J. Orchid Soc. India, 30: 75-87, 2016 *ISSN 0971-5371*

DEVELOPMENT OF MALE AND FEMALE GAMETOPHYTES IN *DENDROBIUM OVATUM* (L.) KRANZ. (ORCHIDACEAE)

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Abstract

The anther in *Dendrobium ovatum* (L.) Kranz. was found to be dithecous and tetrasporangiate. The development of microsporangial wall confirmed to Monocotyledonous type. Mass of densely protoplasmic cells represented the archesporium. The anther wall was 4-layered. Endothecial cells developed one or two ring-like radially disposed thickenings on the inner walls. Tapetal cells were uninucleate and dual in their origin. Simultaneous cytokinesis resulted in tetrahedral, isobilateral and rhomboidal pollen tetrads. At the time of release of pollinia, pollen grains attained 2-celled stage. The ovary was inferior, tricarpellary, syncarpous and unilocular with numerous anatropous, bitegmic and tenuinucellate ovules borne on the parietal placentae. Integuments were developed from the epidermis of the ovular primordium, 2-layered thick; micropyle was formed by the inner integument alone. The development of female gametophyte conformed to monosporic G-3 type. Chalazal megaspore in the triad configuration developed into 6-nucleate embryo sac. The mature embryo sac contained an egg apparatus, secondary nucleus and one antipodal cell. Double fertilization occured in a normal manner.

Introduction

THE ORCHIDACEAE, one of the largest families of angiosperms includes 800 genera and 25,000 species (Chugh *et al.*, 2009). Embryologically, the family is highly interesting as the orchids exhibit great diversity in development and organisation of male and female gametophytes, suspensor and embryo. The first embryological study in the family was done by Muller (1847). Since then several investigations have been carried out. The most comprehensive works have been done by Abe (1972a,b), Paddubnaya-Arnoldi (1967), Schnarf (1931), Swamy (1949), Veyret (1974) and Wirth and Withner (1959). These investigators, in addition to their own observations have published reviews of previous embryological works. Some of the recent works in the area include those of Bhanwra *et al*. (2006), Fredrikson (1990, 1991), Govindappa and Karanth (1980), Gurudeva (2009, 2010, 2011a,b, 2012, 2014), Gurudeva and Govindappa (2008), Kant and Hossain (2010) , Sood (1985a,b, 1986, 1988, 1989, 1992), Sood and Rao (1986a,b), and Swamy *et al*. (2003, 2005).

The genus *Dendrobium* Sw., sub-tribe Dendrobinae, tribe Epidendreae, sub-family Orchidoideae of Dressler and Dodson (1960) comprises nearly 900 species of epiphytic orchids distributed from Indo-Malaysia to Australia. Nearly 102 species are known from India (Kumar and Manilal, 1994); of which Karnataka accounts for 17 species (Rao and Sridhar, 2007). Embryological data in the genus is rather meagre. Pastrans and Santos (1931) studied the life history of *Dendrobium anosmum* and recorded pseudomonosporic type of embryo sac development. Swamy (1949) studied the embryology of *Dendrobium barbatulum, D. graminifolium, D. haemoglossum,* and *D. microbulbon*. Nimoto and Sagawa (1961) and Israel and Sagawa (1964) investigated the ovule development and made an electron microscopic studies on post pollinated ovules in three hybrids of *Dendrobium*. Sundararajan (1971) recorded Polygonum or Allium type of embryo sac development in *Dendrobium macrostachyum*. Govindappa and Karanth (1980) noticed 8-nucleate embryo sac development in *Dendrobium aqueum*. Swamy *et al*. (2003) studied microsporogenesis in *Dendrobium microbulbon*. Embryology of *Dendrobium ovatum* has not been studied so far, hence an attempt was presently made to study the ontogeny and organisation of male and female gametophytes.

Materials and Methods

Dendrobium ovatum (L.) Kranz. is an epiphyte having a sympodial stem with elongated shoots (Fig.1). The *leaves* are alternate, membranous, sheathing at the base, elliptic-oblong or oblong-lanceolate, acute entire and caduceus (Fig. 4). The *inflorescence* is a raceme on a leafless shoot (Figs. 1,2). The *flowers* are cream-yellow, pedicellate and bracteate (Fig. 3). The dorsal *sepal* and lateral *petals* are free and more or less similar. The latter are, however, adnate to the foot of the column. The *lip* is 3-lobed, cream-yellow and the mid lobe of the lip is twice the length of the side lobes. The disc has a tuft of short hairs. The *column* is long

Received: September 15, 2016; Accepted: October 22, 2016

and its foot is broad. The *anther* is conical, obtuse and bears 4-pollinia without *caudicles*.

The flower buds were collected at different stages of development from Hejamadi, Udupi district (Karnataka, India) during January to March, 2013. These were fixed in formalin-acetic-alcohol and stored in 70% ethanol following a thorough wash in running water. Conventional microtechniques were followed. The serial transverse and longitudinal sections at 10- 12μm 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 OF. 1.4 megapixel). Computer images were captured using AV-digitaliser having Grand VCD-200 captured guard.

