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Research Article

EMBRYOLOGICAL STUDIES IN ORCHIDS: AN OBSERVATION ON *CYMBIDIUM PENDULUM* (ROXB.) SW.

[*]L. K. Attri[1], Harsh Nayyar[2], and Ravi Kant[3]
[1]Dolphin PG College of Life Sciences, Chunni-Kalan, Distt. Fatehgarh Sahib, Punjab
[2]Department of Botany, Panjab University, Chandigarh;
[3]Shoolini Institute of Life Sciences and Business Management (SILB), The Mall, Solan.
**Author for Correspondence*

ABSTRACT
The flowers of *C. Pendulum* were hand pollinated in the morning at around 9.00 a.m. The developing ovaries were fixed and the investigations on embryo development have been carried out. It was observed that the fertilization was accomplished at 52-54 days after pollination (DAP), the zygote took a rest of about 20 days after fertilization, divided and re-divided thereafter during 72-110 DAP forming ca (apical cell) on upper side and cb on lower side of proembryo. The cb (basal cell) of pro-embryo gave rise to suspensor initial at 110-120 DAP. As many as five suspensor initials were observed at 130-136 DAP, which on maturity developed into suspensor cells. The ca part of proembryo formed an embryo proper. The striated seed coat has also been observed during these stages of development.

Key Words: *Orchids, Pollination, Fertilization, Zygote, Embryology, Suspensors*

INTRODUCTION
Orchids, known for their myriad of shapes, colors and sizes, embody an order of aristocracy among the flowering plants. These extraordinary plants with a spectrum of floral characteristics and intricate pollination mechanisms represent a fairly young (geologically), highly diverse and successful family, Orchidaceae, which is perhaps one of the largest families of angiosperms, with an estimated number of more than 25,000 species (Atwood, 1986), however, the numerical strength of the family, in terms of the species, has been variously assessed between 17,000 and 35,000 (Garay, 1960; Shultze and Pease, 1963; Willis, 1973). The orchids have out-maneuvered their counterparts by evolving ingenuity and higher levels of specialization in both the vegetative and reproductive traits. It is a monophyletic family in which members share a suit of characters that set them apart from most of the angiosperms. One character shared by nearly all the orchids is the presence of dust likes tunicate seeds (Dahlgren and Clifford, 1982), i.e. only the uni-seriate epidermis of the outer integument persists in the mature seed and forms a hollow space in which the embryo occurs. Although the family has huge number of species wealth, but the embryological studies has restricted only to a handful of species. Importance of the embryological data in predicting a breeding system is well known and the knowledge of the breeding system is a must in taxon like Orchidaceae where consistent improvement of ornamental traits through hybridization is the need. Incidentally, the orchids provide a suitable material for embryological studies because of the presence of large nuclei and numerous ovules within the same ovary at different stages of development. The present work has been carried out to fill this lacuna and is an addition to embryological data; this type of study now days has rarely been taken up. The taxon *Cymbidium pendulum* (Roxb.) Sw., chosen for the present study, belongs to genus *Cymbidiums* L., represented by 20 species in India and distributed in Northeastern, the Himalayan and the peninsular region, in climates ranging from tropical to temperate (Jain and Mehrotra, 1989). *C. pendulum* produces commercially important colorful flowers of attracting appearance and it is extremely important among orchid growers and florists. Development of female gametophyte has already been published by the author for this species and presently, the post fertilization developmental changes have been discussed in relation to time**.**

MATERIALS AND METHODS

Cymbidium pendulum, species under investigation was collected from their natural habitat i.e. from North Eastern areas (mainly Sikkim) and maintained in the Green House, Department of Botany, Panjab University, Chandigarh for further research.

