

## GAMETOGENESIS, SEED DEVELOPMENT AND PERICARP IN *EPIPACTIS LATIFOLIA* (ORCHIDACEAE, NEOTTIEAE)

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The details of gametogenesis, seed development and pericarp in *Epipactis latifolia* Swartz are described. Anther wall development corresponds to the Basic type. Microspore tetrads are of T-shaped, tetrahedral and decussate types. At anthesis the microspores are 2-celled and fibrous thickenings develop in two subepidermal layers. Ovules are anatropous, bitegmic and tenuinucellate. Both the integuments are dermal in origin. Embryo sac development is of the monosporic, Polygonum type. Incipient endosperm of four nuclei is formed. Embryo development conforms to the Asterad type. Mature embryo lacks differentiation. Seeds are minute and nonendospermic and the seed coat is formed entirely by the outer layer of testa. There are three sterile and three fertile valves in the ovary. In the prefertilization stages, valves consist of parenchymatous cells. After fertilization, the sterile valves become sclerenchymatous whereas the fertile valves remain parenchymatous.

**Key Words :** *Epipactis latifolia*, gametophyte, integuments initiation, embryo, pericarp, Orchidaceae.

Embryologically the genus *Epipactis* Zinn. is highly interesting in showing the co-occurrence of three types of embryo sac development, imperfect fertilization and parthenogenesis (see Sato, 1974). In view of the above and with an idea to add to our existing knowledge on the embryology of the family Orchidaceae, the present investigation was undertaken which deals with the structure and development of male and female gametophytes, embryo, and pericarp in *Epipactis latifolia* Swartz.

### MATERIALS AND METHODS

Buds, flowers and fruits of *Epipactis latifolia*, in all stages of development, were collected during June-September, 1993 from Prospect Hill, Shimla, W. Himalayas. The placental columns were excised prior to their fixation in formalin-acetic acid-alcohol, and subsequently stored in 70% ethanol. Dehydration, infiltration and embedding were done in the conventional way. The serial sections were cut at 7-10  $\mu\text{m}$  and stained with safranin-fast green.

### OBSERVATIONS

**Microsporangium, microsporogenesis and male gametophyte :** The anther is tetralocular (Fig. 1). The young anther lobe consists of four or five wall layers out of which the two subepidermal layers are derived from the outer secondary parietal layer (*osp*), whereas the inner one or two layers are derived from the inner secondary parietal layer (*isp*) (Fig. 2). Out of two