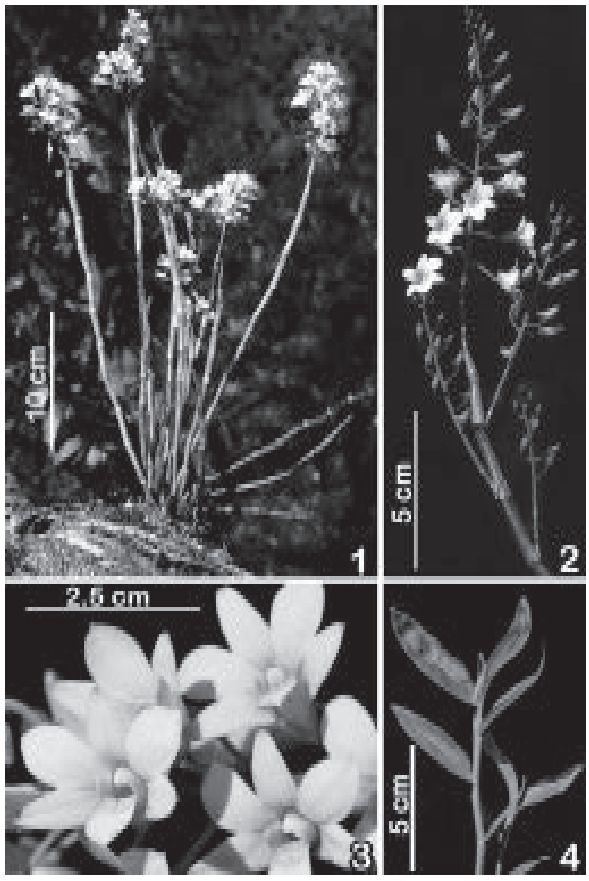
Results

Ontogeny of Microsporangium

The anther was tetrasporangiate. Four hypodermal masses of densely protoplasmic archesporial cells were recognized in a transection of a young anther (Figs. 5, 44). The row of this mass adjoining the epidermis acted as the primary parietal layer and rest of the mass as the sporogenous cells (Fig. 6). Gradually, the cells of the primary parietal layer lost the density of their cytoplasm and therefore appeared more translucent. This layer then divided periclinally and gave rise to two layers of cells (Figs. 7, 8, 45). The outer daughter layer differentiated directly into the endothecium and the inner after another division engendered the middle layer and the glandular tapetum (Figs. 9, 10, 46), often called parietal tapetum. The development of the microsporangium wall thus followed the monocotyledonous type (Davis, 1966). Occasionally, the tapetal cells divided at places and formed double layers (Figs.11, 47). Cells of the connective tissue bordering the sporogenous tissue, meanwhile acquired dense cytoplasm and prominent nuclei to complete the tapetal sheath around the massive sporogenous tissue, this tapetum was called connective tapetum, and therefore, tapetum is of dual in origin. During meiotic divisions of spore mother cells, the uninucleate tapetal cells became very prominent. Nourishment was drawn from these cells for the development of microspores and pollen tetrads and the pollinium. As a consequence, the tapetal cells were depleted of their contents. Ultimately, the cells were broke down alongwith cells of the middle layer. In the mature microsporangium, the wall consisted of two layers of cells namely epidermis made up of tangentially extended thin-walled cells, and the endothecium that became double layered at places because of periclinal division of some of the cells. Cells of this layer were large in size and radially extended. Each of them acquired one or two ring like thickenings on the inner surface of their wall (Figs. 12, 13, 25, 48, 49).

Microsporogenesis and Development of Male Gametophyte

When mitotic divisions in the sporogenous tissue ceased, the cells differentiated into microspore mother cells and divided meiotically. A cell wall was not laid down following the nuclear division (Figs. 14, 15, 16). The resulting dyad nuclei passed through meiosis-II and gave rise to four microspore nuclei (Figs. 17, 18). Cell walls were simultaneously laid down in the mother cells producing the microspore tetrads which may be tetrahedral, isobilateral or rhomboidal depending on the organization of the spindles of the dividing dyad nuclei (Figs. 19, 20). All the above types of tetrad were observed throughout the pollinium.



Figs. 1-4. *Dendrobium ovatum*: 1, Flowering plant on a tree; 2, An inflorescence; 3, Close view of flowers; 4, Leafy shoot.

No apparent changes in the cytoplasm of the microspores occurred before the nuclei divided in the tetrads. The spindles of the dividing nuclei were always disposed in a proximal-distal direction of the spores (Fig. 21). Two unequal cells result following this division of the microspore. The smaller generative cell was always located at the distal end and the larger tube cell laid on the proximal side (Fig. 22) The generative cell later separated itself from the spore coat and entered into the cytoplasm of the tube cell (Figs. 23, 24). All the tetrads of a sporangium stayed intact, as a common sporopollenin wall was laid around the mass organising a pollinium.

The connective tissue extending between the sporangia of an anther half, often designated as the separation layer, was 6-7 layered when the microspore mother cells divided to form tetrads of micropsores (Fig. 50). By the time the pollinia were organised, a small group of cells of the separation layer, located in the sub-epidermal region between the adjacent sporangia broke down. Consequently, an opening in the anther wall between the two sporangia in each of the anther halves was organised, facilitating the release of the pollinia from both the sporangia (Figs. 50, 51, 52). At the final stages, even the separation layer of cells disappeared leaving only the two pollinia in each of the anther halves for final release (Fig. 52).

Development of Megasporangium

The ovary was inferior, tricarpellary, syncarpous and unilocular. After pollination, the three parietal ridges proliferated producing many short protuberances which branch repeatedly in a dichotomous fashion. Each ultimate branch acted as an ovular primordium and consists of a row of 6-8 axial cells protected by the epidermal layer. The terminal cell of the axial row formed a large densely cytoplasmic archesporial cell (Fig. 26). It enlarged further and functioned directly as a megaspore mother cell. The two integumentary primordia arose from the epidermis of the primordium immediately below the level of megaspore mother cell (Fig. 27). Although the inner layer developed faster during the earlier stages, the outer layer soon matched that of the inner while building the two integuments (Figs. 28, 29, 36). Both the integuments were two cell layered throughout the developmental stages of the tenuinucellate antropous ovule. The micropyle was formed by the inner integument alone.

