

A STUDY ON THE EMBRYOLOGY OF *COTULA HEMISPHAERICA* (ROXB.) WALL. EX CLARKE

M.R. VIJAYARAGHAVAN* AND K.C. SUD

Department of Botany, Hans Raj College, University of Delhi, Delhi-110 007

*Department of Botany (Ex Professor), University of Delhi, Delhi-110 007

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In *Cotula hemisphaerica*, anthers are bisporangiate. The male archesporium consists of a single row of 4-7 hypodermal cells. Development of anther wall is according to the Dicotyledonous type. The tapetum corresponds to periplasmodial type. Simultaneous cytokinesis produces tetrahedral, decussate and isobilateral pollen tetrads. The pollen grains at the time of anther dehiscence are three celled. The development of the embryo sac is of the Polygonum type. Mature embryo sac consists of an egg, two synergids, two polar nuclei and variable number of antipodal cells. Rare occurrence of synergid haustoria and germination of pollen grains *in situ* is reported. Fertilization is porogamous. *C. hemisphaerica* is self-sterile. Endosperm development is of the nuclear type, and the embryo development conforms to the *Senecio* variation of the Astered type.

Key Words: Asteraceae, *Cotula hemisphaerica*, Microsporangium, Megasporangium, Endosperm, Embryo.

According to Johanson (1950) "Despite a wealth of investigations on species belonging to Asteraceae, the embryonomy of the family is none too satisfactory a condition". Even today considerable literature available on the embryology of Asteraceae is too insufficient to establish an embryological generalization for this family. Besides the diverse behaviour of endothelium and antipodal cells, the members of the family reveal the different types of embryo sac and endosperm development (Davis, 1966, Pullaiah, 1984). Such variations are not even uncommon at intrageneric level (Pullaiah 1979b, 1984). The present investigation is, therefore, an attempt in this direction and deals with the embryology of *Cotula hemisphaerica* a taxon hitherto embryologically unknown.

C. hemisphaerica (n=20) flowers and fruits from January to March on moist and shady banks of Yamuna river in Delhi. The plant can be recognised by its herbaceous nature, alternate and pinnatisect leaves, and discoid, heterogamous, pedunculated yellow heads. Each head possesses outer (1 or 2 series) florets and inner (disc) florets. The outer florets are not rayed, irregular, epigynous and pistillate. The inner florets are regular, epigynous and bisexual. The head is erect at the time of anthesis but droops at the event of fruit production.

MATERIALS AND METHODS

Small pieces of flower heads at different stages

of development were fixed in formalin-acetic-alcohol for 24 hrs. and alcohol-acetic acid for 18 hrs. The material was subsequently stored in 70% ethanol. Dehydration was done in tert.-butyl alcohol and paraffin embedded material was cut at 8-12 microns thickness. The sections were stained in Heidenhain's iron alum hematoxylin-fast green with a counter stain of fast green.

OBSERVATIONS

Microsporangium: The stamen primordia emerge independently and remain free from each other until the formation of microspore tetrads. At this stage, the adjacent anthers cohere by their epidermal cuticle forming an anther tube. The number of stamens in a disc floret is four (Fig. 1A),

Anther wall development: The anther is bisporangiate (Fig. 1 A-B). An young anther in a cross section appears somewhat elongate and undifferentiated. The archesporium is hypodermal and in longisection reveals a row of 4-7 cells (Fig. 1C-D), and in transsection, a single cell (Fig. 1B),

The archesporial cells undergo periclinal division forming a primary sporogenous layer towards inside and primary parietal layer towards outside. The cells of the primary parietal layer divide periclinally forming the outer and the inner layers, of which the latter matures into the tapetum (Fig. 1 B, E). Subsequent periclinal division of the outer layer gives rise

to the endothecium and the middle layer (Fig. 1B). In structure, the anther wall consists of outer epidermis, fibrous endothecium, middle layer and tapetum. The development of anther wall, thus conforms to the Dicotyledonous type (Davis 1966).