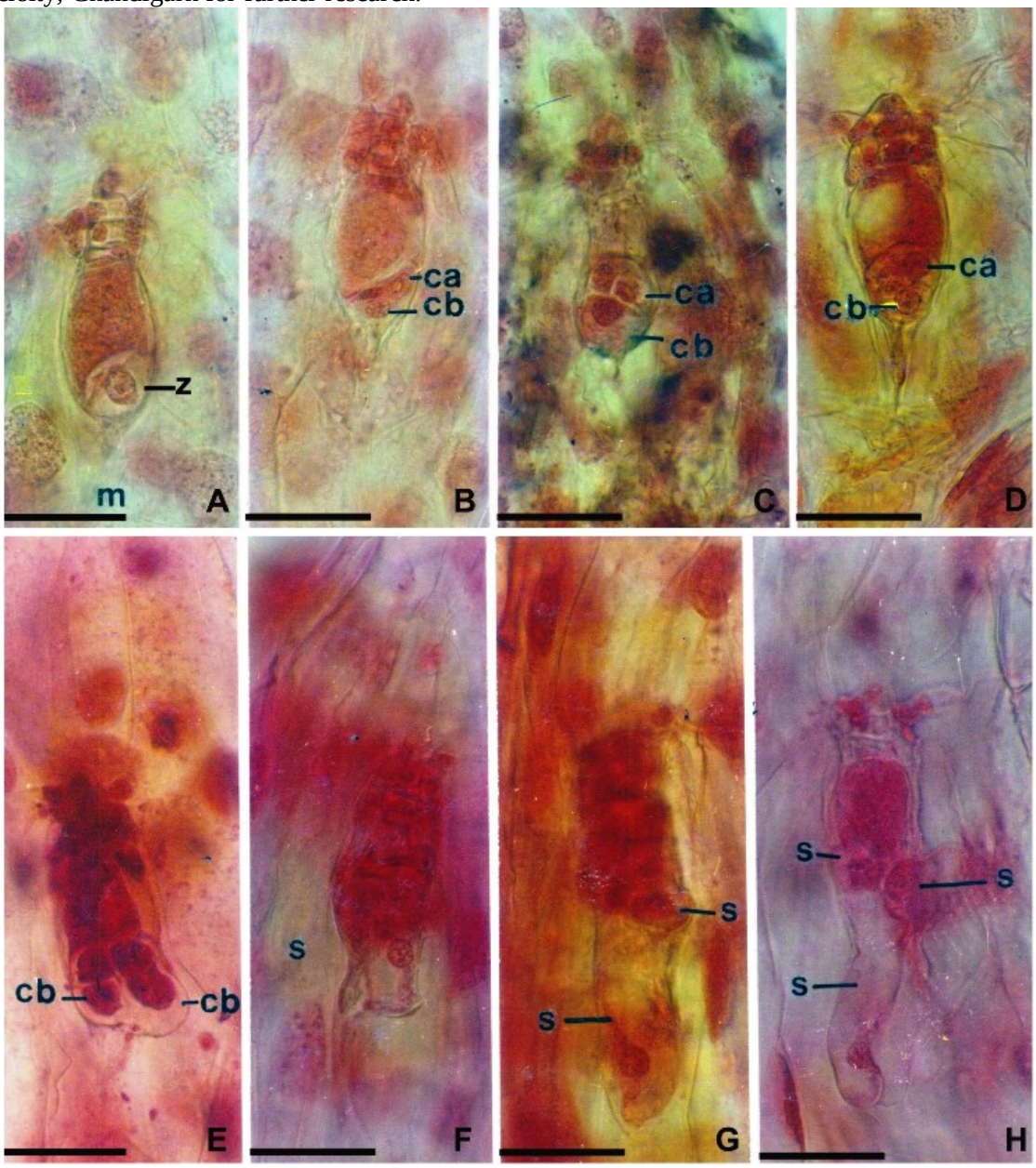

Figure 1(A-O): *Cymbidium pendulum* (Roxb.) Sw. Whole mount of seeds at diffrent stages of embryo development. A, Zygote with dense cytoplasm towards the micropyle origin (72 DAP); B, Oblique division in zygote forming ca and cb (82 DAP); C, D, Longitudinal division in ca forming a 3-celled pro-embryo (94-96 DAP); E, Division in cb forming 4-celled pro-embryo (96-102 DAP); F, Two suspensor initials (110-120 DAP); G, A lateral cell expanded to suspensor (126-130 DAP); H, Three suspensor cells (130-136 DAP). Scale bars=50um.

