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# ONTOGENY OF MICROSPORANGIUM AND DEVELOPMENT OF MALE GAMETOPHYTE IN *MALAXIS VERSICOLOR* (LINDL.) ABEYW. (ORCHIDACEAE)

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The anther in *Malaxis versicolor* (Lindl.) Abeyw. is dithecous and tetrasporangiate. Its wall development conforms to the monocotyledonous type (Davis, 1966). Mass of densely protoplasmic cells represents the archesporium. Each such archesporium develops into pollinium. The anther wall is 4-layered. The endothecial cells develop one or two ring like radially disposed thickenings on the inner walls. Tapetal cells are uninucleate and dual in origin. Simultaneous cytokinesis results in tetrahedral and rhomboidal pollen tetrads. At the time of release of pollnia pollen grain attain 2-celled stage.

**Key words :** Angiosperms, Monocotyledons, Orchidaceae, Microsporogenesis, male gametophyte, *Malaxis*

The Orchidaceae are the largest family of angiosperms and highly evolved group among monocotyledons. The family includes 800 genera and 25,000 species (Chungh *et al.* 2009). Embryologically the family is highly interesting as they exhibit great diversity in development and organisation of male and female gametophytes, suspensor and embryo. Schnarf (1931), Swamy (1949a, 1949b), Abe (1972a, 1972b) and Veyret (1974) have reviewed the previous embryological literature on the family. Some of the recent works in the area includes those of Krishna Swamy *et al.* (2003, 2005), Attri *et al*. (2005, 2007), Bhanwra *et al*. (2006), Gurudeva and Govindappa (2008) and Gurudeva (2009, 2010, 2011a,2011b, 2012, 2014). The genus *Malaxis* Sol ex Sw. (sub-tribe: Liparidinae, tribe : Epidendreae, sub-family:Orchidoideae of Dressler and Dadson, 1960) has 275 species, about 17 species are reported from India and only 4 species of them are occurring in Karnataka (Ananda Rao and Sridhar 2007). *Malaxis saprophyta* and *Malaxis mucifera* were studied embryologically by Sood (1992) and Ravi Kant and Hossain (2010) respectively. Persual of literature indicates no work on the embryology of *Malaxis versicolor,* hence an attempt is made to study the ontogeny of microsporangium and development of male gametophyte.

## MATERIALS AND METHODS

The young flower buds were collected from Manjarabad Fort, Donigal, Sakaleshpura, Hassan district, Karnataka, India, during September and October between 9 am and 12 noon and fixed in Formalin-Acetic acid –alcohol (FAA) for 48 hours and stored in 70% ethanol after a thorough wash in running tap water. Conventional microtechniques were followed. The sections were cut at 10-12 µm and 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 flame. Treated anthers were macerated with Crystal violet and mounted in glycerine. Drawings were made using Cameral Lucida and Meopta microscope. Photomicrographs were taken by using Olympus-CH20I microscope with built in analaogue camera (CM OE 1.4 megapixel) computer images were captured using Av-digitaliser having grand VCD-2000 captured guard.

## OBSERVATIONS

A transection of a young anther shows two distinct lobes each of which lodges two developing microsporangia. Each microsporangium consists of a group of densely protoplasmic cells, the archesporium

