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ONTOGENY OF MICROSPORANGIUM AND DEVELOPMENT OF MALE GAMETOPHYTE IN *PERISTYLUS PLANTAGINEUS* LINDL.

M R Gurudeva

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

Abstract

The anther in *Peristylus plantagineus* Lindl. was dithecous and tetrasporangiate. Its wall development conforms to the monocotyledonous type. Each archesporial cell developed into a block of sporogenous cells before organising into massulae. The anther was 4-layered. The endothecial cells developed ring like tangential thickening on the inner walls. Tapetal cells were uninucleate and of dual origin. The microspore tetrads were tetrahedral, decussate, linear and T-shaped. Pollen were shed at 2-celled stage.

Introduction

THE GENUS *Peristylus* Blume (Sub tribe: Orchidinae; tribe: Orchideae; sub family: Orchidoideae of Dressler and Dodson, 1960) comprises of 70 species distributed in Indo-Malayan regions. The species is characterised by terrestrial tuberoid leafy stem and a terminal raceme. In India, the genus is represented by 28 species and 2 varieties, 8 of which occur in Karnataka (Rao and Sridhar, 2007). Embryological data in the orchid species is rather meagre despite the involvement of several workers (Abe, 1972a, 1972b; Attri *et al*., 2005, 2007; Bhanwra *et al.*, 2006; Govindappa and Karanth, 1980; Gurudeva, 2009, 2010, 2011a, 2011b, 2012, 2014; 2015, 2016a, 2016b, Gurudeva and Govindappa, 2008; Kant and Hossain, 2010; Krishna Swamy *et al.*, 2003, 2005; Poddubnaya-Arnoldi, 1967; Schnarf, 1931; Sood, 1985a, 1985b, 1986, 1988, 1989, 1992; Sood and Mohana Rao, 1986a, 1986b, 1987; Swamy, 1949; Vij *et al.*, 1982; Wirth and Withner, 1959). Earlier, Swamy (1949) studied the male and female gametophytes of *Peristylus spiralis* and *P. stocksii,* and Gurudeva (2015) studied the ontogeny of microsporangium and development of male gametophyte in *Peristylus spiralis.* Information on the embryology of *Peristylus plantagineus* has remained elusive, hence the present communication deals with the ontogeny of microsporangium and development of male gametophyte.

Material and Methods

Peristylus plantagineus is a terrestrial tuberous plant with a terminal raceme. The flower buds were collected at different stages of development from Doddasampige, Biligirangana Hills, Mysuru district (Karnataka, India) during September to October, 2013. 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 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

Microsporangium

A very young anther in transverse section was a two lobed structure and revealed two rows of hypodermal archesporial cells in each lobe. Each row represented the future microsporangium (Figs. 1-2). The number of cells in each row increased by anticlinal divisions and later they divided periclinally giving rise to the primary parietal and primary sporogenous layers (Figs. 3-4). Blocks of sporogenous cells were organised by the division of cells of primary sporogenous layer. The density of cytoplasm in the cells of the primary parietal layer decreased and they divided periclinally and gave rise to two superposed layers namely outer parietal and inner parietal layers. The outer parietal layer directly functioned as endothecium (Figs. 5-6). The inner parietal layer divided periclinally and formed glandular parietal tapetum and the middle layer (Figs. 7-8). The development of microsporangium wall, therefore, conforms to monocotyledonous type of Davis (1966). Meanwhile, cells of the connective tissue adjoining the blocks of sporogenous cells acquired dense cytoplasm and larger nuclei and functioned as connective tapetum in alignment with the layer organised towards the wall side of the microsporangium. The tapetum ensheathing

