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DEVELOPMENT OF POLLINIUM IN *MALAXIS MUSCIFERA* (LINDL.) KUNTZE

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Abstract

Anther primordium of *Malaxis muscifera* (Lindl.) Kuntze initiated as a homogenous mass of meristematic cells is surrounded by protoderm. Two thecae are oriented towards the labellum, each of them consisted of a mass of archesporial cells. The hypodermal layer of sporogenous cells divide periclinally to form an endothecial, a middle layer and a tapetum. Pollen mother cells formed different types of microspore tetrads. Each microspore divides mitotically resulting a generative and a vegetative cell. Degeneration of the sterile septum resulted four naked and clavate pollinia.

Introduction

Orchidaceae is the largest and most evolved family of the flowering plants comprising 25,000 to 35,000 species under 750 to 850 genera (Dressler 1993, Singh 2001, Chowdhery 2001, Lucksom 2007). Orchids have highly specialized pollination system.

The anther is the source of several of the principal characters traditionally used for classification in Orchidaceae, especially in Epidendroideae comprising 80% of the family (Freudenstein *et al*. 2002). The anther features like, morphology of pollinia, whether they are collateral or superposed within the anther; structure of pollinia and the associated caudicle, stipe and viscidium play a significant role in the classification of this family (Lindley 1840).

Malaxis muscifera (Lindl.) Kuntze, a terrestrial orchid is 3 - 25 cm long (Fig. 1A) with ovoid pseudobulb used in the indigenous system of medicine to cure various physical disorders. It is sporadically distributed in some parts of India (Himachal Pradesh and Arunachal Pradesh), Bhutan, China, and Thailand (Deva and Naithani 1986). The natural population of this species is depleting at an alarming rate due to its over exploitation in traditional medicine, destroying pollinators and habitats. Recently it has been reported as endangered species in India (Chauhan *et al*. 2008). Therefore, it is high time to conserve and multiply them to meet up future demand and uphold the ecological harmony. Study of pollinium development would help in systematics and conservation of this species. Although pollinum development in some Spiranthoid, Orchidoid and Epidendroid taxa have been studied (Freudenstein *et al.* 2002, Bhanwra *et al*. 2006a), detailed information regarding the development of anther and differentiation of pollinia is lacking in *Malaxis muscifera*. Considering the importance of anther characters, the present studies were undertaken with a view to adding new information regarding the development and variation in number and structure of pollinia in Orchidaceae.

Materials and Methods

Flower buds and open flowers were collected at different stages of development, during July - August, 2006 from Fagu near Shimla (H. P., India), from an altitude of 2400 - 2480 m. Samples were fixed in 1 ml formalin, 1 ml acetic acid and 18 ml 50% ethanol for 48 hours and subsequently transferred to 70% ethanol for storage. These were dehydrated in ethyl-alcohol-tertiary-butyl-alcohol series (Johansen 1940) and then infiltrated with paraffin wax at 58 - 60°C.

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Thereafter, blocks were made in paraffin wax. Serial sections were cut between 7 and 10 µm with a steel knife on Spenser 820 rotary microtome (American Optical Company, U.S.A.). The sections were stained with safranin-fast-green combination. Photographs were taken by 'Olympus' photomicroscope model 'BH-2'. Voucher specimens of the present taxon have been deposited in the Herbarium of Department of Botany, Panjab University, Chandigarh, India.

Results and Discussion

Anthers in *M. muscifera* showed secondarily erect position and during the early development the anther primordium initiated as a homogenous mass of meristematic cells surrounded by protoderm. It develops two thecae oriented towards the labellum, each with a group of archesporial cells of uniform size and dense cytoplasmic contents. These cells undergo mitotic divisions and formed sporogenous cells (Fig. 1B, C). A similar mode of early anther development has been reported in *Habenaria tridactylites* Lindl., *Ludisia discolor* (Ker-Gawl.) Blume and *Eria javanica* (Swa.) Blume (Freudenstein and Rasmussen 1996), *Cymbidium aloifolium* (L.) Sw. and *Smitinandia micrantha* (Lindl.) Holttum (Bhanwra *et al*. 2006a) and *Epipactis veratrifolia* Boiss. & Hohen. (Bhanwra *et al*. 2006b). Anther position on the column has been considered as an important taxonomic feature (Freudenstein *et al*. 2002). In the subfamilies Cypripedioideae, Spiranthoideae and most Orchidoideae, anther is erect and parallel to the axis of the column. In the subfamily Epidendroideae, the advanced members show much variation regarding the position of anther where anther becomes incumbent (Dressler 1993). The hypodermal layer of sporogenous cells acts as primary parietal layer and undergoes periclinal divisions to form an outer secondary parietal layer and an inner secondary parietal layer (Fig. 1D, E). In the family Orchidaceae, the information about differentiation of anther wall is meager. In *M. muscifera*, the hypodermal layer only forms the anther wall, whereas, pollen grains are formed from the sporogenous cells present inner to the hypodermal layer. Due to more growth in the connective region and upper surface of anther, the two thecae come to lie side by side (Fig. 1F). A septum of sterile cells is formed in the sporogenous mass which is oriented in a plane at right angles to the theca (Fig. 1G). The cells of the outer secondary parietal layer differentiate into endothecium, while the cells of inner secondary parietal layer undergo a periclinal division to form a middle layer towards outer side and tapetum towards inner side (Fig. 2A). This is designated as 'outer tapetum'. The sterile septum grows further and becomes four-celled wide at the sporogenous tissue stage (Fig. 2B). The outermost cells of the sterile septum differentiate into tapetum-like cells, designated as 'inner tapetum'. Inner tapetum gradually becomes continuous with the outer tapetum.

