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The Development of Male and Female Gametophytes in Two Indian Species of *Eriocaulon* L. (Eriocaulaceae)

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Development of male and female gametophytes in *Eriocaulon truncatum* Hamilt. and *E. robusto-brownianum* Ruhl. is described. The microsporangial wall consists of an endothecium, a middle layer and a glandular tapetum in addition to the epidermis. There is a single row of microspore mother cells in each of the microsporangia. The Pollen are 3-celled and spiraperturate at shedding. Embryo sac development conforms to the Polygonum type. The three antipodal cells fuse to form a conspicuous cyst. Soon after the secondary nucleus is formed in the embryo sac, the cyst breaks down and remains as a condensed deeply-stained body below the chalazal pole of the central cell. The previous work on these aspects has been compared and evaluated.

Key Words: Eriocaulaceae, Gametophytes, Development of Antipodal cyst

Introduction

Embryologically the Eriocaulaceae stand significantly apart from all other angiosperms in organizing an antipodal cyst during the development of embryo sac. Although there are 1500 species in the family, published data on the development of male and female gametophytes are available only for six species. Even amongst these, data on four are considered to be incomplete and erroneous (Arekal & Ramaswamy 1980). Investigations have been carried out in this laboratory (Arekal & Ramaswamy 1980, Ramaswamy & Arekal 1980a, b & c, Ramaswamy et al. 1981) with the aim of enlarging our knowledge on the embryology of the Indian and South American taxa of the Eriocaulaceae. The paper describes the development of male and female gametophytes of two species of *Eriocaulon*.

Materials and Methods

The material was collected from the following places of Karnataka State, India: (1) *E. truncatum* Hamilt. marshy fallow fields near Mudigere, Chickmagalur District; (2) *E. robusto-brownianum* Ruhl. marshy banks of small streams and rivulets around Abbe falls, Kodagu District. The voucher specimens have been deposited in the Herbarium of the Post-graduate Department of Botany, University of Mysore, Mysore. Flowering inflorescences at various stages of development were fixed in Formalin-acetic acid-alcohol

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(FAA) and later preserved in 70% ethanol. Individual male and female flowers of different stages of development were separated under a binocular dissecting microscope and were processed for microtomy as per the usual procedure. Serial sections were cut at 8–10 μm thick and stained with Heidenhain's iron alum—hematoxylin using erythrosin in clove oil as a counterstain.

Observations

Eriocaulon truncatum Hamilt.

Microsporangium and male gametophyte

The anther is tetrasporangiate. One or two hypodermal archesporial cells appear conspicuous by their large size and dense protoplasts at the site of young microsporangium, in cross section of very young anther (figure 1A–B). The archesporial cells undergo periclinal divisions producing the primary parietal and primary sporogenous layers. The primary parietal cells undergo a periclinal division producing two layers of cells of which the outer differentiates into the endothecium while the inner appears more conspicuous by dense contents (figure 1C). After a further periclinal division of cells of the inner layer, a tapetum and a middle layer are formed. The anther wall, therefore, consists of an epidermis, an endothecium, a middle layer and the tapetum (figure 1D). During later stages, the tapetal cells enlarge in size and their nuclei become prominent. These cells remain uninucleate (figure 1E–F). The middle layer gets crushed and absorbed. After providing nourishment to the developing pollen, the tapetal cells collapse and their remains could still be discerned in the older stages of the microsporangium. The endothecial cells acquire band-like radial thickenings on the inner side of their radial walls. The epidermal cells appear conspicuous by their large size and granular dark contents (figure 1J).

Meanwhile, the primary sporogenous cells undergo a series of transverse divisions and usually organise a row of sporogenous cells (figure 1C–D) which later give rise to microspore mother cells. Although their primary walls remain intact (figure 1E), these enlarge and their nuclei divide meiotically. Quadripartition of mother cells is of the successive type and the microspore tetrads are isobilateral (figure 1D–G). The microspores enlarge in size, separate and acquire a spherical shape. No vacuolisation of its cytoplasm occurs (figure 1H). The microspore nucleus moves toward one side and divides forming a small densely protoplasmic generative cell and a large tube cell. The generative cell enters into the cytoplasm of the tube cell and gives rise to two male gametes by a mitosis. By this time the adjacent microsporangia fuse together by the breakdown of the cells of the partition wall. The mature pollen grain is spherical and 3-celled. It has a thin intine and a thick minutely warty spiraperturate exine (figure 1I).

Megasporogenesis and female gametophyte

A hypodermal archesporial cell differentiates early in the ovular primordium. It is large, with dense cytoplasm and a prominent nucleus (figure 2A). It enlarges and keeps pace with the elongating ovule (figure 2B). Its nucleus located in the upper part undergoes the usual meiotic division. After meiosis I a transverse wall is laid down and the two dyad cells are formed (figure 2C–E). The nuclei of the dyad cells divide simultaneously. At the end of this division an obliquely T-shaped or a linear tetrad of megaspores is produced depending on the orientation of spindles of the dividing dyad nuclei (figure 2E–G). Soon the chalazal megaspore enlarges and its cytoplasm becomes vacuolate while the upper 3 degenerate (figure 2H). The nucleus of the functional megaspore undergoes a free nuclear division forming two daughter nuclei (figures 2I, 3A).