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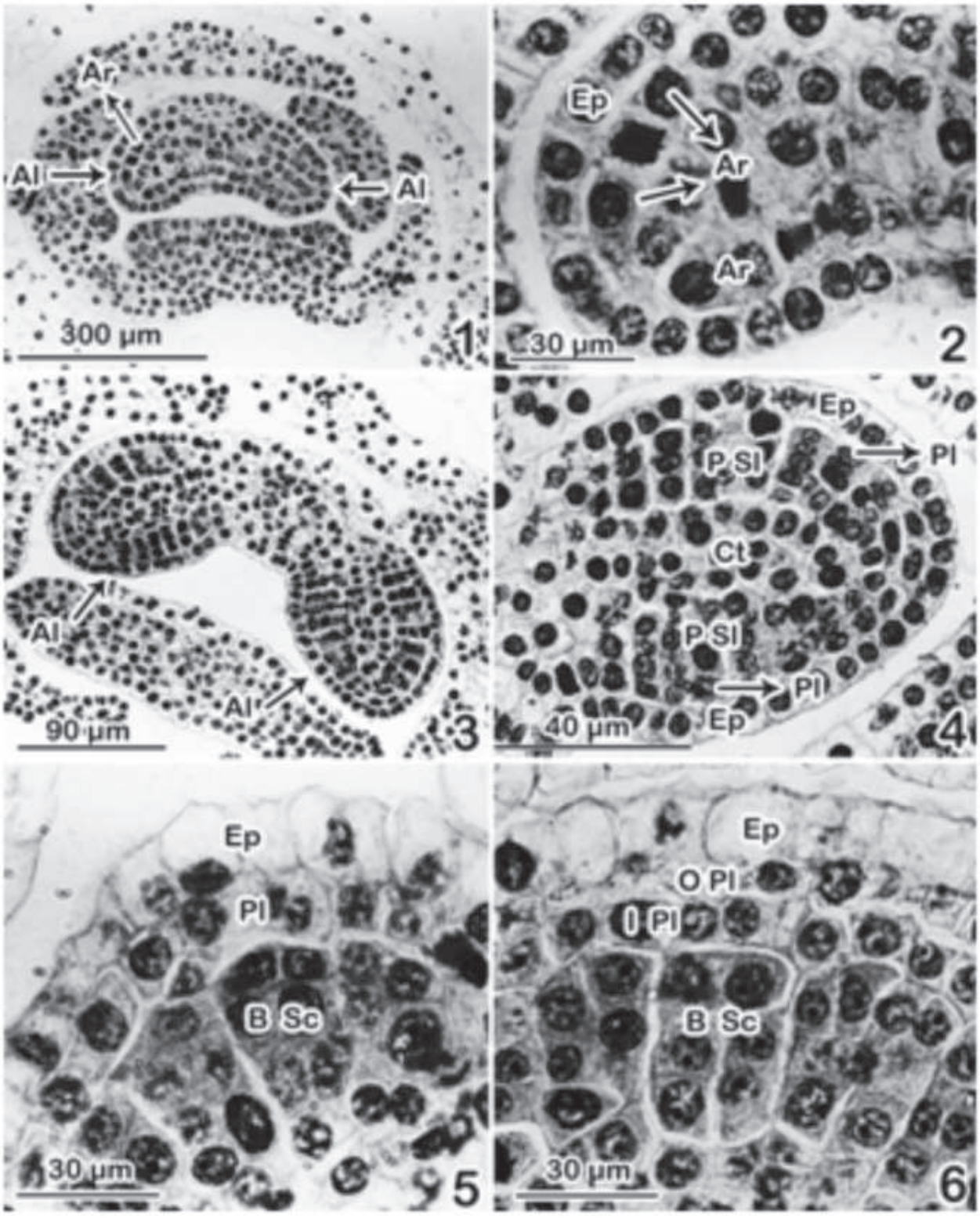
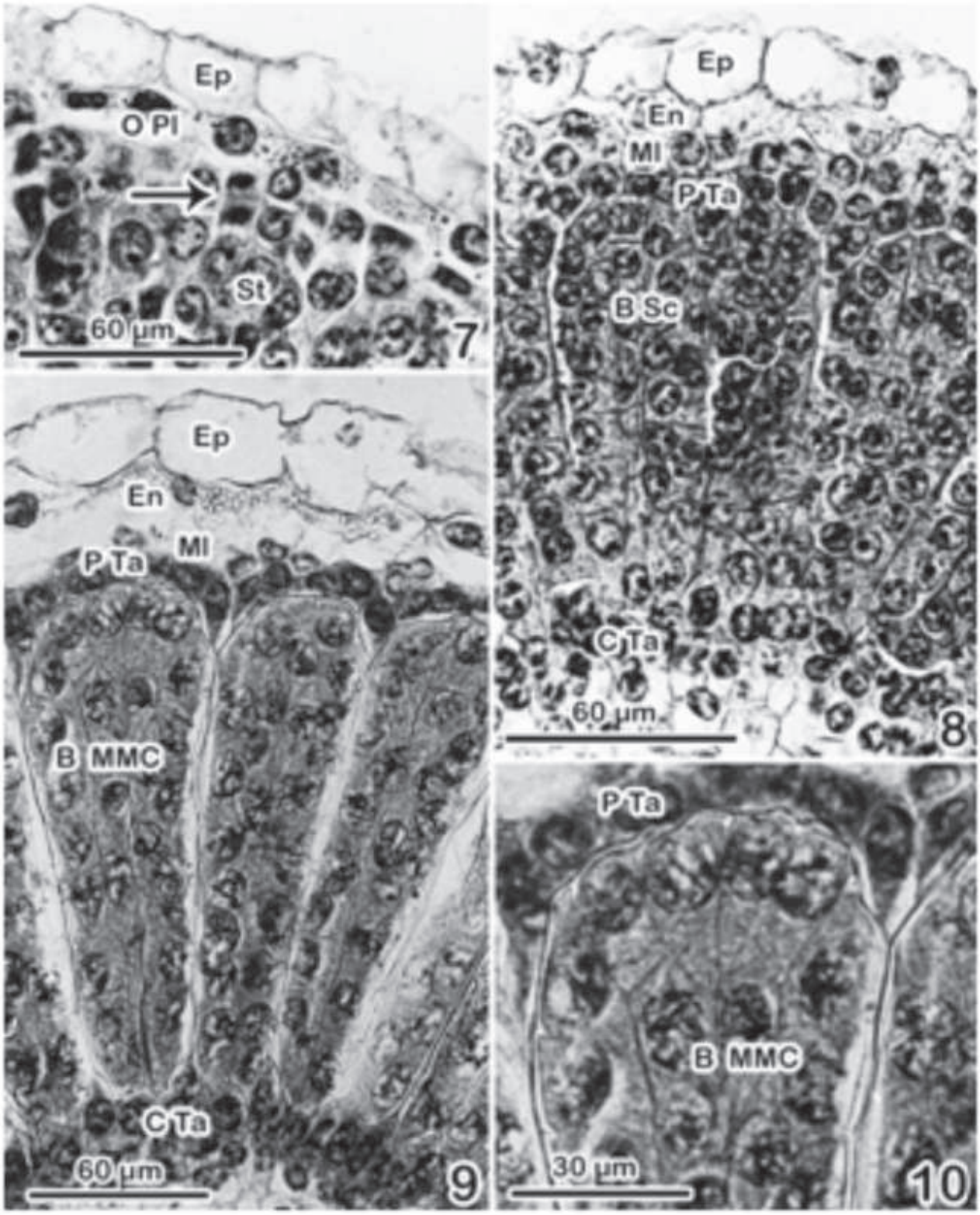
