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Development of pollinium and associated changes in anther of *Calanthe tricarinata* Lindl., an epidendroid orchid

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ABSTRACT: In *Calanthe tricarinata* Lindl., anther primordium develops two thecae each with a mass of densely cytoplasmic archesporial cells surrounded by the protoderm. A six-layered anther wall develops from the archesporial cells of hypodermal layer. Cells in the tapetum are binucleate, only reported in a few primitive orchids. Formation of two septa in each theca results into eight microsporangia which is a primitive character. Pollen mother cells undergo simultaneous cytokinesis and form tetrahedral, rhomboidal, decussate, T-shaped, square and linear type of microspore tetrads. Pollen grains unite to form eight pollinia, which is a primitive feature in the family Orchidaceae. Pollinia unite to the viscidium through long caudicles to form a structure called the pollinarium unit. Pollen grains are monoaperturate with smooth exine. Present observations contribute significantly in the taxonomy and understanding character evolution in the family Orchidaceae.

KEY WORDS: Anther wall, Binucleate tapetum, *Calanthe tricarinata*, Caudicle, Orchid, Pollinium, Septum.

INTRODUCTION

Orchids belong to the largest and highly evolved family of flowering plants called Orchidaceae. The family Orchidaceae have been divided into five subfamilies, i.e., Apostasioideae, Cypripedioideae, Vanilloideae, Orchidoideae and Epidendroideae (Chase *et al.*, 2003). Orchids constitute a peculiar group of plants among angiosperms for their floral diversity, presence of variously modified column and labellum, different types of pollen dispersal units, numerous, smallest and non endospermic seeds, and requirement of a symbiotic association with species specific fungal partner for seed germination in nature (Hossain *et al*., 2013). In the family Orchidaceae, anther hosts several important features which are frequently used in the traditional system of classification, cladistic analysis and to understand the character evolution at the various taxonomic levels. These features include variation in the number of anther wall layers (Arekal and Karanth, 1981; Sood and Rao, 1988; Bhanwra *et al*., 2006a; Kant and Hossain, 2010; Kant *et al*., 2013), pollen grains aggregation into tetrads, massulae, soft or hard pollinia (Dressler, 1993; Singer *et al*., 2008), types of massulae (Dressler, 1993), variation in the number of pollinia (Freudenstein and Rasmussen, 1999), pollen wall sculpturing etc. (Stenzel, 2000).

Literature reveals that orchids show variations regarding the origin and number of anther wall layers. In majority of the taxa, only two-three archesporial cells are differentiated in each anther locule. These cells are divided periclinally into primary parietal cell towards outer side and primary sporogenous cell towards inner side. The primary parietal cells undergo repeated periclinal and anticlinal divisions and form primary parietal layer (Sood, 1986, 1989; Bhanwra and Vij, 2003). In contrast to this, in some other taxa, all the cells present in an anther locule are differentiated into archesporial cells. In these taxa, the archesporial cells of hypodermal layer form the anther wall whereas the archesporial cells present inner to the hypodermal layer form the sporogenous cells by repeated mitotic divisions (Bhanwra *et al*., 2006a; Kant and Hossain, 2010; Kant *et al*., 2013). In most of the orchids, anther wall is four-layered (Sood, 1986). However, in some other orchids, anther wall is five-eight layered (Sood and Rao, 1988). Innermost middle layer develops fibrous thickenings similar to those of endothecium (Bhanwra *et al*., 2006a, b; Kant *et al*., 2013). Tapetum is mostly uninucleate, however, binucleate tapetum has also been reported in a few primitive taxa (Swamy, 1949; Sood, 1992; Bhanwra *et al*., 2006b). Number of pollinia varies from two, two-bipartite, four, six and eight in the family (Dressler, 1993; Stenzel, 2000). In the family Orchidaceae, the structure of exine is highly variable which is employed as an important taxonomic tool (Williams and Broome, 1976; Stenzel, 2000; Kocyan and Endress, 2001; Barone Lumaga *et al*., 2006; Singer *et al*., 2008). In case of primitive genera, exine surface of pollen grains exhibit more variations than the advanced one. These variations are even prominent at the species level and have taxonomic significance (Kapil and Arora, 1990).

Calanthe tricarinata Lindl., also called 'Monkey Orchid' belongs to the subtribe Bletiinae, tribe Arethuseae, subfamily Epidendroideae and family Orchidaceae (Dressler, 1993). In this taxon, plants are 30- 40 cm long and develop in groups (Fig. 1A). Flowers are very beautiful and 2.2- 2.5 cm across (Fig. 1B).

Flowers resemble a monkey and have a single fertile anther. This taxon is distributed in the temperate parts of Western Himalaya including India, Nepal and China at the elevations between 2000-3000 m (Deva and Naithani, 1986). *C. tricarinata* is used in the traditional system of medicine to cure jaundice, sores, eczema and as an aphrodisiac (Joshi *et al*., 2009; Sharma and Samant, 2017). Despite the significance of anther characters in the traditional system of classification, cladistic analysis and understanding the character evolution, information regarding early anther development, origin and differentiation of anther wall layers, microsporogenesis, microgametogenesis, development of pollinium and surface features of pollen grains is lacking in this important taxon. Occurrence of eight pollinia has been reported in this taxon. However, mode of their formaion is not known. Therefore, *C. tricarinata* Lindl. was selected for the present study to investigate these features in detail.

