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# ONTOGENY OF MICROSPORANGIUM AND DEVELOPMENT OF MALE GAMETOPHYTE IN *PERISTYLUS SPIRALIS* A. RICH. (ORCHIDACEAE)

**M R Gurudeva**

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

**Abstract**

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

## Introduction

THE EMBRYOLOGY of Orchidaceae has attracted the attention of several investigators from time to time because of their extreme specialization in exhibiting great diversity in development of male and female gametophytes, suspensor and embryo apart from their vegetative and floral organs as shown by the most comprehensive embryological works of Abe (1972a,b), Levitte (1901), Schnarf (1931), Swamy (1949a,b), Wirth and Withner (1959), and Veyret (1974). These investigators, in addition to their own observations have also given reviews of previous embryological works. Some of the recent embryological works in the area include those of Bhanwra *et al.* (2006), Fredrikson (1990, 1991), Gurudeva (2009, 2010, 2011a,b, 2012, 2014), Govindappa and Karanth (1980), Gurudeva and Govindappa, (2008), Krishna Swamy *et al*. (2003, 2005), Mohana Rao and Sood, (1979a,b, 1986, 1987), Kant and Hossain (2010), Sood (1985a,b, 1986, 1988, 1989, 1992), and Sood and Mohana Rao (1986a,b). The genus *Peristylus* Blume (sub-tribe: Orchidinae; tribe: Orchideae; sub-family: Orchidoideae of Dressler and Dadson, 1960) comprises of 70 species distributed in Indo-Malayan regions. In India, the genus is represented by 28 species and 2 varieties, 6 of which occur in Karnataka (Ananda Rao and Sridhar, 2007) but the embryological studies in the genus are meager. Swamy (1949a) studied the development of male and female gametophyte in *Peristylus spiralis* and *P. stocksii*. The present communication deals with the detailed study on the mode of wall layer development, nature of endothecial thickenings and derivation of massulae from archesporial cells in *Peristylus spiralis*.

### Material and Methods

*Peristylus spiralis* A. Rich. is a terrestrial leafy herb with small oblong tubers. The *stem* is erect, slender and often covered with basal sheaths and the *leaves* are 3 – 5 in number. They are linear-lanceolate, acute, spirally arranged at the base of the stem (Fig. 1). The *inflorescence* is spirally twisted spike. The *flowers* are greenish white and arise in the axil of small bracts. The sepal and petals are sub-equal. The *lip* is variable, longer than the sepals, cuneate and 3-cleft to about


Figs. 1-2. *Peristylus spiralis*: 1, Flowering plant with tubers;2, Close-up of inflorescence.

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the middle. The *median lobe* usually shorter, broader and curved. *Spur* is a minute globose sac. *Column* is short. *Ovary* is inferior and pale green (Fig. 2).

The flower buds were collected at different stages of development from Bhagamandala, Talacauvery, Kodagu district (Karnataka, India) during September to October, These were fixed in formalin-acetic-alcohol and stored in 70% ethanol following a thorough wash in running water. Conventional micro-techniques were followed. The serial transverse and longitudinal sections at 10-12$\mu$m were stained with Heidenhain's iron-alum and haematoxylin. Erythrosin in clove oil was used as 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-1.4MP). Computer images were captured using AV-digitiser having Grand VCD-200 captured guard.

## Results

*Ontogeny of Microsporangium*

A very young anther in transection was two lobed. Each lobe lodged two rows of densely protoplasmic hypodermal archesporial cells (Figs. 3, 4). The location of each row was the site of a microsporangium. Periclinal division of the archesporial cells occured early to delimit the primary parietal layer from the primary sporogenous layer. Primary sporogenous cells underwent both anticlinal and periclinal divisions and organized into a block of sporogenous cells, each block representing the future pollen massula (Fig. 5). After a period of anticlinal divisions, the primary parietal cells divided periclinally to produce two layers of cells (Fig. 6). The outer parietal layer directly developed into endothecium. The inner parietal layer divided periclinally to give rise to the middle layer and the glandular parietal tapetum (Fig. 7). Meanwhile, cells of the connective adjoining the sporogenous tissue acquired dense cytoplasm and organized into a complete sheath of connective tapetum. As a result, the entire tapetal layer around the sporogenous tissue is of dual origin (Fig. 8). The wall layers of microsporangium consists of epidermis, endothecium, middle layer and tapetum.