The sporogenous cells differentiate into pollen mother cells. The fully formed anther wall consists of an epidermis, an endothecium, a middle layer and tapetum (Fig. 2C). At this stage, when considered along other features, the organization of anther wall appears to be a useful feature in the taxonomy of the family. The most primitive condition is anther wall with two-middle layers as reported in the Cypripedioids orchids, e.g. *Cypripedium cordigerum* D. Don (Sood and Rao 1988). During the evolution, number of middle layers reduced from two to only one in the Spiranthoid and Orchidoid taxa, e.g. *Goodyera repens* (L.) R. Br. (Sharma and Vij 1984) and *Habenaria densa* Wall. *ex* Lindl. (Rao and Sood 1979). The Epidendroid orchids show variations regarding the number of middle layers. Primitive Epidendroids have retained the ancestral condition of two-middle layers as in *Epipactis latifolia* (L.) All. (Sood 1997). Some taxa exhibit an increase in the number of middle layers (up to three) e. g. *Pachystoma senile* (Lindl.) Reichbf. (Gupta 2003). The number of middle layers has been even reduced to one as found in *Malaxis saprophyta* (King & Panting) T. Tang & F.T. Wang (Sood 1992, Bhanwra *et al*. 2006b).

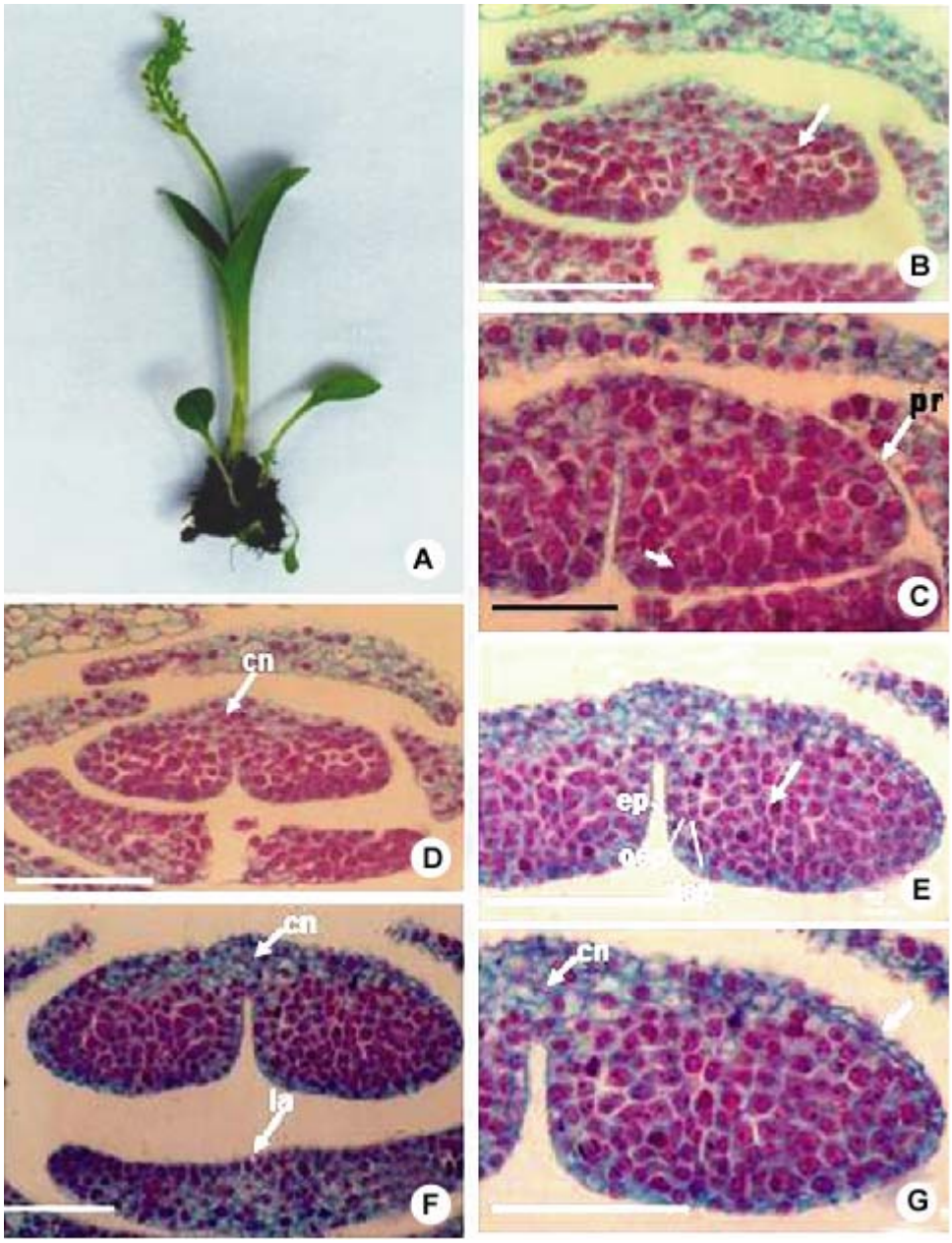

Fig. 1. Development of pollinium in *Malaxis muscifera:* A. A flowering plant stage, B. Young anther with two lateral thecae oriented towards the labellum (arrow indicated sporogenous cells), C. Young anther showing the developement of protoderm (pr), hypodermal cells (arrow indicated periclinal division in a hypodermal cell), D. Young anther at advanced stage of development, E. A part of a young anther showing anther theca with epidermis (ep), outer secondary parietal (osp) and inner secondary parietal (isp) layer and initiation of sterile septum in middle of sporogenous mass (arrow), F. Anther at advanced stage of development showing connective (cn) and labellum (la). G. A portion of a developing anther showing sterile septum and formation of anther wall. Scale bars: B, D, F = 100 µm, C, E, G = 50 µm.

In *M. muscifera* the pollen mother cells were polygonal or hexagonal in outline and compactly arranged. Simultaneous cytokinesis followed meiosis in the pollen mother cells and formed mostly rhomboidal tetrads followed by tetrahedral, isobilateral, decussate, T-shaped and linear microspores (Fig. 2D). In most of the orchids studied so far, cytokinesis is of simultaneous type and forms all type of tetrads, however, their percentage may vary in different

