J. Orchid Soc. India, 39: 49-52, 2025 *ISSN 0971-5371*
https://doi.org/10.64873/JOSI.v39.i1-2. 49-52

MEGASPOROGENESIS AND THE DEVELOPMENT OF FEMALE GAMETOPHYTE IN *HABENARIA CRINIFERA* LINDL.

Soumya Mahabaleshwar Hegde and K Krishnaswamy[1]

Department of Botany, Kuvempu University, Shimoga- 577 201, Karnataka, India
[1]Department of Botany, Sahyadri Science College, Shimoga- 577 201, Karnataka, India

Abstract

Habenaria crinifera is a terrestrial orchid that grows in the dense moist deciduous forest, evergreen forest, and grasslands of the Western Ghats. Megasporogenesis and development of female gametophytes in *H. crinifera* showed that the ovary is inferior and unilocular with many ovules born on the parietal placenta. The ovules were found to be anatropous, bitegmic, and tenuinucellate. The development of the female gametophyte confirms the monosporic 8-nucleate type in *H. crinifera*.

Introduction

THE FAMILY Orchidaceae is one of the largest, highly developed, and widely distributed families of flowering plants. This family constitutes 40% of monocotyledonous taxa (Rasmussen, 1985); it comprises about 693 genera distributed in 29,481 species (POWO, 2025; WFO, 2023). There is a record of 150 species under 70 genera from South India, of which 176 species were reported from Karnataka (Krishnaswamy, 2003; Udupa, 2012). Orchids are economically important plants and their ornamental nature attracts the horticulture and floral industry (Lawrence, 1951). These plants exhibit great diversity in the development and organization of the male and female gametophytes. Schlecter (1926) and Swamy (1943) have given a detailed review of the embryological investigation of orchids; out of 15,000 species of orchids only about 175 species have so far been studied embryologically (Swamy, 1949). Orchids are embryologically highly interesting, as these exhibit great diversity in the development and organization of the male and female gametophyte, suspensor, and embryo. Information about the reproductive biology of a large number of orchid taxa in India is not available.

During the previous studies on the reproductive biology of orchids by Swamy (1943, 1949), significant contribution to the embryo sac development in the Orchidaceae was made. Abe (1967, 1973, and 1977) conducted research on a large number of orchids and suggested new investigations in the field of embryology. Kolomeitseva *et al*. (2021), during their study in *Dendrobium nobile*, reported that the orchid reproductive strategy, including the formation of numerous tiny seeds, is achieved by the elimination of some stages in the early plant embryogenesis and during their study they documented in detail the formation of the maternal tissues (the nucellus and integuments), the structures of female gametophyte (megaspores, chalazal nuclei, synergids, polar nuclei), and embryonic structures. Kimura (1968) noted that *Cypripedium debile* had 8-nucleate embryo sac, whereas Mohan and Sood (1979a) reported that *Spathoglottis plicata* had a monosporic, 8-nucleate embryo sac. Monosporic embryo sac development was reported in *Habenaria densa* and *Satyrium nepalense* (Mohan Rao and Sood 1979a,b). However, Arekal and Karanth (1980) have found a bisporic type of gametophyte in *Zeuxine longilabris*. Sood and Rao (1988) investigated the embryology of the diandrous orchid *Cypripedium cordigerum* and observed anatropous, bitegmic, and tenuinucellate ovule and 6-nucleate and diasporic female gametopyte. Krishnaswamy (2003) observed monosporic 8-nucleate embryo sac development in *Habenaria grandifloriformis* and *Platanthera susannae*.

Habenaria crinifera Lindl. is a small sized terrestrial orchid commonly known as *Narilatha,* doll orchid; it usually grows in the grassland and shade of moist to deciduous forest at elevations from sea level to 1700 m amsl. The tuberous species with a wide range of distribution throughout tropical and subtropical Asia and the West Pacific grows both as an epiphyte and terrestrial plant; it blooms in large clusters during late rainy seasons (Fig 1. A-B). The petals of white flowers have smooth edges. The long, white lip is three times longer than the sepals; it has a long, three-lobed claw with side lobes that resemble wedges, serrated edges on the outside, and a thin tail that grows from the inside

Received: February 9, 2025; Accepted: June 30, 2025

that is as long as the lip. The mid lobe has two long, tail-like, lance-shaped segments that are as long as the side lobes and are clawed. The spur is narrow and curved. Its propagation occurs by tubers. During the present investigation, studies on megasporogenesis and the development of the female gametophyte was studied in *H. crinifera*.

