Investigations on the Megasporogenesis in *Euphorbia rosea* Retz. of Euphorbiaceae

Kadu Sandhya R.
BhartiyaMahavidylaya, Amaravti, 444603.

Abstract:

This study deals with the process of development of megaspore in the plant. *Euphorbia rosea* Retz. One of the herbaceous member of the 'spurge family'. The plant is unisexual and the female flower is roseycolored. The cyathium inflorescence is the characteristic feature of this family. The genus is characterised by the presence of an anatropous, bitegmic and crassinucellate ovules.The nucellus is very much drawn out of the micropyle in form of nucellar beak, there is no micropyle at all in the strict sense. The female archesporium is hypodermal and multicellular integuments.Two celled female archesporium is seen. The megaspore tetrad formed in *Euphorbia* is also variable and the tetrad is linear in *Euphorbiarosea.*

Keywords: Euphorbia, Megaspore,nucellar beak, archesporium, bitegmic, tetrad,

Introduction:

Among the angiosperms, families such as the Euphorbiaceae are notable for their floral and inflorescence. *Euphorbiarosea* is commonly called as 'Rosey spurge'. This plant was commonly found in Afghanistan, peninsular India and Srilanka. It is an ascending herb, upto 50 cm. tall. The leaves are oblong-elliptic. The flowers are rose colored, in lax cluster of about 10 flowers. Sometimes flowers may occur singly in the upper leaves axils. The flowering period is from November to January.The female florets laterally pendulous. Styles deeply bifid, stigma spathulate.

Megasporogenesis is an important process in plant reproduction, which includes several series of developmental stagesfrom sporogenous cells to megaspore. The female gametophyte develops from the megaspore formed in the nucellar tissueof the ovule.The family Euphorbiaceae shown eight different types of embryo sac development. (Banerji, 1951). A comparative histogenic study of ovules of some representatives of the genus Euphorbia reveals a remarkable similarity during the pre-fertilization stages, in post fertilization stages the basal part of the nucellus develops into a podium.

The basal cells of the inner epidermis of the inner integument. The suspensor of the embryo is embedded in the remnants of the nucellar beak which persists in the mature seed (Bor and Kapil, 1975).The nucellus is free from the inner integument up to the base and the nucellar beak does not protrude beyond the base of the endostome as in *Ricinuscommunis* (Singh, 1954).

Review of Literature:

The characteristic development of the inflorescence and seed in Euphorbiaceae has interested phytomorphologists for over a century and a half.For instance, Gaertner (1791) described the mature seed of *Euphorbia peplus* as having a single coriaceous' integuments'. The first neatly illustrated and detailed description of the ontogeny of the ovule of *E. lathyrus*

is by Mirbel (1829). He not only clearly distinguished an outer integument (primine), an inner one (secondine) and nucellus but also recognised the obturator and a conspicuous basal nucellarswelling noticeable only after fertilization.

Lyon (1898) published the observations of Euphorbia corollata describing the binucleate condition of the pollen grains at the time of shedding and development of 8-nucletae normal type of megagametophyte.

Modilewski (1909) found Penaea type magagametophyte in *E. procera* (1909) and *E. palustris* (1911) while he described (1910) the normal type ofmegagametophyte in *E. lathyrus*, *E. silicifolia*, *E. globose*, *E. meloformis*, *E. cyparissias*, *E. corraloides*, *E. variegata*, and *E. helioscopia*.

Dessiatoff (1911) claimed to have observed the Penaea type of megagametophyte in *E. variegata*.Donati (1912) studied the female gametophyte in *E. pulcherrima* and reported it to be at the 16-nucletae type. Schurhoff (1926) described the Penaea type of megagametophyte in *E. procera* and further reported the presence of polyembryony in *E. helioscopia* and *E. platyphylla*.