The tapetum, initially, possesses uninucleate cells with dense cytoplasm (Fig. 1B) but later becomes binucleate due to mitotic divisions (Fig. 1E-F). The tapetum is periplasmodial type. During the formation of microspores, the inner tangential walls of tapetum break down, their protoplasts flow into the anther locule and fuse with the neighbouring protoplasts resulting in the formation of periplasmodium which encircles the microspores (Fig. 1H). Later this amorphous substance is gradually used up by the growing pollen grains and disappears completely prior to the formation of two male gametes (Fig. 1U).

The epidermal cells undergo anticlinal divisions and become greatly stretched and thus keep pace with the enlarging anther (Fig. 1H, U). Its cells, however, persist upto the maturity of the anther. Concomittant with the disappearance of the tapetal periplasmodium, the hypodermal layer acquires characteristic fibrous thickenings (Fig. 1U). The middle layer collapses due to the gradual expansion of sporogenous tissue and the rigid endothecium. The lysed middle layer persists until the uninucleate microspore stage (Fig. 1G). The anther dehisces introrsely and sheds three-celled pollen grains (Fig. 1U).

Microsporogenesis : The primary sporogenous cells divide transversely to form one row of secondary sporogenous cells which directly mature into meiocytes (Fig. 1B, E). These meiocytes undergo two meiotic divisions (Fig. 1I-L). The first meiotic division is free nuclear (Fig. 1B, K) and the second division results in the formation of four nuclei. Cytokinesis is of the simultaneous type. The arrangement of microspores is either tetrahedral (Fig. 1F, L, M) or isobilateral (Fig. 1N). Rarely, tetrads of decussate type are also encountered (Fig. 1O).

The microspores are liberated by the lysis of microspore mother cell walls. The exine in the liberated microspore is conspicuous and spiny. The young pollen grain is non-vacuolate and has a central nucleus (Fig. 1P). Later the pollen grain enlarges and develops a central vacuole which pushes the nucleus towards the periphery.

Male gametogenesis : The mitosis results in two cells namely generative and vegetative cells. The generative nucleus adheres to the wall of the intine (Fig. 1Q). The pollen grain, gradually becomes two-celled by the formation of lenticular generative cell (Fig. 1Q, R). This cell divides to give rise to two male gametes (Fig. 1S). The exine is spinous and intine is smooth and uniformly thick. At shedding stage, the pollen grains are spheroidal, triporate and three-celled (Fig. 1S).

The pollen grains occasionally show precocious germination and germinate to form small pollen tubes where either one or both the male gametes migrate (Fig. 1T).

Megasporangium : The ovular primordium arises as a small protuberance from the base of the ovary and gradually fills the loculus. The ovule undergoes curvature during its development due to the pronounced growth towards one side. At megaspore mother cell stage, the ovule is anatropous, unitegminal and tenuinucellar (Fig. 2A-B).

Megasporogenesis : The hypodermal archesporium is overarched by a single layer of nucellar epidermis (2A) and functions directly as the megaspore mother cell (Fig. 2B). The enlargement of megaspore mother cell is accompanied by a few anticlinal divisions in the cells that overly as the nucellar epidermis.

The meiotic division in the megaspore mother cell results in the formation of megaspore dyad with smaller micropylar and larger chalazal cells (Fig. 2C). Next division in each of two cells leads to the formation of a linear tetrad of megaspores (Fig. 2D).

At the megaspore tetrad stage, the nucellar epidermis eventually degenerates and the inner layer of the integument differentiates as the endothelium (Fig. 2D) which is initially uniseriate but later at two-nucleate embryo sac stage, becomes two-celled thick (Fig. 2F). However, it remains uninucleate throughout its development.

The chalazal megaspore functions whereas three micropylar megaspores degenerate (Fig. 2E). The functional megaspore enlarges, develops vacuoles and is besieged by endothelium (Fig. 2E).

Female gametogenesis : The division in the functional megaspore results in the formation of two-

Table 1. Temporal relationship between female and male stages in a disc floret of *C. hemisphaerica*.