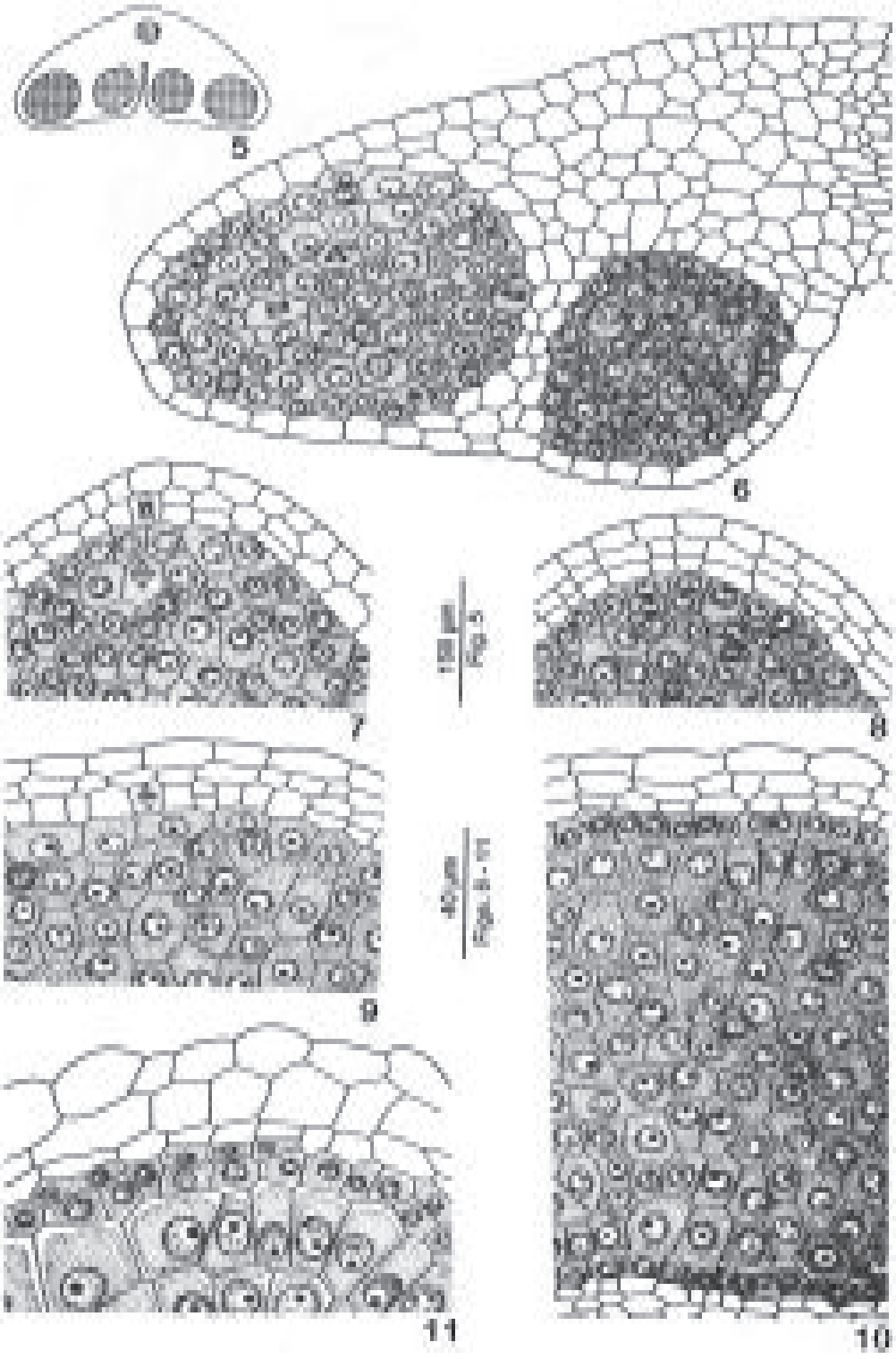
Usually a single megaspore mother cell was organized in the developing ovule. Very rarely two of them have been observed within a common nucellus (Fig. 29). Further development of the second megaspore mother cells could not be recorded.