Material and Methods

The material used for the present study includes flowers of *H. crinifera* at different stages of development. These were collected from the forest of Shimoga (Karnataka) between June 2021 and September 2021. Material was fixed in Formalin-Acetic acid-Alcohol, dehydrated in ethyl alcohol-xylene series and embedded in paraffin wax (52°C). Sections were cut at 10µm to 14µm thickness, stained with iron-alum-hematoxylin and counter-stained with erythrosine in clove oil. Observations were made under a microscope; photographs were taken using a digital camera.

Results and Discussion

In *H. crinifera,* the ovary was inferior, tricarpellary, syncarpous, and unilocular. A transverse section of the ovary showed three placental ridges. The placenta was forked and ovular primordia originated as small protuberances on the placenta. The placental ridges proliferated to produce several short protuberances, each branch acted as an ovular primordium and these were ensheathed by the nucellar epidermis. The terminal cell of the ensheathed cell was densely cytoplasmic and had a large nucleus that acted as an archesporial cell. Ultimately, it increased in size and functioned as a megaspore mother cell. The ovules were tenuinucellate, bitegmic, and anatropous. The integuments have two layers of cells. First meiotic division results in the formation of the dyad; the upper dyad cell degenerates, and chalazal dyad cell were functional. The functional megaspore underwent a second meiotic division to form two megaspores and these were separated by a vacuole to form two nucleate embryo sacs. Further, mitotic division resulted in the formation of a four-nucleate embryo sac. In the following division, eight nucleate embryo sacs have been formed. A mature embryo sac has an egg apparatus consisting of two synergids, an egg; central cell with two polar nuclei (which fuse to form a secondary nucleus), and three antipodal cells.

Orchids have attracted plant embryologists for years concerning their female gametophyte developmental stages. The ovules initiate their development on the placenta only after pollination. Similar observations were made earlier by the previous workers (Ekanthappa, 1981; Swamy, 1949). The ovules were tenuinucellate, bitegmic, and anatropous with the inner integument forming the micropyle as reported earlier in the majority of orchids (Abe, 1977; Haung *et al.*, 1998; Hegde and Krishnaswamy, 2021: Johri *et al.* 1992; Law and Yueng, 1993; Yeung *et al.*, 1994). In *Pholidota imbricata* and *Hateria sichokiana,* both integuments formed the micropyle (Ekanthappa, 1981; Tohda, 1967; Vij *et al.*, 1982). In *Epipogium aphyllum,* micropyle was not organized and ovules were unitegmic (Afzelius, 1954). In *Calanthe triplicate,* two synergids and an egg were found to be in the micropylar part of the embryo sac (Krishnaswamy, 2003). According to Swamy (1947), the two-layered state of the integument is regarded as a derived condition in contrast to multi-layered integuments.

In the present work, ovular primordia developed after pollination. The nucellar filaments comprised 5-8 nucellar cells which were covered by nucellar epidermis. A nucellar cell present at the uppermost end of the nucellar primordium beneath the epidermis was the hypodermal cell with dense cytoplasm and conspicuous nucleus and this acted as an archesporial cell, it enlarged its size and acted as a megaspore mother cell. Similar observations were made in some other orchids by Sood and Sharma (1987) and Zhang and Neil (1993). In the presently studied *H. crinifera*, meiosis-I in the megaspore mother cell resulted in the formation of two individual dyad cells. The second meiotic division occured only in the lower dyad cell. The upper dyad cell degenerated and the chalazal dyad cell was only functional. The second mitotic division produced 4-nucleate embryosac, a vacuole present at the centre of the embryosac. The subsequent division resulted in the formation of 8-nucleate embryo sac (Fig.1 C-G). A completely organized embryo has an egg apparatus with two synergids and an egg, a central cell with two polar nuclei, and three antipodal cells (Fig. 1 H). At the time of double fertilization, polar nuclei fuse to form a secondary nucleus. Fertilization was porogamous; during fertilization one of the synergids was degenerated by the entry of the pollen tube. Double fertilization occured initiating the zygote development, and the other synergid subsequently degenerated.

The present study in *H. crinifera* revealed that the development of the female gametophyte was monosporic, 8-nucleate polygonum type and similar development has been reported in the majority of the orchids. Further studies need to be made in other large number of orchids as the data on megasporogenesis and the development of female gametophyte is deficient.