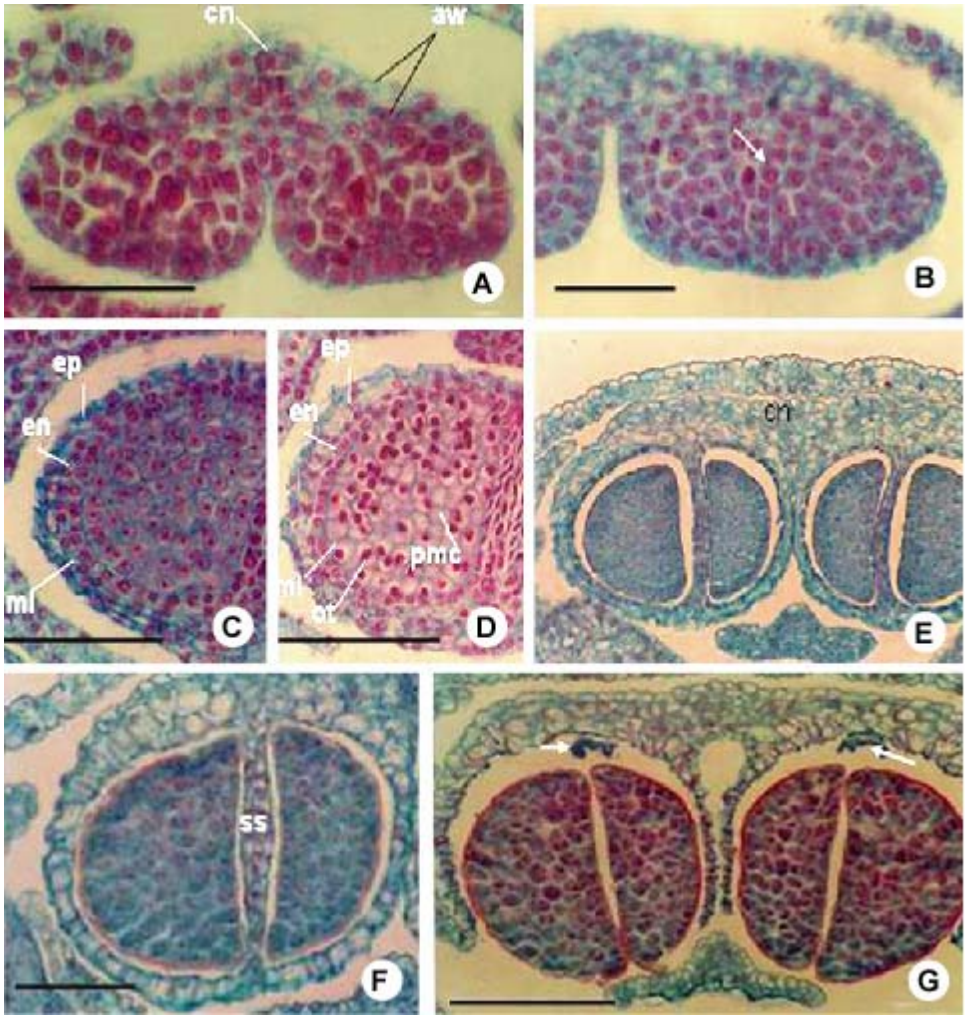

Fig. 2. Transverse sections of anther showing development of pollinium in *Malaxis muscifera:* A-B. Developing anther showing anther wall (aw), connective tissue (cn), sporogenous cells and sterile septum (arrows), C-D. Parts of anthers showing epidermis (ep), endothecium (en), middle layer (ml), tapetum (ot) and pollen mother cells (pmc), E. TS of anther after formation of microspore tetrads, F. Anther section showing epidermis, endothecium with fibrous thickenings, sterile septum (ss) and two pollinia, G. Anther at dehiscence stage showing four pollinia and remains of sterile septum (arrows). Scale bars: A, C, D, E and G =100 µm; B and F = 50 µm.

taxa. However, there are few exceptions regarding the type of cytokinesis. A successive type of cytokinesis has been reported in *Paphiopedilum*, *Selenipedium* and *Cypripedium* (Swamy 1949), and *Epipogium roseum* (Don) Lindl. (Arekal and Karanth 1981). The microspores do not separate out of the tetrad arrangement (Fig. 2E). The nucleus of each microspore is centrally located

which shifted to a peripheral region at the time of mitotic division to form a smaller, lenticular, generative cell and a larger spherical vegetative cell. The generative cell gradually detached itself from the parent wall and moved into the cytoplasm of the vegetative cell. The pollen grains were shed at the two-celled stage. A similar type of pollen grain development was observed in other orchids studied so far (Bhanwra and Vij 2003, Bhanwra *et al*. 2006a, b).

The epidermal cells of *M. muscifera* became compressed during the development of male gametophyte, and persisted as such in the mature anther. In majority of the taxa, the epidermis is persistent, while in some members of the family, the epidermal cells show hypertrophy and possess dense cytoplasmic contents (Rao and Sood 1979, Bhanwra and Vij 2003). The endothecial cells developed ring-shaped fibrous