Megasporogenesis and Organization of Embryo Sac

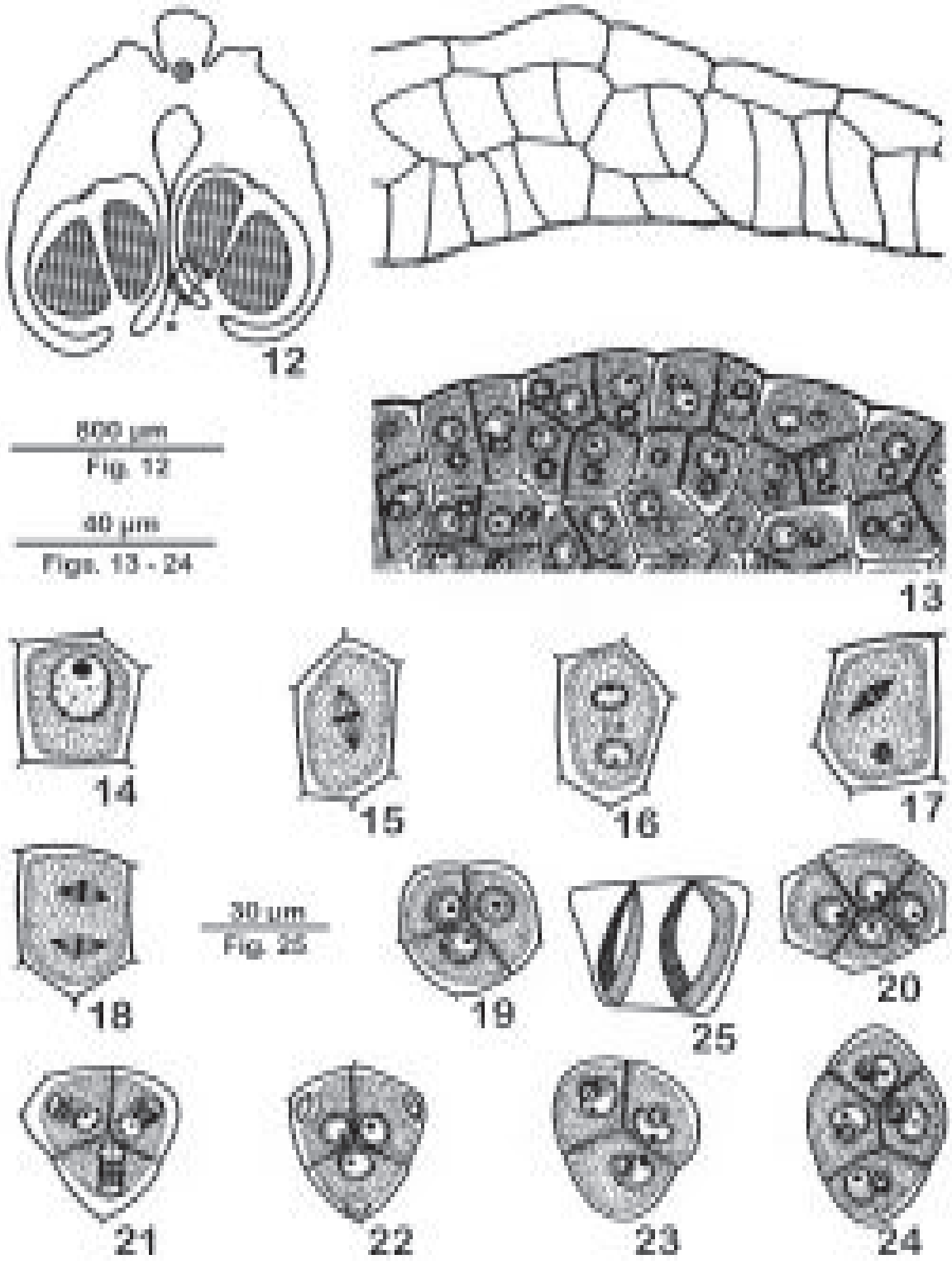
The megaspore mother cell underwent meiosis-I and produced two superposed dyad cells (Figs. 30, 31). Usually the upper smaller dyad cell degenerated. The lower larger one, after meiosis-II, gave rise to two unequal superposed megaspores. Thus a triad was formed consisting of an upper degenerating dyad cell, a small non-functional megaspore and a chalazal larger functional megaspore (Figs. 32, 33). A free nuclear division occurred in the functional megaspore. The resulting daughter nuclei were pushed apart to the opposite poles when a central vacuole was formed in the cytoplasm (Figs. 34, 35). Thus, a 2-nucleate embryo sac was established. The nuclei of the 2-nucleate embryo sac soon divided simultaneously and gave rise to two pairs of nuclei, a pair located at each poles (Figs. 36, 37). A 4-nucleate embryo sac was, therefore, organised (Fig. 37). The young gametophyte enlarged further. The surrounding nucellar cells got crushed. As a result, the gametophyte came in contact with the inner integument. The four nuclei of the sac divided synchronously. During this division the spindles of the nuclei at the chalazal pole coalesced (Figs. 38, 39). After telophase, therefore, a quartet of nuclei at the micropylar pole and only a pair of them at the chalazal end were found (Fig. 40). The micropylar nuclei then contributed to the organization of an egg apparatus and the micropylar polar. One of the chalazal nuclei acted as the chalazal polar and move towards the micropylar polar. The other one stayed *in situ* and represented the sole antipodal without being enclosed by a cell wall (Fig. 41). An embryo sac, therefore, was 6-nucleate. In the mature embryo sac, the egg apparatus consisted of pear shaped, posteriorly vacuolated, juxtaposed synergids and a large egg located behind them. The polar nuclei either remained free or fuse together forming the secondary nucleus. The antipodal was represented by a single nucleus.

Fertilization

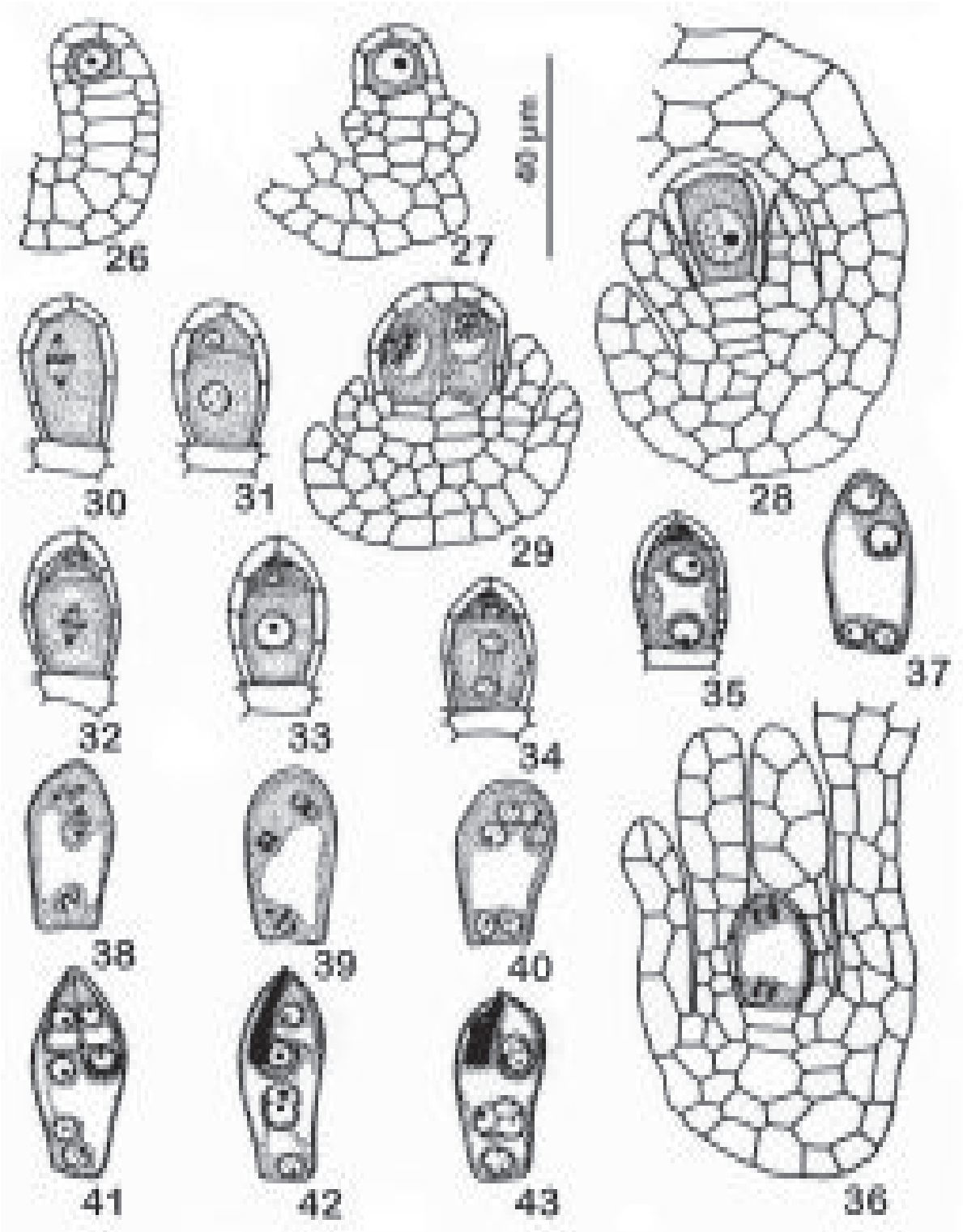
Double fertilization occurred. The pollen tube destroyed one of the synergids during its entry into the embryo sac and released the two male gametes. One of them fused with the egg effecting syngamy and the other united with the two polars or with the secondary nucleus and completed triple fusion (Figs. 42, 43). The process of syngamy was faster than triple fusion. The resulting primary endosperm nucleus was tetraploid and degenerated during post-fertilization stages. The antipodal nucleus and the surviving synergid also degenerated subsequently.



