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EMBRYOLOGY OF ERIOCAULON XERANTHEMUM MART. (ERIOCAULACEAE)

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SUMMARY

The development of microsporangium, male and female gametophytes, endosperm, embryo and seed-coat of *Eriocaulon xeranthemum* Mart. has been studied.

The microsporangium wall consists of an epidermis, an endothecium, a middle layer and a glandular tapetum. The endothecium is fibrous. Quadripartition in the microspore mother cells is successive. At shedding the pollen grains are 3-celled and spiraperturate.

The female gametophyte is of the *Polygonum* type. The three antipodal cells give rise to a conspicuous antipodal cyst, which breaks down before fertilization. The endosperm is *ab initio* nuclear. Both apical and basal cells contribute to the formation of the embryo proper. In the mature seed, the embryo is small with an incipient internal differentiation into cotyledonary and epicotylary sectors. Both integuments contribute to the seed coat. The pericarp is 3-layered, thin-walled and membranous.

1. INTRODUCTION

Recent embryological studies in the Eriocaulaceae have demonstrated the formation of an antipodal cyst during the organization of the female gametophyte, a feature which has never been observed in any of the angiosperms so far investigated (AREKAL & RAMASWAMY 1980; RAMASWAMY & AREKAL 1980b). The genus *Eriocaulon* L. includes 472 species distributed in several parts of the world under varied climatic conditions and habitats (MOLDENKE 1971). So far, in literature, embryological data are available of only four species. The earlier investigations of SMITH (1910), PATEL & PATEL (1964) and BEGUM (1968) do not appear to give an accurate account. The recent work of AREKAL & RAMASWAMY (1980) is the only account which provides a clear picture of the embryology of the genus. The present study is an attempt to enlarge our knowledge of the reproductive biology of this unique genus. It deals with the development of microsporangium, male gametophyte, megasporangium, female gametophyte, endosperm, embryo and seed-coat structure of *Eriocaulon xeranthemum*.

2. MATERIAL AND METHODS

Eriocaulon xeranthemum Mart. is a widely distributed taxon in India, from East to West and from North to South. The material for the present study included very young, flowering, and fruiting capitula. They were collected from plants growing along the marshy regions of Kottigehar, Chikkamagalur District, Karnataka State and fixed in F.A.A. The voucher specimens are deposit-

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ed in the herbarium of the Department of Botany, University of Mysore, Mysore. Whole capitula as well as individual flowers were processed for microtomy following the established xylol-ethanol series. Sections were cut at 8–12 microns thick and stained with Heidenhain's iron alum hematoxylin using erythrosin as a counter stain.

3. OBSERVATIONS

3.1. Microsporangium and male gametophyte

The anther is tetrasporangiate. A cross section of a very young anther appears nearly rectangular and in each of the angles lodges the young microsporangium. Usually two rows of hypodermal archesporial cells become conspicuous early and initiate the microsporangium *(fig. 1 A)*. A periclinal division of the archesporial cells results in the organization of primary parietal and primary sporogenous layers *(fig. 1 B)*. By further periclinal divisions in the primary parietal layer a glandular tapetum, a middle layer and the endothecium are organized *(fig. 1 C, D)*. The tapetal cells enlarge in size and acquire dense protoplasts. They continue to remain uninucleate throughout their existence. After providing nourishment to the microspore mother cells and pollen grains, the entire tapetum breaks down along with the middle layer and in the mature microsporangium it is only discernible as a granular layer *(fig. 1 L, J)*. Meanwhile, the endothecial cells enlarge, become conspicuous and acquire band-like thickenings. The epidermal cells accumulate deepstaining, large, granular substances *(fig. 1 J, K)*.

Although the primary sporogenous layer begins with two juxtaposed rows of cells, only a single vertical row of sporogenous cells finally remains *(fig. 1 D)*. These cells finally function as spore mother cells and undergo the usual meiotic divisions producing isobilateral tetrads of microspores *(fig. 1 E-F)*. Quadripartition of the microspore mother cells is of the successive type. The microspores enlarge, separate and become spherical. A conspicuous central vacuole appears in the cytoplasm of each spore pushing the nucleus to one side *(fig. 1 G)*. Division of the microspore nucleus is followed by the formation of a wall delimiting a densely protoplasmic generative cell and a large vegetative cell. The generative cell separates itself from the microspore wall and enters into the cytoplasm of the vegetative cell *(fig. 1 H)*. A mitotic division of the generative cell results in the production of two male gametes. The two adjacent microsporangia coalesce and release of pollen occurs by a longitudinal opening of the anther wall under the influence of the fibrous endothecium. At shedding the pollen grains are spherical, 3-celled and possess a thin intine and a thick, finely echinulate exine with spiral apertures *(fig. 1 I)*.