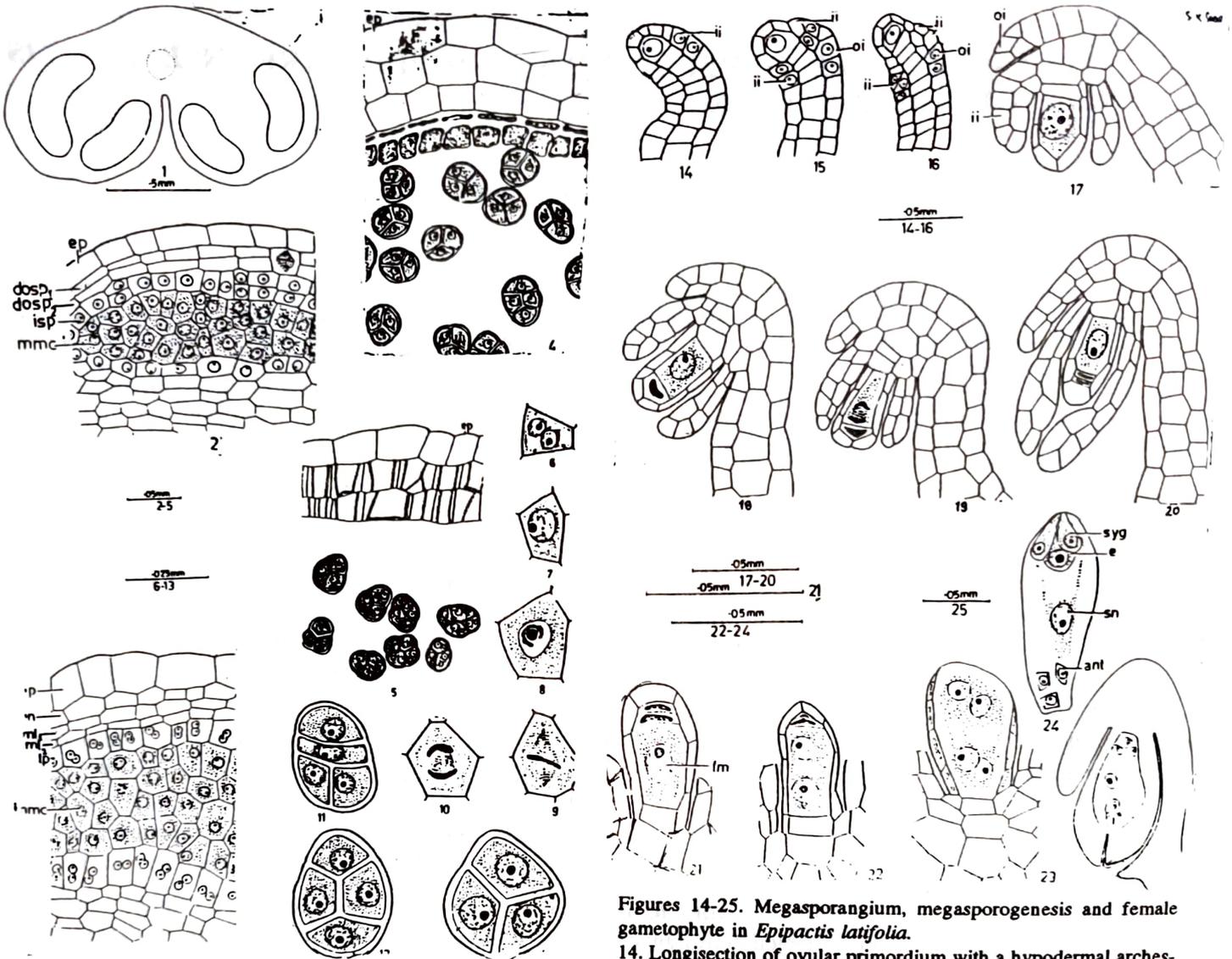
daughter layers of *isp* the inner one develops into the tapetum and the outer one forms the inner middle layer (Fig. 2 and 3). Out of the two daughter layers of *osp*, the outer one forms the endothecium and the inner one forms the outer middle layer (Figs. 2 and 3).

In the mature anther three wall layers persist; epidermis, endothecium and the upper middle layer. Both the endothecial and the upper middle layer cells develop fibrous thickenings. The tapetum and the lower middle layer degenerate (Figs. 4 and 5).

Cytokinesis in the microspore mother cell is of the simultaneous type (Figs. 7-10). T-shaped, decussate and tetrahedral tetrads are formed (Figs. 11-13). The microspores of the tetrads do not separate into individual pollen grains (Fig. 4), but each is 2-celled at anthesis (Fig. 5).

**Megasporangium :** Numerous ovular protuberances develop from the three parital forked ridges (Fig. 42) in the ovarian cavity (Fig. 43). The ovule primordium is composed of a single axial row of cells covered by an epidermal layer (Fig. 14).

The primordium of the inner integument arises from the epidermal layer of ovule primordium (Fig. 14). It is in the form of a ring and consists of two enlarged, neighbouring dermal cells. These cells, by periclinal and anticlinal divisions give rise to a 2-cell thick inner integument (Figs. 15-17). The micropyle



Figures 1-13 Microsporangium, microsporogenesis and male gametophyte in *Epipactis latifolia*.

1. Outline diagram of transection of young anther. 2, 3. Portions of transections of anthers showing wall layers at early and late microspore mother cell stages. 4. Portion of transection of anther at pollen tetrad stage showing degenerated tapetal layer and middle layer 2 ( $ml_2$ ). 5. Portion of transection of anther at 2-celled stage of pollen; note 2-layered endothecium. 6. Binucleate tapetal cell. 7-10. Microspore mother cells in meiosis I. 11-13. T-shaped, decussate and tetrahedral tetrads of microspores. (*dosp<sub>1</sub>*, daughter outer secondary parietal layer 1; *dosp<sub>2</sub>*, daughter outer secondary parietal layer 2; *en*, endothecium; *ep*, epidermis; *isp*, inner secondary parietal layer; *ml<sub>1</sub>*, middle layer 1; *ml<sub>2</sub>*, middle layer 2; *mmc*, microspore mother cell; *tp*, tapetum).