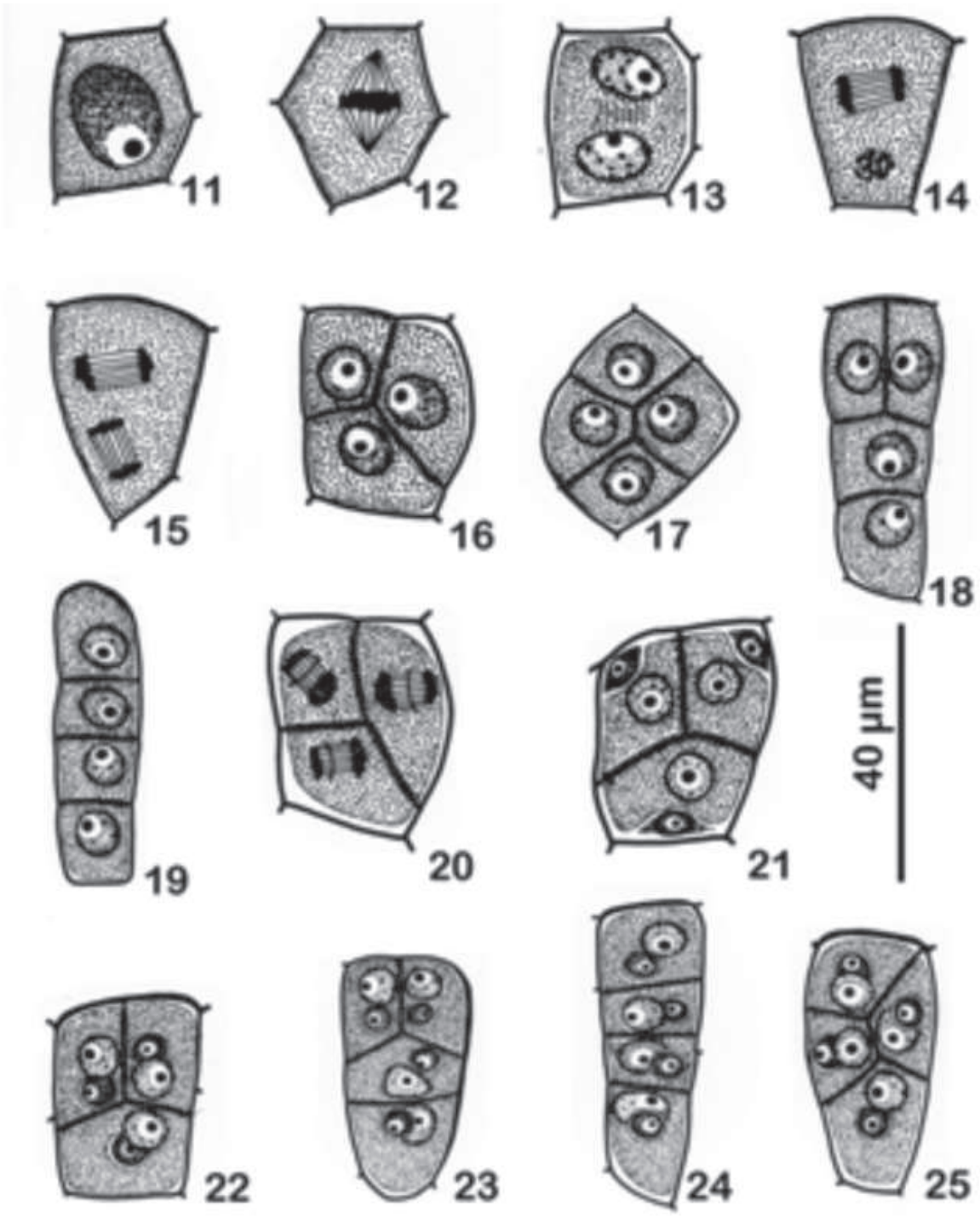


