, initiation of ovules on the placenta occurs only after pollination has taken place (Brown, 1833; Nimoto and Sagawa, 1961; Vij and Sharma, 1986; Yeung and Law, 1989). It may also be recorded here that early placental proliferation is associated with the free pollen bearing plants such as *Paphiopedilum druryi* (Swamy, 1949) and *N. veratrifolia* (Kocyan and Endress, 2001) and its postponement is related to massulae and pollinia bearing plants. Finger-like ovular primordium consists of an axial row of cells ensheathed by a layer of epidermis. This is also true with the orchids that have been previously studied (Abe, 1972a; Gurudeva, 2009, 2010, 2011a, b, 2014; Krishna Swamy *et al.*, 2005; Swamy, 1949). The integumentary initials originated from nucellar epidermal cells. The inner integument was always the first to be differentiated, the first to become active and first to degenerate unlike in *Vanilla planifolia* (Swamy, 1947). The micropyle was organized by the inner integument alone. This is in complete agreement with the earlier findings (Abe, 1972a; Govindappa and Karanth, 1980; Gurudeva, 2016a, b; Mohana Rao and Sood, 1987; Sood and Mohana Rao, 1986; Swamy, 1949).

The archesporial cell enlarged in size and directly functioned as megaspore mother cell. It underwent meiosis-I giving rise to two superposed dyad cells. The ovule was completely anatropous at this stage. The funicle is devoid of vasculature. A similar feature has been observed in most orchids. It was very likely that in other investigated taxa, where the nature of funicle is not designated, would also be of similar type. The smaller upper dyad cell degenerated and the lower one passed through meiosis-II giving rise to a smaller upper non-functional megaspore and a lower functional megaspore. This led to the organization of a triad which is a common feature in all the orchids (Abe, 1972a; Govindappa and Karanth, 1980; Gurudeva, 2009, 2010,

2011a, b, 2016a, b; Mohana Rao and Sood, 1987; Sharp, 1912; Sood, 1992; Swamy, 1949; Ward, 1880). According to Abe (1972b), triad formation is considered to be a derived character.

The nucleus of the functional megaspore divided successively and developed into 8-nucleate embryo sac. The mature embryo sac comprised of egg apparatus (with two synergids and an egg cell), a secondary nucleus and three antipodals. Embryo sac with antipodal nuclei has also been recorded in *Epidendrum* (Gurudeva and Govindappa, 2008) and *Habenaria* (Swamy, 1946). The mode of embryo sac development confirms to monosporic Polygonum type and G-1a according to the classification provided by Abe (1972b).

Clements (1995, 1999) reported bisporic type of embryo sac development in *N. veratrifolia*. In contrast, monosporic type embryo sac development has been recorded in the present investigation, providing evidence for co-existence of both the types in the same taxon. A similar condition has been reported earlier in *Habenaria clavigera* and *H. latilabris* (Sharma and Vij, 1987). It is worthwhile to note that bisporic type of embryo sac development is not restricted to any specific group of orchids, and is recorded in triandrous, diandrous and monandrous orchids (Clements, 1995, 1999; Swamy 1949).

The embryological features recorded in *N. veratrifolia* support the retention of subfamily Apostasioideae within the family Orchidaceae.

References

- Abe, K. 1972a. Contributions to the embryology of the family Orchidaceae. VI. Development of embryo sac in 15 species of orchids. *Sci. Rep. Tohoku Univ.*, **36**: 135-78.
- Abe, K. 1972b. Contributions to the embryology of the family Orchidaceae. VII. A comparative study of the orchid embryo sac. *Sci. Rep. Tohoku Univ. Ser. IV (Biol.)*, **36**: 179-201.
- Afzelius, K. 1954. Embryo sac development in *Epipogium aphyllum*. *Svensk. Bot. Tidskr.*, **48**: 513-20.
- Arekal, D. A. and K. A. Karanth. 1981. The embryology of *Epipogium roseum* (Orchidaceae). *Plant Syst. Evol.*, **138**(1-2): 1-7.
- Brown, R. 1833. Observation on the organs and mode of fecundation in Orchidaceae and Asclepiadaceae. *Trans. Linn. Soc. (N.Y.)*, **16**: 685-45.
- Bruns - Balogh, P. and V. K. Funk. 1986. A phylogenetic analysis of the Orchidaceae. *Smithsonian Contr. Bot.*, **61**: 1-79.
- Clements, M. A. 1995. *Reproductive Biology in Relation to Phylogeny of the Orchidaceae Especially the Tribe*

