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The Embryology of *Epipogium roseum* (*Orchidaceae*)

By

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Abstract: Development of pollen and female gametophyte in *Epipogium roseum* (D. Don) Lindl. has been investigated. The embryo sac conforms to the Apinagia type. The taxonomic position of *Epipogium* within the family is discussed.

The taxonomic position of the genus *Epipogium* R. Br. in the *Orchidaceae* is controversial. It is placed in the *Cephalantherinae* (Ziegenspeck 1936), near *Neottiinae* (Afzelius 1954), in the *Orchideae* (Dressler & Dodson 1960, Garay 1964) and in an independent tribe *Epipogieae* (Dressler 1974). An attempt has been made in the present study to evaluate the taxonomic position of the genus in the family based on the embryology of *E. roseum* (D. Don) Lindl.

E. roseum is a leaf-less, achlorophyllous saprophytic orchid having a wide distribution from the tropical Himalayas to Peninsular India, West Africa, Sri Lanka, Indonesia, and Australia. The taxon appears on thick humus in wet deciduous forest beds protected by a dense canopy. The vegetative part is a small, oval, brown underground rootless tuber which bears the aerial racemose flowering shoot of 15 to 50 cm (Fig. 1 *a*). The flowers are straw-colored, speckled with deep pink spots. After the flowering season the tuber collapses and shrivels. The appearance in known localities is sporadic, and the reproductive phase lasts for about two weeks. Seed setting is rare.

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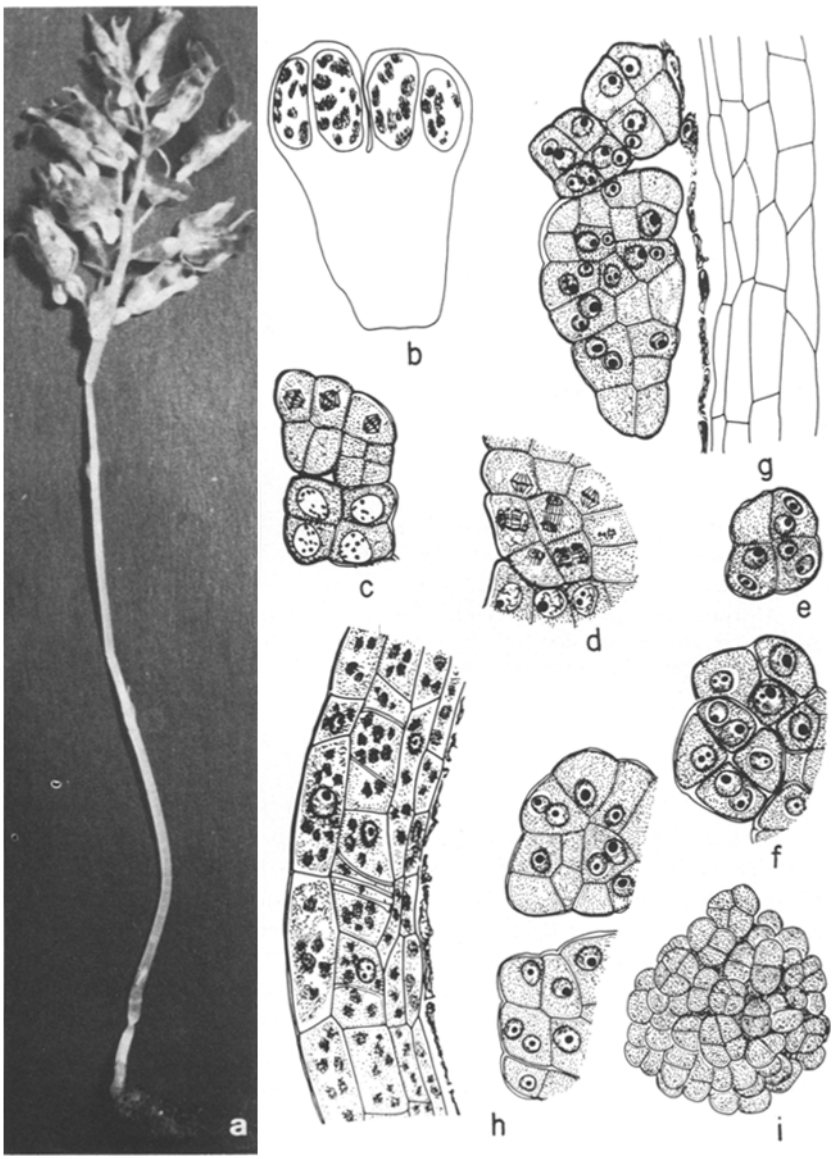