Figs. 1-6. *Peristylus plantagineus*, photomicrographs showing different stages during ontogeny of microsporangium: 1, Young anther with two lobes, note two rows of archesporial cells in each lobe; 2, Anther lobe enlarged to show row of archesporial cells; 3-4, Anther lobe showing parietal layer and primary sporogenous cells; 5, A portion of anther lobe showing primary parietal layer and blocks of sporogenous cells; 6, Portion of anther lobe showing outer parietal layer and inner parietal layers, note blocks of sporogenous cells. (Al, Anther lobe; Ar, Archesporial cells; Ep, Epidermis; Ipl, Inner parietal layer; Opl, Outer parietal layer; Pl, Primary parietal layer; Psl, Primary sporogenous layer; Ct, Connective tissue; B Sc, Blocks of sporogenous cells).



Figs. 7-10. *Peristylus plantagineus*, photomicrograph showing different stages of ontogeny of microsporangium: 7, Portion of microsporangium showing division of inner parietal layer; 8, Part of microsporangium to show the origin of parietal tapetum and connective tapetum; 9-10, Portion of microsporangium to show uninucleate tapetum and microspore mother cells. (BMMC, Blocks of microspore mother cells; C Ta, Connective tapetum; En, Endothecium; Ep, Epidermis; Ml, Middle layer; Opl, Outer parietal layer; P Ta, Parietal tapetum; St, Sporogenous tissue; B Sc, Blocks of sporogenous cells).