In one instance two 2-nucleate embryo sacs were observed in the same ovule (figure 3B). Usually the two nuclei of the 2-nucleate embryo sac move apart to the opposite poles when a central vacuole is formed in the cytoplasm. The two nuclei divide simultaneously and the embryo sac becomes 4-nucleate (figure 3C). After one more simultaneous free nuclear division the embryo sac attains the 8-nucleate stage, the nuclei being distributed in 2 quartets. Organization of the embryo sac then occurs. The micropylar quartet of nuclei contribute to the formation of a 3-celled egg apparatus and a micropylar polar nucleus, while the three elongated antipodal cells and the chalazal polar nucleus are formed from the chalazal quartet of nuclei (figure 3D). A prominent vacuole is present in the central cell. The embryo sac elongates and crushes the nucellar cells in the micropylar region.

The walls of the antipodal cells disintegrate and the protoplasts of the 3 cells unite to form a conspicuous antipodal cyst which extends into the central cell (and a reduction in size of the central cell occurs) and the two polar nuclei are brought closer (figure 3E). The three nuclei of the cyst become arranged in a linear row, enlarge and fuse to form a conspicuous elongated nucleus (figure 3F–I). Meanwhile the two polar nuclei unite to form the secondary nucleus. The central cell enlarges enormously and expands around the antipodal cyst. The mature embryo sac is spindle-shaped. It has an egg apparatus of two large, pear-shaped synergids and an egg, a large central cell lodging the secondary nucleus at its chalazal region and a pyriform antipodal cyst in the nucleus of which the three nucleoli still remain free (figure 3I).

Eriocaulon robusto-brownianum Ruhl.

Microsporangium and male gametophyte

A transection of a young anther is somewhat 4-angled (figure 4A). Each of its corners represents the site of a future microsporangium and it lodges 2 or 3 densely cytoplasmic, large-nucleated, hypodermal archesporial cells. These cells divide periclinally to produce the primary parietal and primary sporogenous layers. The former layer contributes 3 layers to the anther wall after further divisions, the innermost functioning as the tapetum (figure 4B–E). The tapetal cells have a granular cytoplasm and prominent nuclei. They enlarge but remain uninucleate and nourish the pollen mother cells and subsequently the pollen grains. During later stages of anther development the tapetum and middle layers are absorbed and the endothecium develops characteristic fibrous thickenings. Meanwhile dark contents accumulate in the epidermal cells and the anthers dehisce (figure 4K, L).

The cells of primary sporogenous layer give rise to a row of microspore mother cells. The microspore mother cells enlarge, round off and undergo meiotic divisions to produce isobilateral tetrads of microspores (figure 4E, F). Quadripartition of the microspore mother cells is of the successive type. The microspores enlarge, become spherical and separate. A prominent central vacuole appears in the cytoplasm and pushes the microspore nucleus to the periphery (figure 4G). The nucleus divides and cytokinesis follows, resulting in a small generative cell and a large vegetative cell. The former separates itself from the microspore wall and moves into the cytoplasm of vegetative cell (figure 4H). The generative cell divides mitotically to form 2 male gametes. The pollen grains at the time of shedding are 3-celled with a thin intine and a thick spiraperturate, minutely spinescent exine (figure 4 I–J).

Megasporogenesis & the female gametophyte

A hypodermal archesporial cell is organised very early in the ovular primordium, elongates