Female stage	Male stage
Archivesporium	Young microspore mother cells Uninucleate tepetal cells
Megaspore mother cell	Dividing microspore mother cells Bi-nucleate tepetal cells
Dyad of megaspores	Microspore tetrads Amoeboid tapetum
Linear tetrad of megaspores	Young and developing microspores.
Functional chalazal megaspore	Tricolpate, vacuolate, unicellular and uninucleate microspores
Two-nucleate and four-nucleate embryo sac with prominent two-layered endothelium	Two-celled pollen grains Periplasmodium
Mature embryo sac	Three-celled pollen grains Enlarged endothecium showing fibrous thickenings

nucleate embryo-sac. The two nuclei move to the opposite poles due to the development of a large central vacuole (Fig. 2F). The next division in the two nuclei gives rise to the four-nucleate embryo sac (Fig. 2G). One or two vacuoles separate the nuclei from each other. Further divisions in the four nuclei occur simultaneously and eight-nucleate, unorganised embryo sac is formed.

Mature embryo sac : The elongated and cylindrical mature embryo sac has tapering ends and possesses an egg apparatus, two polar nuclei and antipodal cells (Fig. 2H). The egg apparatus consists of two elongated synergids and a pear-shaped egg with dense cytoplasm and eccentrically located nucleus. Each synergid is uninucleate having a basal vacuole. In few instances, one of the synergids elongates deeply into the micropyle, appears slightly swollen and even acts as the haustorium (Fig. 2I). The two polar (Fig. 2I) nuclei fuse before fertilization to form the secondary nucleus.

The antipodal cells are situated in a narrow elongated pouch of the embryo sac. They are usually three in number and show two uninucleate and one binucleate cells (Fig. 2H). In other instances, four antipodal cells are found where uninucleate and binucleate cells alternate (Fig. 2K); and the number of antipodal cells even increase up to six (Fig. 2J, L). Occasionally, the chalazal antipodal cell elongates and reveals

haustorial tendency (Fig. 2J). A temporary relationship between female and male stages in a disc floret of this taxon has been reported (Table 1).

Fertilization : The plant shows porogamous fertilization; and syngamy and triple fusion occur simultaneously (Fig. 3A). The pollen tube persists during the post-fertilization period (fig. 3B). This taxon apparently is self-sterile. Capitula, which did not have access to insects, revealed embryo sac development but were bereft of embryos.

Endosperm : The endosperm development is of the Nuclear type. The primary endosperm nucleus divides earlier than the zygote (Fig. 3B, C). The initial mitotic division and a few subsequent divisions are not followed by wall formation (fig. 3C, D). The endosperm becomes cellular by the time the globular embryo is formed (Fig. 3E). In the mature seed, the embryo is surrounded by only one or two layers of endosperm.

Embryogeny : The zygote is elongate and has a vacuole towards the micropylar end, and an eccentrically placed nucleus towards the chalazal end (Fig. 3F). It divides transversely to form a two-celled proembryo with a terminal cell, *ca*, and a vacuolate basal cell, *ob* (Fig. 3G). A transverse division in the latter results in two cells namely *m* and *ci* while the terminal cell divides longitudinally forming two juxtaposed cells of the tier *q*. Thus, an inverted T-shaped, four-celled proembryo with cells arranged in three tiers, namely *q*, *m*, and *ci*, is formed (Fig. 3H). The cells of the tier *q* divide to form a quadrant of four cells. The cell *m*, meanwhile, undergoes a longitudinal division and *ei* divides transversely to form two cells, namely *n* and *n'*. Thus an eight-celled proembryo is formed (Fig. 3I, J). Next vertical divisions occur in the cells of the tiers *m* and *q*; while the cell *n'* divides transversely to form two cells *o* and *p* (Fig. 3J, K). Thus, by the end of the 4th cell generation, the resultant proembryo becomes 16-celled.