3.2. Megasporogenesis and female gametophyte

The young ovular primordium organises a hypodermal archesporial cell very early. It enlarges and functions directly as the megaspore mother cell *(fig. 2 A-B)*. Its nucleus located at the upper end undergoes meiosis I and two un-

equal dyad cells result *(fig. 2 C-D)*. The dyad cells then divide transversely and give rise to a linear tetrad of megaspores *(fig. 2 D-E)*. The chalazal megaspore functions and the others degenerate *(fig. 2 F)*. After a free nuclear division in the functional megaspore the two resulting daughter nuclei move apart to opposite poles and divide again, thus producing a 4-nucleate embryo sac *(fig. 2 G-H)*. An 8-nucleate embryo sac is formed after the third simultaneous, free nuclear division in the functional megaspore. Meanwhile, the embryo sac elongates towards the micropyle, destroying the nucellar cells at that region, and comes in direct contact with the integument. Cellular organization in the embryo sac sets in. The four nuclei of the micropylar region contribute to the formation of a 3-celled egg apparatus and the micropylar polar nucleus, while the four nuclei at the chalazal end take part in the production of three antipodal cells and the chalazal polar nucleus. The antipodal cells are larger in size compared to the cells of the egg apparatus. The two polar nuclei meet near the antipodal cells *(fig. 2 I)*. Soon afterwards, marked changes occur in the antipodal cells. Their walls break down and their cytoplasms coalesce. The three nuclei become serially arranged and enlarge. They meet as a linear row and gradually fuse to form an elongated, lobed, triploid nucleus *(fig. 3 A-D)*. Usually the upper two nuclei of the row fuse first and subsequently the lower one is added *(fig. 3 C, E)*. The coalesced cytoplasts, meanwhile, become very dense and the surface hardens, thus clearly demarcating the upper central cell from the lower part, and a very conspicuous antipodal cyst becomes established. During its organization the cyst extends towards the egg apparatus and occupies two thirds of the space of the embryo sac, thus reducing the size of the central cell. The polar nuclei which were lodged farther from the egg apparatus, are now brought very near to it *(fig. 2 I; 3 E)*. Then the two polar nuclei fuse to form the secondary nucleus. The formation of the secondary nucleus immediately brings about significant changes within the embryo sac. A downward elongation of the central cell begins. This is also associated with the increase in its size. As a consequence, the length of the antipodal cyst is reduced, and at this stage the triploid nucleus appears to occupy nearly all the space within the cyst *(fig. 3F)*. Finally the cyst degenerates and looks like an opaque, deeply stained mass lying at the chalazal end of the female gametophyte *(fig. 6 A)*.

The mature embryo sac is spindle-shaped and is enveloped by the inner layer of the inner integument, except at the chalazal end. The egg apparatus consists of two pear-shaped, comparatively large synergids and an egg cell. The secondary nucleus is larger and lies above the degenerated antipodal cyst *(fig. 3 F)*.

3.3. Endosperm

Fertilization takes place by porogamy. The primary endosperm nucleus (located at the chalazal end) divides much earlier than the zygote. No cell wall is laid down after this division. The two daughter nuclei produced undergo a series of divisions. Only the first few divisions are simultaneous. Accumulation of additional nuclei and cytoplasm occurs more strongly at the chalazal part than at the micropylar region. A large central vacuole is present in the endosperm.

Cell wall formation in the endosperm begins at the chalazal part and extends upwards. After the first phase of wall formation is completed, a larger amount of endosperm is noticeable at the chalazal part than in the micropylar region *(fig. 4)*. Further growth of endosperm proceeds centripetally by cell divisions. Ultimately the entire embryo sac becomes filled with endosperm cells. A major portion of the endosperm is made up of very large, isodiametric cells and the chalazal portion consists of smaller, densely protoplasmic cells *(fig. 5 B)*. The cells of the outermost layer of endosperm are elongated, narrow and densely protoplasmic. As the seed ripens, the large isodiametric endosperm cells accumulate large quantities of starch, while the densely protoplasmic, peripheral and chalazal cells contain less starch *(fig. 5 A, B)*. Only a very small part of the endosperm is made use of by the embryo in the ripe seed.

3.4. Embryo

The zygote is generally spherical. It enlarges and divides transversely engendering an apical cell *ca* and a basal cell *cb (fig. 6 A, B)*. Soon the apical cell undergoes a vertical division to produce two juxtaposed cells. Subsequently a similar division occurs in the basal cell *(fig. 6 C)*. Periclinal divisions in the two cell tiers delimit the protoderm from an inner group of cells *(fig. 6 D)*. From now on, growth activity is confined to the derivatives of the apical cell; even here cell divisions are restricted to one half of the hemisphere (single hatched in the text figures). As a result, the latter sector acquires a larger number of cells while the former remains relatively quiescent (the nucleated cells in *fig. 6 E-F)*. This step in embryogenesis signifies the activity of the cotyledonary sector, the quiescent sector representing the locus of differentiation of the epicotyl.

The rapidity of cell divisions in the cotyledonary sector spreads to the subadjacent cell tiers. Although exomorphic symmetry is maintained by the developing embryo, this mitotic activity causes a pronounced asymmetry between the cotyledonary and epicotylary sectors as is seen in longitudinal sections passing through the epicotyl-cotyledonary axis *(fig. 6 G. I)*. An internal symmetry parallel to the external one can be demonstrated in longitudinal sections passing through the para-cotyledonary planes *(fig. 6 H)*. In the ripe seed the embryo does not exhibit any histological differentiation of radicle or hypocotyl. The cotyledonary sector does not grow beyond the level of the epicotyledonary locus and its further development only takes place during germination (RAMASWAMY et al. 1981).

