J. Orchid Soc. India, 32: 67-72, 2018 *ISSN 0971-5371*

ONTOGENY OF MICROSPORANGIUM AND DEVELOPMENT OF MALE GAMETOPHYTE IN *BULBOPHYLLUM FIMBRIATUM* (LINDL.) REICHB. F. (= *CIRRHOPETALUM FIMBRIATUM* LINDL.)

M R Gurudeva

Department of Botany, Visveswarapura College of Science, K. R. Road, Bengaluru- 560 004, Karnataka, India

Abstract

The anther in *Bulbophyllum fimbriatum* (Lindl.) Reichb. f. was dithecous and tetrasporangiate. Its wall development conforms to the monocotyledonous type. Mass of dense protoplasmic cells represented the massive archesporium. Each such archesporium developed into pollinium. The anther wall was 4-layered. The endothelial cells developed one or two ring-like radially disposed thickenings on the inner walls. Tapetal cells were uninucleate and dual in origin. Simultaneous cytokinesis resulted in tetrahedral and rhomboidal pollen tetrads. At the time of release of pollnia, pollen grain attained 2-celled stage.

Introduction

THE GENUS *Bulbophyllum* Thouars (Sub-tribe: Dendrobinae, Tribe: Epidendreae, subfamily: Orchidoideae of Dressler and Dodson, 1960) has 900 species distributed in tropical and sub-tropical regions of the world, about 86 species are reported from India and only 7 species have been recorded from Karnataka (Ananda Rao and Sridhar, 2007). Swamy (1949) studied the development of male and female gametophytes in *Bulbophyllum mysorense* and *B. neilgherrense.* Ekanthappa and Govindappa (1977) studied the female gametophyte development in *Cirrhopetalum fimbriatum* (=*Bulbophyllum fimbriatum*) and recorded both monosporic and bisporic types of embryo sac development. The present communication deals with the ontogeny of microsporangium and development of male gametophyte in *Bulbophyllum fimbriatum,* an endemic orchid of Western Ghats.

Material and Methods

Bulbophyllum fimbriatum (Lindl.) Reich. f. is an epiphytic herb with a brown woody *rhizome*, bears yellowish, leafless, *pseudobulb* at the time of flowering (Fig. 1). Two sessile, sub-coriaceous, oblong-lanceolate *leaves* are produced per pseudobulb in the vegetative phase (Fig. 2). *Inflorescence* is an umbellate raceme and is borne on a separate scape arising from the rhizome. The *flowers* radiate in all directions from the apex of the peduncle (Fig. 1). *Anthers* small, 4-*pollinia* are waxy and ovoid. The flower buds were collected at different stages of development from Abbe falls, Madikeri town, Kodagu district (Karnataka, India) during March to May 2012. These were fixed in formalin-acetic-alcohol and stored in 70% ethanol followed by a thorough wash in running water. Conventional micro-techniques were followed. The serial transverse and longitudinal sections at 10-12 µm were stained with Heidenhain's iron-alum and haematoxylin. Erythrosin in clove oil was used as a counter stain. Mature anthers were selected and placed in a watch glass treated with 1N HCl and gently warmed over the flame. The treated anthers were macerated with crystal violet and mounted in glycerine. Drawings were made using Camera Lucida and Meopta microscope. Photomicrographs were taken by using an Olympus-CH20i microscope with built in analogue camera (CM OF. 1.4 megapixel). Computer images were captured using Av-digitaliser having Grand VCD-200 captured guard.