Periolinal divisions in the *m* and octant differentiating the dermatogen, and the vertical division of *n* constitute the next generation (Fig. 3K). Further divisions in all planes in *q*, *m*, *n* and *o* result in the formation of globular embryo which becomes heart-shaped with well-developed cotyledonary loci (Fig. 3L) to form later the dicotyledonous embryo (Fig. 3M). The two cells of the suspensor are derived from the cell *p*.

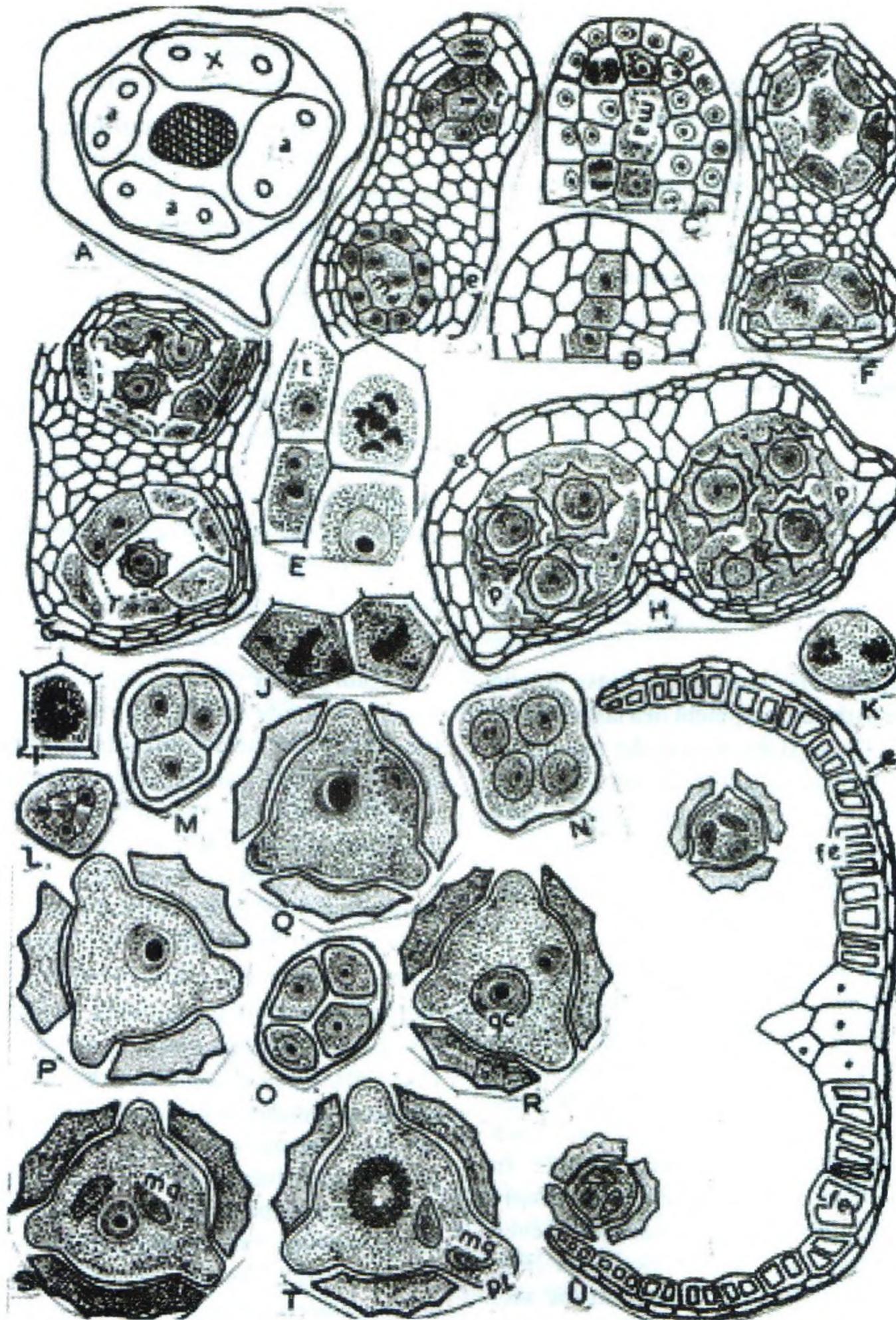


Figure 1A-U Microsporegenesis and microgametogenesis in *Cotula hemisphaerica*.