*Microsporogensis and Pollen Development*

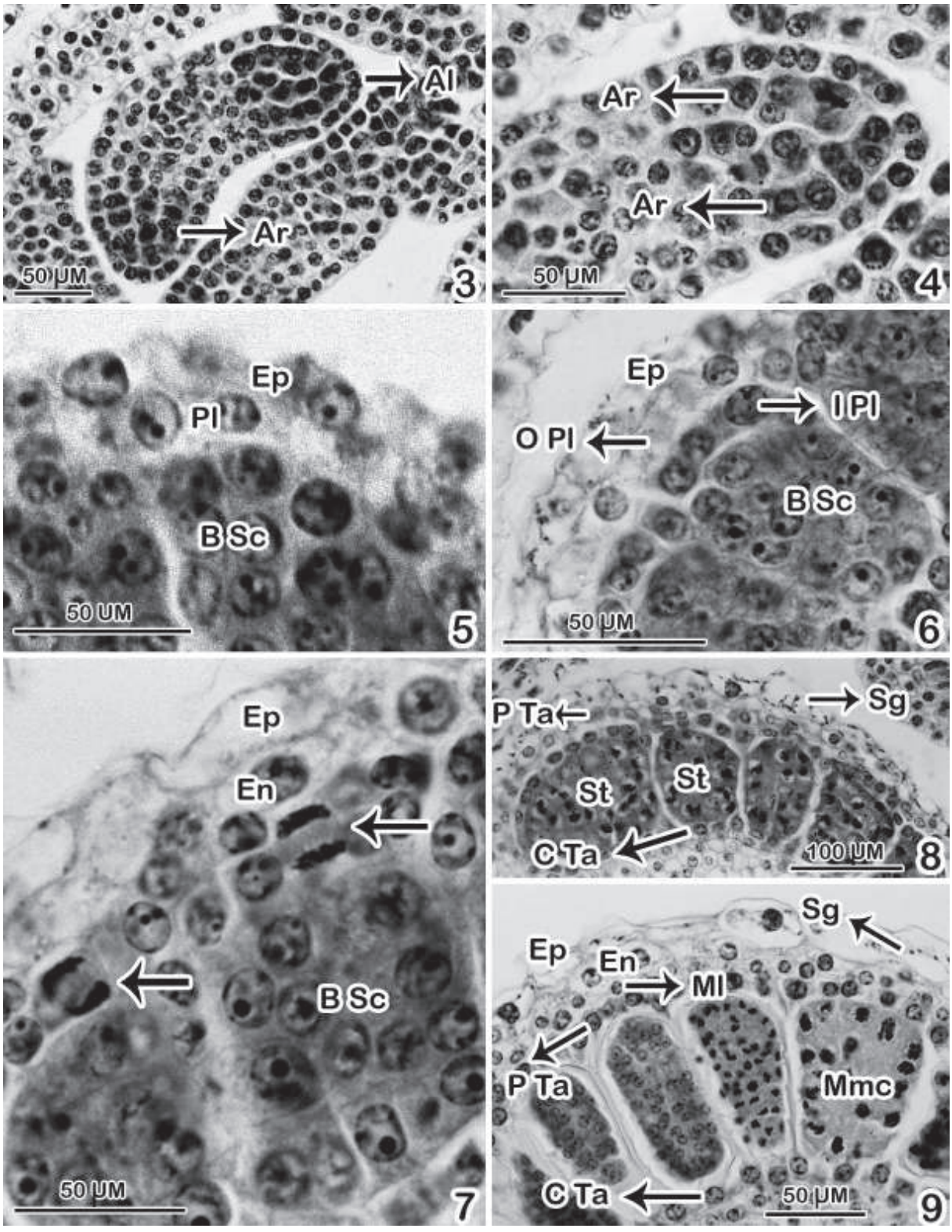
Sporogenous cells in each block differentiated into microspore mother cells (Fig. 9). Meiotic divisions occured in the microspore mother cells. The first nuclear division was not followed by a wall (Figs.10-12). The resulting dyad nuclei divided simultaneously and gave rise to four microspores. The orientation of the spindles of the dividing dyad nuclei varied considerably (Figs. 13, 14). After the simultaneous quadric-partition of the mother cells, tetrahedral, rhomboidal, T-shaped and linear microspore tetrads were formed within a massula (Figs.15-18). The spores of the tetrad did not separate apart, so also the tetrads of a massula. The nuclei of the microspores of the tetrads divided synchronously to render each one of them as bi-celled. The orientation of the spindles of the dividing nuclei especially in the tetrahedral and rhomboidal tetrads were always disposed along the proximal and distal axis (Figs.19-21). The smaller densely protoplasmic generative cell was always cut off towards the distal end adjoining the spore coat (Figs. 22, 23). 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. 24-27). By this time, the pollen massulae were fully covered by a coat of sporopollenin and these appeared as independent structures within the microsporangium.