Results

Ontogeny of Microsporangium

The anther was dithecous and tetrasporangiate. In a transverse section of a very young anther, four hypodermal masses of dense protoplasmic cells represented the massive archesporium (Fig. 3). Each of them was the site of a developing microsporangium. In due course, the cells of the archesporium adjoining the epidermis lost their cytoplasmic density and functioned as the primary parietal layer while the rest in mass continued to retain dense cytoplasm and acted as sporogenous tissue (Fig. 4). Cells of the primary parietal layer divided periclinally and gave rise to outer parietal and inner parietal layers (Fig. 5). The outer parietal layer directly differentiated into endothecium. The cells of the inner parietal layer divided periclinally and produced the middle layer and glandular parietal tapetum (Figs. 6-7). The mode of development of microsporangium wall conformed to the monocotyledonous type of Davis (1966). Simultaneously, cells of the connective tissue bordering the sporogenous cells

Received: December 23, 2016; Accepted: September 30, 2018

acquired dense cytoplasm and functioned as connective tapetum in alignment with the layer towards the wall of the microsporangium. The tapetal layer that surrounds the sporogenous tissue of a sporangium is, therefore, of dual origin (Fig. 7).

Microsporogenesis and Pollen Development

The sporogenous cells in the meantime, increased in number and size and acted as microspore mother cells (Figs. 8-10). Meiosis in microspore mother cells was of simultaneous type. The microspore tetrads formed were generally tetrahedral and rhomboidal types. They remained together in the pollinium and were distributed throughout the pollinium (Figs. 11-15). Microspores of the tetrad divided asymmetrically (Fig. 16) and became 2-celled pollen grains in the pollinium (Fig. 17). During the organization of the microspore tetrads and pollen within the sporangium, nourishment was drawn from the uninucleate cells of the tapetal layer. As a result, the layer ultimately broke down and got absorbed along with the middle layer. In the meantime, common sporopollenin wall was laid around the pollinium. In the mature microsporangium, the wall consists of the epidermis and endothecium. Certain cells of the epidermis were characteristically bulged and densely protoplasmic. Each of the endothecial cells acquired one or two ring-like radially disposed thickenings on the inner walls (Figs. 18-19).

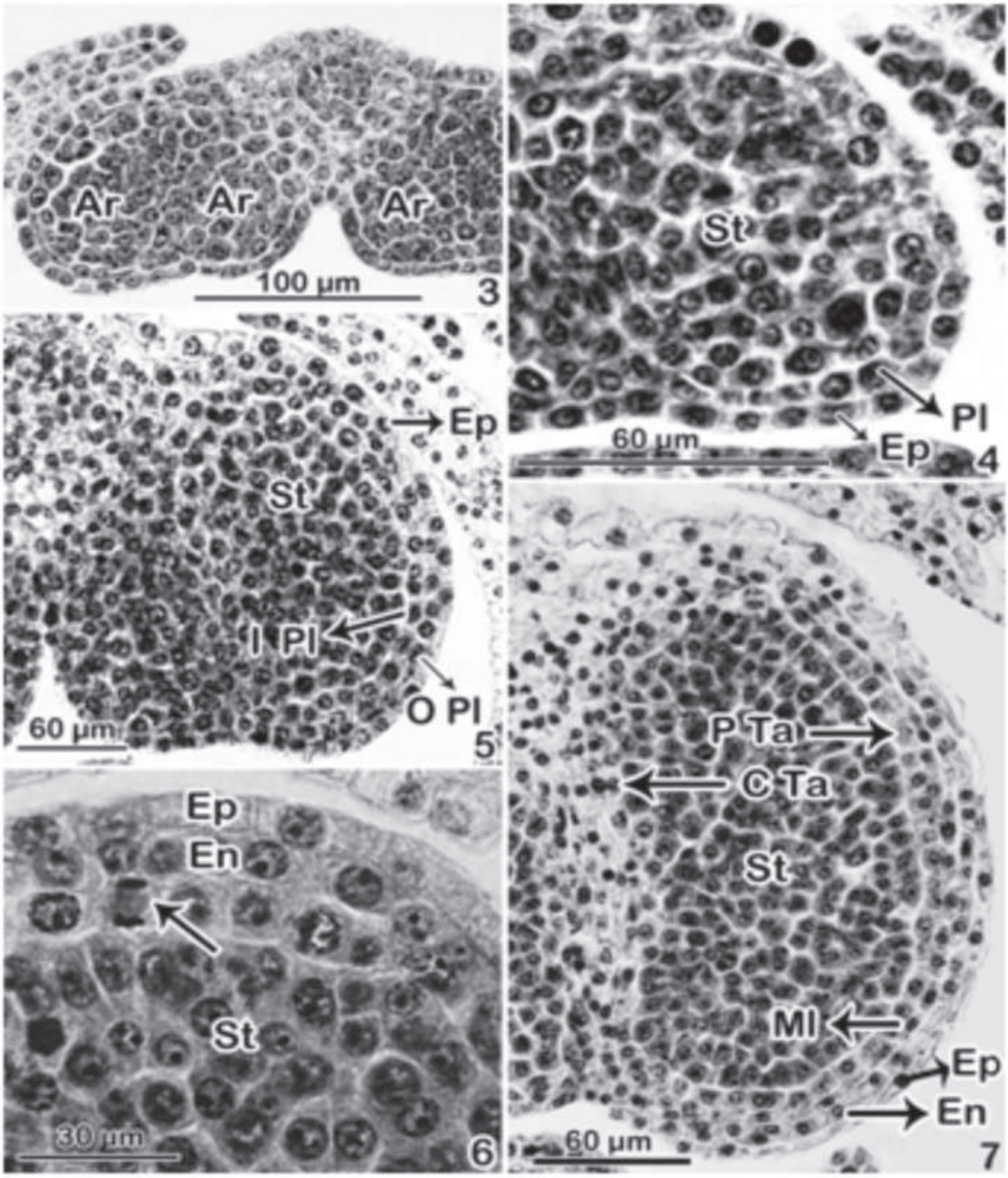
Figs. 1-2. *Bulbophyllum fimbriatum*: 1, Leafless pseudobulb with an umbellate inflorescence; 2, Leaf bearing pseudobulb in vegetative phase.