A. transection of androecium showing four bisporangiate anthers (diagrammatic) X 376. B. transection of bisporangiate anther marked 'X' in Fig. A showing the differentiation of wall layers X 927. C-D. Longisections of anther lobes showing archesporium and developing wall layers X 927. E. Tapetum and microspore mother cells X 927. F. Transection of anther showing wall layers and developing sporogenous cells X 927. G-H. Transection of anthers showing the formation of periplasmodial tapetum and uninucleate pollen grains X 927. I-L. Microspore mother cells in meiosis I and II X 1690. M-O. Tetrahedral, isobilateral and decussate microspore tetrads X 1690. P. Young pollen grain X 2933. Q-R. Two-celled pollen grains X 2933. S. Three-celled pollen grain at anthesis X 2933. T. Mature pollen grain showing a small pollen tube emerging during anthesis X 2933. U. Transection of anther showing epidermis, fibrous endothecium and three-celled pollen grains X 927.

(a, anther; e, epidermis; fe, fibrous endothecium; gc, generative cell, ma, male archesporium; mg, male gamete; p, periplasmodium; pt, pollen tube; t, tapetum).

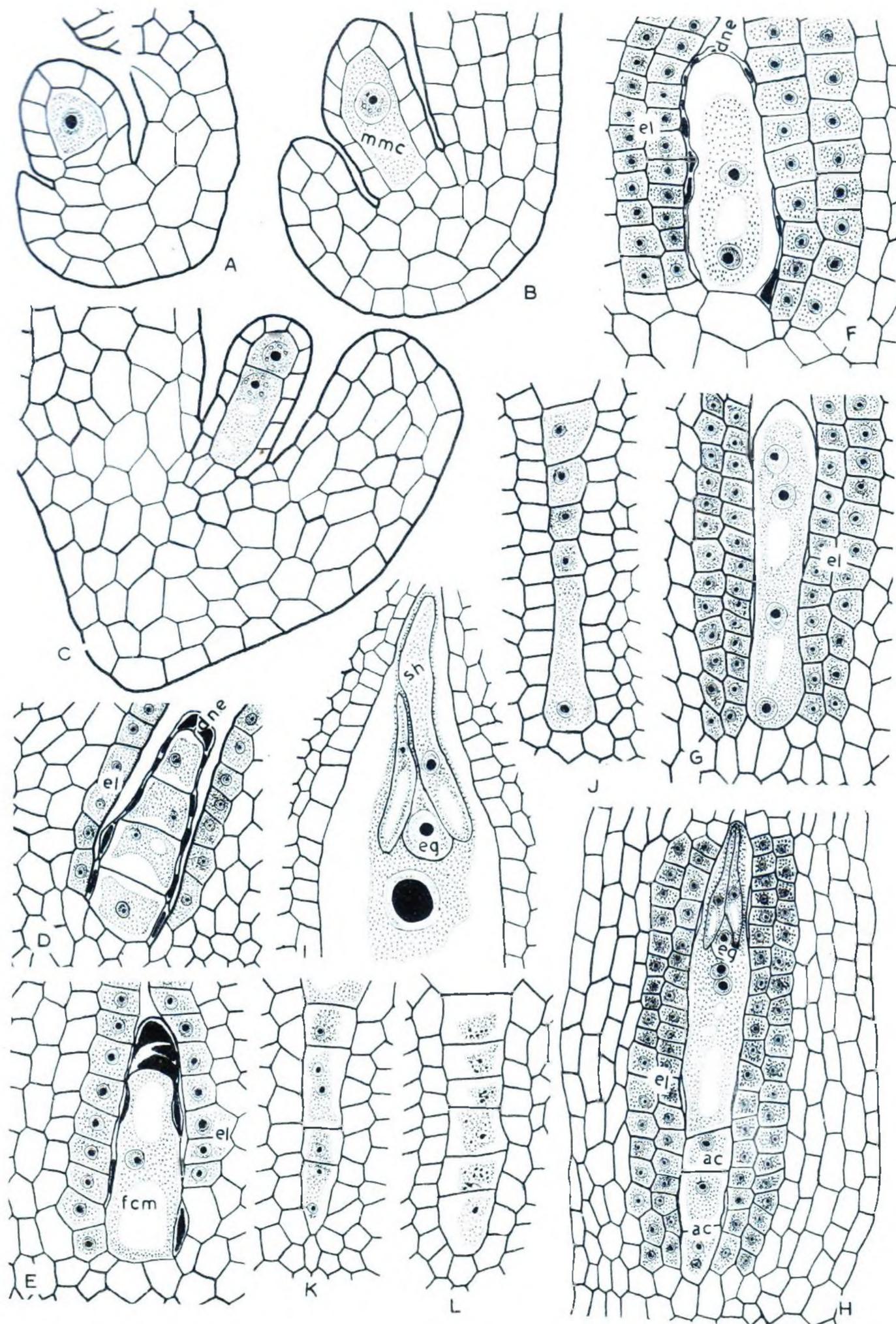


Figure 2A-L. Megasporogenesis and megagametogenesis in *Cotula hemisphaerica*.

A. Longisection of young ovule showing archesporial cell X 1320. B. Megaspore mother cell X 1382. C. Dyad with unequal cells X 1382. D. Linear megaspore tetrad, note the differentiation of endothelium X 1382. E. Functional chelazal megaspore, the remnants of nucellar epidermis and three degenerated megaspores are noteworthy X 1382. F. Part of longisection of ovule showing two-nucleate embryo sac, degenerated nucellar epidermis and two-layered endothelium X 1267. G, H. Same, at four-nucleate and organised embryo sac stages, G X 1267. H X 755. I. Longisection of upper part of ovule showing an elongated synergid haustorium X 824. J-L. Longisection of lower part of embryo sac showing antipodals. The last antipodal cell is unusually elongate in J X 824.

(ac, antipodal cell; dne, degenerated nucellar epidermis; eg, egg; el, endothelium; fcm, functional chelazal megaspore; mmc, megaspore mother cell; sh, synergid haustorium).