According to Carano (1925 and 26) in *E. dulcis*, the egg fails to devlop due to the absence of fertilisationand degenerate without giving rise to the embryo. Synergids also degenerate.Carano (1926) mentioned the Fritillaria type of megagametophyte in *E. dulcis* and considered its development to the intermediate between 8-16 nucleate types. The female archesporial cell was to be 2-3 layers deep in the nucellus. It degenerated without giving rise to the female gametophyte was explained by Bhalla (1941a. b).Kajale and Rao (1943) described the megagametophyte in *E. hirta*.

Landes (1946) studied the seed development very briefly in *E. corollata*, *E. dentata*, *E. marginata*and *E. esula*.In species of *Euphorbia* the vascular bundles enter the chalazal end in a number of short branches below the hypostase. In *E. masculata*the hypostase is not observed till the ovule is nearing maturity.

Gopinath and Gopalkrishnan (1944) worked out the megasporogenesis and female gametophyte in *E. oreophila* and reported that the development of megagametophyte conforms to the Polygonum type.Thathachar (1953) has given a general account on development of female gametophyte in *E. thymifolia*, *E. hypercifoila*and *E. cristata*.

Maheshwari (1955) has given a critical review of the occurrence of bisporic embryo sac in angiosperm in the family Euphorbiaceae.Mukherjee (1957) has described themegasporangium and female gametophyte.Singh (1959) described the structure and a few stages of development of seed in *E. geniculata*.Ovules are anatropous with a nucellar beak, which reaches up-to the inner integument.

Kelkar (1960) investigated *E. tirucalli*the gynoecium is tricarpellary. Each locule has a single ovule, hanging from the top at the axile placenta. The ovules are bitegmic, crassinucellate and anatropous. The micropyle is organised by the outer integument only. The embryo sac development is of the normal 8-nucleate Polygonum type.

Some of the embryological aspects of *E. dulcis* have been described by Kapil (1961). He observed the ovules are borne on axile placenta. Each ovule is bitegmic and crassinucellate. Obturator consists of elongated cells. The development of the female gametophyte confirms to the fritillaria type. Egg apparatus and antipodals are ephemeral. Both syngamy and triple fusion are absent. The endosperm is autonomous and nuclear. A large number of nucellar embryos are produced thereby exhibiting polyembryony.

Mature embryo, seed coat and fruit wall in *E. cristata*have been investigated by Mukherjee (1961b). He has observed that the embryo is dicotyledonous and straight. Ovules are bitegmic and both the integuments take part in the formation of the testa.Venkateswarula and Rao (1963) studied the development of endosperm in *E. geniculata*. In this plant the development of the endosperm follows the nuclear type.

Singh (1969) has investigated the structure and development of seed in *E. helioscopia*. In this taxa, ovules are bitegmic, crassinucellate and anatropous. Endosperm is of nuclear type.Female gametophytes of *E. ligularia* have been described by Patil and Samant (1972). The ovules are bitegmic, crassinucellar and anatropous. Embryo sac development is of polygonum type.Bor and Bouman (1974) studied the development of integument in *E. milli*. Other aspects such as ovule initiation and its subsequent development have also been situated.

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. 29: L. S. ovary showing development of ovules.

Fig. No. 30: Same as above showing development of ovule at 2-nucleate embryo sac stage. Note the development of integuments.

Fig. No. 31: L.S. anatropous, bitegmic, crassinucellate ovule with obturator.

Fig. No. 32: L. S. ovary showing development of ovule at mature embryo stage. Note elongated, finger like nucellar beak(nb) and obturator (ob).

Fig. No. 33: L. S. ovary showing enlarged view of nucellar beak and obturator at mature embryo sac stage.

Fig. No. 34: T. S. of ovule at the tip of nucellar beak region.

Fig. No. 35: T. S. of ovule at the tip of nucellar region. Note the enlarge view of nucellar beak.

Fig. No. 36: L. S. nucellus showing 2- celled hypodermal female archesporium.

Fig. No. 37: Same as above showing megaspore mother cell with one parietal layer.