During the subsequent development, the tapetal cells became conspicuous, remained uninucleate and provided nourishment to the spore mother cells, microspores and pollen grains, while other layers extended laterally. Finally, the large epidermal cells accumulated starch grains. The cells of the endothecium acquired ring-like thickenings, one per cell and tangentially disposed on the inner surface of the cell wall. The tapetum and middle layers got absorbed and the endothecium and epidermis were left when pollen massulae were fully organized and ready for release (Figs. 28-30).

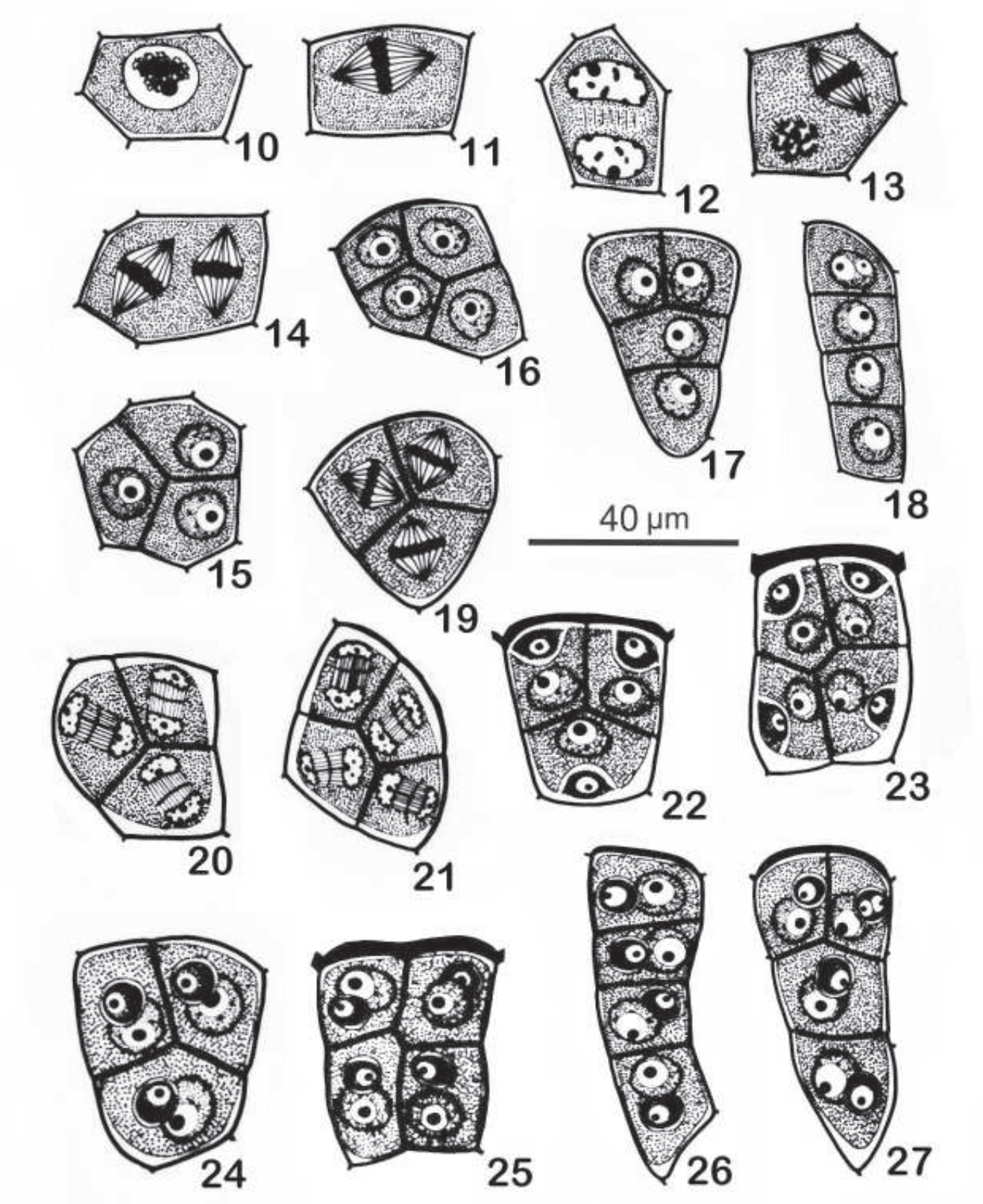
By this time, pollen massulae were fully organized, the group of smaller thin-walled cells belonging to the separating layer (connective cells) between the adjacent microsporangia and the cells located in the sub-epidermal region at the junction of the sporangial walls, were broken down. Aided by the endothecial thickenings, a common opening was created between the adjacent microsporangia, permitting the exit of the pollen massulae (Figs. 31-35).