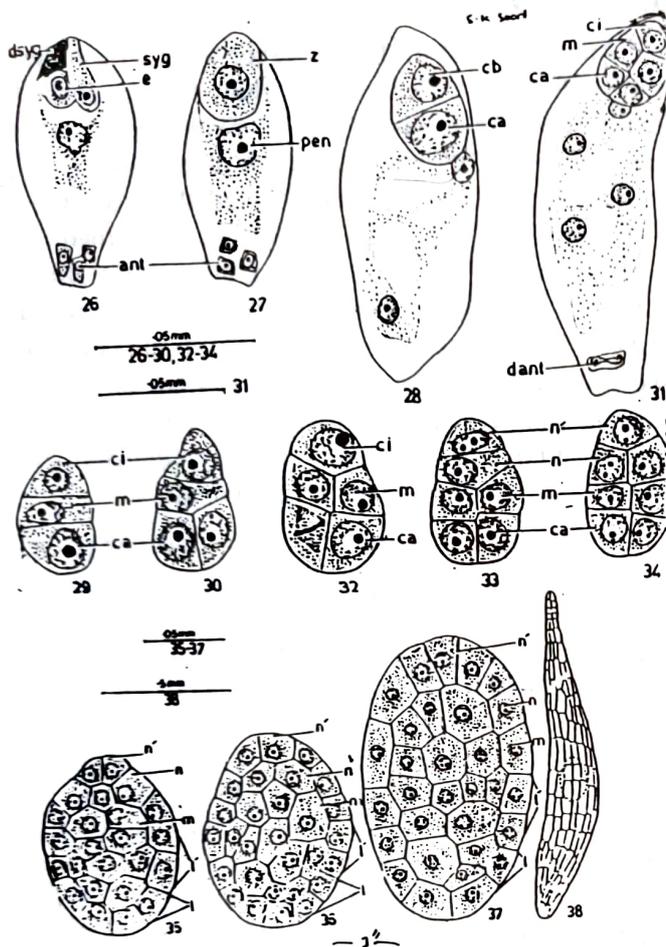
is formed by the inner integument (Fig. 20 and 25). The outer integument is initiated a little later than the inner one (Fig. 15). Its primordium arises next to the base of the inner integument primordium. The two cells of the primordium, in a manner similar to the inner integument, give rise to the 2-cell thick outer

Figures 14-25. Megasporangium, megasporogenesis and female gametophyte in *Epipactis latifolia*.

14. Longisection of ovular primordium with a hypodermal archesporial cell. 15, 16. Young ovules showing the dermark origin of inner and outer integuments. 17. Megaspore mother cell. 18. Dyad; note degenerated micropylar cell. 19. Dyad; chalazal cell in the 2nd meiotic division. 20, 21. Functional megaspore. 22, 23. Two- and 4-nucleate embryo sacs. 24. Mature embryo sac. 25. Longisection of mature ovule which is anatropous, bitegmic and tenuinucellate; note the micropyle is formed by the inner integument. (*ant*, antipodals; *e*, egg; *fm*, functional megaspore; *ii*, inner integument; *oi*, outer integument; *sn*, secondary nucleus; *syg*, synergid).

integument (Fig. 16 and 17). In prefertilization stages the outer integument is far behind the inner one (Figs. 17-20 and 25). However, it grows beyond the inner one in the postfertilization stages. The mature ovule is anatropous, bitegmic and tenuinucellate (Fig. 25).

**Megasporogenesis and female gametophyte:** The hypodermal archesporial cell functions directly as the megaspore mother cell (Fig. 14 and 17). After meiosis I in the megaspore mother cell two unequal dyad cells are formed. The micropylar dyad cell degene-

