A contribution to the embryology of *Cirrhopetalum fimbriatum* Lindl.

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Abstract. The embryo sac of *Cirrhopetalum fimbriatum* is 6-nucleate, generally bisporic, and occasionally monosporic. The reduction of nuclei is due to the 'strike' phenomenon. The two chalazal nuclei receive the micropylar polar. The secondary nucleus is triploid, and comes to lie below the egg. The embryo (in ripe seed) is undifferentiated. The cells \(ca, m, n, n'/\) contribute to the embryo. The suspensor is single-celled, hypertrophied and cap-like.

Keywords. Orchidaceae; Bulbophyllaeae; *Cirrhopetalum fimbriatum*; embryology.

1. Introduction

The Orchidaceae, well known for the high degree of biological specialisation, is one of those few families which possess monosporic, bisporic, and tetrasporic embryo sacs. In many members the female gametophyte shows a tendency towards reduction in the number of nuclei at the chalazal end, either through the phenomenon of "strike" which is more frequent, or by the secondary fusion of nuclei (Abe 1972). The absence of endosperm in most members, the consistent presence of unorganised embryos, and the structural diversity of suspensor are noteworthy features of Orchidaceae.

Of about 1600 Indian species (Roy and Sharma 1972), not many have yet been studied embryologically. Swamy (1948, 1949 a, b) made a significant contribution to the study of Indian Orchidaceae. We have attempted to study the uninvestigated taxa, especially with reference to ontogeny and organisation of female gametophyte and embryo.

2. Materials and methods

The material used in this study includes post-pollinated ovaries and fruits of *Cirrhopetalum fimbriatum* Lindl. (Orchidaceae), collected near Mercara, Karnataka State. The placental columns were excised and fixed in formalin-acetic-alcohol, and stored in 70% ethanol following a wash in running water for about 30 min. Customary methods were followed for dehydration and imbedding. Sections were cut at 7-10\(\mu\), and stained with iron alum-haematoxylin using erythrosin (in clove oil) as counterstain.
3. Observations

The gynoecium is inferior, tricarpellary, and syncarpous. After pollination three placental ridges arise from the inner wall of ovary. Each ridge becomes bipartite, both parts producing finger-like ovular primordia. An ovular primordium is made up of an axial row of 6-7 cells covered by the nucellar epidermis (figure 1). The uppermost cell of the central column is the archesporial cell which directly functions as megaspore mother cell. The inner integument is initiated when the megaspore mother cell is at prophase, and is fully developed by the time a 2-nucleate embryo sac is organised (figures 4-5). Although the initiation of outer integument takes place subsequent to that of the inner integument, it develops faster and encloses the inner integument. The ovule becomes anatropous at the 4-nucleate stage of embryo sac.

The megaspore mother cell enlarges, becomes densely cytoplasmic, and the nucleus appears prominent. The first meiotic division is followed by a transverse wall (figures 2-3), the micropylar dyad cell is smaller and promptly degenerates. The larger chalazal dyad completes second meiotic division forming two free nuclei (figure 4) which occupy polar positions separated by a central vacuole. In some ovules, in the same ovary, occasionally the lower dyad cell gives rise to 2 unequal megaspores. Of these the lower is larger and it gives rise to a 2-nucleate embryo sac, while the smaller upper megaspore degenerates. A 4-nucleate embryo sac develops after a simultaneous division of the two nuclei (figures 5-6). The two micropylar nuclei of the sac are larger and divide again, while the two smaller chalazal nuclei do not divide further (figure 7). The quartet at the micropylar end organises the egg apparatus and upper polar nucleus. The two chalazal nuclei receive the micropylar polar (figure 8), their fusion occurs at the chalazal end, and the triploid secondary nucleus then migrates near the egg. During development of embryo sac the adjoining nucellar cells collapse so that the gametophyte comes in contact with the inner integument (figures 7-9).

The mature embryo sac has a broader micropylar region containing the 3-celled egg apparatus and the lobed triploid secondary nucleus.