3.5. Ovule, seed coat and pericarp

The mature ovule is orthotropous, tenuinucellate and bitegmic. The inner integument alone organizes the micropyle *(fig. 7 A)*. Both integuments are two-layered. The cells of the inner layers of both integuments are shorter than those of the outer layers *(fig. 7 B)*. During the post-fertilization stages, the inner layers of both integuments conspicuously enlarge in size. The outer layers become vertically stretched and appear to be thin. Further, the inner tangential walls of the inner layer of the inner integument, the inner tangential and radial walls

of the inner layer of the outer integument become thick. The thickening is more pronounced in the radial walls of the inner layer of cells of the outer integument. Meanwhile, tanniniferous materials accumulate in copious quantities within the inner layer of cells of the inner integument, while large vacuoles and scanty cytoplasm are conspicuous in the inner layer of the outer integument *(fig. 7 D F)*. The outer cell layers of both integuments gradually become obliterated as the seeds get ready for dispersal.

The ovary wall is made up of three layers of vertically elongated, densely protoplasmic and vacuolate cells *(fig. 7 B)*. During fruit formation the cells enlarge slightly but elongate further and none of the layers either degenerates or acquires fibrous thickenings *(fig. 7 D-F)*. The pericarp, therefore, remains as a thin membrane of 3 layers of cells.

4. DISCUSSION

The development of the microsporangium in the present study is essentially similar to that of other investigated Eriocaulaceae. Nevertheless, in the occurrence of two rows of archesporial cells, *E. xeranthemum* is nearer to *E. cinereum* (PATEL & PATEL 1964) and *E. setaceum* (RAMASWAMY & AREKAL 1980a) than to the other taxa (SMITH 1910; BEGUM 1968; AREKAL & RAMASWAMY 1980) in which a single row of archesporial cells is the rule. The general mode of organization of the microsporangial wall in the present study conforms to the monocotyledonous type (DAVIS 1966). The presence of a fibrous endothecium and the 3-celled pollen at shedding noted in *E. xeranthemum* also hold true for *E. septangulare* (SMITH 1910), *E. cinereum* (PATEL & PATEL 1964), *Leiothrix fluitans* (MONTEIRO-SCANAVACCA & MAZZONI 1978) and *E. hookerianum* (AREKAL & RAMASWAMY 1980). These features appear to be well-established embryological characters within the family (RAMASWAMY 1975).

The female gametophyte development of *E. xeranthemum* conforms to the Polygonum type (MAHESHWARI 1950) as in other species of Eriocaulaceae so far studied (SMITH 1910; PATEL & PATEL 1964; BEGUM 1968; MANTEIRO-SCANAVACCA & MAZZONI 1978; AREKAL & RAMASWAMY 1980 and RAMASWAMY & AREKAL 1980a). The organization of an antipodal cyst such as noted in the present study is similar to the one recorded for *E. mysorense* and *E. hookerianum* (AREKAL & RAMASWAMY 1968, 1980) and *E. setaceum* (RAMASWAMY & AREKAL 1980a).

The endosperm in *E. xeranthemum* is *ab initio* nuclear. The primary endosperm nucleus is located at the chalazal end as in the other taxa of the family so far examined. After the first few free nuclear divisions, there is an accumulation of a large number of nuclei at the chalazal part of the embryo sac as in *E. quinquangulare* (BEGUM 1968) and *E. hookerianum* (AREKAL & RAMASWAMY 1980). In *E. cinereum* (PATEL & PATEL 1964) and *E. setaceum* (RAMASWAMY & AREKAL 1980a), on the other hand, there is a uniform distribution of nuclei within the peripheral cytoplasm of the developing endosperm. Cell wall formation in the endosperm of the present species starts at the chalazal end and gra-

dually the wave extends to the micropylar part as in *E. hookerianum* (AREKAL & RAMASWAMY 1980) and *E. setaceum* (RAMASWAMY & AREKAL 1980a). In *E. septangulare,* however, wall formation starts from both the chalazal and the micropylar end and ceases towards the middle region of the embryo sac (SMITH 1910). After the initial wall formation is complete, three regions can be recognized in the endosperm of *E. xeranthemum*, a greater amount of tissue being found at the chalazal region, a character which has also been recorded in *E. hookerianum* (AREKAL & RAMASWAMY 1980). Histological differences found in the fully developed endosperm of *E. xeranthemum* such as the densely protoplasmic chalazal part with smaller cells virtually without starch and a peripheral layer of endosperm with less starch, whereas the rest of the endosperm tissue is made up of larger cells very rich in starch, are features not recorded for *E. cinereum* (PATEL & PATEL 1964), *Leiothrix fluitans* (MANTEIRO-SCANAVACCA & MAZZONI 1978) and *E. quinquangulare* (BEGUM 1968), and only comparable to those in *E. septangulare* (SMITH 1910), *E. hookerianum* (AREKAL & RAMASWAMY 1980) and *E. setaceum* (RAMASWAMY & AREKAL 1980a).

The development and organization of the embryo is rather similar to that of *E. robusto-brownianum* (RAMASWAMY et al. 1981) and *E. setaceum* (RAMASWAMY & AREKAL 1980a). Although in the ripe seed the embryo is bell-shaped without any exomorphic differentiation, as in all the Eriocaulaceae studied so far, there is a clear indication of ontogenetic and histological differentiation of epicotyledonary and cotyledonary sectors, a feature which has been overlooked by earlier workers.