During the period of development of pollinium in each of the adjacent microsporangium of an anther lobe, the separation layer/connective layer of cell disintegrated and got absorbed, leaving the two pollinia in each of the anther half. An opening in the anther wall between the two sporangia facilitated the exit of both the pollinia (Figs. 20-22).

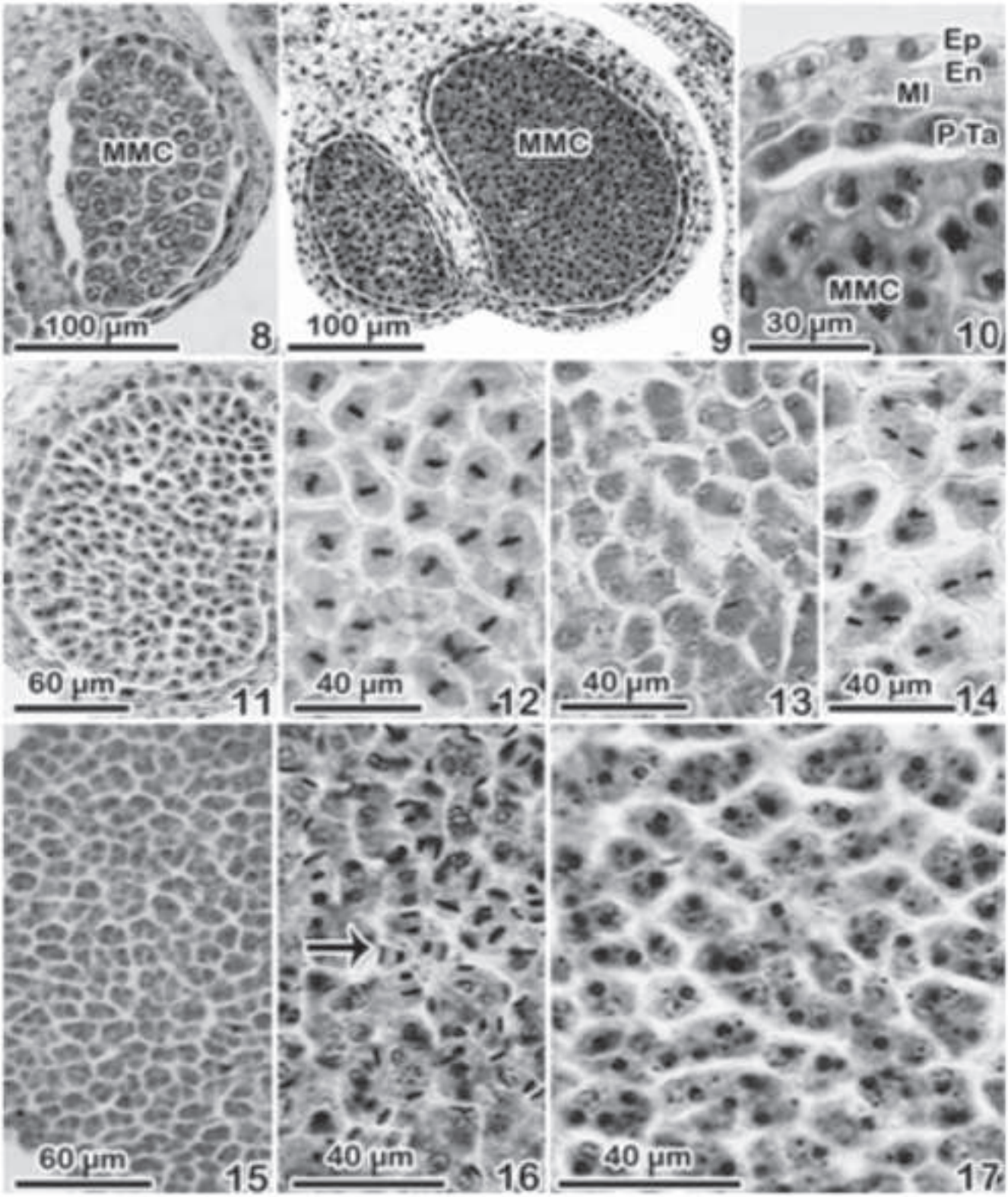
Discussion

The development of the anther wall corresponds to the monocotyledonous type (Davis, 1966). A similar mode of anther wall development has been recorded in many of the orchid taxa (Gurudeva, 2012, 2015, 2016 a,b; Krishna Swamy *et al.,* 2003; Mohana Rao and Sood, 1987; Sood, 1992). The epidermis was single layered. At the time of release of pollinia from the anther, certain epidermal cells became characteristically bulged and densely cytoplasmic. The endothecium was single layered. At maturity, the cells acquired one or two radially disposed thickenings, similar type of endothecial thickenings has been reported in *Aa achalensis* (Cocucci, 1964). Middle layer degenerated when the pollinia attains maturity. Tapetum was single layered, glandular and dual in origin, it is in conformity with the earlier record (Gurudeva, 2012).