MATERIALS AND METHODS

Periodic field trips were made in July- August to the forests of Kufri, at mean sea level of 2720 m (31.10°N' 77.25°E') and Narkanda, at mean sea level of 2710 m (31°16' 77°27E'). Kufri and Narkanda are situated at a distance of 23 km and 60 km from Shimla, the capital city of Himachal Pradesh, India respectively. Flower buds and open flowers at different stages of development were collected and fixed in a solution of formalin-acetic acid-ethanol in 1:1:18 ratio (5 ml formalin: 5 ml acetic acid: 90 ml 50% ethanol) for 24–48 hours and subsequently transferred to 70% ethanol for the long term storage and further use. These floral buds and open flowers were dehydrated in ethanol-tertiary-butyl alcohol series followed by their infiltration with paraffin wax at 60°C. Subsequently blocks were made in the paraffin wax and serial sections were cut with a Spencer 820 Rotary Microtome (American Optical Company, U.S.A.) at a thickness of 5-7 µm. Serial sections were stained with safranin-fast-green series. Permanent slides were prepared through mounting the sections with DPX (a mixture of distyrene, a plastilizer and xylene; HiMedia Mumbai, India) (Johansen, 1940; Berlyn and Miksche, 1976). Slides were observed and micrographs were taken on 'Olympus' photomicroscope model BH-2. For Scanning Electron Microscope (SEM) observations, anthers with anther wall removed and pollinaria units were fixed in 2% glutaraldehyde, 2.5% p-formaldehyde in 0.05 M phosphate buffer (pH 7). This material was then fixed in 1% osmium tetroxide and dehydrated in a graded series of acetone and finally subjected to critical point drying in a CPD 7501 critical point dryer. After drying, gold coating was done on the aluminium stub using double adhesive tape with JFC 1100 sputter (Postek *et al*., 1980). After gold coating, anthers and pollen grains were observed and photographed under a JEOL JSM-600 Scanning Electron Microscope using black and white photographic film on 'Olympus' photomicroscope model BH-2.

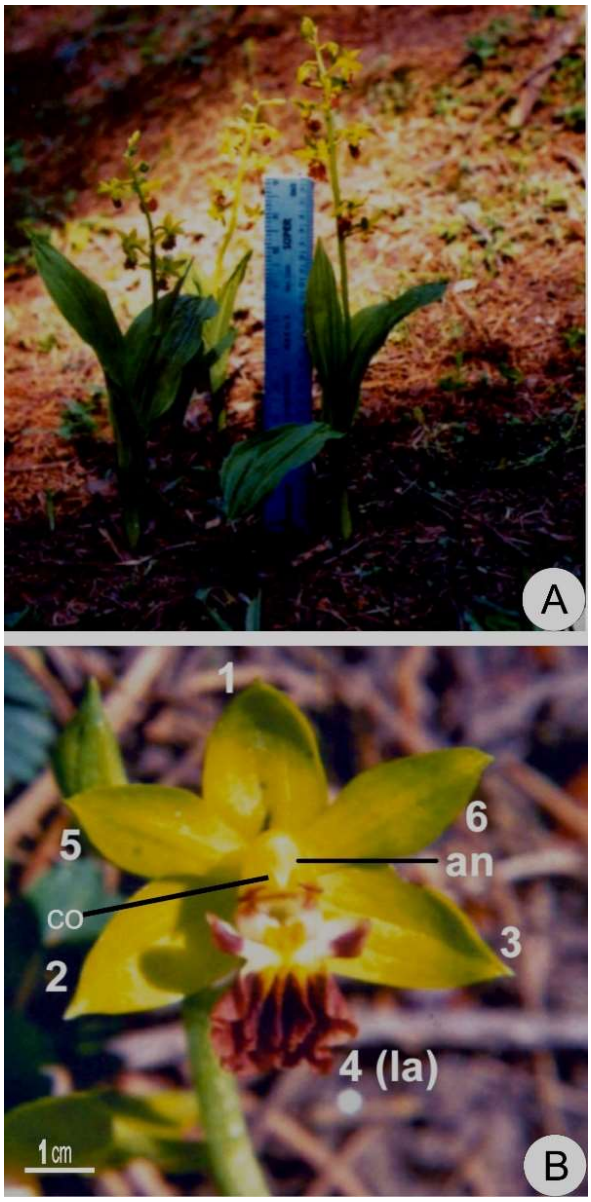

Fig.1. Plants growing in natural habitat and morphology of flower in ***Calanthe tricarinata***. **A.** Plants growing in natural habitat; **B**. A flower showing sepals (1, 2, 3) and petals (4, 5, 6) where la= labellum, an= anther cap, co= column.

RESULTS

Early anther development and differentiation of anther wall

Transverse sections of flower buds and open flowers were observed at different stages of development. It was observed that anther primordium was initiated as a squarish mass of meristematic cells surrounded by the

protoderm (Fig. 2A). In the next stage of development, anther primordium was developed into two lateral thecae oriented towards the labellum (Fig. 2B). Each theca developed a mass of densely cytoplasmic archesporial cells of uniform size as compared to the larger vacuolated cells of connective region. These archesporial cells were increased in number by the repeated mitotic divisions. Out of these cells, the archesporial cells of hypodermal layer were acted as the initials of anther wall layers. These cells were designated as primary parietal cells (ppcs). These cells became organised and formed the primary parietal layer. The cells of the primary parietal layer were divided periclinally into an outer secondary parietal (osp) layer and an inner secondary parietal (isp) layer; however, these divisions were asynchronous (Figs. 2C–D). Due to more growth in the connective region, both the thecae were bent towards the labellum and eventually came to lie side by side. The archesporial cells present inner to the hypodermal layer were divided repeatedly and differentiated into sporogenous cells. During repeated mitotic divisions in the sporogenous cells, a small groove was formed almost in the center of each theca facing the labellum. As development was progressed, two septa were started to develop in each theca, oriented longitudinally to each other (Fig. 2E).

The cells of outer secondary parietal (osp) layer were directly differentiated into endothecium, whereas cells of the inner secondary parietal (isp) layer were divided periclinally into an inner secondary parietal layer$_1$ (isp$_1$) and an inner secondary parietal layer$_2$ (isp$_2$). Out of these two layers, the cells of the inner secondary parietal layer$_1$ (isp$_1$) were divided periclinally and formed middle layer$_1$ towards outer side and middle layer$_2$ towards the inner side. The inner secondary parietal layer$_2$ (isp$_2$) also registered a periclinal division and formed two- layers. Out of these two layers, the outer one was acted as the middle layer$_3$ and the inner layer was differentiated into tapetum (Figs. 2F–H; Figs. 3A–C). This tapetum was designated as 'outer tapetum' (Fig. 3D).