Fig. No. 38: Same as above showing dyad.

Fig. No. 39: Linear megaspore tetrad. Note the degenerated micropylar megaspore.

Fig. No. 40: 2-nucleate embryo sac.

Fig. No. 41: 4- nucleate embryo sac.

Fig. No. 42: Mature embryo sac with egg apparatus, 2 polars and 3 antipodals.

Fig. No. 43: Mature embryo sac. Note linear arrangement of antipodals.

Abbreviations:

Ant- antipodals, e- Egg nucleus, nb- Nucellar beak, ob- Obturator

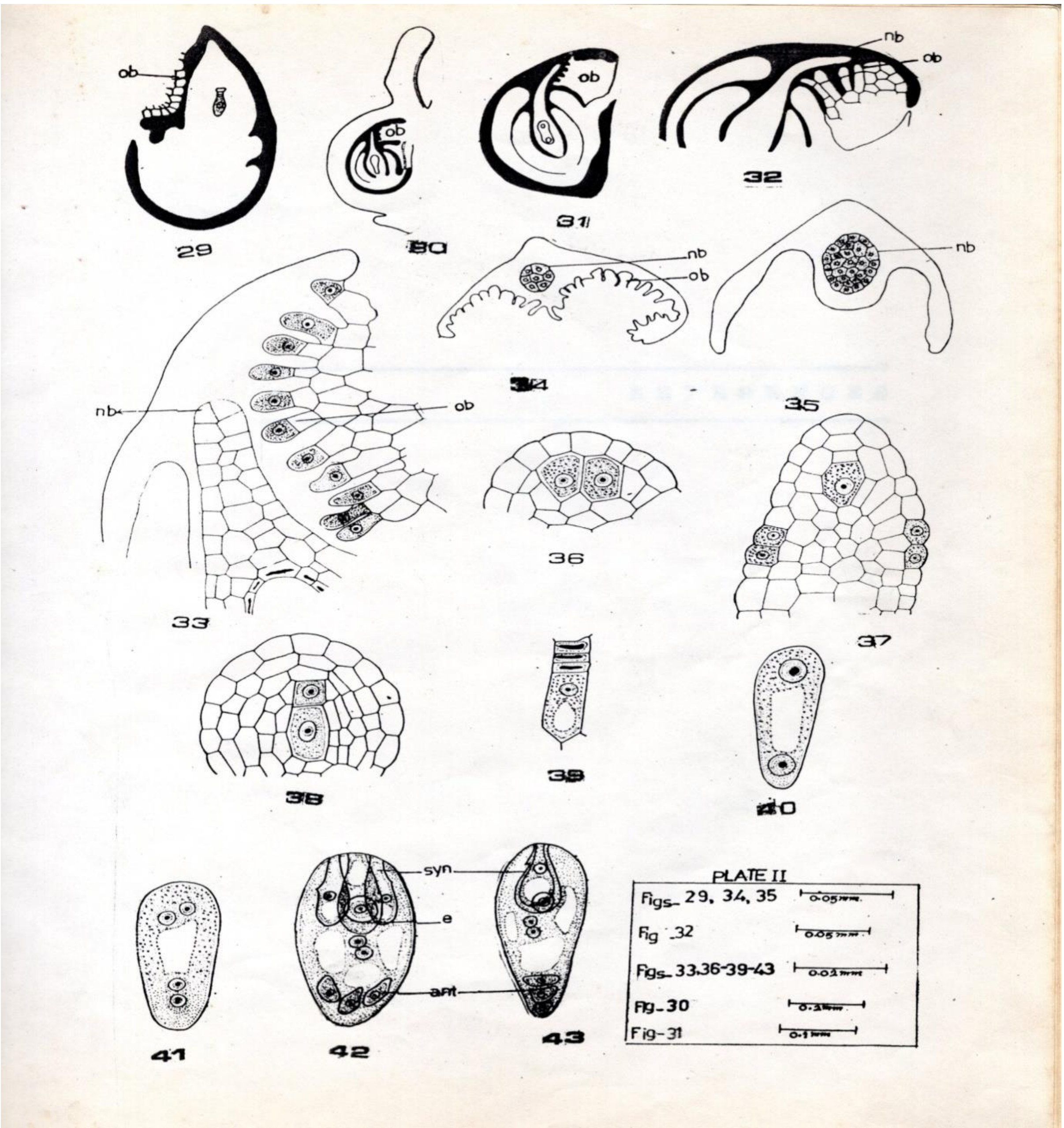

Fig. No. 29-43: Megasporangium, Megasporogenesis and female gametophyte

Ovular primordia arise as a small protuberance of parenchymatous cells. The ovule is attached to the upper and of the axile placentation. At the archesporium and megaspore mother cell stage the ovule primordia is straight. Then it starts bending in the later stage of ovule development. A stronger rate of growth at the convex side of the ovular primordia sets in the curvature ultimately leading to the anatropous condition of the ovule (Fig. No. 30, 31 and 32).

The ovule is further characterised by the presence of long finger like projection of the nucellar beak, which extends beyond the exostome (Fig.No. 30, 31 and 32). The beak (nb) tapers towards the apex and at the tip it consists of 6-7 cells (Fig. no. 34). Gradually it becomes thicker towards the chalaza (Fig. No. 35). The obturator arisesfrom the placenta. To begin with, it consists of closely packed rectangular cells rich in cytoplasm (Fig.No. 29). Later on the obturator cells get separated into loose finger like projections (Fig.No. 33). The nucellar beak (nb) is seen bend over the obturator (Fig.No. 31, 32 and 33).

Each mature ovule is bitegmic and crassinucellate (Fig. no. 30 and 31). At the megaspore mother cell stage both the integument initials are seen at the base of the nucellus (Fig. No. 37). By the time a tetrad is differentiated in the nucellus both integuments are well developed at the base (Fig.No. 29).