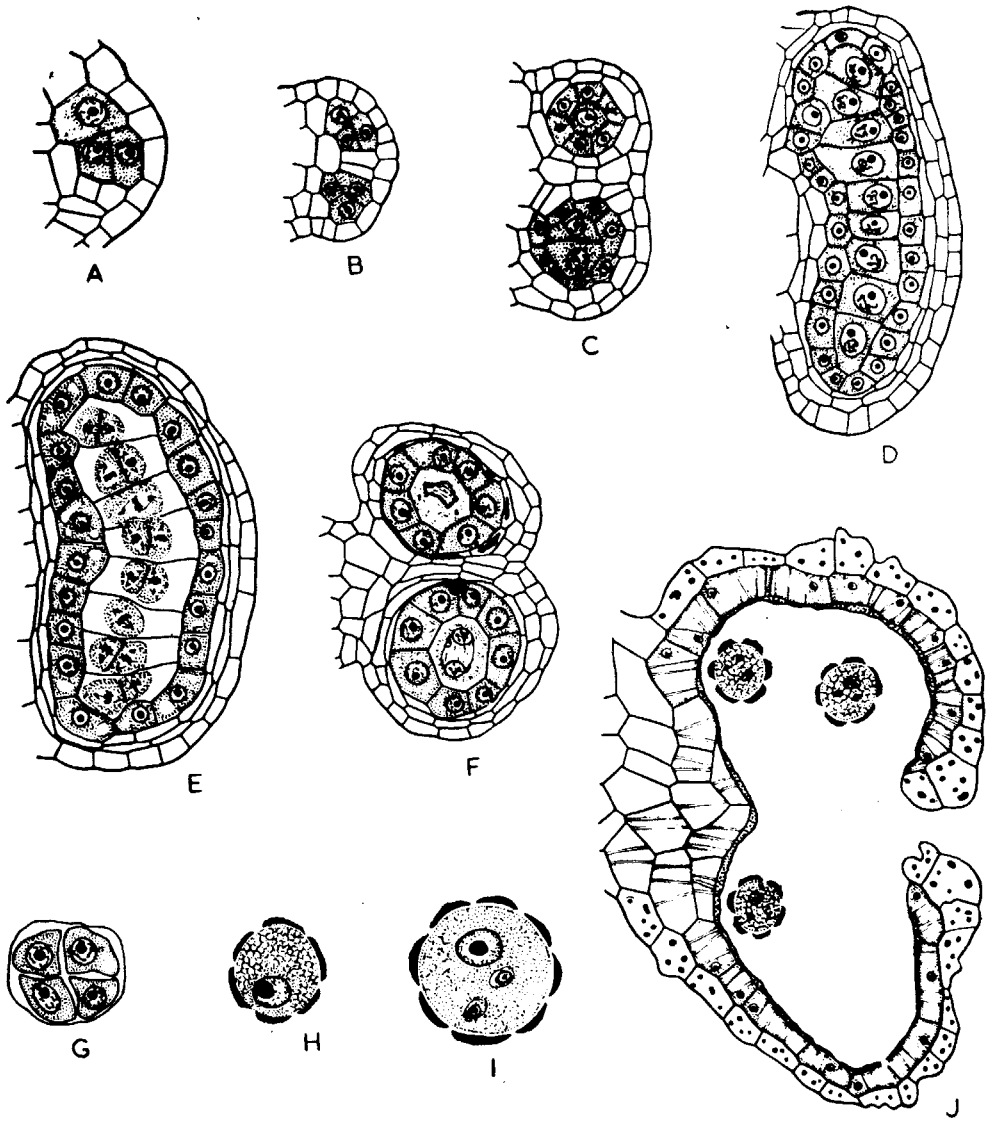

Figure 1 A–J Microsporangium and male gametophyte development in *Eriocaulon truncatum*; *A, B,* transverse sections of anther lobes to show organization of primary parietal and primary sporogenous layers in the developing microsporangia. [*A* (× 600), *B* (× 500)] *C, D,* t. s. and l. s. of anther lobes showing the 4-layered anther wall (× 500); *E–G,* show stages in the organization of Microspore tetrad; [*E, F,* (× 500); *G,* (×1000)]. *H, I,* show stages in the development of a 3-celled pollen grain from microspore (× 1000); *J,* t. s. of a mature anther lobe enlarged to show structural details. (× 400)