Fig. 1. *Epipogium roseum*. *a* Plant, ×1, *b* pairs of microsporangia, ×25, *c–f* pollen mitosis, ×600, *g–h* portions of microsporangia, ×500, *i* massula, ×24

Material and Methods

Flower buds and flowers of *E. roseum*, collected on Jan. 25, 1975 from Shetty Thota, 7 km North-West of Mercara city, Coorg District, Karnataka State, were fixed in Formalin-acetic-alcohol (FAA) and stored in 70% ethanol. They were dehydrated with grades of ethanol, cleared in xylene, and imbedded in paraffin. Microtome sections were cut at 7–12 μm thickness. Staining was done in Heidenhain's iron alum-hematoxylin using erythrosin in clove oil as counterstain.

Results

Microsporogenesis. In a cross section of the anther two pairs of microsporangia, placed side by side, are observed (Fig. 1 *b*). A young microsporangium has a 5-layered wall consisting of an epidermis, an endothecium, two middle layers and a layer of glandular tapetum of uninucleate cells enclosing the spore mother cells. The microspore mother cells are grouped together indicating the future formation of massulae. Tetrad formation is successive. Division of microspore nucleus is synchronous in the tetrads (Fig. 1 *c, d*). At the end of division a small generative cell is cut off towards the distal pole of the spore, while the vegetative cell is larger and occupies a proximal position (Fig. 1 *e*). Gradually the generative cell separates itself from the microspore wall and enters into the cytoplasm of the vegetative cell (Fig. 1 *f-h*). The number and arrangement of pollen tetrads vary (Fig. 1 *g*), resulting in diversity of shape and size of massulae within one and the same microsporangium. The tapetal cells provide nourishment to the developing massulae and in later stages get crushed. The endothecium acquires band-like thickenings while the epidermis develops a thick outer tangential wall. The microsporangium wall cells, in early stages, accumulate a large amount of starch in the form of compound grains. They become gradually depleted during later stage and can be seen even after the disorganization of the tapetum (Fig. 1 *g*). The pollen tetrads are mostly tetrahedral, although isobilateral, decussate, and T-shaped ones are also noted (Fig. 1 *i*). At maturity of the anther and during insect visits the massulae are released. The pollen grains are bi-celled at shedding.

Ovule and Female Gametophyte. The gynoecium is inferior, tricarpellary, syncarpous, and the ovary unilocular. Three placental ridges arise from the inner wall of the ovary. Each ridge later becomes bipartite and many finger-like ovular primordia arise on the surface. The placental proliferation and ovule formation occur before anthesis. The ovules are unitegmic and tenuinucellate. The ovular primordium is composed of an axial row of 4–5 cells surrounded by the nucellar epidermis. The topmost cell of the axial row differentiates into an