Megasporogenesis and female gametophyte

The female archesporium is hypodermal and multicellular (Fig. no. 36). One of the archesporial cells, which are more or less in the median plane, starts developing. It divides periclinally to form a parietal cell and a megaspore mother cell (Fig. no. 37). Soon the other archesporial cells lose their identity and merge with nucellar cells. The megaspore mother cell undergoes meiosis I to form a dyad of equal cells (Fig. no. 38). The two cells of the dyad undergoes meiosis II to form a tetrad. The tetrad thus produced is of linear type (Fig.No. 39). The chalazal megaspore of the tetrad is functional and it develops into an 8 nucleate embryo sac of Polygonum type (Maheshwari, 1950).

The other three megaspore degenerate (Fig.No. 39). The nucleus of the functional megaspore undergoes first mitotic division to form a 2-nucleate embryo sac with a big vacuole (Fig.No. 40). The 2-nucleate embryo sac by second mitotic division forms a 4-nucletae embryo sac (Fig.No. 41). These nuclei undergo the third mitotic division to form an 8- nucleate embryo sac. From the upper 4 nuclei, the egg apparatus and the upper polar are formed while from the lower 4 nuclei, the three antipodals and the lower polar are formed (Fig.No. 42). The egg is flask shaped with a basal nucleus and micropylar vacuole while the synergids have a micropylar nucleus and basal vacuole. The synergids have beaks (Fig.No. 42 and 43). The 3 antipodals are definite cells which may be placed one over the other in a linear manner (Fig.No. 43). Sometimes they are placed in a triangular manner. The mature embryo sac is elongated and spindle shaped (Fig.No. 42 and 43).