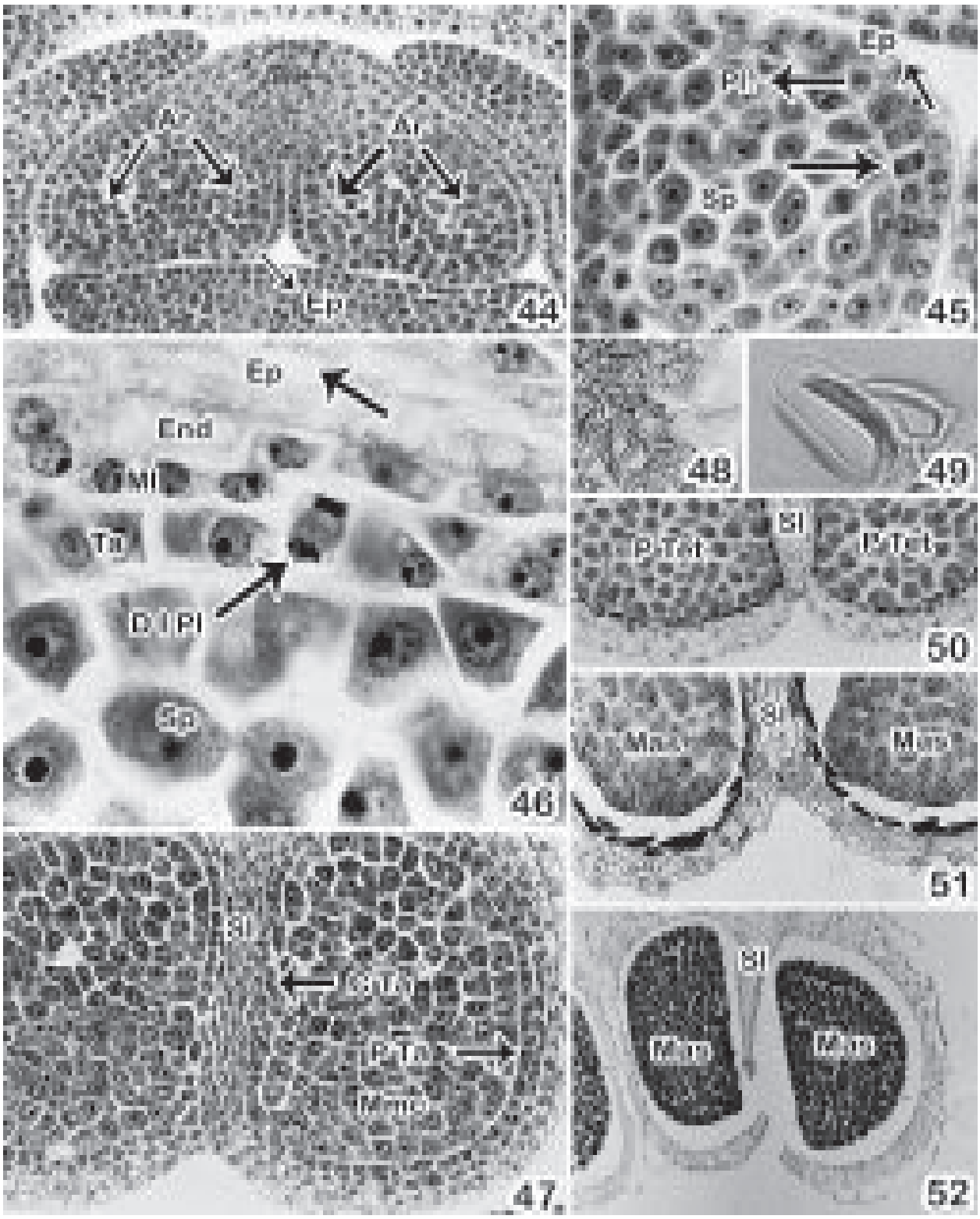
Figs. 5-11. *Dendrobium ovatum*, diagrammatic cross section of developing microsporangium: 5, T.S. of young anther; 6, T.S. of young anther half; note two masses of archesporial cells; 7-8, T.S. part of developing microsporangia to show the origin of two parietal layers; 9-10, T.S. part of mirosporangia to show the origin of tapetum and middle layer from the inner parietal layer; 11, T.S. part of microsporangium; note double layered tapetum at certain places and young spore mother cells with large nuclei.