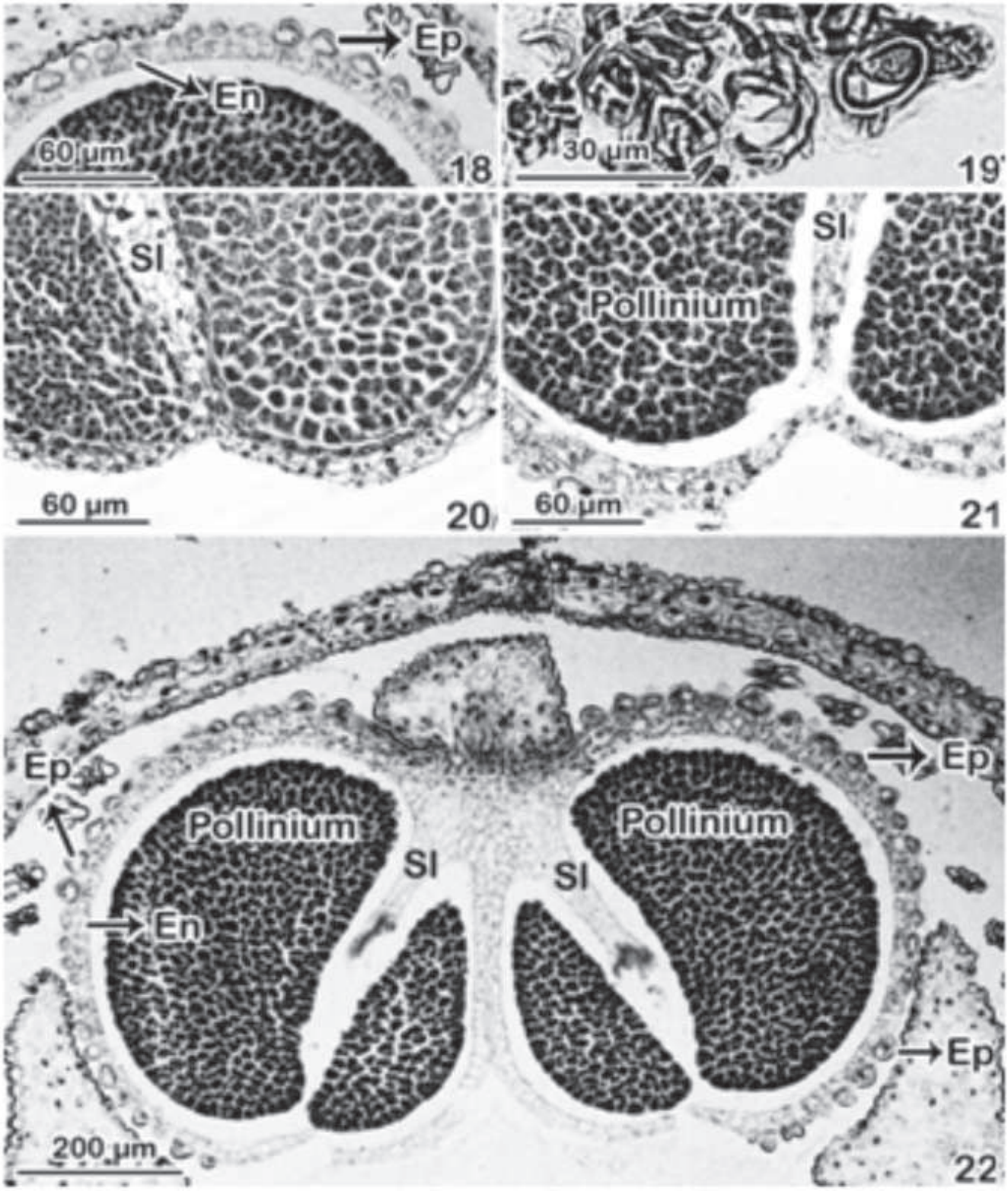
The archesporial cells after producing parietal layer functioned as massive sporogenous tissue which later contributed to the formation of pollinium. Similar feature has been reported by Swamy (1949) and Kant *et al.* (2013). Sporogenous cells enlarged and became microspore mother cells. They underwent usual meiotic divisions and resulted in tetrahedral and rhomboidal tetrads. The nuclear division within the microspore tetrads was synchronous and asymmetrical, in conformity with earlier records (Hagerup, 1938; Mohana Rao and Sood, 1986; Swamy, 1949).



Figs. 3-7. Ontogeny of microsporangium in *Bulbophyllum fimbriatum*: 3, T.S. of young anther showing massive archesporium; 4, Young anther lobe showing primary parietal layer and massive archesporium; 5, T.S. of anther lobe showing outer and inner parietal layers; 6, Part of T.S. of microsporangium to show cell division in inner parietal layer (indicated by the arrow); 7, T.S. of microsporangium showing four wall layers around the massive sporogenous tissue. (Ar, Archesporium; C Ta, Connective tapetum; En, Endothecium; Ep, Epidermis; I Pl, Inner parietal layer; Ml, Middle layer; O Pl, Outer parietal layer; Pl, Parietal layer; P Ta, Parietal tapetum; St, Sporogenous tissue).