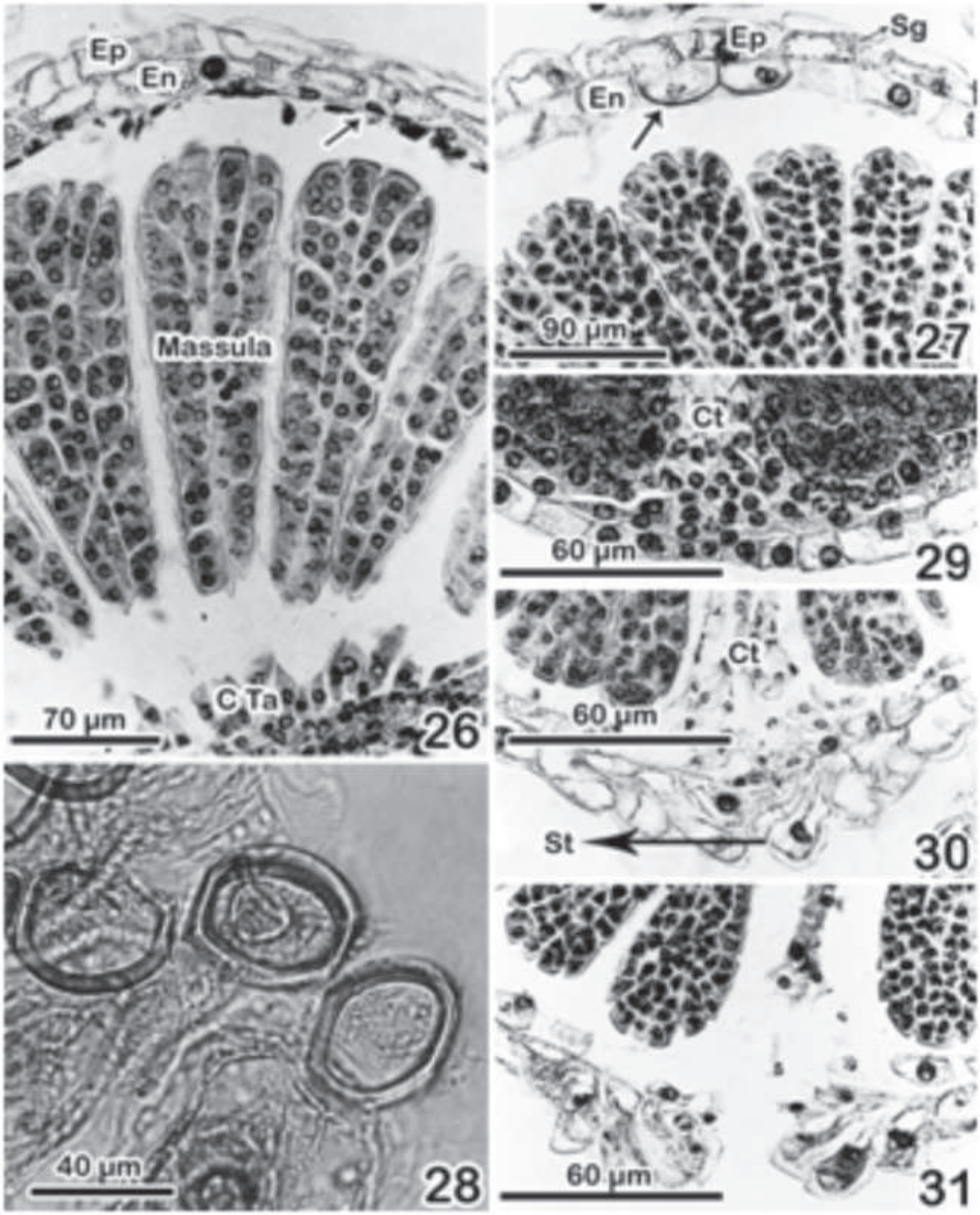


Figs. 11-25. *Peristylus plantagineus*, diagrammatic representation of microsporogenesis and pollen development: 11-15, Show meiosis in microspore mother cells; 16, Tetrahedral microspore tetrads; 17, Rhomboidal microspore tetrad; 18, T-Shaped microspore tetrad; 19, Linear microspore tetrads; 20-21, Show distal origin of generative cell in the pollen grains of tetrahedral tetrads; 22-25, Two celled pollen grains in different kinds of tetrads.

the blocks of sporogenous tissue, therefore, is of dual origin (Fig. 8). Blocks of sporogenous cells increased in size by cell divisions. The final generation of cells in these masses differentiated into blocks of microspore mother cells (Figs. 9-10).

Microsporogensis and Pollen Development

Meiotic divisions occurred in microspore mother cells. The first nuclear division was not followed by wall formation (Figs. 11-13). The two resulting dyad nuclei divided simultaneously and gave rise to four microspore



Figs. 26-31. *Peristylus plantagineus*, photomicrographs showing different stages of microsporangium ontogeny: 26, Part of microsporangium to show degenerating middle layer and tapetum indicated by an arrow; 27, Part of microsporangium to show complete degeneration of middle layer and tapetum, note tangentially disposed endothecial thickenings in the endothecial cell, (indicated by an arrow) and starch grains in the epidermis; 28, Whole mount of ring-like endothecial thickenings; 29-31, Part of anther half to show sequence stages of development of line of dehiscence. (C Ta, Connective tapetum; Ct, Connective tissue; En, Endothecium; Ep, Epidermis; Sg, Starch grains; St, Stomium).

nuclei. The orientation of the spindles of the dividing dyad nuclei varied considerably. As a consequence, after the simultaneous quadripartition of the mother cells, the resulting microspore tetrads were tetrahedral, rhomboidal, T-shaped and linear (Figs. 14-19). The micropore tetrads remained intact within the young massula. In a massula, the peripheral region was mostly occupied by rhomboidal, T-shaped and linear tetrads (Fig. 26). The nuclei of the micropsores of the tetrads divided synchronously to render each of them bicelled. The orientation of the spindles of the dividing nuclei, especially in the tetrahedral and rhomboidal types were always disposed along the proximal and distal axis. The smaller densely protoplasmic generative cell was always cut off towards the distal end and adjoined the spore coat (Figs. 20-21). The generative cell then separated itself from the spore coat and entered into the cytoplasm of the vegetative cell in the microspores of all the types of tetrads (Figs. 22-25). By this time, a conspicuous sheath of sporopollenin was laid down around each pollen massula and the massulae appeared independent within the microsporangium (Fig. 26).