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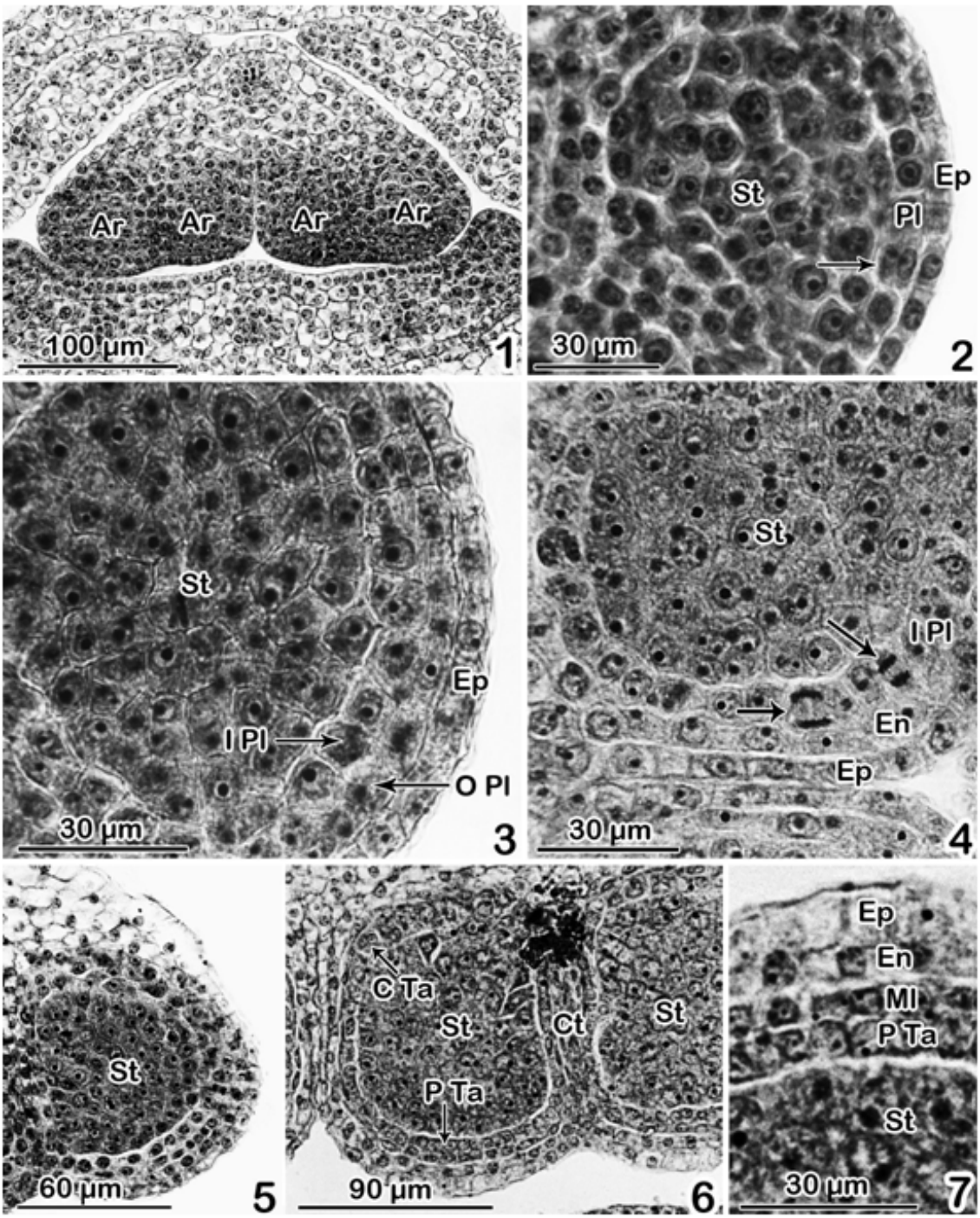


**Plate 1. (Figures 1-7)** ***Malaxis versicolor***

**1 – 7** T.S. of anther; note 4 groups of massive archesporium; Young microsporangium to show primary parietal layer and sporogenous tissue; Portion of microsporangium showing outer primary parietal and inner parietal layers; Portion of microsporangium showing division of cells in inner parietal layer (indicated by the arrow); **6, 7.** Microsporangium showing 4 wall layers; note the organisation of connective and parietal tapetum. Abbreviations: Ar. Archesporium; C Ta. Connective tapetum; Ct. Connective tissue / separating layer; En. Endothecium; Ep. Epidermis; I Pl. Inner parietal layer; Ml. Middle layer; O Pl. Outer parietal layer; P Ta. Parietal tapetum; Pl. Primary parietal layer; St. Sporogenous tissue.