At this stage, the septa were completely formed. Formation of two septa was resulted into four microsporangia in each theca and in turn eight microsporangia in an anther. The sporogenous cells were divided repeatedly through mitotic divisions and differentiated into pollen mother cells. The cells of the septa were increased in number and size. As development was progressed, the outermost layer of septa was differentiated into "inner tapetum". Outer and inner tapetum became continuous with each other. At this stage, the anther wall was fully formed, i.e., epidermis, endothecium, three middle layers and tapetum. Tapetal cells were observed very close to the microspore mother cells (Figs. 3E–F).

Microsporogenesis, microgametogenesis and associated changes in the anther wall

The pollen mother cells were hexagonal or polygonal in shape. The outermost pollen mother cells, i.e. cells close to the tapetum were arranged in a plane at right angle to the tapetal cells. As meiotic divisions were started in the pollen mother cells, the cells in the tapetum were divided mitotically. But, nuclear divisions in the tapetal cells were not followed by the wall formation and resulted into binucleate cells (Figs. 3G–H).

In the pollen mother cells, first meiotic division was resulted into two nuclei but there was no wall formation. Second meiotic division and simultaneous cytokinesis in the pollen mother cells was resulted into tetrahedral, rhomboidal, decussate, T-shaped, square and linear type of microspore tetrads. Majority of the microspore tetrads were tetrahedral in shape. Meiotic divisions were asynchronous in the pollen mother cells which were even lying very close to each other in the same microsporangium (Figs. 5 A–C). A schematic representation of the formation of anther wall and microspores has been presented in Fig. 4.

In each microsporangium, tetrads were not separated from each other. During the microgametogenesis, the microspore nucleus was shifted to the proximal position away from the center where it was divided mitotically into a smaller lens-shaped generative nucleus and a larger spherical vegetative nucleus. Mitotic division was followed by the cell wall formation and resulted into a generative and vegetative cell. Mitotic divisions were asynchronous among different tetrads in a sporangium. During the mitotic divisions, asynchrony was so much prominent that some microspores were at the anaphase stage of mitosis and others were at the resting stage (Fig. 5D). During the development of male gametophyte, middle layers and tapetum were degenerated completely. In the next stage of development, nucleus of the generative cell was migrated into the cytoplasm of vegetative cell. Vacuoles were absent in the cytoplasm of pollen grains. The generative nucleus was lightly stained, whereas, the vegetative nucleus was deeply stained (Fig. 5E). The epidermal cells were become compressed and stretched during the development of male gametophyte.

Development of pollinium

In a microsporangium, pollen grains were not separated from each other. As the development progressed, aggregation of pollen grains in a sporangium was resulted into a clavate pollinium in each microsporangium and in turn eight pollinia in an anther (Fig. 5F). Degeneration of septa was started from the stomium region in each theca and extended towards the connective of anther. The proximal region of each pollinium was narrow and consisted of loosely arranged pollen grains. As the development progressed, these