The seed coat of *E. xeranthemum* is derived from both integuments, but the principal contribution is by the inner layers of the two integuments alone. The inner layer of the inner integument acquires heavy thickenings on its inner tangential walls and its cells become filled with tannin-like substance. Despite the outer layers of cells of the integuments being thin-walled in *E. xeranthemum* as in *E. hookerianum* (AREKAL & RAMASWAMY 1980), the outer tangential walls of the inner layer of the outer integument do not become so conspicuously thickened as they are in *E. setaceum* (RAMASWAMY & AREKAL 1980a). In *E. hookerianum* (AREKAL & RAMASWAMY 1980), the heavy thickenings in the inner layer of the outer integument are limited to the inner tangential walls. Heavy lignification of outer tangential walls of cells of the outer cell-layer of the inner integument recorded by BEGUM (1968) for *E. quinquangulare* and by MONTEIRO-SCANAVACCA & MAZZONI (1978) for *Leiothrix fluitans* respectively, has not been observed in the present study of *E. xeranthemum*. Therefore, there are differences in the finer histological features of the seed coat among members of the Eriocaulaceae.

The development of the pericarp of *E. xeranthemum* begins with three layers of vertically elongated cells as in *E. hookerianum* (RAMASWAMY & AREKAL 1980a) and *E. setaceum* (RAMASWAMY & AREKAL 1980a). Nevertheless, in its final organization it resembles more closely *E. setaceum* (AREKAL & RAMASWAMY 1980) in which the inner layer of the pericarp does not develop band-like thickenings unlike *E. quinquangulare* (BEGUM 1968), *Leiothrix fluitans* (MON-

TEIRO-SCANAVACCA & MAZZONI 1978) and *E. hookerianum* (AREKAL & RAMASWAMY 1980) in which such thickenings are prominently observed.

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REFERENCES

AREKAL, G. D. & S. N. RAMASWAMY (1968): Megasporogenesis and development of the female gamtophyte in Eriocaulon mysorense Fyson, *Proc. 55th Indian Sci. Congr.* Hyderabad, **3**: 338.

— & — (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.* B. **67**: 00–00.

DAVIS, G. L. (1966): *Systematic Embryology of Angiosperms*, New York.

MAHESHWARI, P. (1950): *An introduction to the Embryology of Angiosperms*, New York.

MOLDENKE, H. N. (1971): *A fifth summary of Verbenaceae, Avicenniaceae, Stilbaceae, Dicrastylidaceae, Symphoremaceae and Eriocaulaceae of the world.* **1**: 273–276; **2**: 745–749. New Jersey.

MONTEIRO-SCANAVACCA, W. R. & S. C. MAZZONI (1978): Embryological Studies in Leiothrix fluitans (Mart.) Ruhl. (Eriocaulaceae) *Revt. brasil. Bot.* **1**: 59–64.

PATEL, C. M. & D. S. PATEL (1964): Morphological and embryological studies in Eriocaulon cinereum R. Br. *J. Gujarat Univ.* **7**: 58–70.

RAMASWAMY, S. N. (1975): *Embryology and systematic position* of *Eriocaulaceae*. Ph. D. Thesis, University of Mysore, Mysore.

RAMASWAMY, S. N. & G. D. AREKAL (1980a): Embryology of Eriocaulon setaceum L. *Pl. Syst. Evol.* **135** (in press).

— & — (1980b): On the Behaviour of antipodals in Eriocaulaceae. *Caryologia* (in press).

— SWAMY, B. G. L. & GOVINDAPPA, D. AREKAL (1980): From Zygote to Seedling in Eriocaulon robusto-brownianum Ruhl. *Beitr. Biol. Pflanzen* **55**: 179–188.

SMITH, W. R. (1910): The floral development and embryogeny of Eriocaulon septangulare. *Bot. Gaz.* **49**: 281–289.