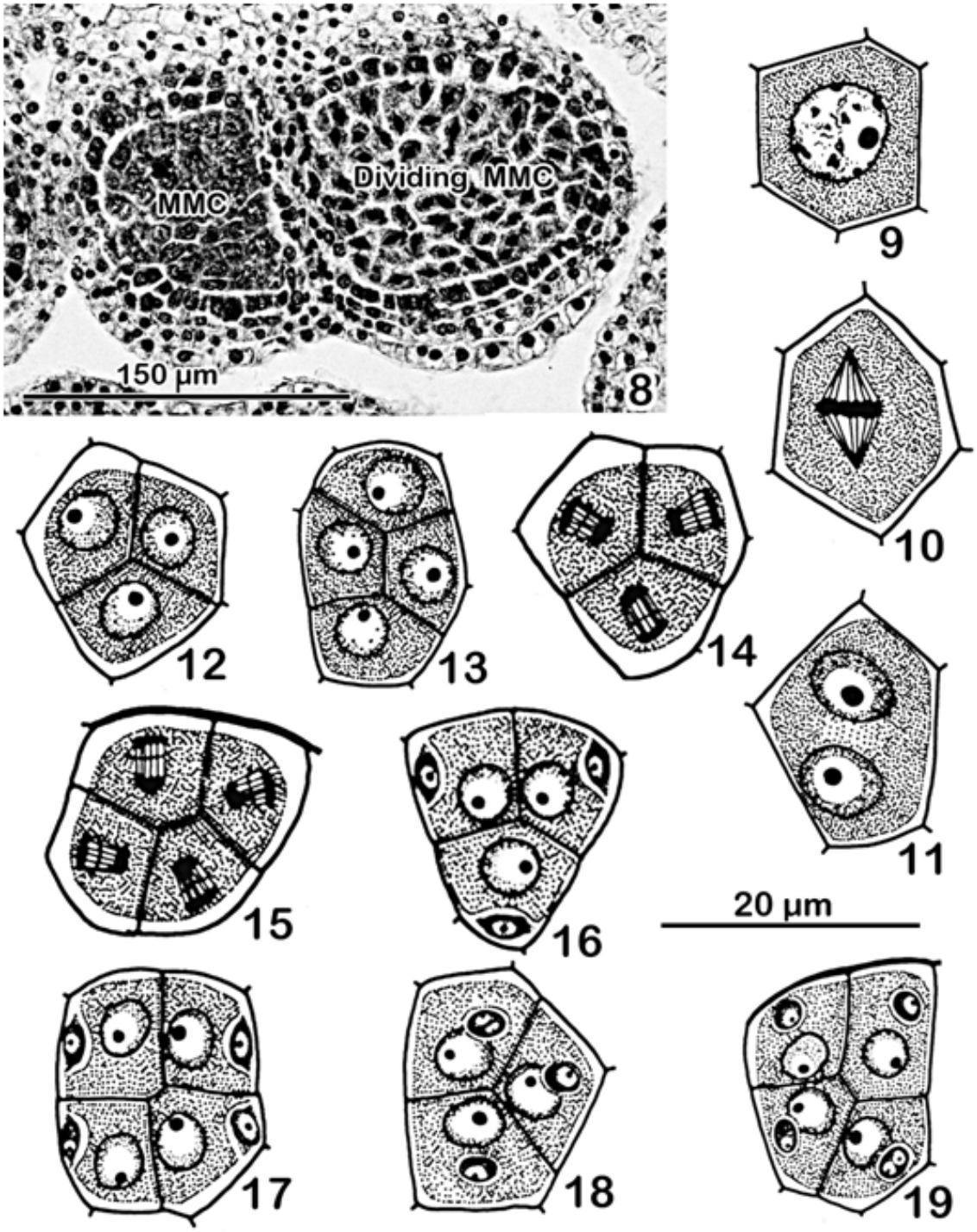


**Plate 2. (Figures 8-19)** ***Malaxis versicolor***

**8.** Anther lobe; note one microsporangium with micro spore mother cells; the other showing meiosis-I in microspore mother cells; **9. 10, 11.** Shows meiosis-I in microspore mother cells; **12.** Tetrahedral microspore tetrad;**13.** Rhomboidal microspore tetrad; **14,15.** Nuclear divisions in microspores of tetrads and note orientation of spindles; **16, 17.** Young 2-celled pollen grains in tetrads; **18, 19.** Mature pollen grains in tetrads. Abbreviations: MMC. Microspore mother cell.