## Discussion

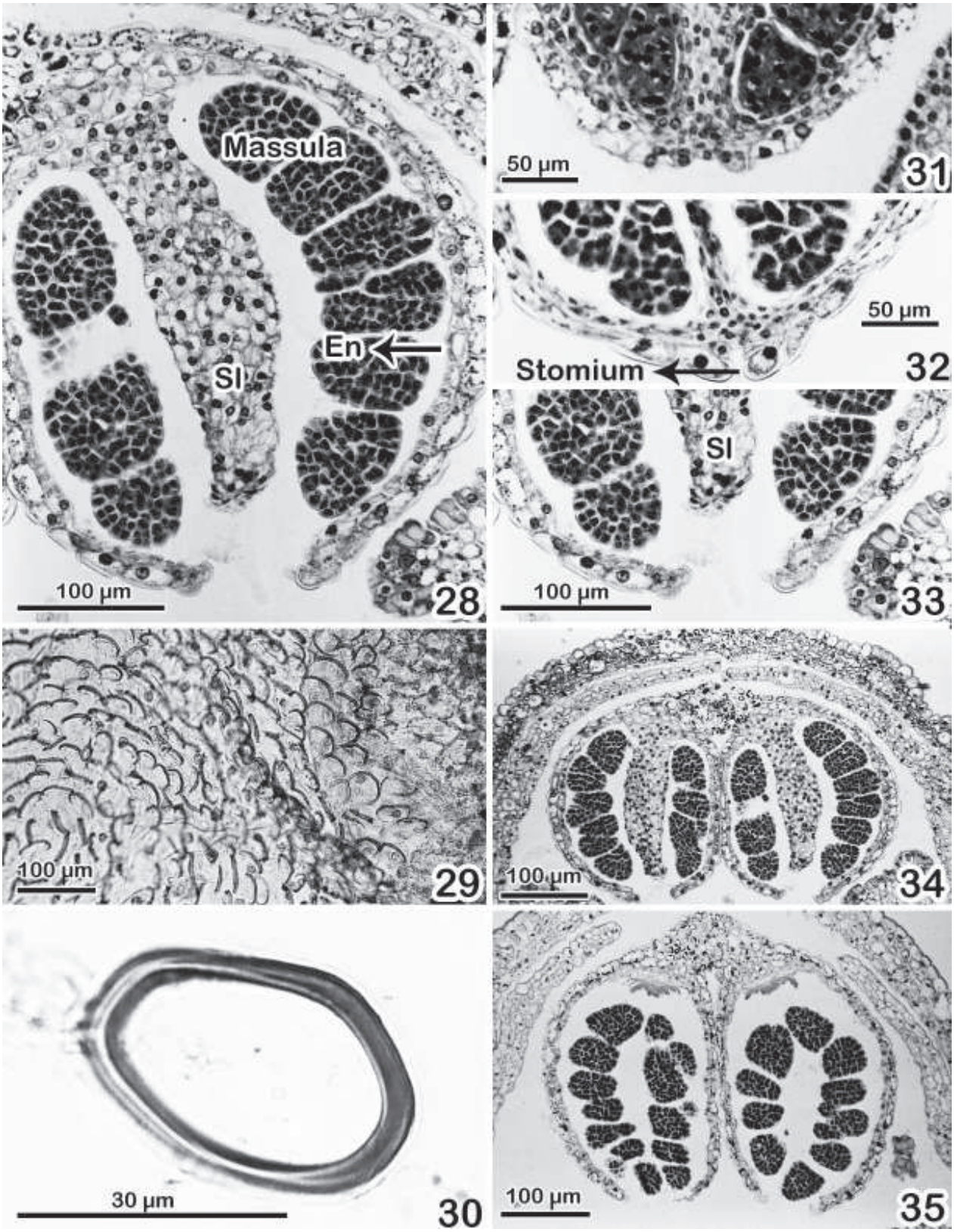
The anther was dithecous and tetrasporangiate. Similar nature of anther has been recorded in most of the orchids (Gurudeva, 2012; Krishna Swamy, *et al.*, 2003; Sood, 1985a, 1989, 1992). The mode of