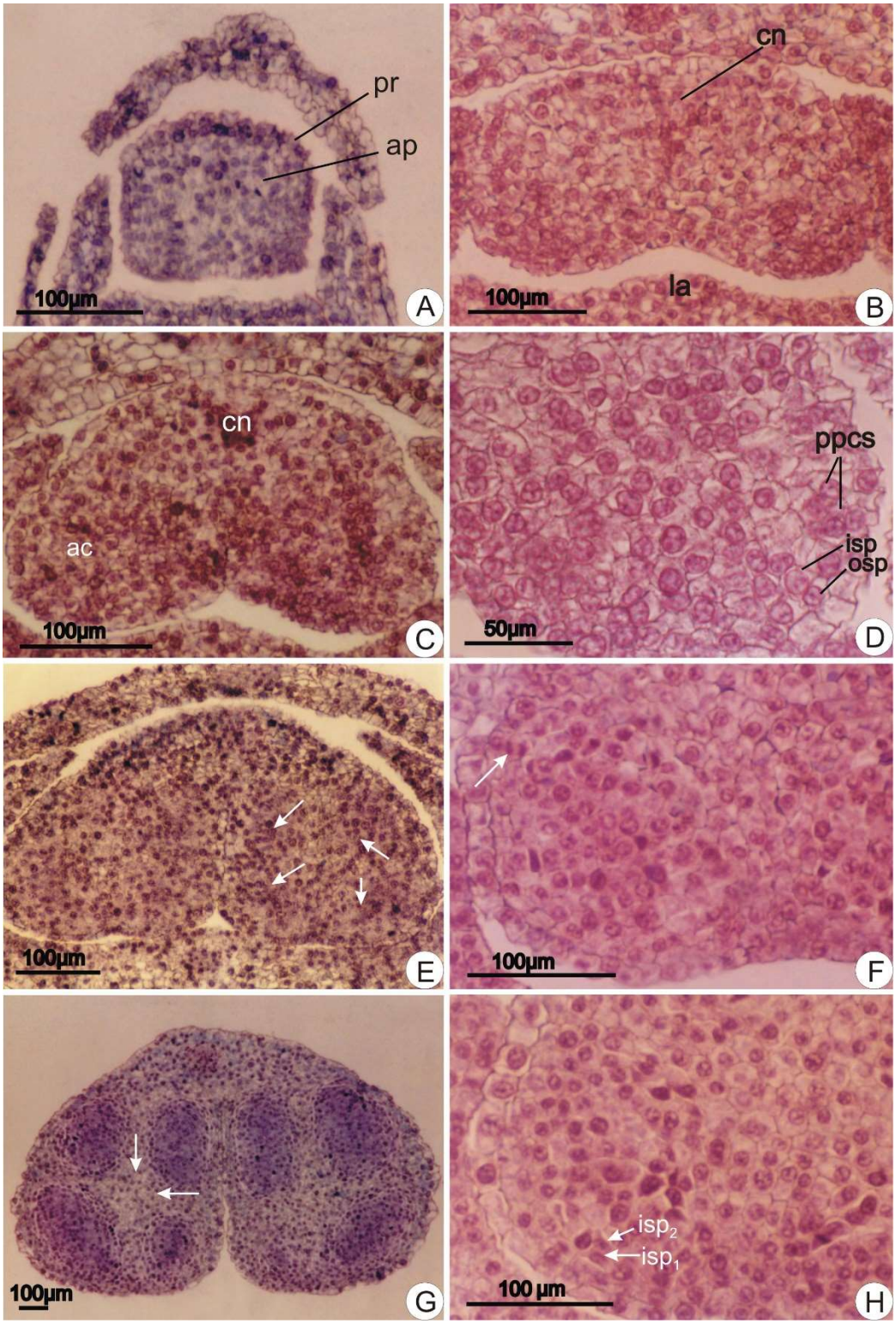

Fig. 2. Transverse sections (T.S) of anther showing anther development in ***C. tricarinata*. A.** Anther primordium (ap) surrounded by protoderm (pr); **B.** Anther primordium showing connective (cn), development of two lateral thecae oriented towards the labellum; **C.** Young anther at advanced stage of development showing archesporial cells (ac) and connective (cn); **D.** Magnified view of Fig. C showing primary parietal cells (ppcs), formation of outer secondary parietal (osp) layer and inner secondary parietal (isp) layer; **E.** Young anther showing four groups of sporogenous cells (arrows) in the right theca; **F**. Mitotic division in the inner secondary parietal layer (arrow); **G.** Two septa oriented longitudinally to each other (arrows); **H**. Formation of inner secondary parietal layer$_1$ (isp$_1$) and inner secondary parietal layer (isp$_2$).

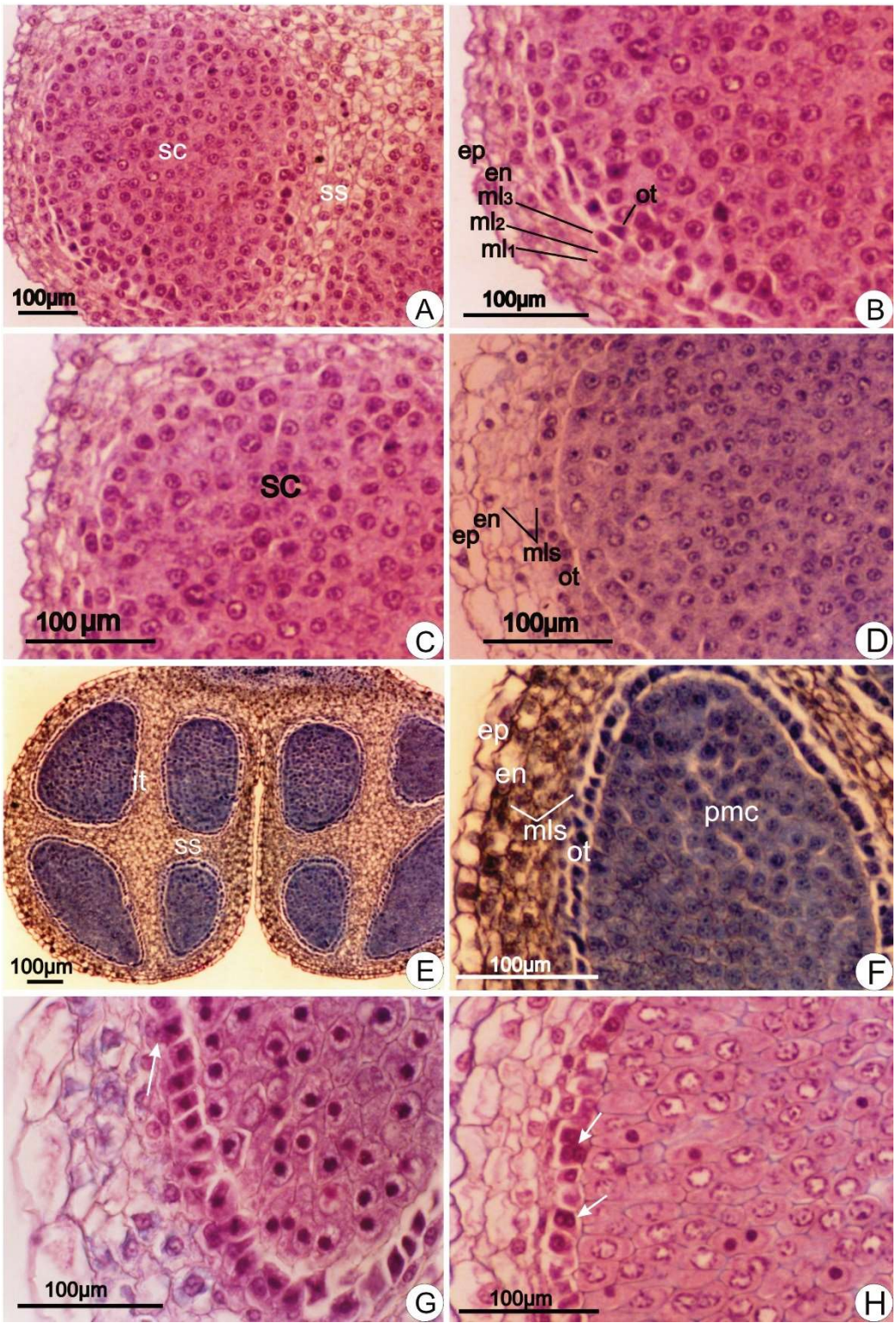

Fig. 3: T.S. showing anther development in ***C. tricarinata***. **A.** Microsporangium showing sporogenous cells (sc) and septum (ss); **B.** Differentiation of middle layers and outer tapetum (ep= epidermis, en= endothecium, ml_1, ml_2, ml_3 = middle layer 1, 2 and 3 respectively, ot= outer tapetum); **C**. Part of sporangium showing sporogenous cells; **D**. Part of anther showing anther wall (ep= epidermis, en= endothecium, mls= middle layers, ot= outer tapetum); **E.** Anther with eight sporangia (two sporangia only partially shown), septum (ss) and inner tapetum (it); **F.** Magnified view showing fully formed anther wall (mls = middle layers) and pollen mother cells (pmc);. **G.** Mitotic division in the tapetal cell (arrow), and fully formed anther wall; **H.** Binucleate tapetal cells (arrows).