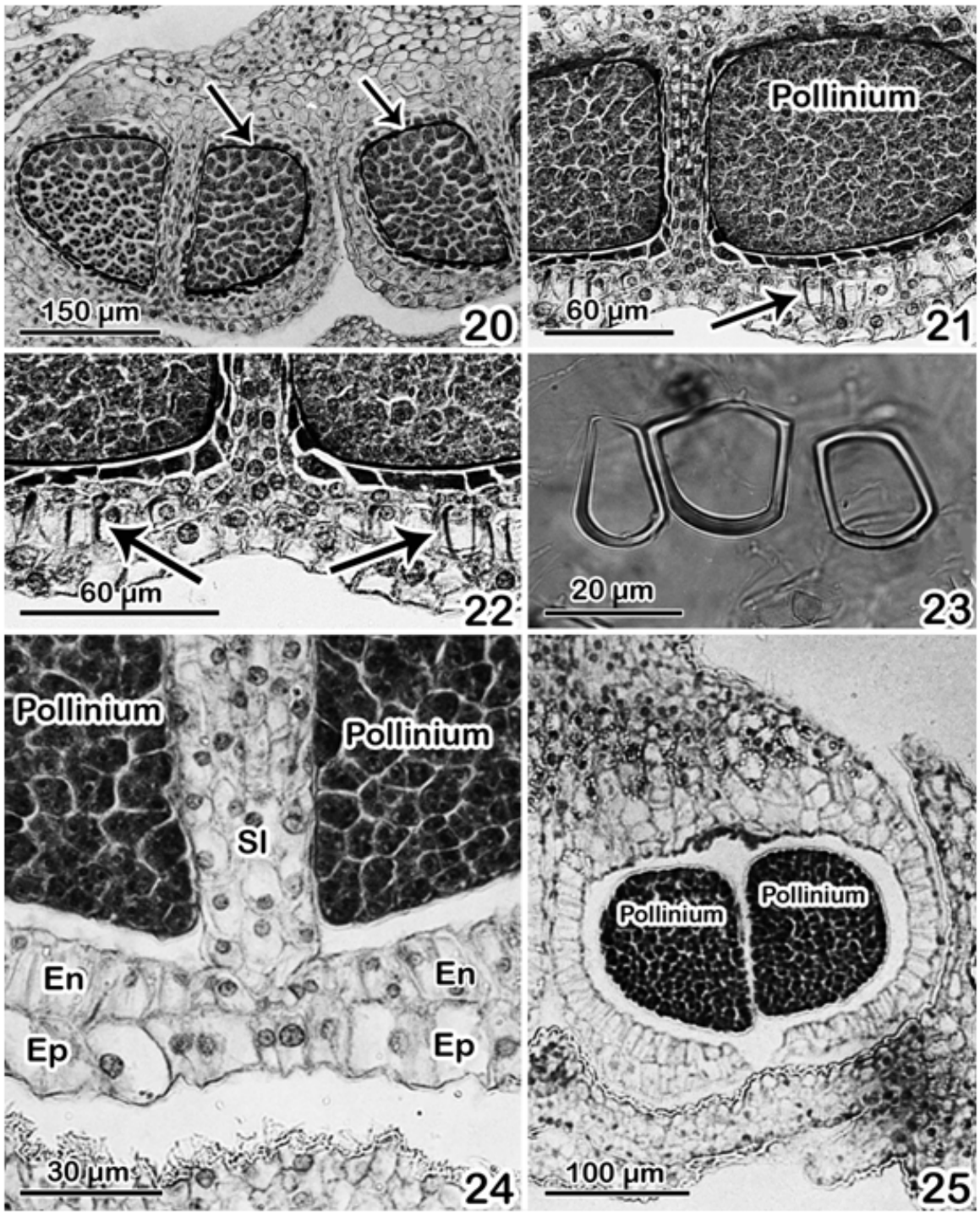


**Plate 3. (Figures 20-25)** ***Malaxis versicolor***

**20.** Anther lobes; note all the tetrads of a sporangium stay intact; note sporopollenin wall is layed around the pollimium; **21, 22.** Endothecial cells with one or two radially disposed thickenings (indicated by the arrow); **23.** Whole mount of endothecial thickenings; **24.** Part of anther lobe after the disintegration of the tapetum and middle layer; note the connective / separating layer between the two pollinia; **25.** Part of anther lobe; note the complete disorganisation of the separation layer and formation of a common opening in the anther wall between the sporangia of an anther half. Abbreviations: Sl. Separation layer; En. Endothecium ; Ep. Epidermis.

(Fig.1). It is protected on one side by the epidermis and on the other by connective tissue. The cells of the hypodermal layer act as primary parietal layer while the rest of the mass in the sporangium functions as the sporogenous tissue (Fig. 2). The primary parietal layer divides periclinally and gives rise to two layers of cells namely outer parietal layer and inner parietal layer (Fig.3). The outer differentiates into endothecium and inner divides periclinally to form glandular parietal tapetum and the middle layer (Fig.4). The ontogeny of microsporangium wall, therefore, is of the monocotyledonous type (Davis 1966). Meanwhile the cells of the connective adjoining the sporogenous cells acquire dense protoplasm and function as the connective tapetum. It remains in alignment with the layer on the wall ward side. The tapetum that surrounds sporogenous tissue, therefore, is of dual origin (Figs. 5, 6, 7). As the changes occur in the wall of the developing microsporangium, sporogenous tissue differentiates into microspore mother cells. Then they undergo meiotic divisions. Meiosis-I is not followed by wall formation (Figs. 8 – 11). After meiosis-II simultaneous quadripartition of the mother cell occurs resulting in tetrahedral and rhomboidal microspore tetrads (Figs. 12, 13). Separation of microspores from the tetrads does not occur and all the tetrads of a sporangium remain intact to develop into a single pollinium. The tetrahedral and rhomboidal tetrads are observed throughout the pollinium.