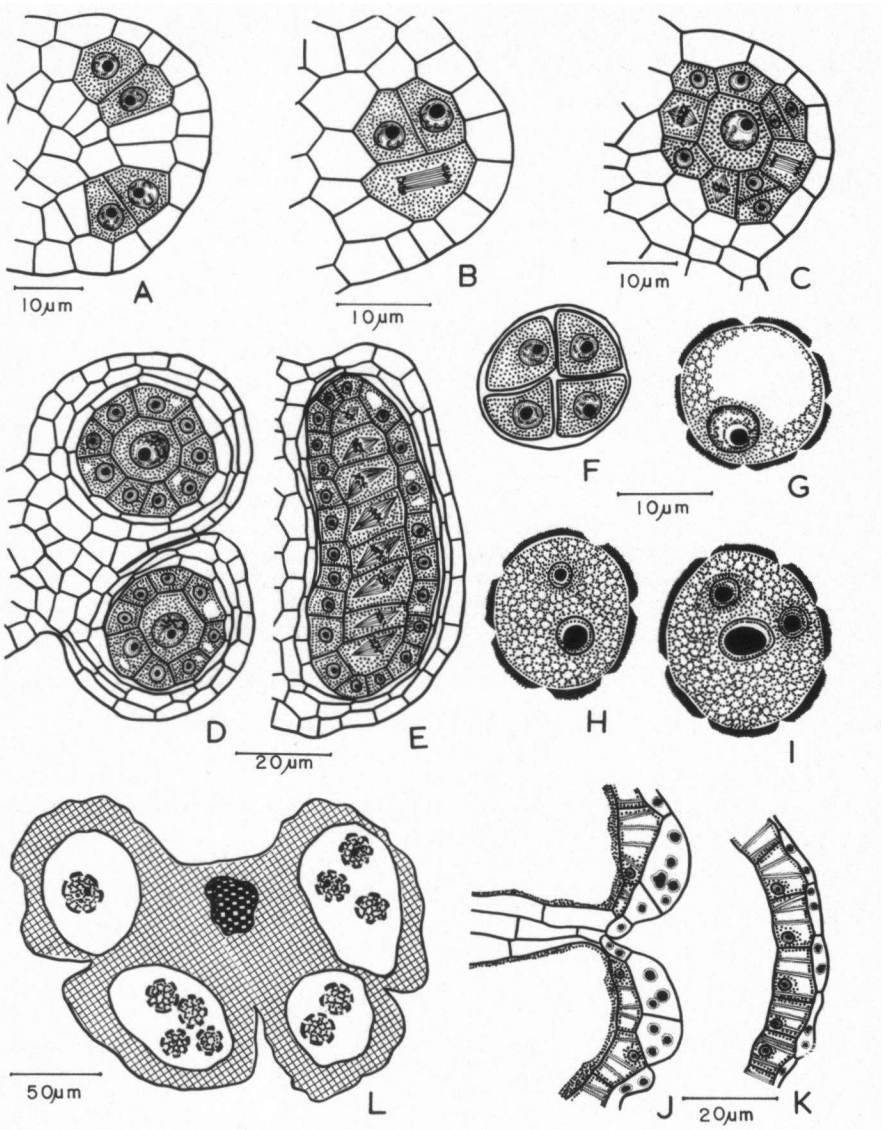

Fig. 1. A-L: Microsporangium and male gametophyte development in *Eriocaulon xeranthemum.*

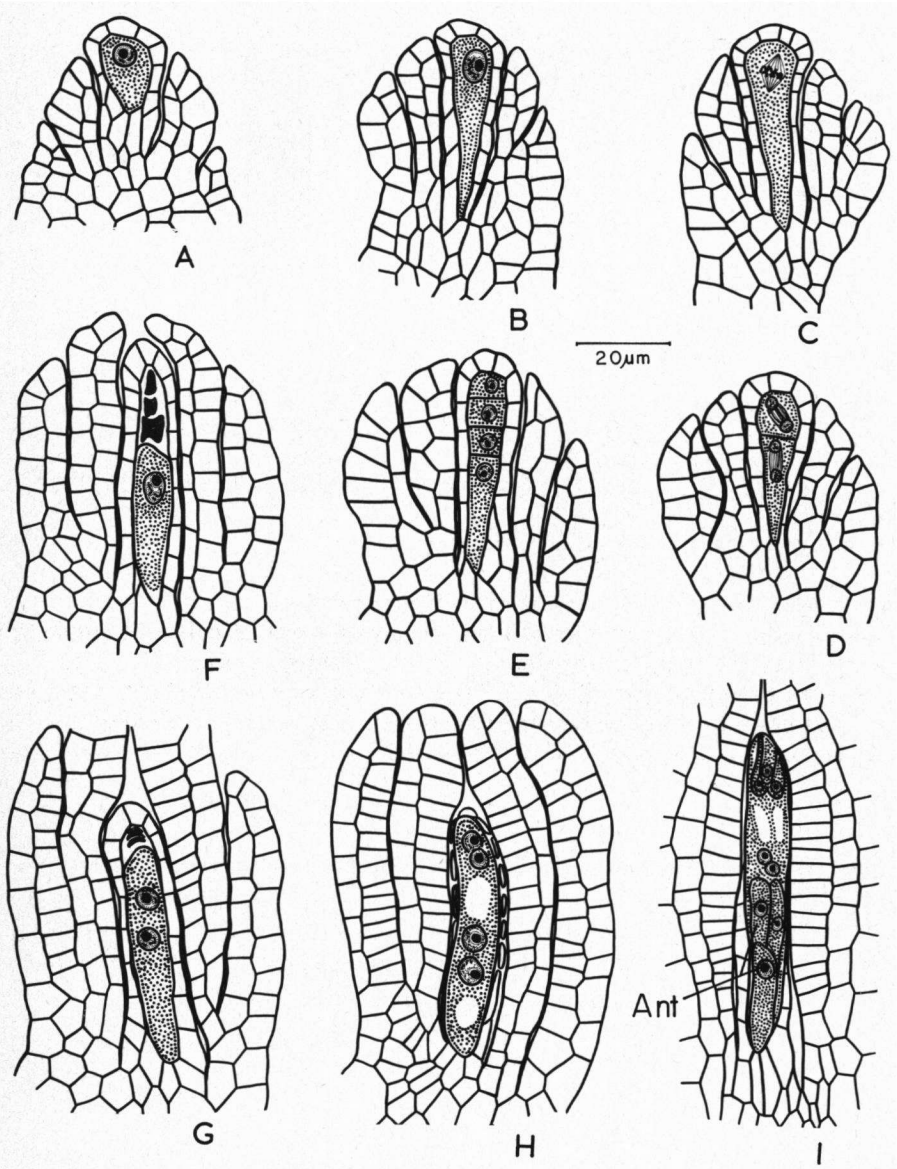

Fig. 2. A-I: Megasporogenesis and development of female gametophyte in *Eriocaulon xeranthemum.*
(Ant = antipodal cells).