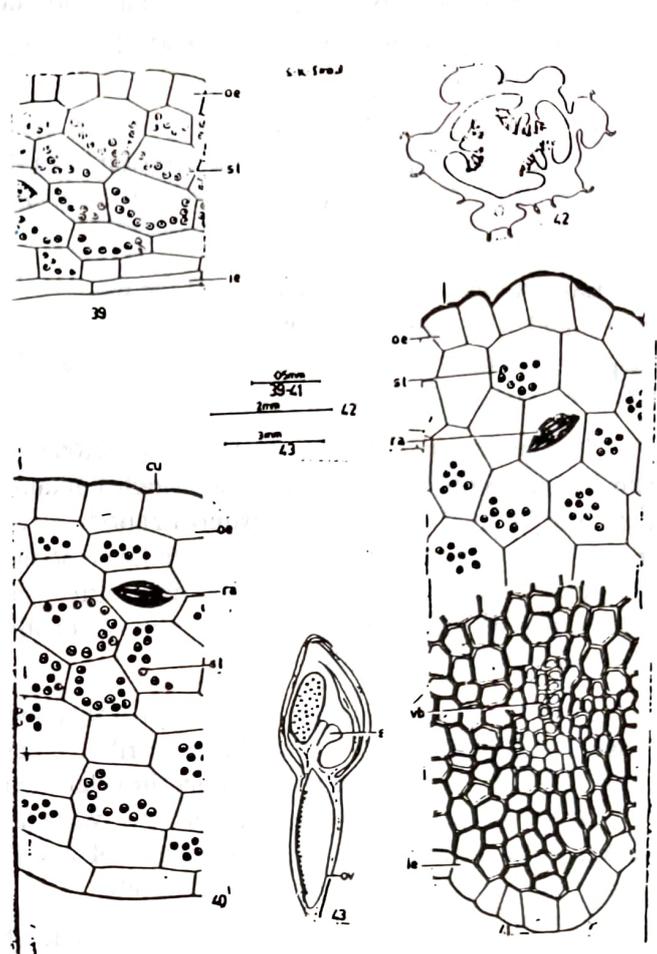
Seed development and pericarp in *epipactis*

Figures 26-38. Fertilization, endosperm and embryogeny in *Epipactis latifolia*.

26. Double fertilization; one male nucleus appressed to the egg nucleus and the other one to the secondary nucleus. 27. Zygote; note enlarged primary endosperm nucleus and antipodal cells. 28. Two-celled proembryo; and 2-nucleate endosperm. 29, 30. Three- and 4-celled proembryos. 31. Five-celled proembryo with vertically divided middle cell; note 4-nucleate endosperm. 32-35. Stages leading to the formation of globular proembryo. 36, 37. Young and mature undifferentiated embryos. 38. Surface view of seed with embryo in the middle part. (*ant*, antipodals; *dant*, degenerated antipodals; *dsyg*, degenerated synergid; *e*, egg; *pen*, primary endosperm nucleus; *syg*, synergid; *z*, zygote).

rates (Fig. 18), while the chalazal one undergoes meiosis II forming two megaspores (Fig. 19 and 20). The nucleus of the chalazal megaspore which is functional (Fig. 21) undergoes three cycles of mitotic divisions to form 2-(Fig. 22), 4-(Fig. 23) and 8-nucleate embryo sacs sequentially. The mature embryo sac contains an egg apparatus, secondary nucleus and three antipodal cells (Fig. 24). Its development conforms to the *Polygonum* type.

**Fertilization** : Double fertilization occurs. One male gamete nucleus fuses with the egg nucleus and



Figures 39-43. Pericarp in *Epipactis latifolia*.

39. Transsection of ovary wall in the region of fertile valve at megaspore mother cell stage. 40. Transsection of pericarp in the region of fertile valve at mature embryo stage. 41. Transsection of pericarp in the region of sterile valve at mature embryo stage. 42. Transsection of ovary showing sterile and fertile valves, and placentae bearing ovules. 43. Median longitudinal section of flower. (*cu*, cuticle; *ie*, inner epidermis; *oe*, outer epidermis; *ov*, ovary; *ra*, raphides; *s*, stigma; *st*, starch; *vb*, vascular bundle).

the other one with the secondary nucleus of the central cell (Fig. 26).

**Endosperm and embryo** : The primary endosperm nucleus undergoes two cycles of mitosis to form two (Fig. 28) and four endosperm nuclei (Fig. 31). These four free endosperm nuclei do not divide further but degenerate.

The zygote (Fig. 27) enlarges and divides transversely to form a terminal cell *ca* and a basal cell *cb* (Fig. 28). The *ca* undergoes a vertical division, while *cb* divides transversely giving rise to a middle cell *m* and a suspensor initial cell *ci* (Fig. 29 and 30). The proembryonal tetrad is T-shaped (Fig. 30). The daughter cells of *ca* divide longitudinally at right

angles to the first division, giving rise to a quadrant (Fig. 32). The quadrant divides transversely forming an octant with two tiers *l* and *l'*.

Meanwhile the cell *m* undergoes a vertical division, while *ci* undergoes a transverse division to form *n* and *n'* (Fig. 33), which in turn divide vertically (Fig. 34 and 35).