Figs. 3-9. Ontogeny of microsporangium in *Peristylus spiralis*: 3-4. T.S. of very young anther showing archesporial layers; 5, Portion of microsporangium showing epidermis, primary parietal layer and block of sporogenous cells; 6, Portion of microsporangium showing outer parietal and inner parietal layers; note blocks of sporogenous cells; 7, Portion of microsporangium showing division in inner parietal cells, indicated by the arrows; 8-9, Portion of microsporangium showing wall layers and microspore mother cells, note starch grains in the epidermal cells. Abbreviations: Al, Anther lobe; Ar, Archesporial cells; B Sc, Block of sporogenous cell; C Ta, Connective tapetum; En, Endothecium; Ep, Epidermis; I Pl, Inner parietal layer; Ml, Middle layer; MMC, Microspore mother cells; Pl, Primary parietal layer; P Ta, Parietal tapetum; Sg, Strach grains; St, Sporogenous tissue.




Figs.10-27. Microsporogenesis and development of pollen in *Peristylus spiralis*: 10-12, Meiosis-I in the microspore mother cells; 13-14, Simultaneous division of dyad nucleus; 15, Three microspores in a tetrahedral tetrad; 16, Rhomboidal tetrad; 17, T-shaped tetrad; 18, Linear tetrad; 19-21. Divisions of microspores in the tetrad; note proximal and distal orientation of nuclear spindles of the dividing nuclei; 22-27, Migration of parietal disposed generative cell in the pollen tetrad.




Figs. 28-35. Dehiscence of microsporangium in *Peristylus spiralis*: 28, Mature microsporangium showing tangentially disposed endothecial thickenings; note the starch grains in the epidermal cells and massulae in the sporangium; 29, Whole mount of endothecial layer; 30, Oval shaped thickening detached from the endothecial cell; 31-35, Show the formation of stomium and stages of microsporangial dehiscence. Abbreviations: En, Endothecium; Sl, Separating layer.

organization of the anther wall conforms to the monocotyledonous type (Davis, 1966). A similar method of wall development has been recorded in *Habenaria edgeworthii, H. elisabethae, H. galeandra, H. intermedia,* and *Neottia listeroides* (Sood, 1984, 1985b, 1986), *Oreorchis foliosa* (Mohana Rao and Sood, 1987), *Epipactis helleborine* and *E. veratrifolia* (Vij and Sharma, 1987). It is very likely that in others where the type of organization has not been studied so far, could also be of similar pattern. In the presently investigated species, the sporangial wall comprised four wall layers namely epidermis, endothecium, middle layer and the tapetum. Similar number of wall layers has been recorded earlier in most of the investigated taxa (Cocucci, 1964; Gurudeva, 2012; Mohana Rao and Rao, 1983, 1984; Sood, 1986; Swamy, 1949a).