At later stages of development, the conspicuous uninucleate tapetal cells provided nourishment to the spore mother cells and pollen massulae and ultimately broke down along with middle layer (Fig. 26). The cells of the endothecium and epidermis extended laterally. The cells of the endothecium acquired prominent ring like tangentially disposed thickenings, one per cell (Figs. 27-28). The epidermal cells accumulated starch grains (Fig. 27).

As the massulae matured, the thin walled group of cells located in the sub-epidermal region at the junction of the adjacent microsporangia broke down. The epidermal cells at the region became enlarged and elongated to form a stomium. Under the influence of wall thickenings of the endothecial layer, an opening was created between the adjacent microsporangia in each of the anther halves, making way for the exit of the massulae of both the sporangia through the opening (Figs. 29-31).

Discussion

The development of anther wall corresponds to the monocotyledonous type (Davis, 1966). A similar mode of anther wall development has also been reported in *Habenaria diphylla* (Gurudeva, 2012), *Malaxis saprophyta* (Sood, 1992) and *Microstylis cylindrostachya* (Sood, 1985a). The anther wall comprised of epidermis, endothecium, middle layer and glandular tapetum. Similar feature has been reported in many of the investigated taxa (Sharma and Vij, 1984; Sood, 1992). The epidermis was single layered and showed the presence of starch grains, indicating thereby the role of this layer in nutrition besides protection. Nutritive role of epidermis has also been reported in *Epipogium roseum* (Govindappa and Karanth, 1981), *Habenaria diphylla* (Gurudeva, 2012) and *Zeuxine longilabris* (Karanth *et al.,* 1979). The endothecium was single layered. At maturity, the cells acquired ring-like tangentially disposed thickenings on the inner surface of their walls. This type of endothecial thickenings corresponds to Type-II of Freudenstein (1991). Similar type of tangentially disposed endothecial thickenings were recorded in *Habenaria clavigera* (Sharma and Vij, 1987) and *Habenaria diphylla* (Gurudeva, 2012). Different types of endothecial thickenings in orchids have been recorded by Untawale and Bhasin (1973) and classified by Freudenstein (1991). The middle layer was single layered, degenerated when the massulae attained the stage of maturity. Tapetum was glandular and dual in origin. Similar observation has been made in *Habenaria edgeworthii, H. elisabethae* and *H. galeandra* (Sood, 1986), *Habenaria diphylla* (Gurudeva, 2012) and *Zeuxine strateumatica* (Kant and Bhanwra, 2010). Tapetal cells remained uninucleate throughout and in conformity with several members of orchids (Krishna Swamy, 2003; Sood, 1985a, 1985b). The archesporial cells after cutting off parietal layer functioned as sporogenous tissue. The sporogenous cells belonging to a massula were derived from single archesporial cell. Similar condition has been reported in *Calanthe veratrifolia, Neottia ovata* and *Orchis maculata* (Guignard, 1882); *Habenaria diphylla* (Gurudeva, 2012); species of *Habenaria* and *Peristylus* (Swamy, 1946, 1949) and *Himantoglossum hircinum* (Heusser, 1915). The sporogenous cells enlarged in size and became microspore mother cells. They underwent usual meiotic divisions and resulted in tetrahedral, decussate, T-shaped and linear tetrads. Quadripartition of microspore mother cell was simultaneous in conformity with other investigated orchid taxa (Gurudeva, 2012; Krishna Swamy, 2003; Mohana Rao and Sood, 1986; Prakash, 1973; Sood, 1992; Swamy, 1946, 1947, 1949). The nuclear division within the microspore tetrad was synchronous and asymmetrical in conformity with the earlier records (Gurudeva, 2012; Hagerup, 1938; Sharma and Vij, 1987; Swamy, 1949). The pollen grains were two celled when massulae were ready for pollination. At the time of anther dehiscence, a well-developed stomium formed at the junction of the two adjacent microsporangia, disorganized leading to the formation of a vertical slit in each of the two anther lobes which facilitated to carry the massulae.

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