Later divisions are irregular in all the tiers, *l*, *l'*, *m*, *n* and *n'* resulting in an embryo which lacks suspensor, polar meristems and cotyledon (Figs. 35-37). The mature embryo is ovoid (Fig. 37).

**Seed and seed coat :** The mature seed is elongate and has a central distended portion which contains the embryo (Fig. 38). In the postfertilization stages the 2-layer thick tegmen and the inner layer of the testa degenerate. The seed coat is formed entirely by the outer layer of the testa. It contains vertically elongate, thin-walled cells.

**Pericarp :** A transection of young ovary shows three ovuliferous (fertile) and three sterile valves (Fig. 42). The former valves are much broader than the sterile ones. Each valve contains one vascular bundle. The outer epidermis of both types of valves gives rise to uniseriate trichomes (Fig. 39).

The fertile valve of the young ovary is made of six or seven layers of parenchyma (Fig. 39). Most of the cells contain starch grains and some cells contain raphides also. In the postfertilization stages the thickness of the valve increases to ten layers and the outer wall of the inner epidermis becomes thickened (Fig. 40).

In the prefertilization stages the sterile valve is 20-cell thick. Its cells are parenchymatous and thin-walled. In postfertilization stages the number becomes 25 and the outer half of the pericarp becomes sclerenchymatous. The outer wall of the outer epidermis develops a thick cuticle (Fig. 41).

In the fertile valves the inner epidermal cells are transversely arranged, whereas in the sterile valves these are longitudinally arranged (Fig. 40 and 41). As the capsule dries the fertile valves contract in the longitudinal direction and the sterile valves shrink in the transverse direction. This results in the longitudinal splitting of the sterile valves. Through these splits, the dust-like seeds escape.

## DISCUSSION

Although most of the earlier reports on the anther wall studies in the Orchidaceae show four layers and Monocotyledonous type of wall development (Mohana Rao and Sood, 1979; Wirth and Withner, 1959), the present study shows five layers, and the pasic type of development (Davis, 1966), a feature recorded for the first time for Orchidaceae. Another unique structural feature of the anther wall of *Epipactis latifolia* is the development of endothelial thickenings in the two subepidermal layers. Swamy (1949a) stated that the binucleate tapetum is characteristic of the diandrous orchids. *Epipactis latifolia* which is a monandrous orchid also develops binucleate tapetum.

The present investigation clearly elucidates the occurrence of only Polygonum type of development of embryo sac whereas the co-occurrence of mono- and bisporic types of development in *Epipactis latifolia* (Vermoesen, 1911), *E. papillosa* var. *sayekiana* (Sato, 1974); and mono-, bi- and tetrasporic types in *E. pubescens* (Brown and Sharp, 1911) have been recorded. Six-nucleate mature embryo sac of *E. pubescens* results due to the fusion of the two mitotic spindles at the chalazal pole in the four-nucleate embryo sac stage (Brown and Sharp, 1911). In *E. latifolia*, however, the spindles at the chalazal pole do not fuse and the mature embryo is eight-nucleate. The primary endosperm nucleus in orchids usually degenerates. In some cases it undergoes mitotic divisions to produce 2-16 nuclei (see Kimura, 1971; Sood and Mohana Rao, 1988; Veyret, 1974; Wirth and Withner, 1959). In *Epipactis latifolia* four endosperm nuclei are formed. The present study and all the earlier studies which report the incipient development of endosperm refute the view that endosperm is absent in Orchidaceae.

Webster (1887) observed only a small quantity of seeds in the capsules of *Epipactis latifolia* due to lack of fertilization in many ovules. In the same taxon Hagerup (1945) recorded parthenogenetic embryo in some seeds and zygotic embryo in others. However, in our material the process of fertilization, the development of embryo and the number of seeds formed per capsule are normal; parthenogenesis does not occur.

According to Johansen (1950) in the embryo of

*Epipactis palustris* the suspensor is absent or is represented by a single cell. In the present study the suspensor is absent, as all the derivatives of suspensor initial cell take part in the organisation of mature embryo. Like the embryo in most other previously investigated taxa, in *E. latifolia* the embryo lacks differentiation of meristems and organs (Veyret, 1974; Wirth and Withner, 1959). The embryogeny conforms to the Group 'A' or Asterad type of Swamy (1949 b).

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