Presently, the epidermis was always single-layered, its cells were generally larger in size and tangentially extended and was basically protective in function and remained persistent even at anthesis. A similar observation has been made in *Aa achalensis* (Cocucci, 1964), and *Neottia listeroides, Microstylis cylindrostachya* and *Habenaria* species (Sood, 1984, 1985a, 1986). Epidermis showed the presence of starch grains which indicated that this layer was concerned with nutrition besides its usual function of protection. Nutritive role of epidermis has also been recorded in *Zeuxine longilabris* (Karanth, *et al.,* 1979), *Epipogium roseum* (Govindappa and Karanth, 1981) and *Habenaria diphylla* (Gurudeva, 2012). As this behaviour of the epidermis of the microsporangial wall has not been recorded so far in any of the angiosperms, gymnosperms and pteridophytes studied so far, appears unique.

Presently, the endothecium was single layered. At maturity, its cells acquired thickenings on the inner surface of their walls and these thickenings were ring-like, single and tangentially disposed. This type of endothecial thickenings corresponds to Type-II of Freudenstein (1991). Similar type of tangentially disposed endothecial thickenings were earlier recorded in *Habenaria diphylla* (Gurudeva, 2012) and *Habenaria clavigera* (Sharma and Vij, 1987). Different types of endothecial thickenings in orchids have been recorded by Untawale and Bhasin (1973) and were classified by Freudenstein (1991). Further it is worthwhile to investigate the exact functional role of the different kinds of thickenings, especially in connection with the opening of the anther lobe at the time of release of massulae / pollinia release.

The middle layer consists of single row of thin-walled tangentially extended cells. During microsporogenesis, when the tapetal cells become very conspicuous and active, this layer gets gradually crushed and absorbed. Similar observation has been made in *Aphyllorchis montana, Dendrobium microbulbon, Platanthera susannae,* and *Sirhookera latifolia* (Krishna Swamy *et al.,* 2003). Persistence of middle layer and acquisition of thickenings along with the endothecium and their role assisting in opening of anther lobe has been recorded in *Arundina graminifolia* (Rao, 1967), *Bromheadia finlaysoniana* (Jayanayaghy and Rao, 1966), and *Spathoglottis plicata* (Prakash and Lee-Lee, 1973).

The inner most layer of the sporangium wall was the tapetum. Because of its dual origin, it was completely surrounded by the sporogenous tissue. It was of glandular type. Similar feature has been recorded in majority of orchids (Gurudeva, 2012; Kant *et al*. 2013; Krishna Swamy *et al.*, 2003; Sood and Mohana Rao, 1986a, 1986b; Sood and Sham, 1987; Swamy, 1949a). Tapetal cells remained uninucleate throughout and it is in conformity with several orchids (Krishna Swamy *et al.*, 2003; Mohana Rao and Sood, 1987; Sood, 1985a,b; Sood and Mohana Rao, 1988). Finally the tapetal layer breaks down leaving its remnants within the confines of the locule. In addition to the nutritional role, it is generally believed that tapetal cells play a role in exine formation by secreting sporopollenin precursors, which are then polymerised during maturation of the pollen in angiosperms (Heslop-Harrison, 1971).

The archesporial cells after producing a parietal layer functioned together as sporogenous tissue. Presently, in current investigation in *Peristylus spiralis,* the sporogenous cells belonging to a massula are derived from a single archesporial cell. A similar condition has been reported in *Himantoglossum hircinum* (Heusser, 1915), *Calanthe veratrifolia, Neottia ovata,* and *Orchis maculata* (Guignard, 1982), and in several species of *Habenaria* and *Peristylus* (Swamy, 1946, 1949a). The sporogenous cells enlarge and become microspore mother cells in all the species so far studied (Blackmen and Yeung, 1983; Swamy, 1949a; Wirth and Withner, 1959) including the present investigation. The microspore mother cells underwent the usual meiotic divisions and resulted in different types of microspore tetrads. Quadri-partition of microspore mother cells is simultaneous in most of the taxa investigated so far (Coccuci, 1967; Prakash and Lee-Lee, 1973; Swamy, 1941, 1946, 1949a; Vij and Sharma, 1987) including the present study. The tetrads may be arranged in different patterns. The type of microspore tetrads was dependent on the orientation in which the walls were deposited during meiotic division. The orientation of

microspores in tetrad has been described as tetrahedral, isobilateral, rhomboidal, linear and T-shaped tetrads. The location of the type of tetrads within the massulae was variable. Usually, the per cent of linear, T-shaped, isobilateral and rhomboidal tetrads are more at the periphery than at the centre of the massula, whereas tetrahedral tetrads were more common at the centre of the massula.