Figs. 12-25. Microsporogenesis and pollen development: 12, Outline T.S. of mature anther; 13, Portion marked 'x' in fig. 12 enlarged to show endothecium with thicknings and part of pollinium; 14-16, Meiosis-I in microspore mother cell; 17-18, Meiosis-II in microspore mother cells; 19, Tetrahedral tetrad; 20, Rhomboidal tetrad; 21, Nuclear division in spore tetrad; 22, Young 2-celled pollen in tetrad; 23-24, Mature pollen tetrads; 25. An endothecial cell with two radially disposed ring like thickenings.



Figs. 26-43. Development of embryo sac in *Dendrobium ovatum*: 26, Ovular primordium showing hypodermal archesporial cell; 27, Ovular primordium with megaspore mother cell; note the initiation of integuments; 28, Ovule with megaspore mother cell; 29, Ovule with double juxtaposed megaspore mother cells; 30, Megaspore mother cell at meiosis-I; 31, Unequal dyad cells; 32, Shows degenerating upper dyad cell and lower dyad at meiosis-II; 33, A triad; 34, Nuclear division in the functional megaspore; 35, 2-nucleate embryo sac; note degenerating nucellar cells; 36, Synchronous nuclear division in two nucleate embryo sac; note the inner integument alone forming the micropyle; 37, 4-nucleate embryo sac; 38-39, Nuclei in 4-nuvleate embryo sac in division; note the fusion of spindles at chalazal end; 40, 6-nucleate embryo sac; 41, Organized embryo sac; 42-43. Show the stages of double fertilization.



Figs.44-52. *Dendrobium ovatum*, photomicrograph showing different stages during microsporangium ontogeny: 44, T.S. of young flower bud with young anther at the centre. ×247; 45, Part of T.S. of microsporangium; note the perilinal division of primary parietal layer, indicated by the arrow. ×265; 46, Part of microsporangium showing division in the inner parietal layer. ×700; 47, Microsporangium, to show double layered tapetum. ×330; 48-49, Whole mount of endothecial thickenings. ×780, 1000; 50-52, Part of anther halves to show the organisation of the line of openings between the adjacent microsporangia. ×282, 234, 126.

Discussion

The anther was with four microsporangia. The archesporium appear as 4-hypodermal mass of densely protoplasmic cells. The row of this mass adjoining to the epidermis gradually loses the density of their cytoplasm and therefore appears translucent. This layer acts as primary parietal layer and contributes the wall layers of microsporangium. The mode of organisation of the sporangial wall conforms to the monocotyledonous type (Davis, 1966) since tapetum and middle layer are sister layers. A similar type of wall development has been recorded in *Satyrium nepalense* (Rao and Sood, 1979b), *Zeuxine strateumatica* (Vij *et al*., 1982), *Habenaria edgeworthii, H. elisabethae, H. galeandra, H. intermedia, Microstylis cylindrostachya,* and *Neottia listeroides* (Sood, 1984, 1985a,b, 1986).

The epidermis was always single-layered. The cells were generally larger in size and tangentially extended. It is basically protective in function and remains persistent even at anthesis. A similar observation has been made in *Aa achalensis* (Coccucci, 1964), *Arundina graminifolia* (Rao, 1967), *Habenaria densa* and *Satyrium nepalense* (Rao and Sood, 1979 a,b), *Epidendrum ibaguense* (Blackman and Yeung, 1983). 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).

The endothecium is generally single layered in a majority of orchids studied so far (Bhanwra *et al*., 2006; Kant and Bhanwra, 2010; Kant and Hossain, 2010; Sood, 1992; Sood and Rao, 1986). It becomes double layered at places because of periclinal division of its cells in *Zeuxine strateumatica* (Kant and Bhanwra, 2010) and *Dendrobium ovatum* of the present study. Chardard (1971) noted a double layered endothecium as a regular feature in *Cleistostoma racemifera* (= *Sarcanthus pallidus*). Similar feature has been reported in *Epipactis* (Vij and Sharma, 1987). Guignard (1882) recorded three layers of endothecium in *Limnodorum*. It should be pointed out that multi-layered condition should be regarded as primitive and it represents the ancestral character.

The cells were generally thick-walled and vacuolated when young, and at maturity, these acquired thickening on the inner surface of their wall. These thickenings may be fibrous as in *Vanilla planifolia* (Swamy, 1947), *Polystachya flavescence* (Swaminathan, 1967), *Habenaria densa, Cephalanthera ensifolia,* and *Oerorchis foliosa* (Rao and Sood, 1979a, 1986, 1987), *Herminium angustifolium* (Sood and Rao, 1986a) and *Zeuxine strateumatica* (Kant and Bhanwra, 2010), U or V shaped fibrous thickenings in *Malaxis saprophytica* (Sood, 1992), ring like one or two and radially disposed as in *Aa achalensis* (Cocucci, 1964), *Malaxis mucifera* (Kant and Hossain, 2010) and *Dendrobium ovatum* (present study) or single and tangentially disposed is recorded in *Habenaria clavigera* (Sharma and Vij, 1987) and *Habenaria diphylla* (Gurudeva, 2012). Different types of endothecial thickenings in orchids have been recorded (Untawale and Bhasin, 1973) and classified by Freudenstein (1991). Swamy (1949) opined that endothecial thickenings are more pronounced in the epiphytic species compared to the terrestrial forms. On the other hand the previous study (Gurudeva, 2012) and present study indicates that the thickenings are well developed in pollinia bearing taxa compared to the massulae producing species. Further studies in this regard will throw more light on this aspect.

