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MEGASPOROGENESIS AND THE DEVELOPMENT OF FEMALE GAMETOPHYTE IN *GEODORUM DENSIFLORUM* (LAM.) SCHLTR.

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Abstract

Geodorum densiflorum, a terrestrial orchid, grows in the shade of dense moist deciduous forests, evergreen forests, and grasslands of Western Ghats. During the present study, megasporogenesis and development of female gametophyte in this species showed that ovary was inferior and unilocular with many ovules borne on the parietal placenta. The ovules were anatropous, bitegmic, and tenuinucellate. The development of the female gametophyte confirmed to the monosporic 8-nucleate type in *G. densiflorum*.

Introduction

ORCHIDACEAE IS one of the largest, highly evolved, wide spread families of flowering plants. Orchidaceae constitute a total 40% of monocotyledons taxa (Rasmussen, 1985). The family comprises about 24,484 species distributed in 60-800 genera (Govaerts *et al.,* 2017). Abraham and Vatsala (1981) recorded 150 species under 70 genera from South India, in which 176 species are from Karnataka (Krishna Swamy *et al.,* 2004; Udupa *et al*., 2011). Orchids are economically highly important species; their ornamental nature attracts the horticulture and floral industry (De and Pathak, 2020; Janakiram and Baskaran, 2018; Lawrence, 1951; Prakash and Pathak, 2020b; Thammasiri, 2020). The extraordinary taxonomic and morphological diversity of orchids is accompanied by a remarkable range of pollinators and pollination systems (Gaskett, 2011; Pal *et al*., 2019; Prakash and Pathak, 2020a). Orchids have been used since ages for curing various diseases like dysentery, tuberculosis, malaria, hypertension, inflammation, obesity, asthma, depression, heart problem, bone fractures, uterine diseases, lung and liver diseases (Balkrishna *et al.,* 2020; Kumar *et al.,* 2018; Kumar *et al.,* 2019; Kumari and Pathak, 2020; Prakash and Pathak, 2019). Orchids exhibit great diversity in the development and organization of the male and female gametophyte, suspensor, and embryo (Gurudeva, 2014, 2016a,b, 2018, 2019). Schnarf (1931) and Swamy (1943) has given a detailed review of the embryological investigation on orchids. Information about the reproductive biology of a large number of taxa in the India is, however, meagre.

Previous studies on the reproductive biology of orchids were made by Swamy (1943, 1949); the author made a significant contribution to the embryo sac development in the Orchidaceae. Abe (1967, 1973, 1977) conducted research on a large number of orchids and suggested new investigations in the field of embryology. The orchid reproductive strategy, with the formation of numerous tiny seeds, is achieved by the elimination of some traits in the early plant embryogenesis (Kolomeitseva *et al.,* 2021). Kimura (1968) observed 8-nucleate embryo sac in *Cypripedium debile* and Prakash and Aow (1973) reported monosporic 8-nucleate embryo sac in *Spathoglottis plicata.* Mohana Rao and Sood (1979 a,b) studied embryology of *Habenaria densa* and *Satyrium nepalense* and observed monosporic embryo sac development. Arekal and Karanth (1980) have found a bisporic type of gametophyte in *Zeuxine longilabris*. Sood and Mohana Rao (1988) investigated embryology of the diandrous orchid, *Cypripedium cordigerum* and observed anatropous, bitegmic, tenuinucellate ovule and 6-nucleate bisporic female gametopyte. Krishnaswamy *et al.* (2005) observed monosporic 8-nucleate embryo sac development in *Habenaria grandifloriformis* and *Platanthera susannae*. The development of bisporic 8-nucleate type of embryo sac in *Calanthe triplicata* was confirmed by Krishna Swamy *et al*., 2005.

Presently, *Geodorum densiflorum* (Lam.) Schltr., an endangered orchid has been investigated to study the megasporogenesis of the female gametophyte. Plant usually grows in the grassland and shade of moist to deciduous forest at elevations of 1700 m amsl. It has a wide range of distribution throughout tropical and subtropical Asia and West Pacific. The plant has spherical underground *pseudobulb* with 2-5 leaves, *leaves* are leathery, thin textured, and petiolate in nature. The plant blooms during April-May. The *inflorescence* showing

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prominent *Umbrella hand shape* hanging in downward direction is erect, with clustered flowers. *Flowers* are pinkish white with purple lip and dark lines. Propagation is mainly carried out by seeds (Fig. 1A-B).

