Assessment of Microsporogenesis in *Euphorbiarosea* Retz.

Kadu Sandhya. R.
BhartiyaMahavidyalaya, Amravati, 444603 (M. S.)

Abstract:

The family Euphorbiaceaethe‘spurge family’iscosmopolitan. It shows a wide range of habits. The family is predominantly a tropical one as very few plants are seen the temperate climates. The family has a wide range of morphological, embryological and palynological characters. The plants are dioecious. They may be trimerous or pentamerous. The genus *Euphorbia* is characterised by cyathium inflorescence.

In the genus *Euphorbia* the male and female flowers are extremely reduced. The male flower consists of a single stamen situated on a short pedicel with a definite articulation.

Keywords: Euphorbiaceae, cyathium, dioecious, embryology, microsporogenesis.

Introduction:

Euphorbiaceae, the spurge family. The genus *Euphorbia*is one of the largest genera among flowering plants. It is distributed mainly in tropical, subtropical and warm temperate regions around the world, comprises some 1,836 species and 84 species indigenous or naturalised and 3 species cultivated as garden plants in India (Balkrishnan,*et al*., 2012). The flowers are typically dioeciousand are borne in a characteristics cluster known as a cyathia. The species contain milky latex, and some are useful as a source of oil or wax.The family has a wide range of morphological, embryological and palynological characters. The flowers may be trimerous or pentamerous. In the genus,*Euphorbia* the male and female flowers are extremely reduced. The male flower consists of a single stamen situated on a short pedicel with a definite articulation.

In angiosperms, the male reproductive development requires the formation of stamen, including the differentiation of anther tissues. In the anther (microsporangium) the male meiosis produces microspores, which develops into pollen grains. The anther dehisces, when the pollen grains are mature.

Review of Literature:

The characteristic development of the inflorescence and seed in Euphorbiaceae has interested phytomorphologists for over a century and a half. The first description of the structure and anatomy of Euphorbiaceae seed.

Early reference on the embryology of the Euphorbiaceae was given by Baillon (1858). The binucelate condition of the pollen grains at the time of shedding in *E. corollata* was described by Lyon (1898).Modilewski(1909) worked out on the sporogenesis and gametophyte of *E. procera* and mentioned the occurrence of 3-nucleate pollen grains at the time of anthesis.

Schurhoff (1924) observed the tapetal cells in *E. procera*having a tendency to grow inside the anther locule but with no formation of true periplasmodium. It was observed that the pollen grains were 3-celled at the shedding stage in *E. procera, E. nicaensis*and*E. aphylla*.

Schnarfreviewed critically the literature of family prior to (1929) in his monumental work “ Vergleichen de Embryologie der Angiosperm” published in (1931).

Cooper (1933) mentioned the presence of binucleatetapetal cells in *E. splendens*. In *E. rosea* the polyembryony was reported by Robyns and Louis (1942). Johansen (1950) in his book "Plant Embryology" has only dealt with a concise account of the types of embryo development in the family Euphorbiaceae. Sharma (1955) studied in a very brief manner the sporogenesis and gametogenesis in *E. pulcherrima*andand *E. heterophylla*.

Thathachar (1953) has been given general account on microsporogenesis in *E. thymifolia*, *E. hypericifolia*and*E. cristata*. The anther tapetum is of the secretory type with 2 or more nuclei in its cells in the later stages. The mature pollen grainsare 3-celled in the above mentioned species.

The reinvestigation of *E. hypercifolia* was done by Mukherjee (1957). He described the morphology of cyathium, microsporangium and male gametophyte.

Some of the embryological aspects of *E. dulcis* have been describedbyKapil (1961). The inflorescence revealed considerable reduction in the number of male flowers. The anther shows epidermis, endothecium, middle layer and glandular tapetum. Pollen tetrads are formed but none of the pollen grains are fertile.

Mukherjee (1961a) studied the embryology of *E.* d*racunculoides* and *E.microphylla*. The anther wall comprises of epidermis, fibrous endothecium, ephemeral middle layer and tricolporate pollen grains in *E. microphylla*andtricolpate in *E. dracunculoides.*

Rao (1970) made a review of the embryological work in the family Euphorbiaceae.Some of the embryological features of *E. vermiculata* have been described by Rao and Devi (1974). In this plant, the anthers are tetrasporangiate. The anther wall consists of an epidermis, endothecium, single middle layer and the secretory tapetum. The anther wall development confirms to the dicot type. The pollen grains are tricolpate and 2-celled.