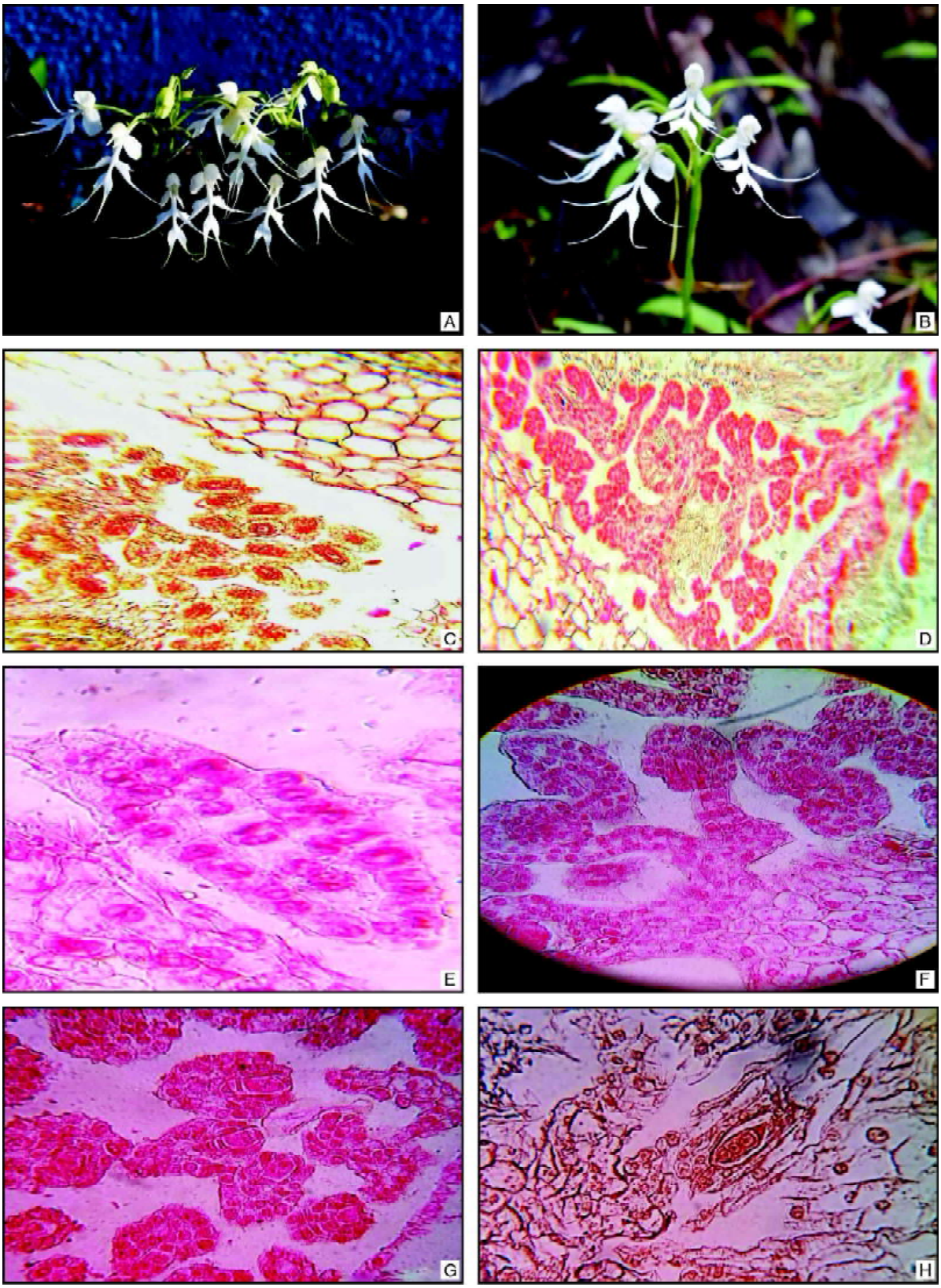

Fig. 1. A-H. Studies on Megasporogenesis in *Habenaria crinifera* Lindl.: A-B, Plants in bloom; C, Archesporial initial cell; D, An ovular primordium; E, Two nucleate stage; F, Archesporial cell enlarged; G, Four nucleate embryo sac; H, Ovule showing megaspore.

Acknowledgements

The first author would like to thank Dr K Krishnaswamy, Supervisor, for his encouragement and support during the present study. The authors wish to thank the authorities of the Karnataka Forest Department for permitting collection of the specimens from the study site. Help and cooperation from the research colleagues is also acknowledged.

References

Abe, K. 1967. Contribution to the embryology of the family Orchidaceae I. Development of embryo sac in *Cymbidium goeringii* Reichb.f. *Sci. Rep. Tohoku Univ*. Ser. VI (Bio.), **33**: 79-82.

Abe, K. 1973. Contribution to the embryology of the family Orchidaceae Viii, Development of embryo sac in *Luisia teres*. *Sci. Rep. Yamaguchi Univ.*, **20**: 19-117.

Abe, K. 1977. Development of embryo sac in *Amitostigma kinoshttae* (Makino) Schltr. (Orchidaceae). *Ann. Bot.* (London). **41**: 513-20.

Afzeius, K. 1954. Embryo sac development in *Epipogium aphyllum*. Svensk. *Bot. Tidskr*. **48**: 513-20.