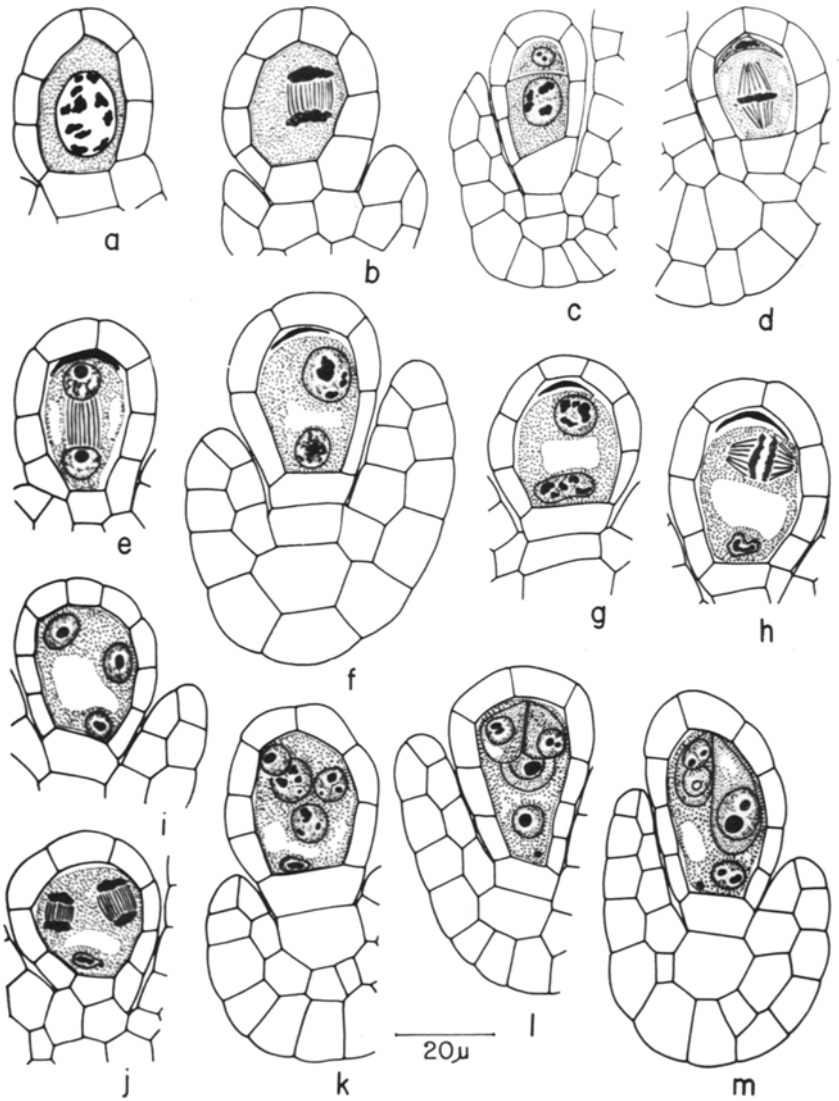

Fig. 2. *Epipogium roseum*. *a* Longi-section of young ovule showing archesporial cell, *b* dividing megaspore mother cell, *c* dyad, *d-e* divisions in the chalazal dyad, *f-g* two-nucleate embryo sac: note smaller degenerating chalazal nucleus, *h-i* division of primary micropylar nucleus and degenerated primary chalazal nucleus, *j-k* division of the two micropylar nuclei and degenerated chalazal nucleus, *l-m* organized embryo sacs with two synergids, an egg and a polar cell

archesporial cell. It enlarges in size and directly functions as the megaspore mother cell (Fig. 2 *a*). Simultaneously, the funiculus curves towards the placenta and the integumentary initials appear. The ovules become anatropous at the megaspore mother cell-state. The megaspore mother cell after meiosis-I produces two dyad cells (Fig. 2 *b, c*) of which the micropylar one degenerates promptly, while the chalazal one functions and exhibits a nuclear division (Fig. 2 *d, e*). This is not followed by wall formation, and a 2-nucleate embryo sac results (Fig. 2 *f, g*). Of the two nuclei, the one at the chalazal end begins to degenerate, while the micropylar one alone divides to produce a quartet of nuclei (Fig. 2 *h, i, j, k*). These four nuclei contribute to the organization of the egg apparatus with 2 synergids, an egg, and an upper polar. Sometimes the remnants of the primary chalazal nucleus persist in the organized embryo sac, thus rendering it 5-nucleate (Fig. 2 *l, m*). The integument does not cover the nucellus even at the organized embryo sac stage of the ovule, and a few ovular cells located at the chalazal region enlarge many times beyond their original size.