Tiwari (1976) studied the different phases of embryology of *E. nerifolia* and *E. panchganiensis*. Male archesporium is hypodermal and multinucellar. Pollen grains are tricolporate and bicelled at anthesis.

Sathianathan and Mukherjee (1983a) studied the reproductive ontogeny in the members of Euphorbiaceae. The plants selected are *E. corrigiloides* and *E.perbracteata.* Anthers are tetrasporangiatebecomingbisporangiate due to dissolution of walls between the thecae. Anther wall development confirms to dicot. Microspore tetrad are tetrahedral and rarely decussate. Pollen grains are 3-celled at anthesis.

The gametophyte of *E. clarkeana* are studied by Zanwar (1994). According to him the anthers are tetrasporangiate. The anther development is of dicot type. Pollen grains are shed at 3-celled stage.

Mukherjee and Sathianathan (1985) studied the embryology of *E. serpens*. The male archesporium is multicellular and hypodermal. Four layered anther wall development confirms to dicot type.Tapetum is of secretory type.

Materials and Methods:

The plants were collected from Nanded and their distribution is not seen in Nagpur. The plants were found in abundance from August to December. The plants were fixed in FAA and Nawaschin fixatives.

For the study of microsporogenesis the material was processed to microtomy. Mostly sections were cut 10-12 microns thick. The ribbon containing the section were stained with iron-alum-heamatoxylin and destained in a saturated solution of picric acid. The counterstaining of erythrosin and fast green was tried for better staining.

Finally, the slides were mounted in Canada balsam.

Observations:

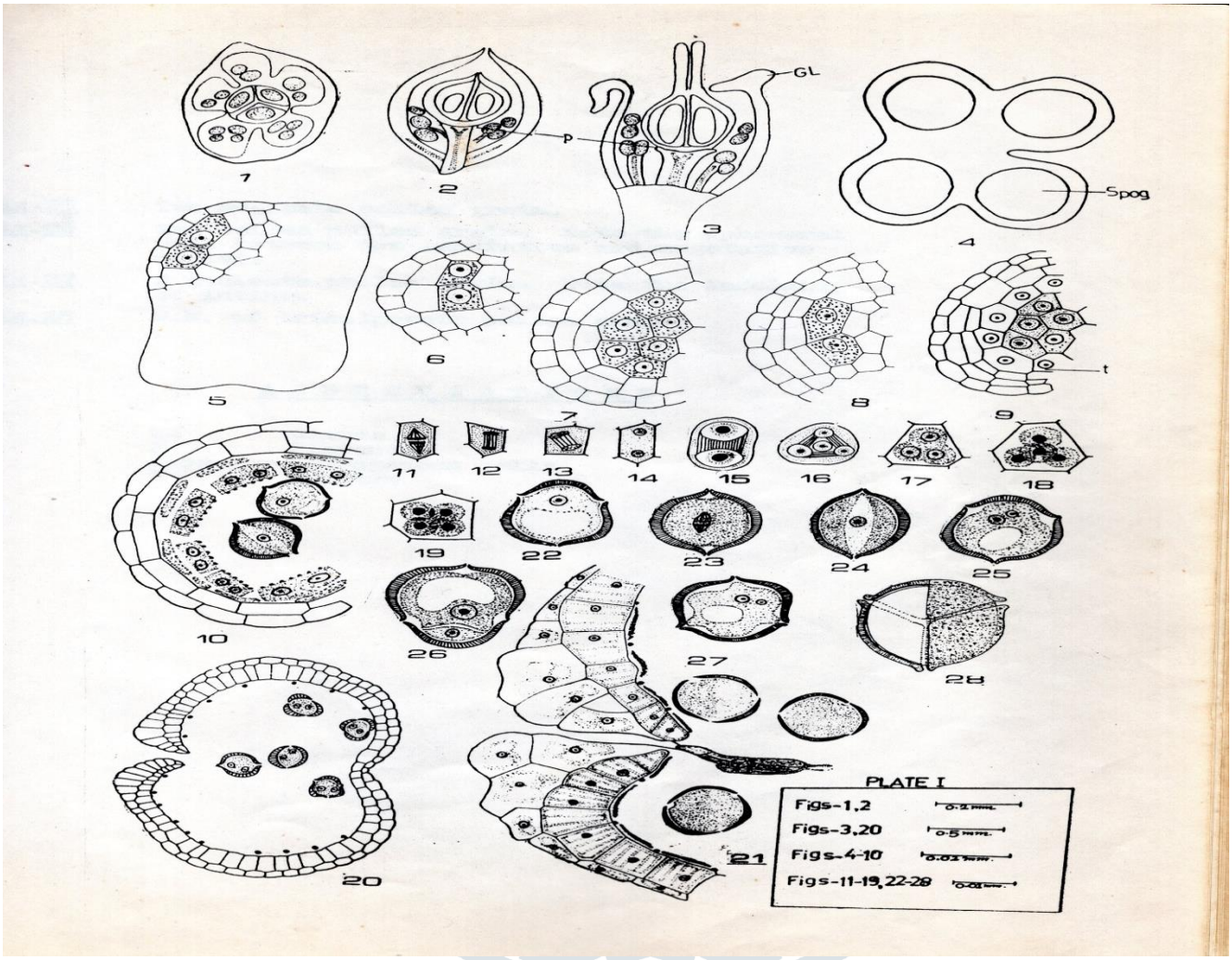

Fig. No. 1-28: Microsporangium, Microsporogenesis and Male Gametophyte

Fig. No. 1: T. S. Cyathium showing a trilocular central female flower surrounded by 5 groups of male flowers and an involucre.

Fig. No. 2: V. S. Cyathium

Fig. No. 3: V. S. Cyathiumshowing a central female flower and numerous male flowers, represented by individual stamens. Note the vestigial perianth (P) and gland (gl)

Fig. No. 4: T. S. anther showing tetrasporangiate condition

Fig. No. 5: T.S. part of young anther showing multicellular hypodermal archesporium

Fig. No. 6: T. S. young anther showing one layer anther wall

Fig. No.7-8:T. S. young anther showing 2 and 3 layered anther wall respectively and central sporogenous tissue

Fig. No. 9: 4-layered anther wall, showing epidermis hypodermis, middle layer, tapetum and central sporogenous tissue

Fig. No. 10: 4- layered anther wall, surrounding the sporogenous cells. Note the degenerating middle layer.

Fig. No. 11-14: Division in tapetal cells showing metaphase, anaphase and telophase.

Fig. No. 15: Meiosis I in pollen mother cell

Fig. No. 16-17: Cytokinesis in pollen mother cell by centripetal furrow

Fig. No. 18-19: Tetrahedral and decussate tetrad respectively

Fig. No. 20: T. S. Anther showing dissolution of wall between the thecae forming bisporangiate anther. Note the stomium

Fig. No. 21: Stomium enlarged at 2-nucleate stage of pollen grain. Note the Ubisch granules on the hypodermal cells.

Fig. No. 22: Uninucleate pollen grain.

Fig. No. 23: Division of pollen nucleus and orientation of spindle in pollen grain

Fig. No. 24: Uninucleate pollen grain showing colpi. Note single nucleus in between the colpi

Fig. No. 25: Two nucleate pollen grain

Fig. No. 26: Two celled pollen grain. Note the ephemeral wall between the generative and vegetative nuclei

Fig. No. 27: 2- nucleate pollen grain. Note the infolding of intine

Fig. No. 28: W. M. of tricolporate pollen grain

Abbreviations: (Gl- glands, P- placenta, Spog- Sporogenous cells, t- Tapetum)

The male flowers are borne in a cup like involucre surrounding a single naked female flower. This constitute a cyathium (Fig. No. 2 and 3). The male flowers are borne in five distinct groups surrounding the female flower situated in the centre of cup. The cup is incompletely partitioned towards the base (Fig. No. 1). Each group consists of 1 to 3 flowers. In the beginning there is a single protuberance but as growth proceeds, it splits up into different initials, and each one develop separately into a male flower.

The male flower exhibited different stages of development in the cyathium. The young male flowers are at the base of cup while the oldest are in the upper part of the cyathium. This is judged by the size of the male flowers in the cyathium (Fig. 2 and 3).