Material and Methods

The material used for the present study included flowers of *G. densiflorum* at different stages of

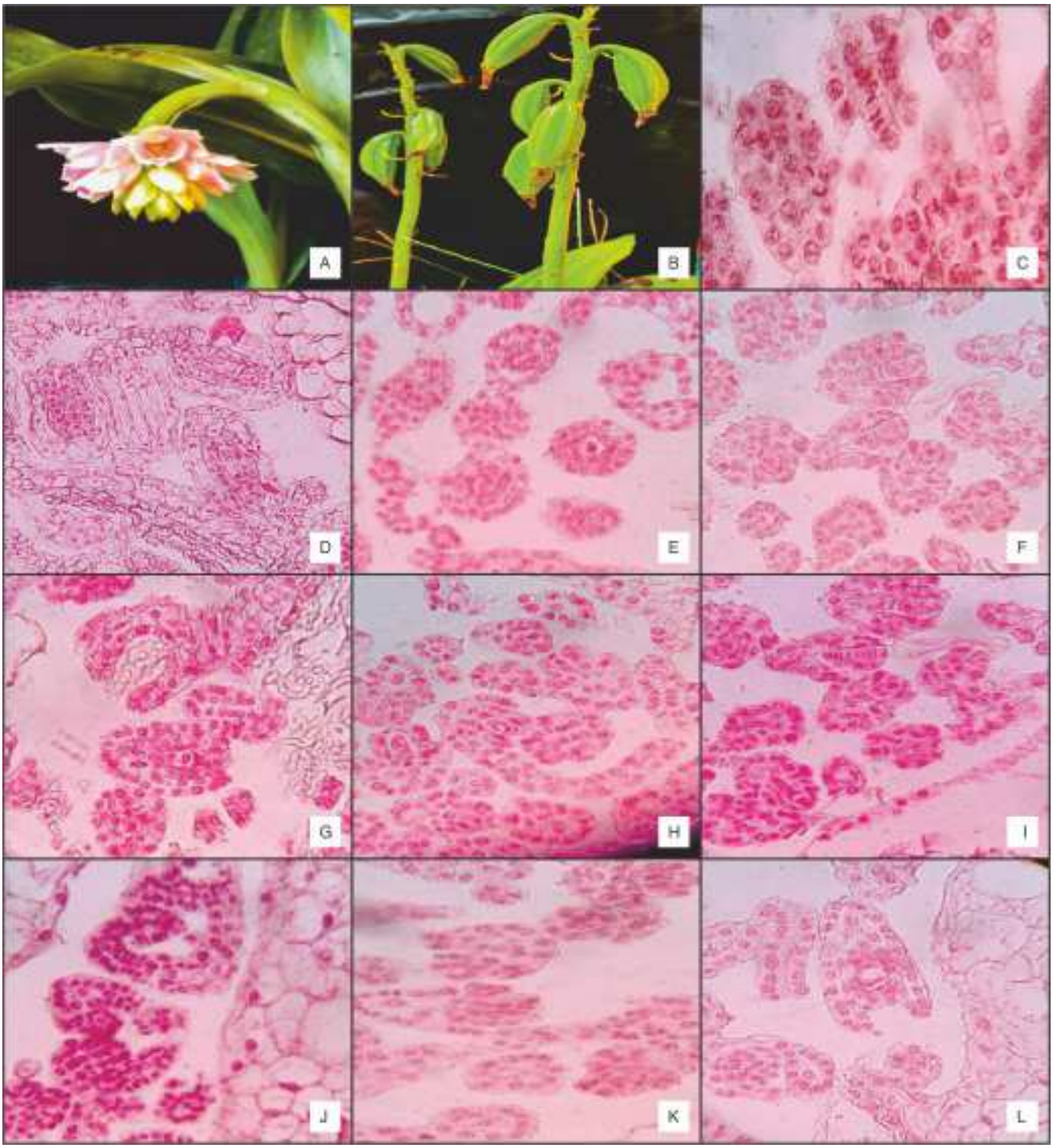

Fig. 1. A-L. Megasporogenesis and development of female gametophyte in *Geodorum densiflorum*: A, Flowers; B, Fruits; C, Megaspore mother cell at meiosis-I; D, Ovular primordia with hypodermal archesporial cells; E, A part of transverse section (T.S.) of ovule showing megaspore mother cell; F, 8- nucleate embryo sac; G, Dyad; H, 2-nucleate embryo sac; I, 4-nucleate embryo sac; J, A part of T.S. ovary showing placental ridges with ovular primordia; K, A part of T.S. of ovule showing megaspore mother cell; L, A part of L.S. of ovule showing organised embryo sac.