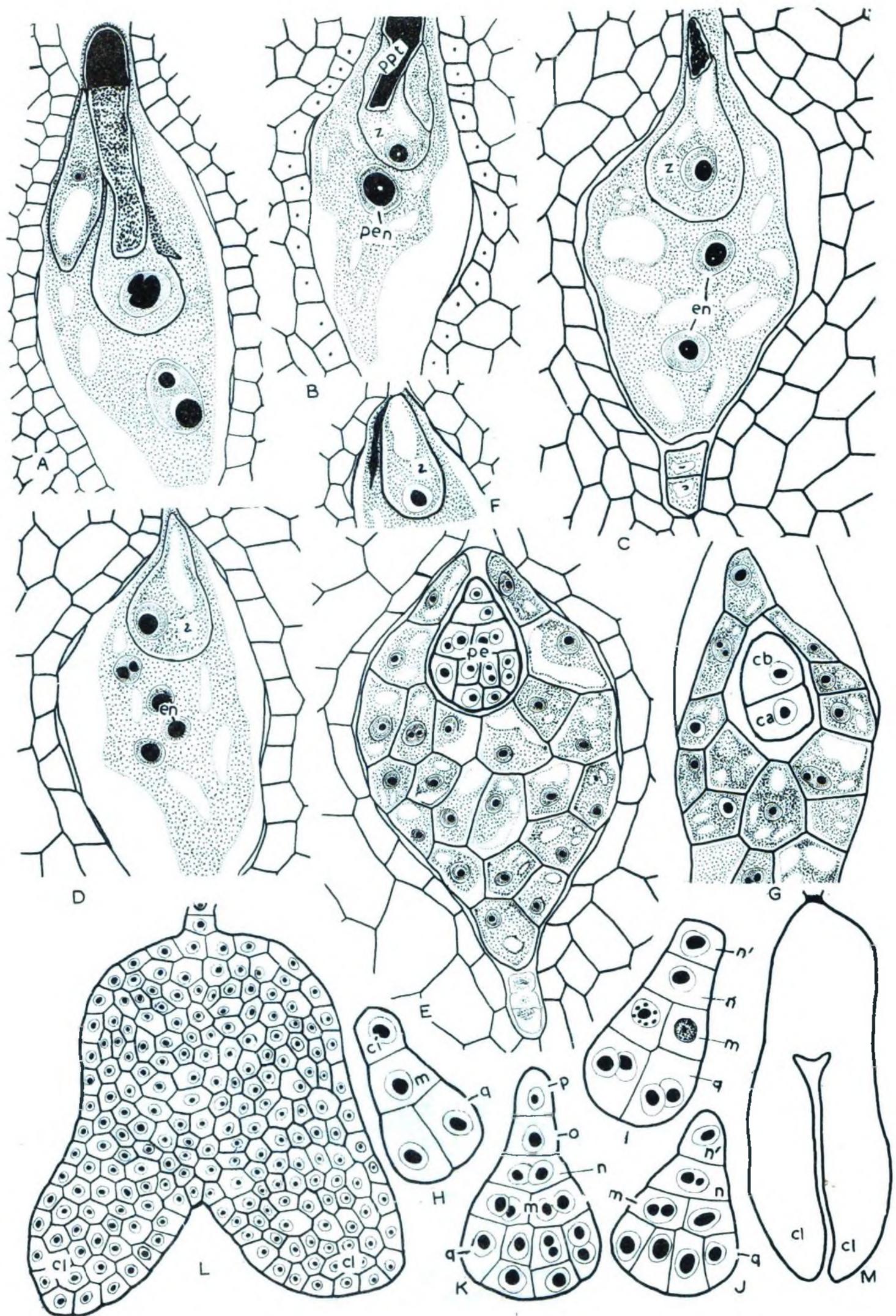
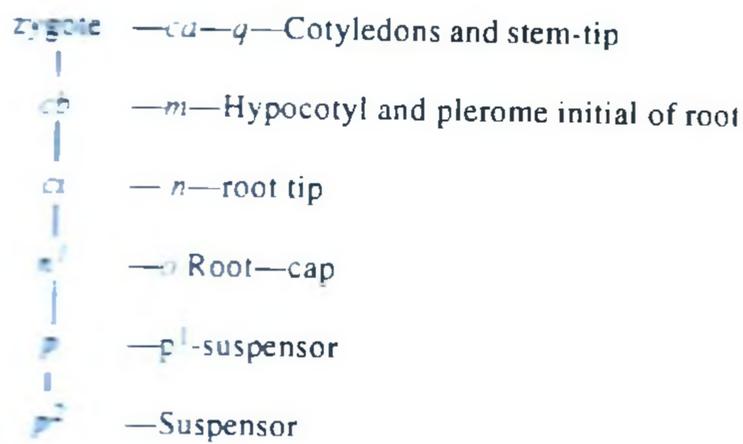


Figure 3A-M. Fertilization and stages in development of endosperm and embryo in *Cotula hemisphaerica*. A. Part of longisection of ovule showing double fertilization X 1018. B. Same, showing zygote, primary endosperm nucleus and persistent pollen tube X 755. C-D. Same, showing zygote, two and four free endosperm nuclei C X 933, D X 755. E. Proembryo with cellular endosperm X 755. F. Zygote X 1267. G-H. Two-, and four-celled proembryos G X 755, H X 1267. I-K. Progressive stages in the formation of globular proembryo X 1267. L. Young dicotyledonous embryo X 755. M. A mature embryo with well developed cotyledons X 128. (cl, cotyledon; en, endosperm nuclei; pe, proembryo; pen, primary endosperm nucleus; ppt, persistent pollen tube; z, zygote).