Figs. 8-17. Microsporogenesis and pollen development in *Bulbophyllum fimbriatum*: 8-10, Microsporangium with microspore mother cells; 11-14, Meiotic division in microspore mother cells; 15, Part of pollinium showing microspore tetrads; 16, Nuclear division in microspore tetrads, note proximal and distal orientation of spindles (indicated by the arrow); 17, Part of pollinium showing mature pollen tetrads. (En, Endothecium; Ep, Epidermis; Ml, Middle layer; MMC, Microspore mother cell; P Ta, Parietal tapetum).



Figs. 18-22. Dehiscence of microsporangium in *Bulbophyllum fimbriatum*: 18, Portion of mature microsporangium showing epidermis and endothecium, note the bulged epidermal cells on outer side of the microsporangium; 19, Whole mount of endothecial thickenings; 20-22, Portion of anther lobe showing the development of line of dehiscence. (En, Endothecium; Ep, Epidermis; Sl, Separating layer).

At the time of anther dehiscence, cells of the wall at the junction of the two adjoining microsporangia disorganised, leading to the formation of a vertical slit in each of the anther lobe which facilitated pollinators to carry the pollinia.

References

Ananda Rao, T. and Sridhar. 2007. *Wild Orchids in Karnataka*. INCERT Seshadripuram, Bangalore, India.

Cocucci, A. E. 1964. The life history of *Aa achalensis* Schlechter (Orchidaceae). *Phytomorphology,* **14**: 588-97.

Davis, G. L. 1966. *Systematic Embryology of Angiosperms*. John Wiley and Sons, New York, USA.

Dressler, R. L. and C. H. Dodson. 1960. Classification and phylogeny in the Orchidaceae. *Ann. Missouri Bot. Gard*., **47**: 25-68.

Ekanthappa, K. G. and D. A. Govindappa. 1977. A contribution to the embryology of *Cirrhopetalum fimbriatum* Lindl. *Proc. Indian Acad. Sci.,* **86b**(4): 211-16.

Gurudeva, M. R. 2012. Ontogeny of microsporangium and development of male gametophyte in *Habenaria diphylla* Dalz. *J. Orchid Soc. India,* **26**(1-2): 93-99.

Gurudeva, M. R. 2015. Ontogeny of microsporangium and development of male gametophyte in *Peristylus spiralis* A. Rich. (Orchidaceae). *J. Orchid Soc. India*, **29**: 39-46.

Gurudeva, M. R. 2016a. Development of male and female gametophytes in *Habenaria ovalifolia* White (Orchidaceae). *J. Orchid Soc. India,* **30**: 1-10.

Gurudeva, M. R. 2016b. Development of male and female gametophytes in *Dendrobium ovatum* (L.) Kranz. (Orchidaceae). *J. Orchid Soc. India,* **30**: 75-87.

Hagerup, O. 1938. A peculiar asymmetrical mitosis in the microspore of *Orchis*. *Heriditas,* **24**: 94-96.

Krishna Swamy, K., H. N. Krishna Kumar, and S. N. Ramaswamy. 2003. Contributions to the microsporogenesis in few orchid taxa. *J. Orchid Soc. India,* **17**(1-2): 31-41.

Mohana Rao, P. R. and S. K. Sood. 1986. Embryology of *Cephalanthera ensifolia* (Orchidaceae). *Acta Bot. Ind.,* **14**: 38-44.

Mohana Rao, P. R. and S. K. Sood. 1987. Embryology of *Oreorchis foliosa* (Orchidaceae). *Phytomorphology,* **37**(1): 1-8.

Kant, Ravi, M. M. Hossain, and L. K. Attri. 2013. Pollinium development in *Spiranthes sinensis* (Pers.) Ames. and *Cymbidium pendulum* Sw. A comparative study. *Bangladesh J. Bot.,* **42**(2): 307-14.

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.

Swamy, B. G. L. 1949. Embryological studies in the Orchidaceae I gametophytes. *Am. Midl. Nat.,* **41**: 184-201.