The middle layer was generally single layered. Occasional occurrence of double middle layers has been recorded in *Zeuxine sulcata* and *Vanilla planifolia* (Swamy, 1946, 1947) and *Spathoglottis plicata* (Prakash and Aow, 1973). On the other hand, consistent occurrence of two middle layers has been observed in *Paphiopedilum druryi* (Swamy, 1949), *Epipogium roseum* (Govindappa and Karanth, 1981), *Cephalanthera ensifolia* (Rao and Sood, 1986), and *Cypripedium cordigerum* (Sood and Rao, 1988).

The innermost layer of the sporangium wall was the tapetum. Because of its dual origin, it completely surrounded the sporogenous tissue. It was of glandular type. Similar feature has been recorded in a majority of orchids (Gurudeva, 2012; Kant *et al*., 2013; Sood and Rao, 1986a, b; Sood and Sham, 1987; Swamy, 1949; Swamy *et al*., 2003). Although tapetum is made up of single layer of cells, in a majority of orchids (Sood, 1985 a,b, 1986, 1992; Sood and Rao, 1986a, b; Sood and Sham, 1987). In *Dendrobium ovatum* of the present study, it becomes two layered at places a feature also recorded in *Eulophia epidendraea* and *Vanilla planifolia* (Swamy, 1943, 1947). Tapetal cells remained uninucleate throughout and it is in conformity with several members of orchids (Rao and Sood, 1987; Sood, 1985 a,b, 1989; Sood and Rao, 1988; Swamy *et al*., 2003). Binucleate tapetal cells have also been reported in several species such as *Paphiopedilum druryii* (Swamy, 1947), *Spathoglottis plicata* (Prakash and Aow, 1973), *Epipactis latifolia* (Sood, 1997) and *Habenaria diphylla* (Gurudeva, 2012). Finally the layer brakes down leaving its contents within the confines of the locule.

The archesporial cells after producing a parietal layer function as sporogenous tissue. The entire mass of archesporium produces a massive sporogenous tissue which later contributes to the formation of pollinium. Similar feature has been reported by Bhanwra *et al*. (2006), Kant *et al*. (2013) and Swamy (1949). The sporogenous cells enlarged and behaved as microspore mother cells. They underwent usual meiotic divisions and resulted in tetrahedral, isobilateral and rhomboidal tetrads, as observed throughout the pollinium. However, there are reports on the occurrence of linear and T-shaped tetrads at the periphery of the pollinium of *Gyrostachys* (Pace, 1914) and species of the tribe Bulbophyllinae, Dendrobiinae and Sarcanthinae (Swamy, 1949).

The nuclear division within the microspore tetrad was synchronous and asymmetrical, in conformity with the earlier records (Hagerup, 1938; Prakash and Aow, 1973; Rao and Sood, 1986). The small newly formed generative cell was initially adpressed to the wall of the microspore. Later it separateed itself from the microspore wall and entered into the cytoplasm of the vegetative cell. The generative cell appeared to become located anywhere within the cytoplasm of the vegetative cell.

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

The pollinium of *Dendrobium ovatum* (present study) and *Malaxis mucifera* (Kant and Hossain, 2010) do not possess carrier mechanism and should be regarded as primitive when compared to the others in which there is caudicle and an attaching glandular disc to facilitate the transference of pollinium (Bhanwra *et al*., 2006; Kant and Bhanwra, 2010).

The gynoecium was inferior, tricarpellary, syncarpous and ovary was unilocular, having numerous anatropous ovules on the divided parietal placentae. The ovule initiation on the placenta was triggered usually after pollination. The observation is in conformity with the earlier records on this aspect (Brown, 1833; Fredrikson, 1991; Govindappa and Karanth, 1980; Gurudeva, 2009, 2011 a,b, 2014; Mayer *et al*., 2011; Niimoto and Sagawa, 1961; Vij and Sharma, 1986; Yeung and Law, 1989).

Organisation of a large number of finger-like ovular primordia consisting of an axial row of cells ensheathed by a layer of epidermis was observed in the present study. A similar feature has been reported in a majority of orchids (Rao and Sood, 1987; Sharma and Vij, 1987; Sood and Rao, 1986a) except in *Vanilla planifolia* (Swamy, 1947), *V. rosheri* (Krupko *et al*., 1954) and *Bletilla stricta* (Abe, 1972a), where 2-3 irregular axial rows of cell ensheathed by a layer of epidermis is reported.

The integumentary initials originated from nucellar epidermal cells. This observation is in conformity with that of previous workers (Abe, 1972a; Sood, 1986, 1987; Swamy, 1949; Wirth and Withner, 1959).

The bending of funiculus, the differentiation of megaspore mother cell and the origin of integumentary primordia occurred almost simultaneously. The inner integument was always the first to be differentiated, the first to become active and invariably first to degenerate. In *Vanilla planifolia* (Swamy, 1947) and *V. roscheri* (Krupko *et al*., 1954), it is interesting to note that the inner integument persisted, even in the mature seed. The outer integument lagged behind the inner, upto the organisation of embryo sac in the ovule, but subsequently out grew the inner, after fertilization. This is in complete agreement with the earlier findings (Abe, 1972a; Govindappa and Karanth, 1980; Gurudeva, 2014; Sood, 1986, 1987; Swamy, 1949) on the contrary in *Aerides maculosum* (Gurudeva, 2009), *Bulbophyllum mysorense* (Swamy, 1949), *Epidendrum radicans* (Gurudeva and Govindappa, 2008), *Trias stocksii* (Gurudeva, 2010) and *Disperis neilgherrensis* (Gurudeva, 2011b), though the outer integument was initiated later, it grew faster and overarched the inner even before the organization of the embryo sac.