The microspore nuclei of a tetrad divide synchronously. The spindles of the dividing nuclei are disposed in a proximal and distal direction within the tetrad (Figs. 14, 15). Of the two resulting cells the smaller generative cell occupied the distal end near the sporecoat while the large vegetative / tube cell is located at the proximal side (Figs. 16, 17). Generative cell soon separates itself from the sporecoat end enters into cytoplasm of the vegetative cell (Figs. 18, 19). All the tetrads of a sporangium stay intact as a common sporopollenin wall is laid around the mass organising a pollinium. (Figs. 20, 21).

During the course of pollen development, nourishment is drawn from the tapetum. As a consequence the tapetal cells are deplete of their content and ultimately the cells breakdown along with middle layer. In the mature microsporangium the wall consists of epidermis and endothecium. Endothecial cells are larger in size and radially extended. Each of them acquires one or two ring like radially disposed thickenings on the inner walls (Figs. 22, 23).

During this period of development of pollinium in each of the adjacent microsporangia of an anther lobe, the separation layer / connective layer of cells disintegrates and get absorbed leaving the two pollinia in each anther half. An opening in the anther wall between the two sporangia facilitates the exit of both the pollinia (Figs. 24, 25).

**DISCUSSION**

The development of anther wall corresponds to the monocotyledonous type (Davis 1966). A similar mode of wall development has been reported in *Malaxis saprophyta* (Sood 1992). The anther wall comprises of epidermis, endothecium, middle layer and tapetum. Similar feature has been reported in most of the investigated orchid taxa (Mohana Rao and Sood 1987, Krishna Swamy *et al*. 2003, Gurudeva 2012). The epidermis is single layered. The endothecium is also single layered. At maturity the cells acquire one or two radially disposed thickening, similar type of endothecial thickenings has been reported in *Aa achalensis* (Cocucci 1964) and *Malaxis mucifera* (Ravi Kant and Hossain 2010). However 'U' or 'V' shaped thickenings on the inner tangential and radial walls of *Malaxis saprophyta* was reported by Sood(1992). Different types of endothecial thickenings in orchids has been recorded (Untawale and Bhasin 1973) and classified by Freudenstein (1991). Swamy (1949a) opined that endothecial thickenings are more pronounced

in epiphytic species compared to the terrestrial forms. On the other hand, previous study (Gurudeva 2012) and the present study indicates that the thickenings are well developed in pollinia bearing taxa compared to the massulae producing species. Further work in this regard will through more light on this conclusion. Middle layer is single layered protective in function and degenerates along with the tapetum when the pollinia attain maturity. Tapetum is single layered, glandular and dual in origin and it is in conformity with earlier records (Ravi Kant and Bhanwra 2010, Ravi Kant and Hossain 2010, Gurudeva 2012).

The archesporial cells after producing parietal layer functions as sporogenous tissue. The entire mass of archesporium produce a massive sporogenous tissue which later contributes to the formation of pollinium. Similar feature has been reported by Swamy (1949a), Bhanwra *et al.* (2006) and Ravi Kant *et al.* (2013). Sporogenous cells enlarge and become microspore mother cells. They undergo usual meiotic divisions and results in tetrahedral and rhomboidal tetrads. The nuclear division within the microspore tetrad is synchronous and asymmetrical, in conformity with earlier records(Hagerup 1938, Swamy 1949a, Prakash and Aow 1973, Mohana Rao and Sood 1986).

At the time of anther dehiscence, wall cells at the junction of the two adjoining microsporangia disorganise leading to the formation of vertical slit in each of the anther lobes which facilitates pollinators to carry the pollinia.

The pollinium of *Malaxis vesicolor* (present study) and *Malaxis nucifera* (Ravi Kant and Hossain 2010) do not possess carrier mechanism 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, Ravi Kant and Bhanwra 2010)

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