development. These were collected from forest of Shimoga (Karnataka state) during June-September, 2021. Flowers were fixed in Formalin-Acetic acid-Alcohol solution, dehydrated in ethyl alcohol-xylene series and embedded in paraffin wax (52°C-54°C). Sections were cut at 10 µm to 14 µm thickness and stained with iron-alum-haematoxylin and counter stained with erythrosine in clove oil. Observations were made under microscope and photographs were taken using digital camera.

Results and Discussion

In *Geodorum densiflorum,* the ovary was inferior, tricarpellary, syncarpous, and unilocular. A transverse section of ovary showed three placental ridges. The placenta was forked and ovular, primordia originated as small protuberances from the placenta and these were en-sheathed by the nucellar epidermis. The terminal cell of the ensheathed cell was densely cytoplasmic and had large nucleus that acted as an archesporial cell, ultimately it increased in size and functioned as megaspore mother cell. The ovules were tenuinucellate, bitegmic, and anatropous. The integuments had two layers of cells. First meiotic division resulted in the formation of dyad; the upper dyad cell degenerated and chalazal dyad cells were functional. The functional megaspore underwent second meiotic division to form two megaspores and they were separated by vacuole to form two nucleate embryo sac. A nuclear mitotic division resulted in the formation of a four nucleate embryo sac. In a following division, 8- nucleate embryo sac was formed. Mature embryo sac was with an egg apparatus consisting of two synergids and an egg, a secondary nucleus (fusion product of two polar nuclei), and three antipodal cells.

Orchids have attracted the plant embryologists for years with reference to their female gametophyte developmental stages. The ovules develop on the placenta only after pollination. Comparable observations have been made by the previous workers (Ekanthappa, 1981; Swamy, 1949). The ovules were tenuinucellate, bitegmic, and anatropous with the inner integument forming the micropyle as in majority of orchids (Abe, 1977; Haung *et al.,* 1998; Johri *et al*., 1992; Law and Yueng, 1989; Yeung *et al.,* 1994). In *Pholidota imbricata* and *Hataeria skikokiana*, both integuments formed the micropyle (Ekanthappa, 1981; Tohda, 1967). In *Epipogium aphyllum,* mycropyle was not organised and ovules were unitegmic (Afzelius, 1954). In *Calanthe triplicata,* two synergids and an egg was formed at the micropylar end of the embryo sac (Krishna Swamy *et al.,* 2005). Two-layered condition of the integument is considered to be derived condition when compared to multi-layered integuments (Swamy, 1947).

In the present study, ovular primordia were developed after the pollination. The nucellar filaments comprised of 5-8 nucellar cells that were covered by nucellar epidermis. A nucellar cell was present at the uppermost end of the nucellar primordium. Beneath the epidermis was hypodermal cell with dense cytoplasm and conspicuous nucleus that acted as an archesporial cell; it enlarged in size and directly developed into a megaspore mother cell. Similar characters were observed in some other orchids (Ekanthappa and Arekal, 1977; Sood and Sham, 1987). In *G. densiflorum*, meiosis-I in the megaspore mother cell resulted in the formation of two individual dyad cells. The second meiotic division occurred 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 embryo sac; a vacuole was present at the centre of the embryo sac. The subsequent division produced 8-nucleate embryo sac (Fig. 1C-I). A complete organized embryo sac had an egg apparatus with two synergids and an egg, a central cell with two polar nuclei and three antipodal cells (Fig. 1L). At the time of double fertilization, polar nuclei fused to form a secondary nucleus. Fertilization was porogamous; during fertilization, one of the synergid was destroyed by the entry of pollen tube. Double fertilization occurred initiating the development of zygote, the surviving synergid degenerated soon.

The present study revealed that the development of the female gametophyte in *G. densiflorum* was monosporic 8-nucleate polygonum type. These observations are in accordance with earlier reports in majority of orchids. The development of female gametophyte is very unique in orchids.

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