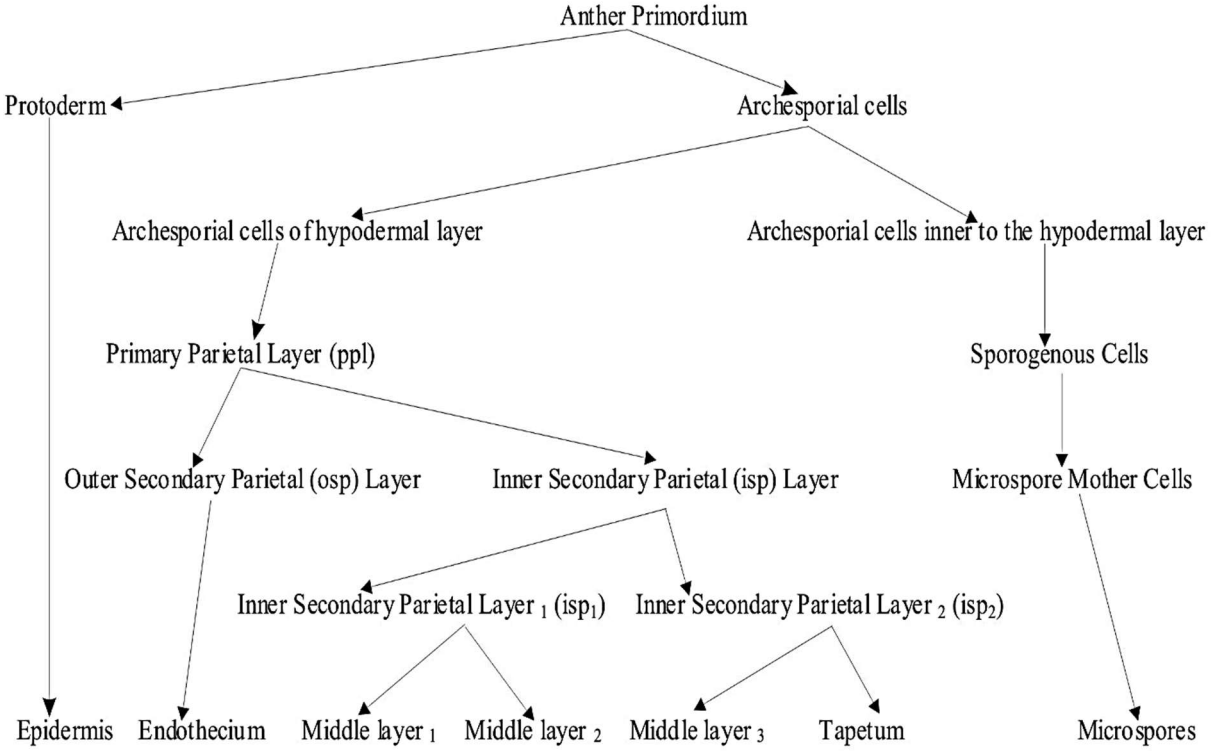

Fig. 4. Schematic representation of formation of anther wall and microspores in ***C. tricarinata.***

pollen grains were got united and formed a granular caudicle (Fig. 5G). Ring-shaped fibrous thickenings were visible in the degenerating endothecium (Fig. 5H). Only remains of the septa were persisted at the connective region in the mature anther (Fig. 5I).

Observations on pollen grains

Pollen grains were at two-celled stage at the time of anther dehiscence. On falling on the stigma, pollen grains were got germinated with single pollen tube came out of the center of a germinating pollen grain. Asynchrony was even observed during the germination of pollen grains (Figs. 5J–K). As shown by the SEM studies, anthers were triangular in shape. Each pollinium was connected to the viscidium through long granular caudicle (Figs. 6A–B). Exine of pollen grains was smooth with small pits and a large aperture in the center (Figs. 6C–D).

DISCUSSION

Early anther development and differentiation of anther wall

In *C. tricarinata*, anther primordium develops two lateral thecae with densely cytoplasmic archesporial cells of uniform size. Due to more growth in the connective region, two lateral thecae are developed. Such observations are also corroborated to other orchids from the subfamilies Orchidoideae and Epidendroideae (Swamy, 1949; Freudenstein and Rasmussen, 1996; Bhanwra and Vij, 2003; Vij *et al*., 2005; Bhanwra *et al*., 2006a, b; Kant and Bhanwra, 2010; Kant and Hossain, 2010; Kant and Goel, 2013; Kant *et al*., 2013; Gurudeva, 2015). In the present taxon, anther wall develops from the archesporial cells of hypodermal layer whereas sporogenous cells are developed from the archesporial cells present inner to the hypodermal layer as also reported in many orchids (Freudenstein and Rasmussen, 1996; Bhanwra *et al*., 2006a; Kant and Hossain, 2010; Kant *et al*., 2013; Gurudeva, 2015). Thus in the present taxon, archesporial cells of hypodermal layer and those present inner to the hypodermal layer have different fate and functions. However, in other orchids, both anther wall and sporogenous cells are the product of archesporial cells present in the hypodermal layer only (Sood, 1986, 1989; Bhanwra and Vij, 2003; Aybeke, 2012).