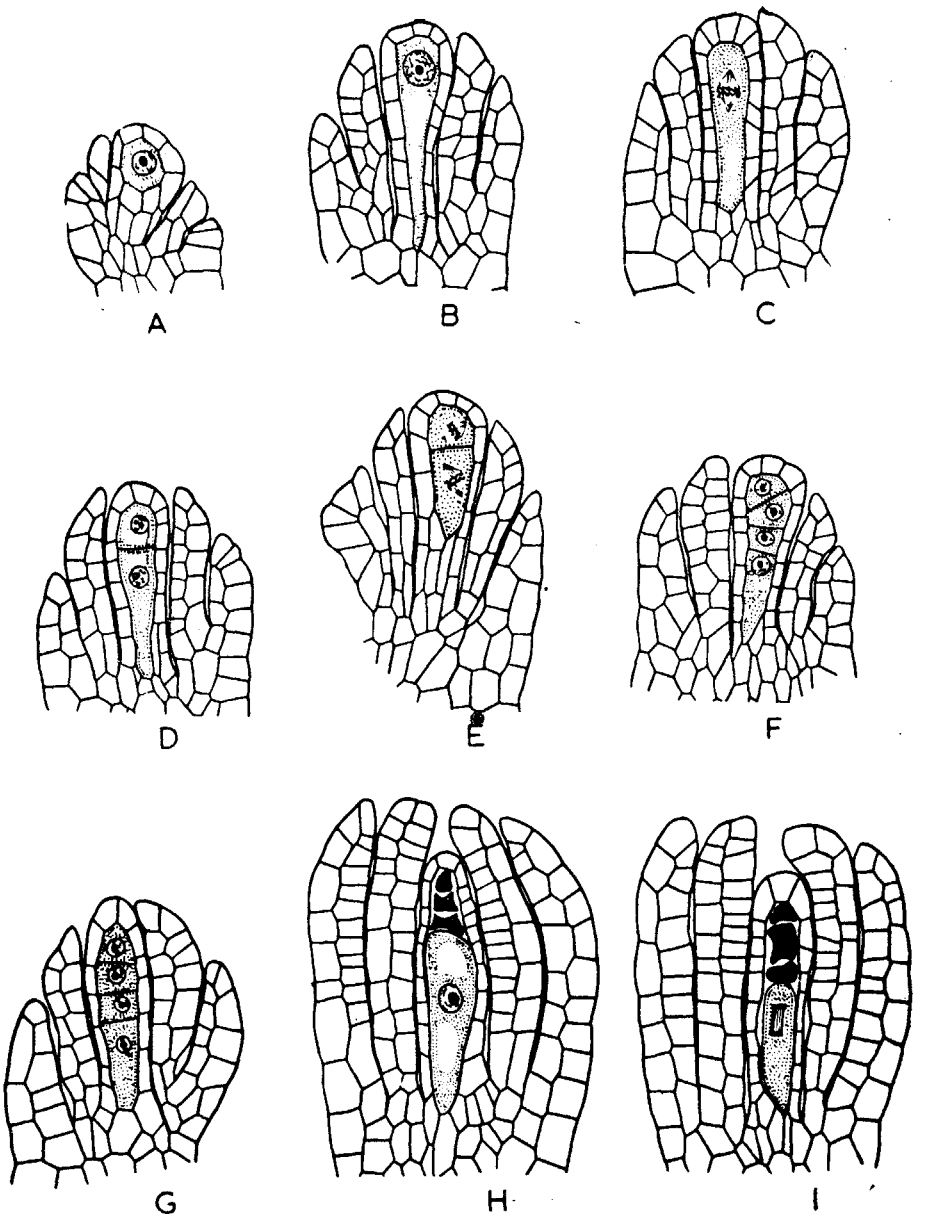

Figure 2 A Megasporogenesis in *E. truncatum* (× 750); *A*, ovular primordium containing a hypodermal archesporial cell; *B*, megaspore mother cell; *C*; nuclear division in megaspore mother cell; *D*, dyad cells; *E*, division of dyad cells; *F*, *G*, oblique T-shaped and linear megaspore tetrads, respectively; *H*, functional megaspore of a linear tetrad; *I*, nuclear division in the fucnctional megaspore

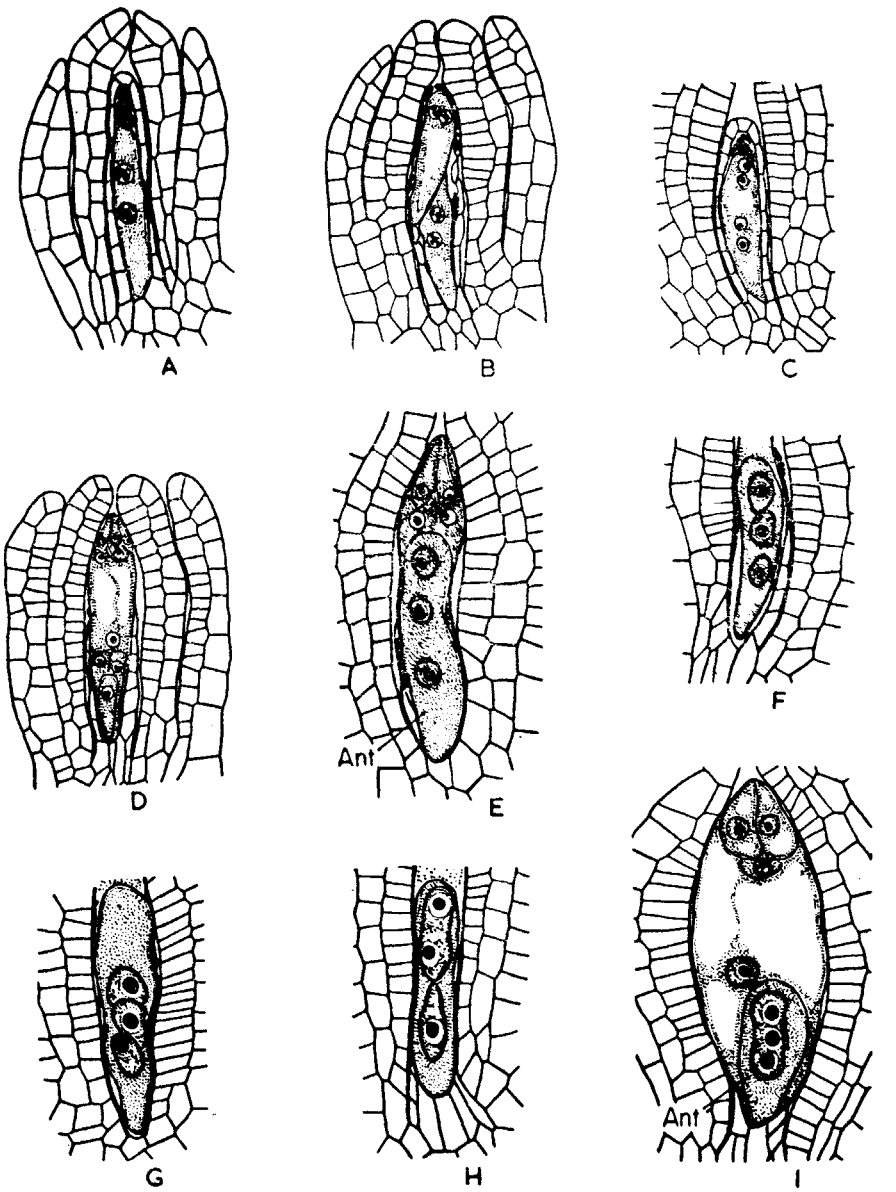

Figure 3 A–F Female gametophyte development in *E. truncatum* (× 500) (**Ant**, antipodol cyst). *A*, an young 2-nucleate embryo sac; *B*, double 2-nucleate embryo sacs; *C*, 4-nucleate embryo sac; *D*, organized embryo sac; *E*, 8-nucleate embryo sac with an elongated antipodal cyst; *F, G*, basal parts of embryo sac with superposed antipodal nuclei in the cyst; *H*, antipodal cyst showing three fusing nuclei; *I*, mature embryo sac; note polyploid antipodal nucleus in the cyst