The microspores were with dense cytoplasm and a large centrally located nucleus. The nuclear division within the microspore tetrad was synchronous and asymmetrical in conformity with earlier records (Hagerup, 1938; Mohana Rao and Sood, 1986; Prakash and Lee-Lee, 1973). The small newly formed generative cell was initially adpressed to the wall of the microspore, later it separated itself from the microspore wall and entered into the cytoplasm of vegetative cell. The pollen grains were 2-celled when massulae were ready for pollination in all the species as studied earlier by many workers (Gurudeva, 2012; Pace, 1909; Prakash and Lee-Lee, 1973; Sood, 1986; Swamy, 1949a).

At the time of anther dehiscence, a well-developed stomium was formed at wall cells at the junction of the two adjacent microsporangia which got disorganized leading to the formation of vertical slit in each of the two anther lobes so as to facilitate carrying of the massula.

## 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.

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

Bhanwra, R.K., S.P. Vij, V. Chandel, R. Kant, and S. Dutt. 2006. Development of pollinium in two epidendroid orchids. *Curr. Sci.*, **90** (10): 1384 -88.

Blackman, S. J. and E. C. Yeung. 1983. Structural development of the caudicle of an orchid (*Epidendrum*). *Amer. J. Bot.*, **70** : 97-105.

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. Gdn.*, **47**: 25-68.

Fredrikson, M. 1990. Embryological study of *Herminium monorchis* (Orchidaceae) using confocal scanning Laser Microscopy. *Amer. J. Bot.,* **77** (1): 123-27.

Fredrikson, M. 1991. An embryological study of *Platanthera bifolia* (Orchidaceae) *Pl. Syst. Evol.,* **174** (3-4): 213-20.

Freudenstein, J. V. 1991. A systematic study of endothecial thickenings in Orchidaceae. *Amer. J. Bot.,* **78** (6): 766-81.

Govindappa, D. A. 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 Publisher, New Delhi, India.

Govindappa, D. A. and K. A. Karanth. 1981. The Embryology of *Epipogium roseum* (Orchidaceae). *Pl. Syst. Evol.*, **138** (1-2): 1-7.

Guignard, L. 1882. Recherches sur la development de lanthere et du pollen chez les Orchidees. *Ann. Sci. Nat. Bot.,* VI. **14**: 26-45.

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. (Ochidaceae) *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. 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. 2014. Ontogeny and organization of female gametophyte in *Goodyera procera* (Ker-Gawl.) Hook. (Orchidaceae) *J. Orchid Soc. India*, **28**: 75 -77.

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.

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

Heslop-Harrison, J. 1971. The pollen wall: Structure and development. *In: Pollen: Development and physiology* (ed. J. Heslop-Harrison) pp.75-98. Butterworths, London, UK.

Heusser, K. 1915. Die Entwicklung dergenerativen Organe von *Himantoglossum hircinum* Spr. (= *Loroglossum hircinum* Rich.). *Beih. Bot. Centralbl.*, **32**: 218 -77.

Jayanayaghy, S. and A. N. Rao. 1966. Flower and seed development in *Bromheadia finlaysoniana. Bull. Torrey Bot. Club,* **93**(2): 97 -103.

Karanth, K. A., P. K. Bhatt, and D. A. Govindappa, 1979. Tapetum like anther epidermis in *Zeuxine longilabris* (Lindl.) Benth. ex Hk. Orchidaceae. *Curr. Sci.,* **48** (12): 542-43.

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.

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.

Levitte, R. G. 1901.Notes on the embryology of some New England Orchids. *Rhodora*, 361-63, 202-05. (cited by Coulter and Chamberlain in "Morphology of Angiosperms, 1912, New York, USA.)

Sharma, M. and S. P. Vij. 1987. Embryological studies on Orchidaceae. VI: *Habenaria* Willd. *Phytomorphology,* **37** (4): 327-35.

Mohana Rao, P. R. and K. M. Rao. 1983. Embryology of *Liparis veridiflora*. *Acta Bot. Indica*, **11**(2): 228 -34.