Disussion:

Ovule and megagametophyte

The genus *Euphorbia* is characterised by the presence of an anatropous, bitegmic and crassinucellate ovules. Anatropous ovules are seen in a vast number of species like *E.geniculata*(Singh, 1959); *E. tirucalli* (Kelkar, 1960); *E. dracunculoides*; *E. peltata* (Mukherjee, 1962a, 1975); *E. helioscopia* (Singh, 1969); *E. Thymifolia*(Pal, 1971); *E. ligularia*(Patil and Samant, 1972); *E. Vermiculata*(Rao and Devi, 1974) and *E. peplus* (Pal and Khan, 1978).

In this taxon, as the nucellar beak is present, there is no micropyle at all in the strict sense.Micropyle being formed by the integuments is seen in *E. heterophylla*, *E. pulcherrima*(Sharma, 1955); *E. dracunculoides* (Mukherjee, 1961a); *E. peltata* (Mukherjee, 1965); *E. rothiana*(Venkateswarlu and Rao, 1973).

The micropyle is organized by the outer integument alone in *E. parbracteata* and *E. prostrata* (Sathianathan and Mukherjee, 1983); *E. pilosa* (Singh and Jawaharlal, 1965) and *E. maddeni*and *E. nivulis* (Bhanwara, 1987).

Obturator

Obturator has been found in many species of Euphorbia. Compact type of obturatoris seen in *E. serpens*. *E. pulcherrima*(Sathianathan, 1983) while a loose type is seen in *E. perbracteata* and *E. prostrata* (presently studied), *E. microphylla* (Mukherjee, 1961a); *E. hypercifolia* (Mukherjee, 1957); *E. helioscopia* (Singh, 1969); *E. peltata* and *E.dracunculoides*(Mukherjee, 1961a).

Nucellus

Two distinct tendencies have been noticed with regard to the behaviour of the nucellus in this family. The nucellus remains inside the integuments, *E. pulcherrima*, *E. perbracteata*, *E. prostrata* (Sathianathan, 1983); *E. rothiana* (shrivastava, 1952); *E. tiruccalli* (Kelkar, 1960), *E. dracunculoides*, *E. peltata* (Mukherjee, 1961a); *E. nerifloia*; *E. panchganensis* (Tiwari, 1976).

The nucellus is very much drawn out of the micropyle in form of nucellar beak. This was observed in *E. rosea*(Present study), *E. corrigioloides*, *E. serpens*. Such a structure has also been observed in *E. hirta* (Kajale and Rao, 1943); *E. nutens*, *E. maculate* (Landes, 1946); *E. hypercifolia* (Mukherjee, 1957); *E. helioscopia* (Singh, 1969a); *E. thymifolia*(Pal, 1971).

The shape of the nucellar beak varies. It may be finger like as in *E. hirta* (Kajale and Rao, 1943), beaklike in *E. helioscopia* (Singh, 1969). Nucellar beak is bent in *E. thymifolia* (Pal, 1971); *E. marginata*(Devi, 1975).

Megasporogenesis and female gametophyte

The female archesporium is hypodermal and uni-or multicellular. Two celled female archesporium is seen in *E. rosea* (Presently studied). However, 3-celled type is observed in *E. pulcherrima* (Sathianathan, 1983). Hypodermal nature of the archesporium is an established feature in the family Euphorbiaceae and that too in the various species of Euphorbia (Kajale and Rao, 1943; Shrivstava, 1952; Thathachar, 1953; Mukherjee, 1957, 65; Pal, 1971; Devi, 1975 etc.).

The megaspore tetrad formed in *Euphorbia* is also variable. In majority, the tetrad is linear as in *Euphorbiarosea* (present study), *Euphorbia thymifolia* (Mukherjee, 1957; Pal, 1971); *E. tirucalli*(Kelkar, 1960); *E. rothiana* (Venkateswarlu and Rao, 1973). The frequency of occurrence of T-shaped tetrad is less. *E. dracunculoides*, *E.microphylla* (Mukherjee, 1961a).

The female gametophyte in the present investigation is of monosporic, 8 nucleate polygonum type.

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