Arekal, G. D. and K. A. Karanth. 1980. Contribution to the embryology of Orchidaceae. *In: Current Trends in Botanical Research* (eds. M. Nagaraj and C. P. Malik) pp. 19-33. Kalyani Publishers, New Delhi, India.

Ekanthappa, K. G. 1981. *Studies in the Orchidaceae.* Ph.D. Thesis. University of Mysore, Karnataka, India.

Huang, B. Q. X. L. Ye, E. C. Yeung, and S. Y. Zee. 1998. Embryology of *Cymbidium sinense*: The microtubule organization of early embryos. *Ann. Bot.*, **81**: 741-50.

Hegde, S. M. and K. Krishnaswamy. 2021. Megasporogenesis and the development of female gametophyte in *Geodorum densiflorum* (Lam.) Schltr. *J. Orchid Soc. India*, **35**: 153-57.

Johri, B. M., K. B. Ambegaokar, and P. S. Srivatsava. 1992. *Comparative Embryology of Angiosperms II*. Springer-Verlag, Berlin, Germany.

Kimura, C. S. 1968. On the embryo sac of *Cypripedium debile* Reichb.*f. Sci. Rep. Tohoku* Univ. Ser. IV **34**: 67-74.

Kolomeitseva, G. L., A. V. Babosha, and A. S. Ryabchenko. 2021. Megagametogenesis, and embryogenesis in *Dendrobium nobile* (Orchidaceae). *Protoplasma,* **258**: 301-17.

Krishnaswamy, K. 2003. *Studies on the Orchidaceae*. M.Sc. Thesis. University of Mysore, Karnataka, India.

Law, S. K. and E. C. Yeung. 1993. Embryology of *Cypripedium passerium* (Orchidaceae): Ovule development. *Lindleyana*, **8**(3): 139-47.

Lawrence, G. H. M. 1951. *Taxonomy of Vascular Plants.* Mc Millian Co. Ltd. New York, U.S.A.

Mohan, R. P. R. and S. K. Sood. 1979a. Embryology of *Habenaria densa* (Orchidaceae). *Bot. Not.,* **132**: 145-48.

Mohan, R. P. R. and S. K. Sood. 1979b. Life history of *Satyrium nepalense* (Orchidaceae). *Norw. J. Bot.,* **26**: 285-94.

POWO. 2025. *Plants of the World Online*. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet. http://www.plantsoftheworldonline.org.

Rasmussen, F. N. 1985. *Families of Monocotyledons. In: Structure, Evolution and Taxonomy* (eds. R. M. T Dahlgren, H. Clifford, and P. F. Yeo). Pp. 249-76. Springer-Verlag, Berlin, Germany.

Schlechter, R. 1926. Das system der orchidaceen. *Notizblatt des Botanischen Gartens und Museums zu Berlin Dahlem,* **9:** 563-91.

Sood, S. K. and N. Sharma. 1987. Gametophytes, embryogeny and pericarp of *Rhynchostylis retusa* Blume (Epidendreae, Orchidaceae). *Phytomorphology*, **37**(4): 307-16.

Sood, S. K. and Rao P. R. Mohana. 1988. Studies in the embryology of the diandrous orchid *Cypripedium cordigerum* (*Cypripedium*, Orchidaceae). *Pl. Syst. Evol.,* **160**: 159-68.

Swamy, B. G. L. 1943. Gametogenesis and embryogeny of *Eulophia epidendrea* Fischer. *Proc. Natn. Inst. Sci. India*, **9:** 59-65.

Swamy, B. G. L. 1947. On the life history of *Vanilla plantifolia*. *Bot. Gaz.,* **108**: 449-56.

Swamy, B. G. L. 1949. Embryological studies in the Orchidaceae. L. Gametophytes. *Am. Midl. Nat.*, **41**(1): 184-201.

Tohda, H. 1967. An embryological study of *Hataeria sichokiana,* a saprophytic orchid in Japan. *1* IV Scr. (Biol.), **33**: 83-95.

Udupa, K. E. 2012. *Studies on Distribution and Biology of Orchids in Chikmagalur District, Karnataka.* Ph.D. thesis, Kuvempu University, Karnataka, India.

Vij, S. P., M. Sharma, and N. Shekhar. 1982. Embryological studies in Orchidaceae II. *Zeuxine strateumatica*. *Phytomorphology*, **32**: 257-64.

WFO. 2023. *World Flora Online*. Published on the Internet; http://www.worldfloraonline.org.

Yeung, E. C., S. Y. Zea, and X. L. Ye. 1994. Embryology of *Cymbidium sinense*; Ovule development. *Phytomorphology*, **44**(1-2): 55-63.

Zhang, X. S. and S. D. O'Neill. 1993. Ovary and gametophyte development are coordinately regulated by auxin and ethylene following pollination. *The Plant Cell*, **5**(4): 403-18.