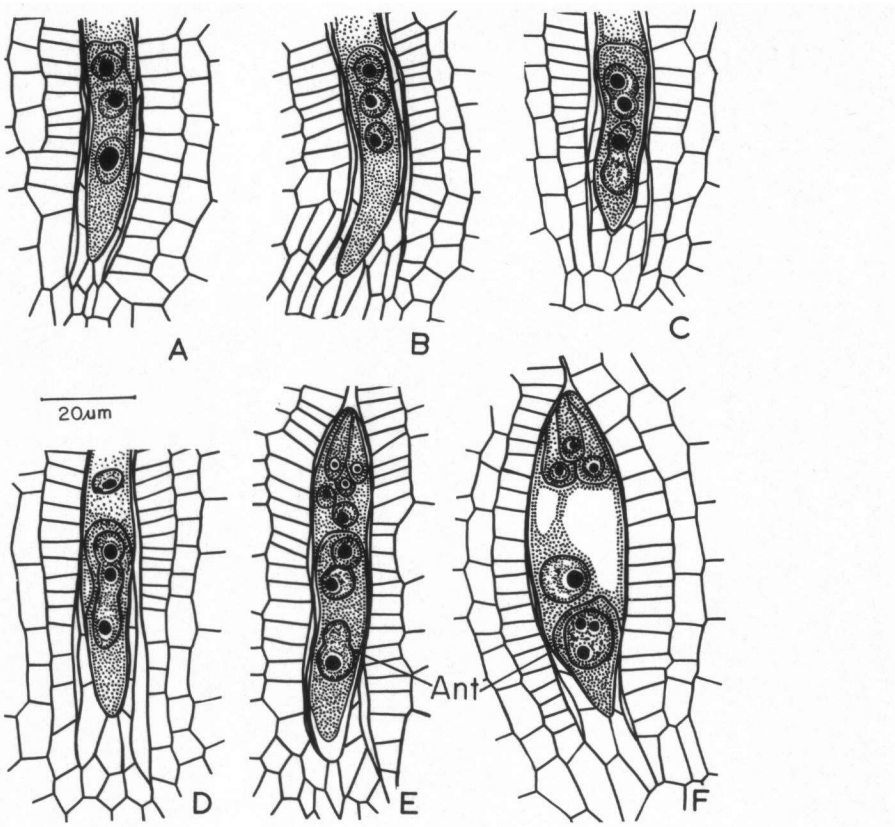

Fig. 3. A-F: Stages of nuclear fusion in the antipodal cyst of the female gametophyte in *Eriocaulon xeranthemum*.
(Ant = antipodal cyst).