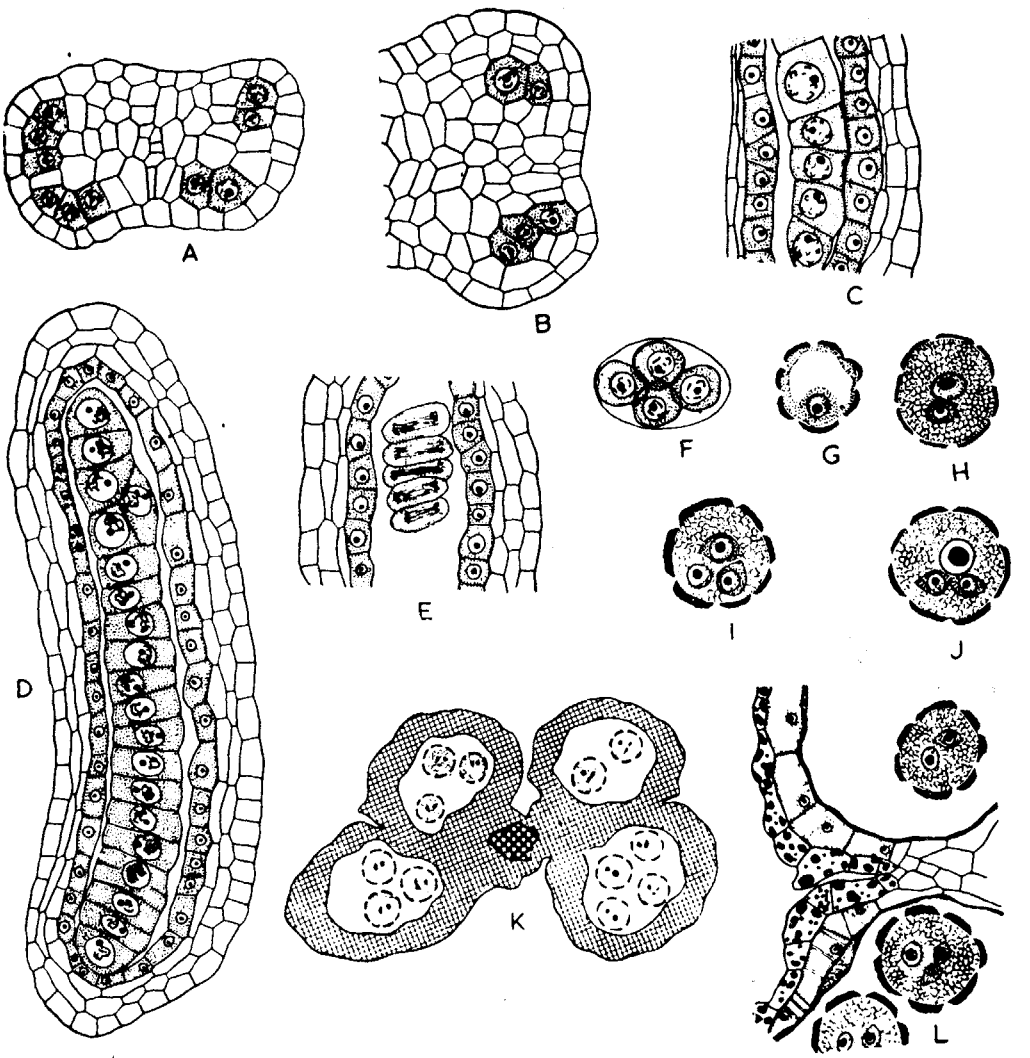

Figure 4 A–L Microsporanguim and male gametophyte development in *Eriocaulon robusto-brownianum* *A*, t.s. of an young anther showing 2–3 hypodermal archesporial cells in each developing microsporangium (×500); *B*, t.s. of anther lobe slightly at an older stage than that of Figure A(×500); *C*, *D*, part and whole longitudinal sections of microsporangia respectively showing a 4-layered anther wall surrounding spore mother cells (×500); *E*, *F*, stages in the organization of a microspore tetrad [*E*(×500), *F*(×1000)]; *G–J*, stages in the development of 3-celled pollen grain from microspore (×1000); *K*, t.s. of old anther, semidiagrammatic. (×200); *L*, t.s. of a part of the anther lobe at stomium region to show structural details (×400)