- Diurideae*. Ph.D. Thesis, Australian National University, Canberra, Australia.
- Clements, M. A. 1999. Embryology, Vol. I. In: *Genera Orchidacearum* (eds. A. M. Pridgeon, P. J. Cribb, M. W. Chase, and F. N. Rasmussen). Oxford University Press, Oxford, U.K.
- Dressler, R. L. and C. H. Dodson. 1960. Classification and phylogeny in the Orchidaceae. *Ann. Miss. Bot. Gard.*, **47**: 25-68.
- Gurudeva, M. R. 2009. Embryo sac development in *Aerides maculosum* Lindl. (Orchidaceae). *J. Orchid Soc. India*, **23**(1-2): 15-18.
- Gurudeva, M. R. 2010. Embryo sac development in *Trias stocksii* Benth. ex Hook f.- An endemic orchid. *J. Orchid Soc. India*, **24**(1-2): 1-3.
- Gurudeva, M. R. 2011a. Development of embryo sac in *Zeuxine gracilis* (Breda) Bl. (Orchidaceae). *J. Indian Bot. Soc.*, **90**(1-2):191-94.
- Gurudeva, M. R. 2011b. Ontogeny and organization of female gametophyte in *Disperis neilgherrensis* Wight (= *Disperis zeylanica* Trimen). *J. Orchid Soc. India*, **25**(1-2): 1-4.
- Gurudeva, M. R. 2014. Ontogeny and organization of female gametophyte in *Goodyera procera* (Ker-Gawl.) Hook. (Orchidaceae). *J. Orchid Soc. India*, **28**: 75 -77.
- Gurudeva, M. R. 2016a. Development of male and female gametophyte in *Habenaria ovalifolia* Wight (Orchidaceae). *J. Orchid Soc. India*, **30**: 1-10.
- Gurudeva, M. R. 2016b. Development of male and female gametophyte in *Dendrobium ovatum* (L.) Kranz. (Orchidaceae). *J. Orchid Soc. India*, **30**: 75-87.
- Gurudeva, M. R. and D. A. Govindappa. 2008. Ontogeny and organization of female gametophyte in *Epidendrum radicans* Pavon. ex Lindl. (Orchidaceae). *J. Orchid Soc. India*, **22**(1-2): 73-76.
- Govindappa, D. A. and K. A. Karanth. 1980. Contribution to the embryology of Orchidaceae. In: *Current Trends in Botanical Research* (eds. M. N. Nagaraj and C. P. Malik). Kalyani Publishers, New Delhi, India.
- Kocyan, A. and P. K. Endress. 2001. Floral structure and development of *Apostasia* and *Neuwiedia* (Apostasioideae) and their relationships to other Orchidaceae. *Int. J. Plant Sci.*, **162**(4): 847-67.
- Krishna Swamy, K., H. N. Krishna Kumar, and S. N. Ramaswamy. 2005. Contributions to the megasporogenesis and megagametogenesis in *Habenaria grandifloriformis* Lindl. and *Platanthera susannae* (L.) Lindl. (Orchidaceae). *Ad. Plant Sci.*, **18**(11): 439-48.
- Sharma, Madhu and S. P. Vij. 1987. Embryological studies on Orchidaceae. VI: *Habenaria* Willd. *Phytomorphol.*, **37**(4): 327-35.
- Mohana Rao, P. R. and S. K. Sood. 1987. Embryology of *Oreorchis foliosa* (Orchidaceae). *Phytomorphol.*, **37**(1): 1-8.
- Nimoto, D. H. and Y. Sagawa. 1961. Ovule development in *Dendrobium*. *Amer. Orchid Soc. Bull.*, **30**: 813-19.
- Sato, Y. 1974. Embryological studies in the Japanese *Epipacis* (Orchidaceae). *Sci. Rep. Tohoku Univ. Ser. IV (Biol.)*, **37**: 33-45.
- Sharp, L. W. 1912. The orchid embryo sac. *Bot. Gaz.*, **54**(5): 372-85.
- Sood, S. K. 1992. Embryology of *Malaxis saprophyta* with comments on the systematic position of *Malaxis* (Orchidaceae). *Plant Syst. Evol.*, **179**(1-2): 95-105.
- Sood, S. K. and P. R. Mohana Rao. 1986. Development of male and female gametophytes in *Herminium angustifolium* (Orchidaceae). *Phytomorphol.*, **36**: 11-15.
- Swamy, B. G. L. 1949. Embryological studies in the Orchidaceae. I. Gametophytes. *Am. Midl. Nat.*, **41**: 184-201.
- Swamy, B. G. L. 1946. Embryology of *Habenaria*. *Proc. Nat. Inst. Sci. Indian*, **12**: 413-26.
- Swamy, B. G. L. 1947. On the life history of *Vanilla planifolia*. *Bot. Gaz.*, **106**: 449-56.
- Vij, S. P. and M. Sharma. 1986. Embryo sac development in Orchidaceae. In: *Biology, Conservation and Commercialization of Orchids* (ed. S.P. Vij) pp. 31-49. East-West Affiliated Press, New Delhi, India.
- Vogel, de E. F. 1969. Monograph of the tribe Apostasioeae (Orchidaceae). *Blumea*, **17**(2): 313-50.
- Ward, H. M. 1880. On the embryo sac and development of *Gymnadenia conopsea*. *Q. J. Microsc. Sci.*, **20**: 1-18.
- WCSP. 2019. *World Checklist of Selected Plant Families*. Royal Botanic Garden, Kew, U.K.
- Yeung, E. C. and S. K. Law. 1989. Embryology of *Epidendrum ibaguense*. I. Ovule development. *Can. J. Bot.*, **67**(8): 2219-26.