Similar to *C. tricarinata*, formation of eight microsporangia have been reported in a few taxa only in the subfamily Epidendroideae, e.g., members of the tribe Arethuseae, Coelogyneae, Epidendreae etc. (Dressler, 1993; Freudenstein and Rasmussen, 1996). In these tribes, two septa are formed in each theca and results into eight microsporangia that also supports the present investigation. Two septa are either formed "longitudinally" as in the present study and *Appendiculata hexandra* (J. Konig) J. J. Sm. or "perpendicular" to each other as in *Eria javanica* (Sw.) Blume (Freudenstein and Rasmussen, 1996). However,

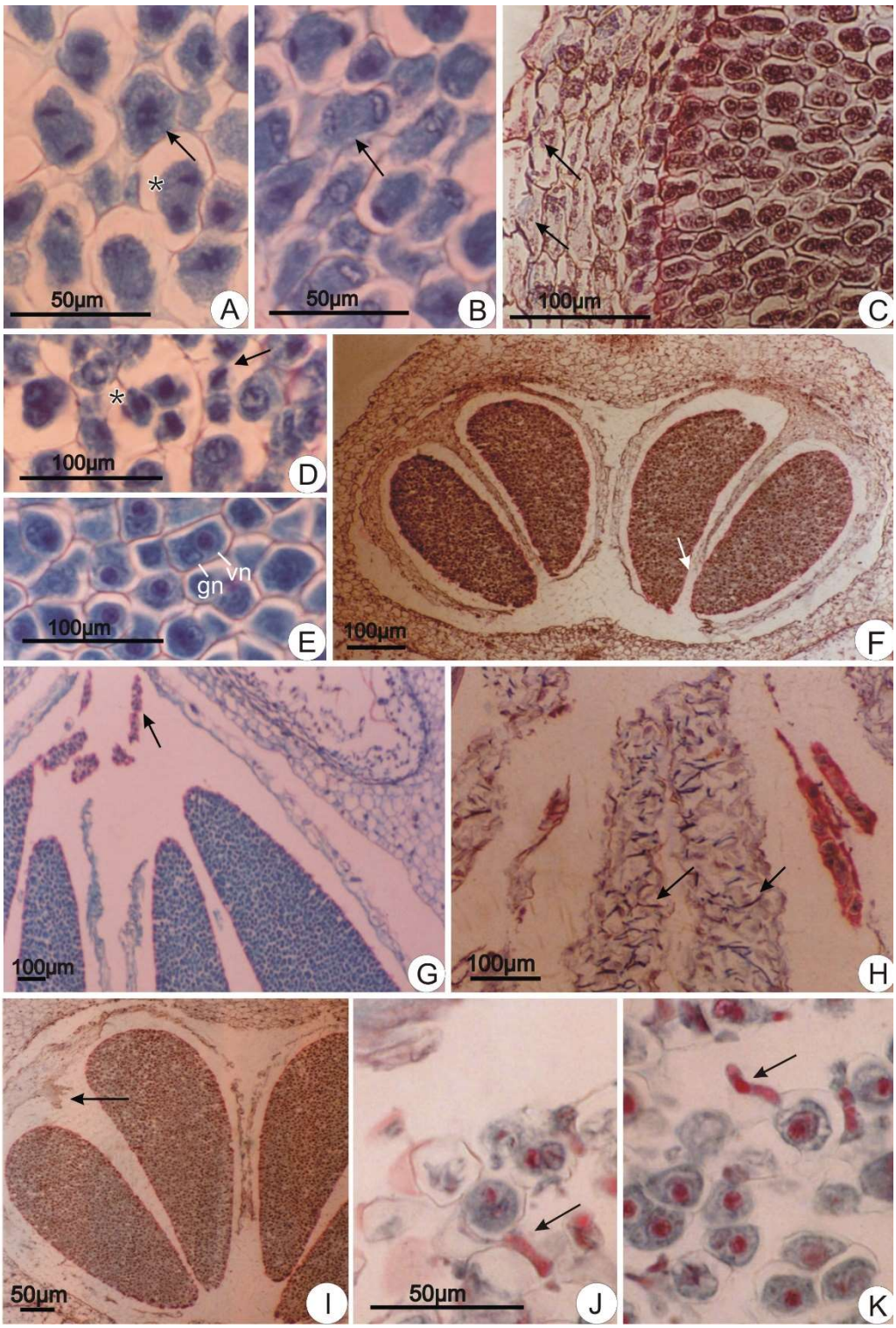

Fig. 5: Microsporogenesis, development of anther and pollen tube formation in ***C. tricarinata***. **A**. Pollen mother cells at late metaphase (arrow) and anaphase (asterisk) stage of meiosis-I (arrow); **B**. Pollen mother cells at telophase stage of meiosis-I (arrow); **C.** Anther wall at microspore tetrad stage with starch grains in endothecium and middle layers (arrows); **D.** Microspore tetrads at metaphase (arrow) and anaphase (asterisk) stage of mitosis; **E.** Pollen grain showing vegetative nucleus (vn) and generative nucleus (gn) lying close to each other; **F.** Anther showing partially degenerated septum (arrow); only four pollinia (po) are visible; **G**. Anther showing caudicle composed of pollen grains (arrow); **H**. Ring shaped fibrous thickenings (arrows); **I.** Anther showing completely degenerated septum (arrow); **J-K.** Germination of pollen grains and development of pollen tubes (arrows).