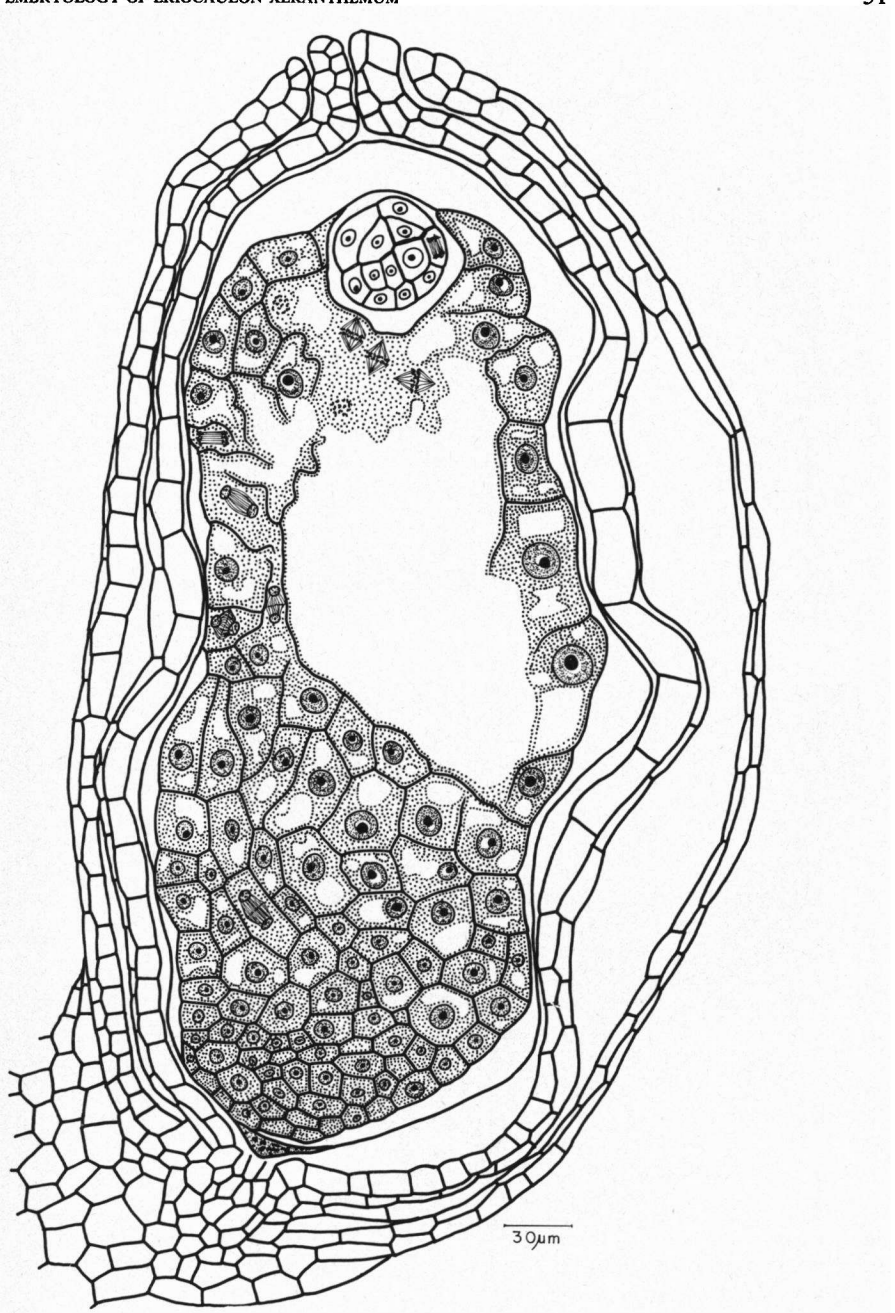

Fig. 4. L.s. young seed to show Endosperm in *Eriocaulon* xeranthemum.

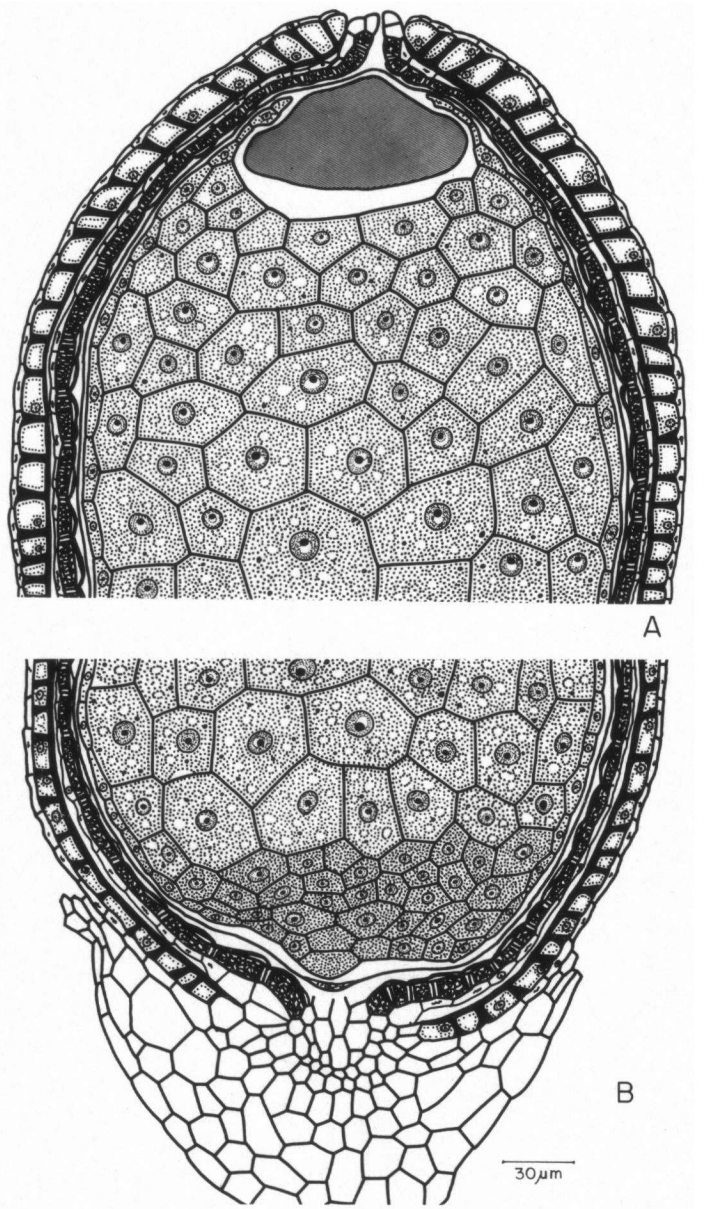

Fig. 5. A-B: Longisections of the Upper and Lower parts of a ripe seed. (Single hatched - Embryo).