Mohana Rao and K. M. Rao. 1984.Embryology of *Habenaria pectinata*. *Phytomorphology,* 34: 237-42.

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

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

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

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

Pace, L. 1909. Gametophyte of *Calopogon*. *Bot. Gaz.,* **48**:126-37.

Prakash, N. and Aow Lee-Lee. 1973. Life history of a common Malayan orchid *Spathoglottis plicata*. *Phytomorphology*, **23** : 9-17.

Rao, A. N. 1967. Flower and seed development in *Arundina graminifolia*. *Phytomorphology*, **17**: 291-300.

Ravi Kant and M. M. Hossain. 2010. Development of pollinium in *Malaxis mucifera* (Lindl.) Kuntze. *Bangladesh J. Bot.*, **39** (2): 193-98.

Ravi Kant, 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.

Schnarf, K. 1931. *Vergleichende Embryologie der Angiospermen*. Gerbruder Borntraeger, Berlin.

Sood, S. K. 1984. An embryological study of *Neottia listeroides*, a saprophytic orchid in India. *J. Plant Anat. Morph.*, **1**: 69-75.

Sood, S. K. 1985a. Gametogenesis, integuments initiation, and embryogeny in *Microstylis cylindrostachya* (Orchidaceae, Epidendreae). *Proc. Indian Acad. Sci.* (Plant Sci.), **95** (6): 379-87.

Sood, S. K. 1985b. A contribution to the embryology of *Habenaria intermedia* (Orchidaceae). *J. Plant Anat. Morphol.,* (Jodhpur), **2** (2): 31-40.

Sood, S. K. 1986. Gametogenesis, integuments initiation, and embryogeny in three species of *Habenaria* (Orchidaceae, Orchideae), *Proc. Indian Acad. Sci.* (Plant Sci.), **96** (6): 487-94.

Sood, S. K. 1988. Development of gametophytes, embryogeny, and pericarp in *Goodyera repens* (Orchidaceae, Neottieae). *Proc. Indian Acad. Sci*. (Pl. Sci.), **98**: 149-56.

Sood, S. K. 1989. Embryology and systematic position of *Liparis* (Orchidaceae). *Pl. Syst. Evol.,* **166** (1-2): 1-9.

Sood, S. K. 1992. Embryology of *Malaxis saprophyta* with comments on the systematic position of *Malaxia* (Orchidaceae). *Pl. Syst. Evol.,* **179** (1-2): 95-105.

Sood, S. K. and P. R. Mohana Rao. 1986a. Development of male and female gametophytes in *Herminium angustifolium* (Orchidaceae). *Phytomorphology*, **36**:11-15.

Sood, S. K. and P. R. Mohana Rao. 1986b. Gametophytes, embryogeny and pericarp of *Microstylis wallichii* Lindl. (Orchidaceae). *Bot. Mag.* Tokyo, **99**: 351-59.

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

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

Swamy, B.G.L., 1941. The development of male gamete in *Cymbidium bicolor*. *Proc. Indian Acad. Sci.,* (B) **14**: 454-60.

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

Swamy, B. G. L. 1946. Embryology of *Habenaria*. *Proc. Nat. Inst. Sci. India*, **12**: 413-26.

Swamy, B. G. L. 1949a. Embryological studies in the Orchidaceae. I. Gametophytes. *Amer. Midl. Natur.*, **41**: 184-201.

Swamy, B. G. L. 1949b. Embryological studies in the Orchidaceae. II. Embryogeny. *Amer. Midl. Natur.*, **41**: 184-201.

Untawale, A. G. and R. K. Bhasin. 1973. On the endothecial thickenings in some monocotyledonous families. *Curr. Sci.*, **42**: 398-400.

Veyret, Y. 1974. Development of embryo. *In: The Orchids Scientific Studies*. (ed. C.L.Withner) pp. 223-265. John Wiley and Sons, New York, USA.

Vij, S. P. and M. Sharma. 1987. Embryological studies in Orchidaceae. V. *Epipactis* Adams. *Phytomorphology*, **37** (1): 81-86.

Wirth, M. and C. L. Withner. 1959. Embryology and development in the Orchidaceae. *In: The Orchids: A Scientific Survey*, (ed. C.L.Withner) pp. 155-88. Ronald Press, New York, USA.