Discussion

There is no earlier report on the development of the male gametophyte on *Epipogium*. AFZELIUS (1954), after investigating the embryology of *E. aphyllum*, stated he lacked the early stages of pollen development in the investigated material. The anther wall in *E. roseum* (present study) consists of 5 layers of cells of which the epidermal one remains persistent. The tapetum is of the secretory type. The pollen grains are united into massulae as in many members of the *Orchidaceae*, *Asclepiadaceae*, and *Mimosaceae*. The pollen tetrads are mostly of the tetrahedral type.

The ovules are unitegmic and the integument does not organize a micropyle. Development of the embryo sac conforms to the bisporic, 5-nucleate type and it becomes ripe prior to pollination. The reduction of nuclei number at the chalazal end of the embryo sac, either by fusion or by suppression of divisions ("strike phenomenon") leading to a reduced number of antipodals, is a common feature in orchids (ABE 1972, SAVINA 1978). In the present taxon, this trend culminates in that the chalazal nucleus never divides; its remnants may or may not be seen in the organized embryo sac. Such a type of embryo sac ontogeny is generally observed in members of the *Podostemaceae* and is designated as the Apinagia type (BATTAGLIA 1971, NAGENDRAN & al. 1976). The 4-5-nucleate embryo sacs reported earlier in the *Orchidaceae* (see literature cited in ABE 1972), with the exception of *Cypripedium debile* (KIMURA 1968), cannot be equated to the Apinagia type of ontogeny, because some of them are monosporic, while the bisporic types show

divisions of the antipodal nuclei at least once. Further, the previous reports have been reinterpreted or questioned (Carlson 1945, Swamy 1945, Abe 1972).

A relationship of *Epipogium* with the *Cephalantherinae* based on the number and morphology of chromosomes has been suggested (Ziegenspeck 1936). But pollen grains are free in *Cephalanthera*, while in *Epipogium* they are united into massulae.

Based on exomorphic features Dressler & Dodson (1960) and Gary (1964) conclude that the genus *Epipogium* should be placed in the *Orchideae*. Further, in the *Orchideae*, the embryo sac development normally is monosporic and 8-nucleate (Schnarf 1929), whereas in *Epipogium aphyllum* (Afzelius 1954, Geitler 1956), it is monosporic 5- or 6-nucleate, and in *E. roseum* it is bisporic and 4- or 5-nucleate.

From an embryological study of *E. aphyllum*, Afzelius (1954) considered the genus to be closer to the *Neottiinae*. The features of resemblance are the pollen tetrads remaining together in massulae, and the monosporic embryo sac with a reduced number of nuclei at the antipodal end. However, *Epipogium roseum* differs from the investigated taxa of the *Neottiinae* in the persistence of the anther epidermal cells till maturity, successive divisions of pollen mother cells, unitegmic ovules without micropylar organization, and bisporic, 4- to 5-nucleate female gametophyte. Vermeulen (1965) stated that the placement of *Epipogium* in the *Orchideae* is as anomalous as in the *Neottiinae*. Recently, based on pollen and other associated features, Dressler (1974) suggested that *Epipogium* should be elevated to an independent tribe *Epipogieae*.

A brief flowering phase, unitegmic condition of the ovule, lack of a distinct micropyle, organization of the embryo sac prior to pollination and degeneration of chalazal nucleus of the embryo sac noted in the present study are features of saprophytic orchids (Tohda 1967, Abe 1976). The Apinagia type of embryo sac development recorded in *E. roseum* is a feature so far noticed only in one species—(*Cypripedium debile*)—among the members of the *Orchidaceae* though not designated as such. However, a detailed embryological work on other uninvestigated species of *Epipogium* would throw light on the question of elevation of *Epipogiinae* to *Epipogieae*.

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