The ovule ultimately became anatropous and bitegmic, with the micropyle being organized by the inner integument alone. This feature is constant in most of the orchids (Abe, 1972a; Attri *et al*., 2005; Govindappa and Karanth, 1980; Gurudeva, 2014; Sood, 1986, 1987; Sood and Rao, 1988; Swamy, 1947; Vij and Sharma, 1987; Wirth and Withner, 1959). However, the production of unitegmic ovules without a micropyle has been reported in *Gastrodea elata* (Abe, 1976; Kusano, 1915), *Epipogium aphyllum* (Afzelius, 1954) and *E. roseum* (Govindappa and Karanth, 1981). According to Afzelius (1954), the reduction in the structure of ovules in these taxa is due to their propagation, usually by vegetative means rather than through the regular sexual method.

The micropyle is organized by the inner integument alone. This feature is also observed in a majority of orchids (Abe, 1972a; Fredrikson, 1990; Govindappa and Karanth, 1980; Gurudeva, 2014; Swamy, 1949; Swamy *et al*., 2005). However, in *Hetaria* (Tohda,

1967), micropyle is organized by the contribution of both the integuments. In *Gastrodea elata,* (Kusano, 1915), *Epipogium aphyllum* (Afzelius, 1954) and *E. roseum* (Govindappa and Karanth, 1981), a micropyle is never organized in the ovule.

Single hypodermal archesporial cell organized in the finger like ovular primordium. Archesporial cell enlarged in size and directly functioned as the megaspore mother cell. Similar observations have been made in several other investigated species (Abe, 1972a; Gurudeva, 2009, 2010, 2011a; Mayers *et al.*, 2011; Rao and Sood, 1979a; 1986, 1987; Sood, 1985a, 1986; Swamy, 1949). Occurrence of two megaspore mother cell in a single nucellus has been reported in *Calopogon puchellus* (Pace, 1909), *Goodyera procera* (Gurudeva, 2014). A similar feature has been noticed in *Dendrobium ovatum* of the present study. In species with double megaspore mother cells, these cells may develop further but ultimately only one of them functions.

The megaspore mother cell underwent meiosis-I and gave rise to two superposed dyad cells, the upper of which was invariably smaller. The smaller upper dyad cell promptly degenerated and the lower one passed through meiosis-II giving rise to a smaller upper non-functional and lower functional megaspores. This lead to the organisation of a triad. Similar triad formation has been reported in a majority of orchids (Abe, 1972a; Attri *et al.*, 2007; Govindappa and Karanth, 1980; Gurudeva 2009, 2010, 2011 a,b, 2014; Sood, 1989; Swamy, 1949).

The chalazal megaspore invariably became functional. The nucleus of the functional megaspore divided twice to form 4-nucleate embryo sac. The 4 nuclei took part in third free nuclear division. The nuclear spindles of the chalazal region fused to produce two diploid nuclei instead of four haploid nuclei. Hence, a six nucleate embryo sac was formed. This feature is occasionally observed in *Epipactis pubescens* (Brown and Sharp, 1911) and *Paphiopedilum insigne* (Afzelius, 1916). Similar type of 6-nucleate embryo sac formation is a common feature in *Bulbophyllum neilgherrense* (Swamy, 1949) and *Polystachya flavescence* (Ekanthappa and Govindappa, 1977a), and *Goodyera procera* (Gurudeva, 2014). A 6-nucleate female gametophyte could also be attained through the phenomenon of strike at the chalazal end. In *Trias stocksii* (Gurudeva, 2010), the two chalazal nuclei of the 4-nucleate embryo sac never took part in the next nuclear division. As a consequence a 4 + 2 arrangement of nuclei results. A similar feature has also been recorded in few orchid taxa (Abe, 1972b; Sood, 1992). Similar to monosporic category, even in bisporic type, formation of 6-nucleate embryo sac has been recorded either by the fusion of spindle at the dividing nuclei of the chalazal end or by strike phenomenon in *Cirrhopetalum fimbriatum* (Ekanthappa and Govindappa, 1977b), *Cypripedium cordigerum* (Sood and Rao, 1988), and *Zeuxine gracilis* (Gurudeva, 2011).

The mature embryo sac was oval with broad micropylar part. The egg apparatus was with 2 juxtaposed pear-shaped synergids and a large egg located behind the synergids. These aspects are consistently similar in other orchid taxa investigated so far (Abe, 1972a; Swamy, 1949). The two polars of the embryo sac generally fuse together forming secondary nucleus. The nature of antipodals in the embryo sac of the investigated taxa appears to be interesting. In *Aerides maculosum* (Gurudeva, 2009), *Disperis neilgherrensis* (Gurudeva, 2011b), *Goodyera repens* (Sood, 1988*), Oreorchis foliosa* (Rao and Sood, 1987), *Liparis* (Sood, 1989), the mature embryo sac contains three antipodal cells. However, in *Epidendrum radicans* (Gurudeva and Govindappa, 2008) three antipodal nuclei are recorded. It is being postulated that the presence of 3 antipodal cells is the basic feature. The next step in simplification is the elimination of walls of antipodal cells observed in *Epidendrum radicans* (Gurudeva and Govindappa, 2008). On the other hand, the number of antipodal cells was reduced from three to one in *Goodyera procera* (Gurudeva, 2014). Further, with the elimination of wall of antipodal cell and only one antipodal nucleus is arrived at as in *Dendrobium ovatum* of the present study, *Trias stocksii* (Gurudeva, 2010) and *Zeuxine gracilis* (Gurudeva, 2011a). It is obvious therefore, that functionally the chalazal end of the embryo sac is not as important as the micropylar end.

The mode of embryo sac development conforms to monosporic and G 3 type of Abe (1972b).

Entry of pollen tube was porogamous. After entry of the pollen tube, one of the synergids degenerated and the two male gametes released. Syngamy occurred earlier than triple fusion. Primary endosperm nucleus promptly degenerated. This is in conformity with most of the earlier observation on this aspect (Swamy, 1949).

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