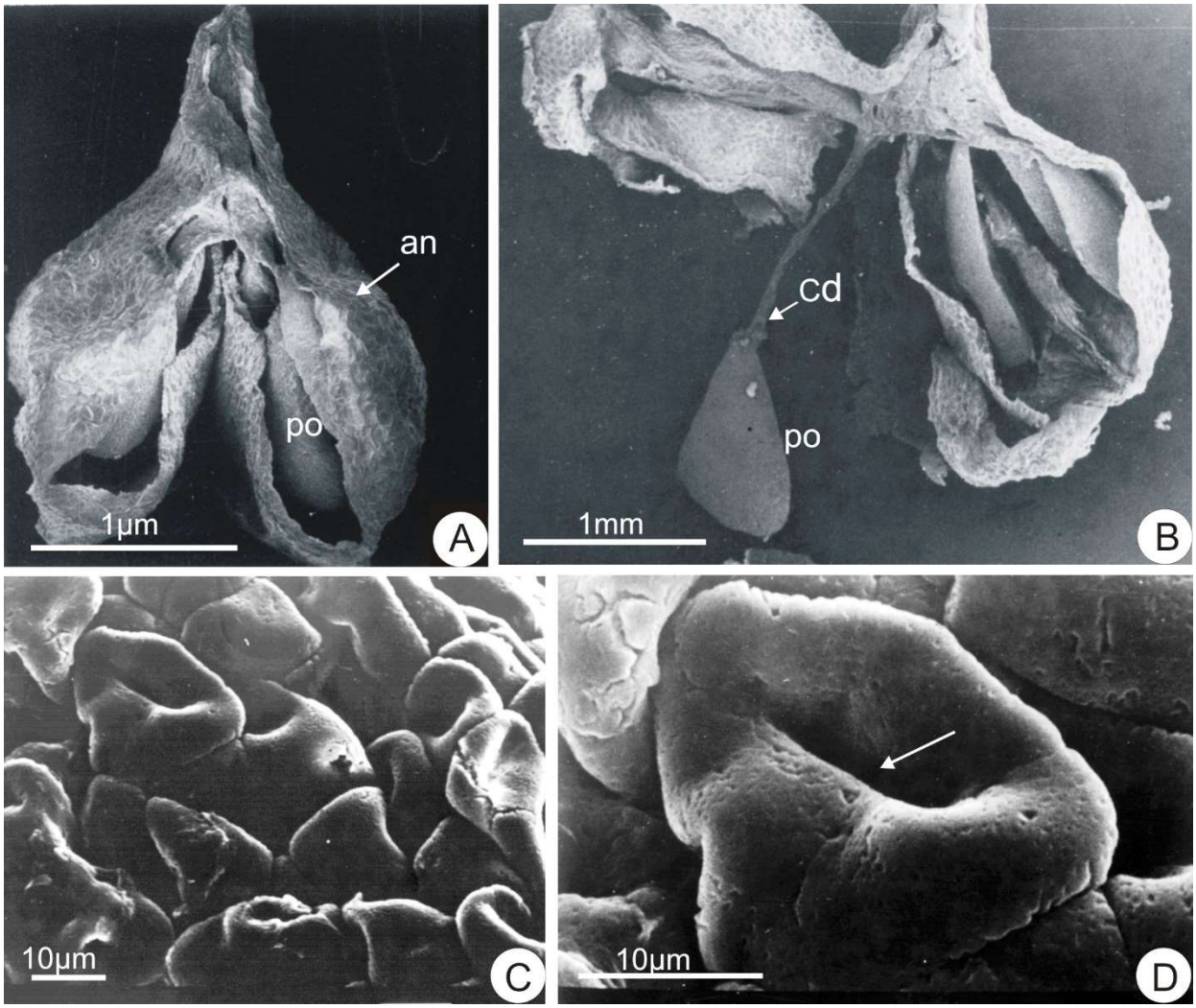

Fig.6. Showing SEM images of pollinium and pollen grains in ***C. tricarinata***. **A.** Dissected anther (an) with enclosed pollinia (po); **B.** Pollinium (po) showing attached caudicle (cd); **C.** Mature pollen grain tetrads; **D.** Pollen grain showing smooth exine, small pits and a single aperture (arrow) in the center.

in some other taxa including *Cymbidium aloifolium* (L.) Sw. (Bhanwra *et al*., 2006a) and *C. pendulum* (Roxb.) Sw. (Kant *et al*., 2013), anther remains bisporangiate due to the incomplete development of a mass of tissue in each theca which divides a theca partially. Unfortunately, most of the earlier studies were conducted at the pollen mother cell stage. At this stage, formation of septa is already complete; therefore, anther was suggested to be tetrasporangiate by most of the earlier orchid embryologists (Swamy, 1949; Sood, 1985, 1986, 1992, 1997; Vij *et al*., 1999). Actually anther is bisporangiate at the initial stages of development. It becomes tetrasporangiate due to the formation of a complete septum in each theca which is a common phenomenon in the family Orchidaceae (Dressler, 1993; Kant and Hossain, 2010; Kant *et al*., 2013; Gurudeva, 2015).

In the subfamily Apostasioideae, information is lacking regarding the development and number of anther wall layers. Occurrence of a single-layered tapetum has been observed in the present taxon. Mostly, tapetum is single-layered in the family (Sood, 1997; Gurudeva, 2015). However, two-three layered tapetum has also been reported in *Cypripedium cordigerum* D. Don (Sood and Rao, 1988; Kant, 2011) and a two– layered in *Zeuxine strateumatica* (Lindl.) Schltr. (Vij *et al*.,1982). Tapetum of dual origin, i.e. outer tapetum towards the anther wall and inner tapetum facing the septum, has been observed in the present taxon. Such studies are limited to a few taxa only in the family Orchidaceae, where tapetum of dual origin has been reported (Vij *et al*., 1982; Kant and Hossain, 2010; Kant, 2011; Kant *et al*., 2013; Gurudeva, 2015). In fact, earlier workers have given very little attention towards the dual origin of tapetum in the family. Similar to *C. tricarinata*, development of six-layered anther wall has only been reported in *Pachystoma senile* (Lindl.) Rchb. f. (Gupta,

2003), *Smitinandia micrantha* (Lindl.) Holtt. (Bhanwra *et al*., 2006a) and *Cymbidium pendulum* (Roxb) Sw. (Kant *et al*., 2013). In many orchids, anther wall is only four-layered (Sood, 1986). Anther wall is generally six-eight layered in the subfamily Cypripedioideae, four-five layered in Vanilloideae and Orchidoideae. Notable variations have been reported in the subfamily Epidendroideae where anther wall may be four, five or six-layered which is due to increase in the number of middle layers and tapetal layers (Sood and Rao, 1988; Sood, 1997; Kant *et al*., 2013).