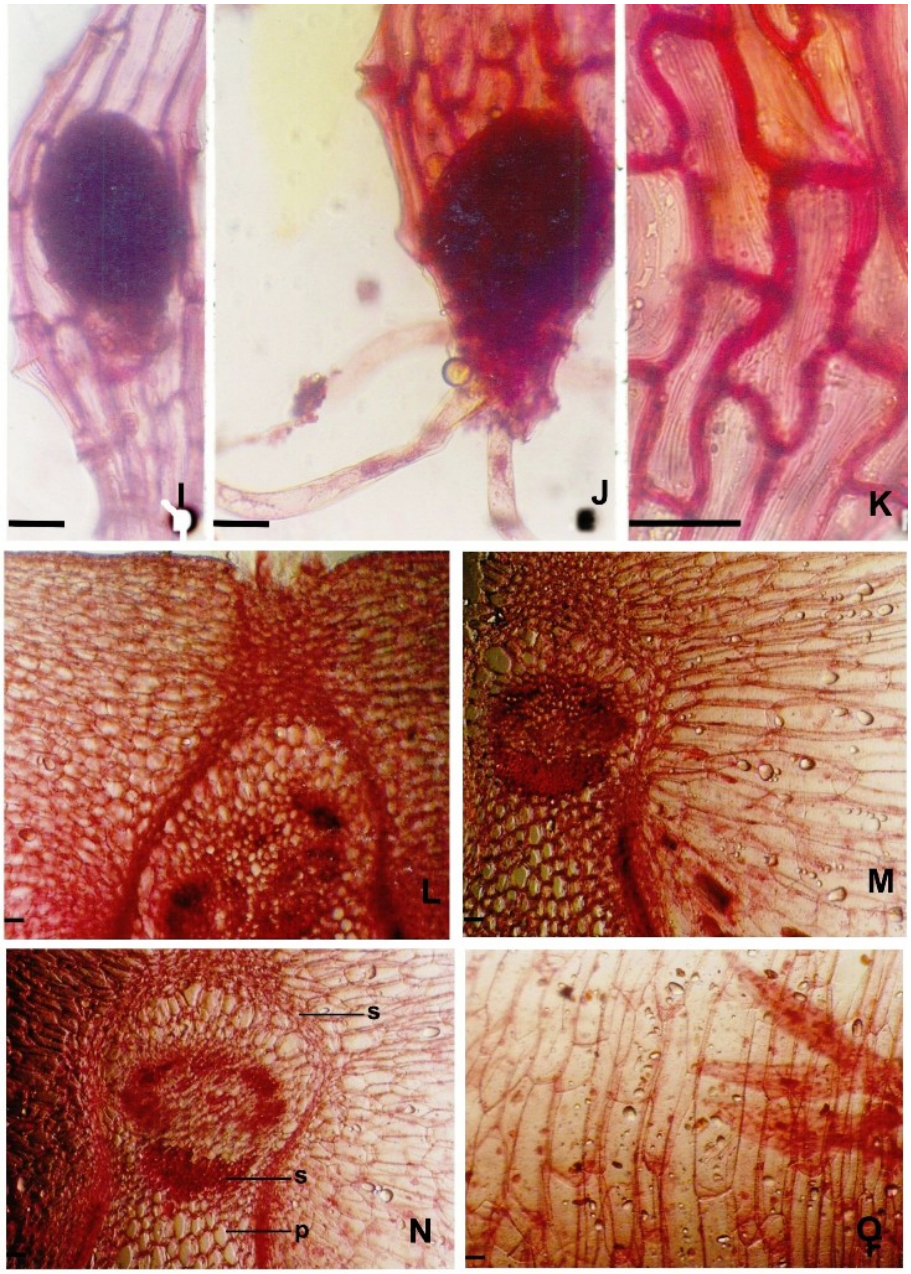

Figure 1(A-O): *Cymbidium pendulum* (Roxb.) Sw. (Contd.). I-J, Embryo proper with suspensor cells (110-136 DAP); K, magnified view of seed coat showing striations (136 DAP); L, M, A segment of ovary showing outer epidermis, ground parenchyma (p), and vascular bundle having xylem and phloem surrounded by scalarenchymatous sheath followed by parenchymatous (2-3 layers) and sclarenchymatous cells (s) extended in the outer epidermis (38-54 DAP); N,O, Pericarp showing increase in number of ground parenchyma layers with large cells in the middle and smaller cells towards the upper and lower epidermis (82-136 DAP). Scale bars: I-J= 50um and K-O= 100um.

The flowers were pollinated at anthesis and fixed in FAA (70% ethanol: glacial acetic acid: formalin = 18:1:1) for 36 hours and subsequently stored in 70% ethanol for further used. The material was fixed at the interval of couple of days observed the post pollination developmental changes especially at and after fertilization.

The whole mounts were prepared and stained in safranin to see the development of embryo and suspensor cells and photographs were taken with good quality of microscope with best attached camera.

Hand sections were cut with the help of sharp blade and stained in safranin to observe the growth of pericarp.

RESULTS AND DISCUSSION

In the present studies, the syngamy and triple fusion occurred in normal manner and resulted in the formation of a zygote (Fig. 1A) at around 54 days after pollination (DAP) and primary endosperm nucleus. The synergids were ephemeral. The zygote was ovoid in outline and the nucleus occurred in the middle while the cytoplasm showed small vacuoles (Fig. 1A). There is a good deal of variation in the time interval between pollination and fertilization in the orchidaceae. The period is generally short in the terrestrial orchid of the subfamily Spiranthoideae and Orchidoideae this duration is only eight to ten days in *Habenaria* sp. and *Satyrium napalense* while this duration varies from five days to two to three months in the epidendroid taxa e.g. fifteen days in *Spathoglottis plicata*, thirty days in *Vanilla planifolia*, forty five days in *Cymbidium bicolor* and *Eulophia epidendrea*e, sixty to seventy five days in *Bulbophyllum mysorense*, *Dendrobium* sp., *Phalaenopsis pulcherrima*, *Geodorum densiflorum* (Niimoto and Sagawa, 1961, 62; Swamy, 1949) and *Phalaenopsis* sp. (Zhang and O'Neill,1993) while 50-54 days in *Cymbidium pendulum* and *C. aloifolium* (Attri *et al*., 2005, 2007). Each orchid thus seems to have stabilized its time interval for different developmental stages in relation to the environment and the availability of species – specific pollinators.