Schematic details of the development of the embryo are as follows :



Since both the cells *ca* and *cb* contribute to the formation of the embryo and the cell *m* forms the entire hypocotyl and plerome initial of the root, the embryo development, therefore, conforms to the *Senecio* variation of the Asterad type.

DISCUSSION

The tetrasporangiate condition of anthers is common in Asteraceae (Davis, 1966). *Cotula hemisphaerice* (present study) shows bisporangiate anthers. Earlier, this infrequent feature was also reported in few members of the family (Sundara Rajan, 1974; Rangaswamy and pullaiah, 1984). However, Kaul (1973) reported trisporangiate anthers in *Youngia japonica*. In the present report, the anther tapetum is of the periplasmodial type which is in conformity with earlier reports for the family (Pullaiah, 1984; Villari, 1987). Rarely, secretary tapetum has been observed in *Ainsliaea aptera* (Kapil and Sethi, 1962), and *Prenanthes brunoniana* (Sood and Thakur, 1984). Tapetal cells are plurinucleate in *Helichrysum rupestre* var. *errerae* (Villari, 1987). Similarly, polyploid nuclei of various sizes were reported in the tapetal cells of *Senecio candicans* (Rangaswamy and Pullaiah, 1986). However, the present data indicated uni-, and bi-nucleate tapetal cells. Such differences may be due to the variability in the amount of the nutritive materials passing into the cells. As in majority of Asteraceae (Davis, 1966; Pullaiah, 1984), the present work indicates the presence of fibrous thickenings in the endothecium of a mature anther.

Archesporium is single layered and the primary sporogenous cells divide transversely in this taxon. Earlier, a vertical or transverse division of primary sporocytes was noted in *C. australis* (Davis, 1962). As is true for Asteraceae, the present study also shows the simultaneous type of cytokinesis. Brewbaker (1967) maintains that the Asteraceae, at anthesis, have only

three-celled pollen grains. The present investigation supports this earlier generalization. Anthesis at four-celled stage has, however, been reported in a rare instance (Rangaswamy and Pullaiah, 1986). In *C. hemisphaerica*, (Present study), a few pollen grains germinate *in situ*, which condition was earlier known only in *Caesulia axillaris* (Deshpande, 1962).

As usual, the female archesporium is unicellular in *C. hemisphaerica* (present data). Mono-, bi-, and tetrasporic types of embryo sac development occur in the family Asteraceae (Davis, 1966; Pullaiah, 1984), including diplospory of the Antennaria type in *Eupatorium tanacetifolium* (Rozenblum *et al.*, 1988). The development of the embryo sac in the present study follows the monosporic, Polygonum type.

Endothelium is biseriate in *Pluchea tomentosa* (Ram, 1986). Rarely, it is uniseriate at the apex and multiseriate at the chalazal end in *Tithonia rotundifolia* (Pullaiah, 1978). However, *C. hemisphaerica* (present data) clearly shows uniseriate endothelium which later becomes biseriate.

Both synergid and antipodal haustoria occur in *Elephantopus scaber* (Pullaiah, 1979a). Rarely, a gametophyte shows the co-existence of three types of haustoria namely, synergid, embryo sac and antipodal (Maheswari Devi and Padma, 1985). The present investigation, however, shows only synergid haustoria in the gametophyte of *C. hemisphaerica*.

Asteraceae show the occurrence of both Nuclear and Cellular types of endosperm development (Davis, 1966; Pullaiah, 1984). Nuclear ontogeny in *C. hemisphaerica* (present study) is observed. In contrast, Davis (1962) recorded cellular ontogeny for *C. australis*. Earlier, both Nuclear and Cellular endosperm formation are reported for the genus *Blumea* (Pullaiah, 1979b). The embryo development follows *Senecio* variation of Asterad type (Johanson, 1950) in this family including *C. hemisphaerica*.

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