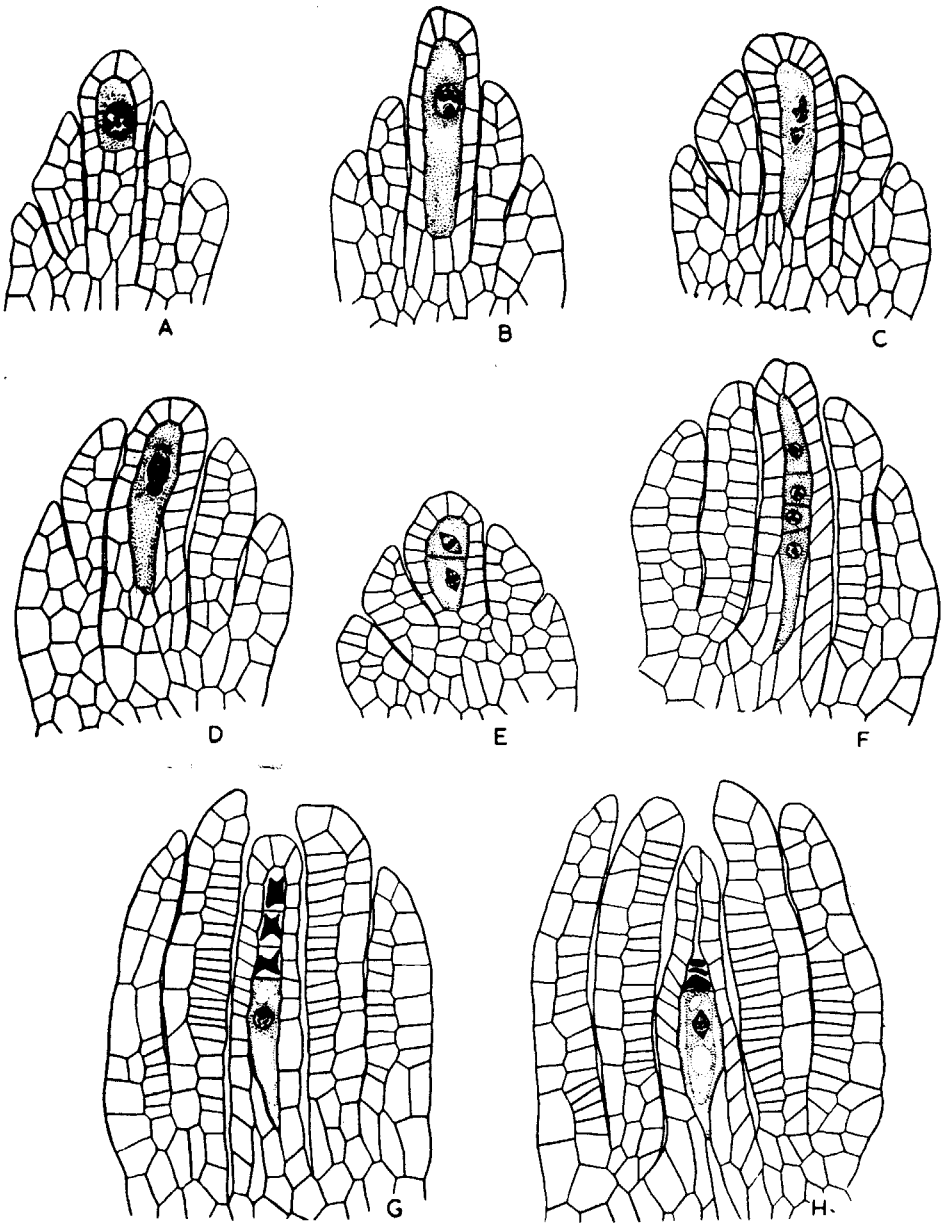

Figure 5 A–H Megasporogenesis in *E. robusto-brownianum* (×500). *A*, an ovular primordium containing a hypodermal archesporial cell; *B*, a megaspore mother cell; *C*, *D*, nuclear division in megaspore mother cells; *E*, nuclear division in dyad cells, F, A Livear tetrad of megaspores; *G*, functional megaspore of a linear tetrad; *H*, nuclear division in the functional megaspore