Occurrence of binucleate tapetum in *C. tricarinata* is a new report for the tribe Arethuseae. Cells in the tapetum are mostly uninucleate in the family (Sood and Rao, 1988; Kant *et al.*, 2013) except some primitive taxa, viz., *Paphiopedilum druryi* (Bedd.) Pfitzer (Swamy, 1949), *Epipactis latifolia* (L.) (Sood, 1997), *Spathoglottis plicata* Blume (Prakash and Lee, 1973), *Cephalanthera ensifolia* (Sw.) Rich. (Sood, 1986) and *Epipactis veratrifolia* Boiss & Hohen. (Bhanwra *et al*., 2006b), where binucleate tapetal cells have been reported. Binucleate tapetal cells contribute more nutrients to the developing microspores and pollen grains as compared to the uninucleate tapetum (Pacini *et al*., 1985).

Microsporogenesis, microgametogenesis and associated changes in the anther wall

Similar to the present taxon, cytokinesis is mostly of the simultaneous type in the family Orchidaceae. Only in a few taxa, cytokinesis is of the successive type (Swamy, 1943; Aybeke, 2012). Occurenece of tapetal cells very close to the developing microspores in the present taxon and other angiospems has been considered an advanced feature (Pacini *et al*., 1985). During the microsporogenesis and microgametogenesis, asynchronous divisions in *C. tricarinata* are most likely due to the weak cytoplasmic connections between the pollen mother cells and microspores respectively, as also reported in other orchids (Heslop-Harrison, 1968).

Development of pollinium

At the maturity of anther, due to the degeneration of septa and aggregation of pollen grains, eight clavate pollinia are formed in *C. tricarinata*. In the family Orchidaceae, eight pollinia have only been reported in members of the tribe Arethuseae including present taxon, Coelogyneae, Epidendreae and subtribe Pleurothallidinae, all belong to the subfamily Epidendroideae (Dressler, 1993; Stenzel, 2000). The subtribe Pleurothallidinae is characterised by eight, six, four and even two pollinia. Occurrence of eight pollinia has been considered the most primitive feature in the family (Dressler, 1993; Stenzel, 2000). Formation of four pollinia is the predominant condition in the family. (Rasmussen, 1982; Freudenstein and Rasmussen, 1996).

As development progresses, pollen grains aggregate to form a structure called caudicle which connect the pollinium with the viscidium in the present taxon. A caudicle is either formed by the sticky material or sterile pollen grains, and has been reported in the subtribe Pleurothallidinae (*Dryadella* Luer, *Acianthera* Scheidw) and Laeliinae (*Cattleya* Lindl., *Pseudolaelia* Porto & Brade) (Singer *et al*., 2008). A caudicle represents a weak region in the pollinarium unit. During the pollination process, pollinarium unit get broken at the region of caudicle and in turn pollinium gets attached on the stigma of another flower visited by the pollinator (Dressler, 1993). The work of Singer *et al*. (2008) is worth mentioning here. These workers have studied the structure of pollinia and pollinarium unit in many taxa, and significance of these structures in the taxonomy. According to Singer *et al*., (2008), true pollinia are only present in the advanced subfamilies, i.e., Orchidoideae and Epidendroideae where pollen grains are shed in the form of a compact mass. Whereas, true pollinia are lacking in the subfamilies Apostasioideae, Vanilloideae and Cypripedioideae. In these subfamilies, pollens grains are free, loosely arranged and shed as single grains.

Observations on pollen grains

In *C. tricarinata,* mature pollen grains are monoaperturate, have smooth exine and small pits on their surface. Pollen surface features are highly variable in the family Orchidaceae (Williams and Broome, 1976; Schill and Pfeiffer, 1977; Burns-Balogh and Hesse, 1988; Kocyan and Endress, 2001; Kant *et al*., 2013). In the subfamily Apostasioideae, pollen grains are monoaperturate and exine is reticulate. In the subfamily Cypripedioideae, pollen grains have tectate exine with two-three elongate furrows. In the subfamily Orchidoideae and primitive genera of the subfamily Epidendroideae including the present taxon, pollen grains are mostly monoaperturate and exine sculpturing varies from reticulate, semitectate reticulate, reticulate heterobrochate, tectate perforate, foveolate, rugulate, psillate to verrucate, psillate-scabrate or baculate (Kapil and Arora, 1990; Barone Lumaga *et al*., 2006). In the advanced epidendroids, pollen grains are mostly inaperturate and exine is tectate reticulate, tectate perforate or tectate type (Zavada, 1990). Therefore, occurrence of aperturate pollen grains in the presently studied taxon also confirms its primitive character.

CONCLUSION

C. tricarinata shows some primitive characters including binucleate tapetal cells, complete absence of fibrous thickenings in the middle layers and their poor

development in the endothecium, formation of eight pollinia and aperturate pollen grains, whereas advanced features include presence of three-middle layers, simultaneous type of cytokinesis in the pollen mother cells, close proximity of microspores with the tapetum, aggregation of pollen grains into a compact mass called pollinium and formation of caudicle in the pollinarium unit. The results of the present investigation play a significant role in the taxonomy, cladistics and understanding character evolution. As Orchidaceae is one of the largest and most evolved families, however, detailed early anther development, differentiation of anther wall and development of pollinium has been studied in only a handful of taxa. Therefore, to make the classification system more efficient, similar studies in other taxa is also required. In this context, the subfamilies Apostasioideae and Cypripedioideae require special attention where information on these features is completely lacking.

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