thickenings during the development of male gametophyte. Similar thickenings are also present in the two layers of the connective facing the locule. In other members of the family, endothecial layer develop fibrous thickenings, but their type may be different (Dressler 1993). The middle layer and tapetum degenerated during the development of male gametophyte (Fig. 2F). Middle layer is also ephemeral in the subfamilies Cypripedioideae, Spiranthoideae and Orchidoideae. The members of the subfamily Epidendroideae show much variation regarding the fate of middle layer/s. In taxa having a single middle layer, it is ephemeral, while in those genera having two - three middle layers, all the layers developed fibrous thickenings similar to those of endothecium (Bhanwra *et al*. 2006b).

Complete degeneration of sterile septum at the maturity of anther resulted into four pollinia per anther in *Malaxis muscifera*. Orchids are able to produce a variety of number of pollinia which depends on presence/absence or degeneration of septum. If septum is absent, two pollinia are formed as reported in *Stelis* (Dressler 1993) while, in presence of partial septum, two lobed or hollow pollinia develop as reported in *Cymbidium aloifolium* (Bhanwra *et al*. 2006a). When the septum completely degenerates, four pollinia are formed, which is possibly the most common feature in this family. However, eight pollinia can also form either by exclusively longitudinal septation as in *Calanthe rubens* Ridl. or a combination of longitudinal and transverse septation as in *Thelasis pygmaea* (Griff.) Blume (Freudenstein and Rasmussen 1996). Anther dehiscence is introrse and the four pollinia lie almost naked in the anther (Fig. 2G). Anther dehiscence is also introrse in Cypripedioids, Spiranthoids and Orchidoids, while in the Epidendroids, it may be introrse or latrorse (Freudenstein *et al*. 2002).

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