The anthers are tetrasporangiate, the sporangia being equal in size (Fig. no. 4). Each male flowers develops as a parenchymatous protuberance and soon it becomes four lobed (Fig. no. 5). At each corner of the lobe,2 or 3 longitudinal rows of cells below the epidermis become conspicuous by their bigger size and denser cytoplasm. This becomes multicellular, hypodermal archesporium (Fig. no. 5). Soon these cells by periclinal division cut off primary layer below the epidermis and primary sporogenous layer on the inner side (Fig. no. 6). The primary parietal layer divides periclinally to form 2 parietal layers (Fig. no. 7 and 8). Out of these 2 parietal layers, the outermost again divides (Fig. no. 9). So that the anther wall becomes 4-layered including epidermis. These layers are designated as epidermis, hypodermis, middle layer and the tapetum (Fig. no. 9). Thus the anther development is of dicotyledonous type of Davis (1966). The middle

layer is ephemeral and soon starts degenerating. The innermost layer is the tapetum which in the beginning is uninucleate and densely cytoplasmic (Fig. no. 9). But in the later stages of meiosis I and II of pollen mother cells, it becomes binucleate due to simple mitotic division (Fig. no. 11-14). When the young pollen grains are formed, yellow staining granules are seen on the inner tangential wall of the tapetum. By the time the uninucleate pollen grain has developed a vacuole, the tapetum starts degenerating. The tapetal cells become disjointed, their cell walls are dissolved and the Ubisch granules are seen on the inner cytoplasm of the tapetal cells (Fig. no. 10). At the binucleate stage of pollen grains, the tapetum has degenerated (Fig. no. 20). As the tapetum degenerate at its own place, it is of secretory type.

During the development of the anther, the common partition wall between the two sporangia takes place (Fig. no. 20 and 21). Thus at the mature stage the anther looks to be bisporangiate though its ontogeny is of tetrasporangiate type.

The primary sporogenous cells divide mitotically once or twice to increase the number of pollen mother cells. Each pollen mother cell is polygonal and undergoes two meiotic divisions (Fig. no. 15 and 16). During meiosis the cytoplasm recedes from the cell wall and the space is occupied by mucilage. The mucilage takes a red stain with erythrosine. The four nuclei formed after meiosis arranged in tetrahedral manner (Fig. no. 16). Cytokinesis takes place by peripheral furrows and four microspores are formed (Fig. no. 17). Mostly they show tetrahedral arrangement (Fig. no. 18). Sometimes iso-bilateral arrangement is also seen (Fig. no. 19).

The microspores are liberated in the anther locule by the dissolution of the microspore mother cell wall. The young microspores are full of cytoplasm. Soon a vacuole arises and the nucleus is pushed to the periphery (Fig. no. 22).Here it divides (Fig. no. 23). The division results into small generative cell and a bigger vegetative cell. These two cells are separated by an ephemeral wall (Fig. no. 26). The wall soon disappears and the pollen grains become 2-nucleate (Fig. no. 25), but actually they are two celled. At this stage, they are liberated from the anther locule. The mature pollen grains are spheroidal and trizonocolpate (Fig. no. 24 and 28). The exine differentiates into sexine and nexine. The former appears striated in section. It is thinnest along the margin of the colpi (Fig. no. 22 and 26). The nexine between the colpi is thickened longitudinally in the form of 2 prominent ridges parallel to the polar axis which in transvers section presents a lenticular appearance (Fig. no. 25 and 26).

The outermost layer of the microsporangium is the epidermis. It consist of rectangular cells in the early stages of development (Fig. no. 6-9). Later on they start tangentially flattening to accommodate the developing microsporangia (Fig. no. 10). The layer below the epidermis is the hypodermis. This is the outermost parietal layer. Its cells become radially elongated and develop fibrous bands directing from the inner tangential wall to the outer wall. This forms the fibrous endothecium (Fig. no. 20 and 21). The cells of the endothecium do not present a uniform dimension. Over a short distance they become progressively smaller towards the line of dehiscence on either side. A reverse change over a corresponding distance takes place in epidermal cells and they become tangentially elongated. Both these layers show the presence of nuclei in their cells up to mature stage of the anther (Fig. no. 21).

Discussion:

The present embryological investigations showed that in the genus *Euphorbia*, the anthers are uniformly tetrasporangiate but becomes bisporangiate prior to dehiscence of anther due to the dissolution of the common partition wall and subsequent confluence of the adjacent sporangia.

The male archesporium is hypodermal and multicellular in all the species of *Euphorbia*.Archesporial cell undergo periclinal division to form a primary parietal layer and primary sporogenous layer. The primary parietal layer divides periclinally again to form two secondary parietal layers. The inner secondary parietal layer does not divide any further and forms the tapetum. The outer secondary parietal layer generally divides once periclinally to form a middle layer and an outer layer.