After a resting period of 20 days, the zygote underwent a transverse or oblique division and resulted in a 'terminal cell' (ca) and 'basal cell' (cb) (Fig. 1A). Oblique division in the zygote was also seen occasionally (Figure 1B). The basal cell underwent a longitudinal division and so was the plane of division in the terminal cell, which resulted in four cells of same size at 99-104 DAP (Fig. 1C-E). The three micropylar cells increased in size and developed small vacuoles while the terminal cell appeared densely cytoplasmic. One of the cell formed by the division in cb grew towards the micropyle and formed a suspensor at 116-120 DAP (Fig.1F). This suspensor cell eventually extended beyond the micropyle formed by inner integument and grew into the lumen formed by the outer integument. The other two cells also elongated at 126-130 DAP (Fig. 1G). The nucleus of the each suspensor occupied a position near the tip of the cell (Fig.1G). Two more suspensor cells were formed from the base of the filamentous proembryo which grew towards the chalazal end of the seed prior to the growth of the embryo proper at 136 DAP (Figure 1H). At maturity, nearly five suspensor cells were seen which were in close proximity with the seed coat (Fig. 1I-J). In orchidaceae, the suspensor shows a good deal of variation in the number of constituent cells and their organization (Swamy, 1949). It may be: (a) single celled, hypertrophied to assume a sac like, conical or tubular form (*Cyperipedium, Dendrobium*); (b) uniseriate filament of five-ten cells which grows beyond the micropyle so as to reach the placenta where it gives rise to haustorial branches (e.g. *Habenaria, Ophrys, Satyrium*); (c) appears as a cluster of grapes (*Epidendrum, Sobralia*); (d) the suspensor occurs in the form of an apical cap of eight- cells which partially enclosed the embryo (*Cottonia, Luisia, Vanda*, *Rhynchostylis*) and (e) six-ten cells towards the micropylar end elongate and form tubular extensions e.g. *Cymbidium, Eulophia*. The embryo proper developed from one of the cells of ca.

The two integuments enclosed the young embryo; each of the two was 2-celled. The inner tangential and radial wall of the outer layer of the outer integument showed secondary deposition. The mature embryo was surrounded by a single layer of cells derived from the outer layer of the outer integument. The seeds in species under study were mono-embryonate (Fig. 1I, J). The inner integument and the inner layer of the

outer integument degenerated after fertilization. The cells of the outer layer of the outer integument lost their protoplast, became transparent and differentiated into a single-layered seed coat. The individual cells of the seed coat were elongate in a plane parallel to the long axis of seed. The cells of seed coat exhibited striations (Fig. 1K). The seed coat is formed by the outer layer of the outer integument but in taxa like *Apostasia wallichii, Neuwiedia hollingeri*, *N. veratrifolia* and *Vanilla planifolia*, the seed coat is formed by the crushed cells of the inner integument and the outer integument whose cells become sclerotic and completely opaque due to deposition of a dark material (Swamy, 1947, 1949). The shape of the seeds and characteristic of the individual cells of the seed coat has been found to be useful in the systematics of the family (Dressler, 1993).

At thirty days after pollination, the pericarp exhibited an outer epidermis which was thick- walled and showed cutinization of the outer tangential walls, a parenchymatous ground tissue about 30 layers deep and an inner epidermis (Fig. 1L). There were six vascular bundles, one each in the fertile and the sterile lobe (Fig. 1M). In each vascular bundle, the xylem and the phloem were enclosed in small sclerenchyma cell, followed by large parenchyma cells which in turn were enclosed in two to three layers of small sclerenchyma cells which showed an extension towards the outer epidermis (Fig. 1N). During further development, the ground parenchyma cells proliferated further and exhibited a zone of 10-12 layers of large, tangentially elongated cells in the middle and smaller cells towards the outer epidermis and as well as near the inner epidermis (Fig. 1O).

In *Cymbidium pendulum*, the primary endosperm nucleus, surrounded by dense cytoplasmic contents does not divide further. It presents for some time during stages of development of the embryo but it is not discernible in later stages of the proembryo. A similar fate if the primary endosperm nucleus has been reported in *Cymbidium bicolor* (Swamy, 1949) and *C. sinense* (Yeung *et al.*, 1994). The primary endosperm nucleus degenerates as such in a number of orchid taxa, while there are others which shows the formation of two, four, eight or up to a maximum of sixteen endosperm nucleus as in *Vanilla planifolia* (Abe, 1972; Swamy, 1947, 1949; Vij and Sharma, 1988; Yeung and Law, 1992; Yeung *et al.*, 1997).

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