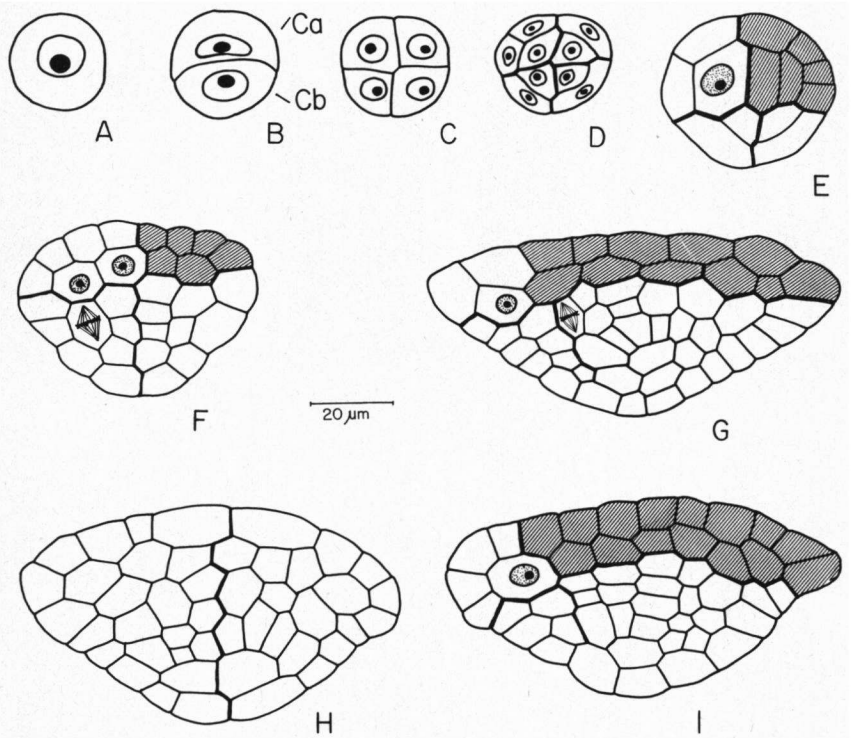

Fig. 6. A-I: Development of embryo in *Eriocaulon xeranthemum*.
(Ca = apical cell; Cb = basal cell).

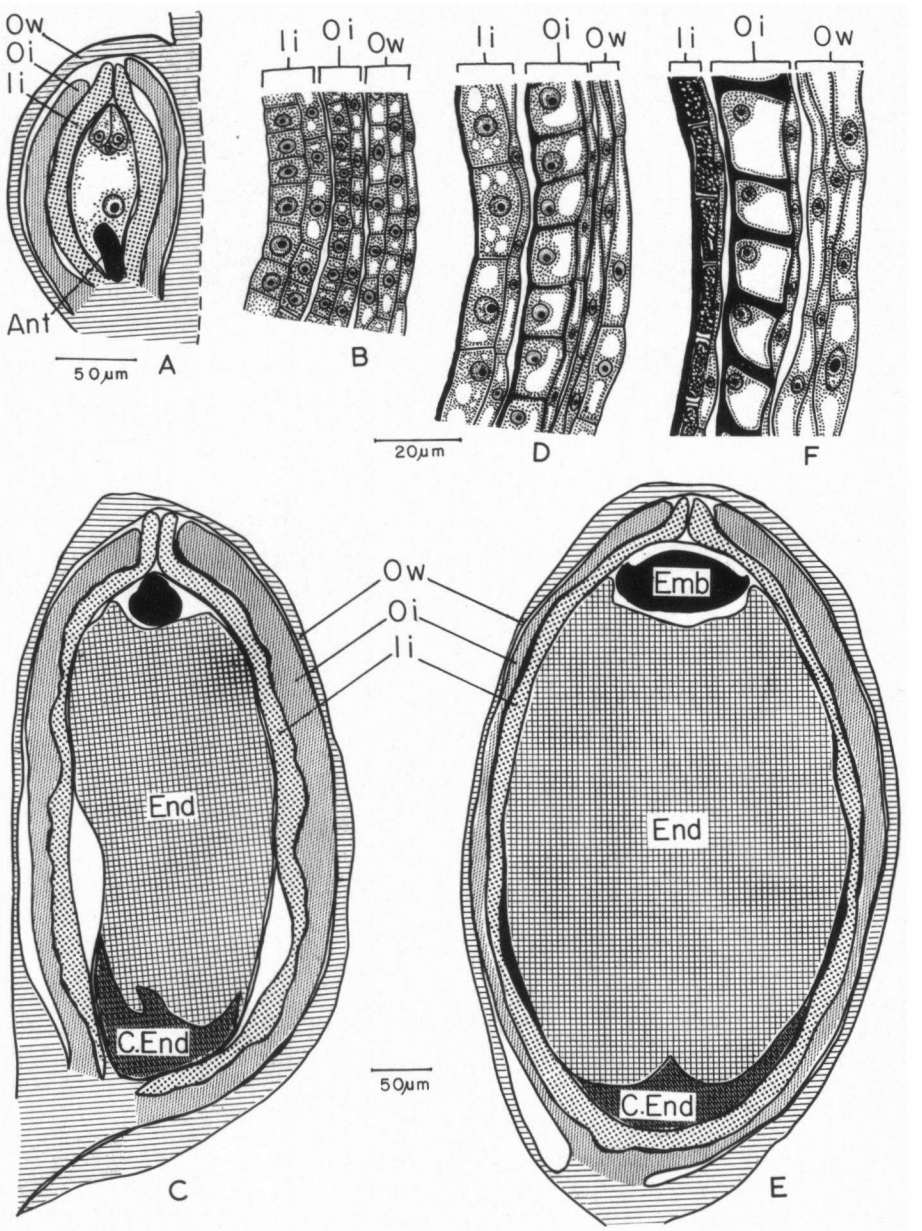

Fig. 7. A-F: Development of seed coat and pericarp in *Eriocaulon xeranthemum*.
A: Longitudinal section of an ovule at the mature embryosac stage, semidiagrammatic; B: Portion of integuments and ovary wall of Fig. A enlarged to show cellular details; C: L. s. of an young seed, diagrammatic; D: A portion of the seed coat and the pericarp of C. enlarged to show details; E: L. s. of ripe seed; F: A portion of seed coat and pericarp enlarged to show their detailed structure.