` In *E. pulcherrima* (Sathianathan,1981) the outer secondary parietal layer undergoes more than one periclinal divisions to form 2-3 middle layers. Due to periclinal and anticlinal divisions in the parietal cells, the anther wall is formed the anther wall is composed of four layers of cells, in this taxon, viz., epidermis, hypodermis, middle layer and tapetum. In *E. pulcherrima* (Sathianathan, 1981) which has 5-6 layers of cells. Four layered anther wall is very common. It is present in the majority of the species of Euphorbiaceae so far investigated as in *E. hirta* (Kajale and Rao, 1943); *E. hypericifolia*, *E. cristata*, *E. thymifolia* (Thathachar, 1953); *E. dracunculoides* (Mukherjee, 1961a); *E. peltata* (Mukherjee, 1965); *E. vermiculata* (Rao and Devi, 1974); *E. marginata*(Devi, 1975); *E. splendens* (Chodha and Bhatnagar, 1976). There has been a single report about the presence of a 3-layered anther wall by Sharma (1955) in *E. heterophylla* and *E. pulcherrima*.

The dehiscence mechanism of the anther is controlled by the epidermis and the fibrous endothecium. Certain cells of the epidermis near the line of dehiscence (stomium) get enlarged while the cells of the endothecial layer undergo reverse change over the corresponding region, so that they become smaller in size. These structural changes bring about the dehiscence of the anther. This situation is also seen in *E. hypericifoila* (Mukherjee, 1957); *E. dracunculoides* (Mukherjee, 1961a); *E. peltata*(Mukherjee, 1965) and in the present investigation.

The tapetum is uniformly of the secretory type. Majority of the species belonging to this family, have a secretory tapetum. However, Schurhoff (1924)while studying E. procera, reported that eventhough the tapetum is of secretory type, the tapetal cells have a tendancy to invade the sporogenous area and lodge between the pollen grains. this is a partial periplasmodium.

The quadripartitioning of the microspore mother cell and the mode of reduction division is of the simultaneous type and constant throughout the family. Simultaneous division and cytokinesis by furrow formation has been observed in species of *Euphorbia*. Cytokinesis results into tetrads of spores. Tertrahedral arrangement is most common in *E. dracunculoides* (Mukherjee, 1961); *E. vermiculata*(Rao and Devi, 1974). However, rarely decussate and isobilateral tetrads are also seen. The later condition is reported in *E. dracunculoides*(Mukherjee, 1961a).

Bicelled mature pollen grains are reported in *E. corollata* (Lyon, 1898); *E. helioscopia*and*E. royleana* (Bhalla, 1941a,b); *E. pulcherrima*(Sharma, 1955); *E. marginata* (Devi, 1975) and *E. splendens* (Chodha and Bhathatnagar, 1976) and *E. nivulia* (Bhanwara,1987).

However, 3-celled mature pollen grains are seen in *E. procera*, *E. nicaensis*, *E. aphylla*

(Schurhoff, 1924); *E. hirta*(Kajale and Rao, 1943); *E. hypericifolia* (Thathachar, 1953b; Mukherjee, 1957); *E. microphylla*, *E. dracunculoides* (Mukherjee, 1961a); *E.peltata* (Mukherjee, 1965); *E. rothiana*(Venkateswarlu and Rao, 1973); *E. maddeni*(Bhanwara, 1987) and in the present investigated plant.

Uninucleate pollen grains at anthesis are seen in *E.nutans* (D'Amato, 1946), which looks to be doubtful.

Thus the pollen grains in the genus *Euphorbia* present an interesting array of spore type differing from in respect of colpi, pore, exine, stratification etc. this shows that the family is distinctly eurypalynous (Erdtman, 1952).

Acknowledement:

I am grateful to Dr. P. K. Mukherjee, (PGTDept. Of Botany, Nagpur University, Nagpur) for his keen supervision on the M. Phil. Thesis during the year (1987-1988). I am also thankful toDr. M. D. Padhye, Professor and Head Dept. of Botany, Nagpur University, Nagpur, who was kind enough to extend all facilities and constant encouragement. I record my thanks to Shri. C. D. Zanwar, Research Scholar, Dept. of Botany.