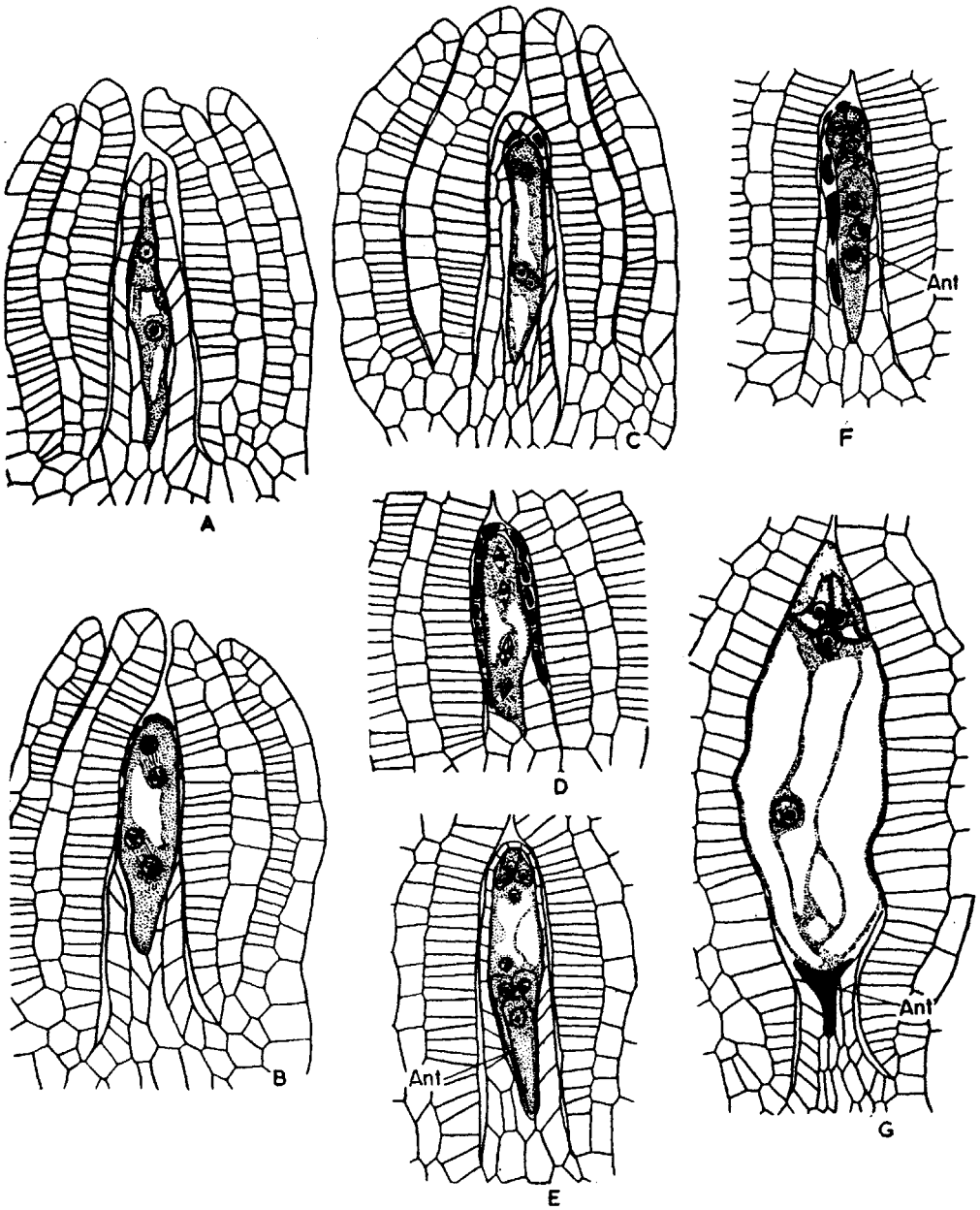

Figure 6 A–G Female gametophyte development in *E. robusto-brownianum*, [*A–F* (×500), *G*, (×400). (**Ant**, antipodal cyst *G* and *F*). *A*, 2-nucleate embryo sac; *B–E*, stages in the organization of an 8-nucleate embryo sac; *F*, 8-nucleate embryo sac with the antipodal cyst extended towards the micropylar part; *G*, mature embryo sac; note the enlargement and elongation of the central cell and degenerated antipodal cyst

considerably and functions directly as the megaspore mother cell (figure 5 A–B).

The megaspore mother cell directly gives rise to a linear tetrad of megaspores (figure 5 C–F). The upper three megaspores of the tetrad degenerates and the chalazal one functions (figure 5G, H). Although these developmental changes are going on, the ovules sometimes remain small in size (figure 5E). The functional megaspore becomes vacuolate and enlarges. It undergoes a free nuclear division and the two-nucleate embryo sac thus produced elongates and becomes spindle-shaped (figure 6A). The 2 nuclei gradually move apart to opposite poles as the central vacuole increases in size. After two more free nuclear divisions an 8-nucleate embryo sac is produced (figure 6B–D) the nuclei being disposed in 2 quartets. During subsequent organisation of embryo sac, the 3 nuclei of the micropylar quartet contribute to the egg apparatus consisting of an egg and 2 synergids, while 3 of the chalazal quartet take part in the organisation of antipodal cells. The remaining nuclei of both the quartets function as the two polars (figure 6E).

The three pyriform antipodal cells are larger than the cells of the egg apparatus. Even before the fusion of the polar nuclei, the walls of antipodal cells disorganise and their protoplasts fuse to form a very conspicuous, densely cytoplasmic cyst as observed in *Eriocaulon truncatum*. The antipodal cyst elongates towards the micropyle pushing the chalazal polar nucleus close to the egg apparatus (figure 6F). The three nuclei in the cyst fuse together to form a large irregular triploid nucleus.

The two polar nuclei fuse to form the secondary nucleus. The nucellar cells enveloping the micropylar half of the embryo sac become crushed by the expansion of gametophyte.

A marked change occurs within the embryo sac after the formation of the secondary nucleus. The central cell enlarges enormously (figure 6G) and concomitantly, the antipodal cyst degenerates. The cyst can be seen as a dark mass (figure 6G).

The mature embryo sac (figure 6G) is large, spindle-shaped and lies in direct contact with the inner layer of the inner integument. The egg apparatus consists of 2-curved synergids with basal vacuoles and a pear-shaped egg. The central cell is conspicuously vacuolate and lodges the secondary nucleus in the centre.

Discussion

In almost all the investigated members of the Eriocaulaceae the basic structure of the anther wall is uniform and comprises the epidermis, a fibrous endothecium, a middle layer and the glandular tapetum. Previous errors in the reports on the mode of development of the wall layers and the pollen (Smith 1910, Patel & Patel 1964, Begum 1968) have been pointed out by Arekal and Ramaswamy (1980).

As in the other investigated taxa (Smith 1910, Patel & Patel 1964, Begum 1968, Monteiro-Scanavacca & Mazzoni 1978, Arekal & Ramaswamy 1980), a transection of a very young anther is invariably 4-lobed and each lobe lodges a hypodermal archesporium. The number of rows of archesporial cells is 1 or 2 in *E. truncatum*. *E. robusto-brownianum* is more like *E. setaceum* (Ramaswamy & Arekal 1980a) in having 3 or 2 rows of archesporial cells. The occurrence of a massive archesporium such as reported for *E. cinereum* by Patel and Patel (1964) was not observed by us.

We found that irrespective of the number of the initial rows of archesporial cells in the young microsporangia, invariably only one row of microspore mother cells is eventually organised as also noted in *E. setaceum* and *E. xeranthemum* (Ramaswamy & Arekal

1980a, 1980c). However, Patel and Patel (1964) have observed two rows of microsporocytes.