Fertilisation is porogamous. The pollen tube enters the embryo sac by destroying one of the synergids and liberates two male gametes. One of these fuses with the egg, and the other with the secondary nucleus (figures 9-10). The primary endosperm nucleus degenerates by the time the proembryo attains the quadrant stage.

4. Development of the embryo

The zygote undergoes a transverse division to form the apical cell $ca$, and basal cell $cb$ (figures 11-13). $cb$ divides transversely producing $m$ and $ci$, while $ca$ divides vertically forming two juxtaposed cells (figures 14-16). The proembryonal tetrad conforms to the $A_2$ category of Souèges (1939). $ci$ divides transversely into cells $n$ and $n'$ (figures 17-18). The two juxtaposed cells of $ca$ undergo a vertical division, at right angles to the previous one, forming quadrant $q$ (figure 19). Meanwhile, $m$ gives rise to two juxtaposed cells after a vertical division (figure 19). These undergo another similar division at right angles to the first, resulting in a tier of circumaxial cells (figure 20). The tier $q$ divides transversely forming regions $l$ and $l'$, leading to the octant stage (figure 21). Periclinal divisions follow in tiers $l$, $l'$, and $m$ delimiting...
Figures 1-10. Development of embryo sac; 1. L.s. Ovular primordium, archesporial initial; 2. L.s. Ovule, megaspore mother cell at metaphase I; 3. Dyad cells; 4. 2-nucleate embryo sac; 5. Division of nuclei; 6-7. 4 and 6 nucleate embryo sacs; 8. Organised embryo sac; 9-10. Stages in double fertilisation. All figures × 400.
Figures 11-25. Development of the embryo. All figures ×400.
the dermatogen de (figure 22). Figures a, b, c, d, e, and f are transections of an embryo similar to one shown in figure 22, and illustrate the disposition of cells at levels A, B, C, D, E and F respectively (figure 22). The subsequent divisions appear to be irregular finally giving rise to the globular proembryo (figure 23). Meanwhile, the cell \( n' \) enlarges, and forms a hood-like suspensor with a hypertrophied nucleus (figure 24). The cell \( n \) undergoes a vertical division, the derivatives integrate with the embryonal mass. The mature embryo (in ripe seed) is morphologically undifferentiated (figure 24).

During development of seed, the cells of entire inner integument and of inner layer of outer integument lose their contents, become translucent, and their walls acquire characteristic thickenings. They constitute a single-layered seed coat (figure 25).

5. Discussion

The occurrence of bisporic embryo sacs has been consistently noticed in all species of Diandraceae, Neottia nidus-avis (Modilowski 1918), Cymbidium bicolor (Swamy 1942), Hetaeria nitida (Olsson 1967), Pogonia japonica (Abe 1968), occasionally in Epipactis pubescens (Brown and Sharp 1911), Gyrostachys cernua and G. gracilis (Pace 1914), Spiranthes australis (Maheshwari and Narayanaswamy 1951), Oreohtris patens (Abe 1971b), Orchis aristata (Abe 1971c), Orchis sambusiana and Oncidium pratetum (Afzelius 1916). The reduction of nuclei at the chalazal end has also been recorded in several species of both Monandrae and Diandraceae, and is due to the phenomenon of "strike". This is not due to the fusion of spindles of the dividing nuclei as reported in Epipactis pubescens (Brown and Sharp 1911), Paphiopedilum insigne (Afzelius 1916), and Polystachya flavescens (Ekanthappa and Arekal 1977).

A single large suspensor cell derived from \( ci \) is also formed in species of Peristeria and Spathoglottis (Swamy 1949b). Swamy (1949b), who reviewed the embryogeny of orchids, states "only the first two or three cell generations in the zygote are consistent, the subsequent divisions take place with no definite pattern". We noticed that even after the third cell generation in the proembryo cell divisions are regular. The development of the embryo, therefore, essentially conforms to "Group B" of Swamy (1949b).

The essential similarities noted in the embryo sac and embryo development of Cirrhopetalum and Bulbophyllum justifies their placement in the tribe Bulbophylleae.

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