References:

Baillon, B. H. (1858): Etude general du grupe des Euphorbiaceae. Rev. in Bull. Bot. Soc. France 5: 776-780

Balkrishnan, N. P., Chakrabarty, T., Sanjappa, M., Lakshminarsimhan, P. and Singh, P. (2012): Flora of India, Vol. 23

Bhalla, V. (1941a): Life history of *Euphorbia helioscopia* L. *Proc. Ind. Sc. Cong*. 161

Bhanwra, R. K. (1987): Embryology of *Euphorbia maddeni* and *Euphorbia nivulia*. *Curr. Sci*. 56; 20, 1063-64

Chodha, S. P. and Bhatnagar, A. K. (1976): Embryological studies in *Euphorbia splendens*Boj. *Proc. Ind. Sc. Cong.*

Cooper, D. C. (1933): Nuclear division in the tapetal cells of certain angiosperms. *Amer. J. Bot*. 20: 358-365.

D' Amato, F. (1939): Ricercheembryologicheacaryologichesulgerere*Euphorbia*. Nuovo. Giorn. Bot. ital. 46: 470-509.

Devi, R. R. (1975): Embryology of *Euphorbia marginata*. *Proc. Ind. Sc. Cong*. 79

Johanson, D. A. (1940): Plant Microtechnique. Mc. Graw Hill Book Co., New York

Kajale, L. B. (1954): Fertilization and development of embryo and seed in Euphorbia hirta L. *Proc. Nat. Inst. Sci.* India 4: 353-360

Kajale, L. B. and Rao, G. V. (1943): Pollen and embryo sac development of two Euphorbiaceae. *J. IndianBot. Soc*. 22: 229-235

Kapil, R. N. (1961): Some embryological aspects of *Euphorbia dulcis* L. Ibid. 11: 24-36

Lyon, F. M. (1898): A contribution to the life history of *Euphorbia corollata*. Bot. Gaz. 25: 418-426

Modilewski, J. (1909): ZurEmbryobildungVon*Euphorbiaprocera*. Ber. Dtsch. Bot. Ges. 27: 21-26

Modilewski, J. (1910): WeitreBeitrangeZurEmbryobildungeinigerEuphorbiacean. Ibid. 28: 413-418

Mukherjee, P. K. (1957): Studies in the embryological of *Euphorbia hypercifolia* L. *Bull. Bot. Soc*. Univ. Sagar 9: 7-18

Mukherjee, P. K. (1961a): Embryological of two Euphorbiaceae. *Proc. Indian Acad. Sci*. 43: 217-229

Mukherjee, P. K. (1965): Contribution to the embryology of *Euphorbia peltata*Roxb. Ibid. 35: 327-337

Mukherjee, P. K. and Sathianathan, K. N. (): Embryological studies in Euphorbia serpens. VIIIth All India Bot. Conf. Hyderabad 72

Rao, P. N. (1970): Euphorbiaceae. Bull. Indian Nat. Sci. Acad. $1: 136-141

Rao, D. S. and Devi, R. R. (1974): Some embryological feature of Euphorbia vermiculata Rat. Curr. Sci. 43(8): 257-59

Robyns, W. and Louis, A. (1942): Eeschouwingen over PolyembryonieenPolysperiebij de Bedektzadigen. Verh. Kon. Vlaam. Acad. Wentensch. Belg. 4: 5-112.

Sathianathan, K. N. and Mukherjee, P. K. (1983a): Reproductive ontogeny in the two members of Euphorbiaceae. *Proc. Ind. Sc. Cong*. 61.

Schnarf, K. (1929): Embryologie der Angiosperm. Berlin.

Schurhoff, P. N.(1924): ZytologischeUntersuchungen in der Reihe der Geraniales. Jb. Wiss. Bot. 63: 707-759

Sharma, G. (1956): Studies in the family Euphorbiaceae I. The gametophyte of Chrozophorarottleri A. Juss. *J. Indian Bot. Soc*. 35: 189-193

Thathachar, T. (1953): Morphological studies in Euphorbiaceae. *J. Mysore Univ*. 31: 363-399.

Tiwari, H. N. (1976): Embryology of *Euphorbia nerifolia* L. and *Euphorbiapanchganiensis* Blatt. And McCenn. In Recent trends and contacts between Cytogenetics, Embryological and Morphology, U.G.C. Seminar, Nagpur 33.

Zanwar, C.D. (1984): Gametophytes of *Euphorbia clarkeana*. Abst. *Proc. Indian Sci. Cong*. 118.