Our findings show that microsporogenesis is successive and the tetrads are isobilateral as in the other investigated taxa of the Eriocaulaceae (Smith 1910, Patel & Patel 1964, Monteiro-Scanavacca & Mazzoni 1978, Arekal & Ramaswamy 1980, Ramaswamy & Arekal 1980a, c). Organisation of a large vacuole within the cytoplasm of the enlarging microspore pushing the nucleus to a side such as observed in *E. robusto-brownianum* has also been a regular feature in all the investigated species of *Eriocaulon* except in *E. truncatum*, where, such a vacuole is not organised.

The two species resemble certain other investigated taxa as regards pollen development and mode of anther dehiscence (Begum 1968, Arekal & Ramaswamy 1980, Ramaswamy & Arekal 1980a, c).

In all the investigated taxa of the Eriocaulaceae, the gynoecium is tricarpellary, syncarpous, trilocular with three pendulous, bitegmic and tenuinucellate ovules on an axile placenta. The differentiation of a hypodermal archesporial cell and its direct development into a megaspore mother cell is uniform in the entire family. But in the subsequent development and organisation of the embryo sac, there are slight variations. The megaspore mother cell in *E. truncatum* is nearly club-shaped as in *E. xeranthemum* (Ramaswamy & Arekal 1980c). In *E. robusto-brownianum*, however, it is almost cylindrical. After the usual meiotic divisions, a megaspore tetrad is organised. The spore tetrad is consistently linear in *E. robusto-brownianum* as in *E. septangulare* (Smith 1910) and *E. xeranthemum* (Ramaswamy & Arekal 1980c). Both linear and obliquely T-shaped megaspore tetrads occur in *E. truncatum*. Such a feature has also been recorded for *E. setaceum* (Ramaswamy & Arekal 1980a). However, *E. hookerianum* is more like *E. cinereum* (Patel & Patel 1964) and *E. quinquangulare* (Begum 1968) in producing both T-shaped and linear tetrads of megaspores.

The taxa so far investigated show Polygonum type of embryo sac development. Occasionally two megaspores of a tetrad show signs of further development in *E. hookerianum* although they do not develop into embryo sacs (Arekal & Ramaswamy 1980). In *E. truncatum*, the rare occurrence of double 2-nucleated embryo sacs has been observed in the same ovule, evidently being derived from 2 megaspores of a single tetrad.

An unusual feature in *Eriocaulon* is the organisation of a very conspicuous antipodal cyst. The arrangement and behaviour of the 3 nuclei in the cyst varies (see Ramaswamy & Arekal 1980b). In *E. truncatum* two nuclei fuse first and the third follows suit. This is quite similar to the state noted in *E. xeranthemum* (Ramaswamy & Arekal 1980c). On the other hand, all the 3 nuclei may fuse simultaneously forming a triploid, hypertrophied nucleus in *E. robusto-brownianum* as in *E. quinquangulare* (Begum 1968) and *E. hookerianum* (Arekal & Ramaswamy 1980). In both the taxa studied presently the cyst occupies 2/3 the space of the embryo sac and is very prominent. Although their figure 5 reveals the existence of an antipodal cyst in *Leiothrix fluitans*, Monteiro-Scanavacca and Mazzoni (1978) failed to recognize it.

No sooner the secondary nucleus is organised in the embryo sac, the central cell starts extending towards the chalazal pole. The cyst degenerates and is pushed down by the elongating central cell. A similar feature has also been recorded for *E. hookerianum* (Arekal & Ramaswamy 1980), *E. setaceum* and *E. xeranthemum* (Ramaswamy & Arekal 1980a, c). Such an unusual behaviour of antipodal cells recorded in this study (also see Arekal & Ramaswamy 1980, Ramaswamy & Arekal 1980b) namely loss of cell walls,

organisation of a cyst, enlargement of its nuclei before their subsequent fusion to form a polyploid nucleus and the final breakdown of the nucleus along with the surrounding cytoplasm has not been reported so far in any of the angiosperms investigated.

References

Arekal G D and Ramaswamy S N 1980 Embryology of *Eriocaulon hookerianum* Stapf and the systematic position of Eriocaulaceae; *Bot. Notiser* **133** 295–309

Begum M 1968 Embryological studies in *Eriocaulon quinquangulare* Linn.; *Proc. Indian Acad. Sci.* **B67** 148–156

Monteiro-Scanavacca W R and Mazzoni S C 1978 Embryological studies in *Leiothrix fluitans* (Mart.) Ruhl. (Eriocaulaceae); *Revista. brasil. Bot.* **1** 59–64

Palm B 1920 Preliminary notes on pollen development in tropical monocotyledons; *Svensk bot. Tidskr.* **14** 261–266

Patel C M and Patel D S 1964 The morphological and embryological studies in *Eriocaulon cinereum* R. Br.; *J. Gujarat Univ.* **7** 58–70

Ramaswamy S N and Arekal G D 1980a Embryology of *Eriocaulon setaceum* L.; *Pl. Syst. Evol.* **135** (In Press)

_____1980b On the behaviour of antipodals in Eriocaulaceae; *Caryologia*

_____1980c Embryology of *Eriocaulon xeranthemum* Mart. (Eriocaulaceae); *Acta Bot.* Neerl. **29** (In Press)

Ramaswamy S N, Swamy B G L and Arekal G D 1981 From zygote to seedling in *Eriocaulon robusto-brownianum* Ruhl. (Eriocaulaceae); *Beiter. Biol. Pflanzen* **554** 179-188

Smith R W 1910 The floral development and embryogeny of *Eriocaulon septangulare*; *Bot. Gaz.* **49** 281–289