EUPHORBIA GENICULATA – OVULE TO SEED

J. BOR[1] and R. N. KAPIL

Department of Botany, University of Delhi, Delhi-110007, India

SUMMARY

A comparative histogenetic study of ovules of some representatives of the genus *Euphorbia* reveals a remarkable similarity during the pre-fertilization stages. The inner integument is initiated first. Mitotic activity in the subdermatogen causes the initiation of integuments as well as the apical growth of the nucellus. Subsequent mitotic activity in the dermatogen contributes to the formation of the multi-layered nucellar flanks, the distal region of integuments, and the caruncle. During the post-fertilization stages the basal part of the nucellus develops into a podium. The basal cells of the inner epidermis of the inner integument elongate appreciably, whereas its proximal cells add to the formation of a podium. The funicular vascular supply extends into the base of the inner integument. The suspensor of the embryo is embedded in the remnants of the nucellar beak which persists in the mature seed. Enucleate cytoplasmic vesicles project into the central vacuole of the nuclear endosperm. The dermal cells of the placental obturator elongate radially and form uni- and multi-cellular hairs. Development of the female gametophyte follows the Polygonum type.

1. INTRODUCTION

Euphorbia, the largest genus of the family Euphorbiaceae, exhibits a great variety in its growth habit, morphology, anatomy, cytology and embryology (MAHESHWARI 1942). About 15 papers have been published on the structure and development of the ovules, but most of these do not discuss the initiation and ontogeny of the ovular primordium and the integuments, or the characteristic development of the nucellus, obturator, and caruncle (see, *e.g.*, S.P. SINGH 1959). The ovular primordia of several euphorbiaceous taxa are simply described as being composed of a "*homogeneous mass of parenchymatous cells*" (R. P. SINGH 1962). BOR & BOUMAN (1974) have, however, shown that in *Euphorbia milii* and *Codiaeum variegatum* they consist of a dermatogen, subdermatogen, and central core. Their study reports for the first time that in these genera the inner integument is initiated subdermally.

   The purpose of this investigation is to clarify some developmental controversies and to establish if, and to what extent, *Euphorbia geniculata* (an annual herb) and *E. milii* (a perennial, sturdy, bushy plant) agree in the initiation and development of their ovules.

---

[1] Permanent address: Hugo de Vries-Laboratorium, Plantage Middenlaan 2A, Amsterdam A1-004, The Netherlands.

2. MATERIALS AND METHODS

Euphorbia geniculata Orteg. was collected in August 1955 at Delhi, fixed in
F.A.A., dehydrated in an ethanol-xylene series, and embedded in paraffin (of
56–58 °C melting point). Serial sections (5–13 μm) were stained with safranin
and fast green. Schultze's technique of maceration (see CHAMBERLAIN 1901)
was employed for examining sclereids. The initial work was undertaken to
investigate the embryology, but the slides were re-examined for histogenetic
studies.

3. EARLY OVULE DEVELOPMENT AND MEGASPOROGENESIS

The initiation and development of the nucellus, integuments and obturator
agree with those in *E. milii* (BOR & BOUMAN 1974). The ovular primordia are
initiated simultaneously on the flanks of the floral apex (*fig. 2A*). Subsequently,
3 zones, viz., a dermatogen, subdermatogen, and central core become distin-
guishable (*fig. 3*: l_1, l_2, l_3.). The cells of the inner zone (l_3) are initially oriented
in longitudinal rows, but mitotic activity in various planes soon destroys this
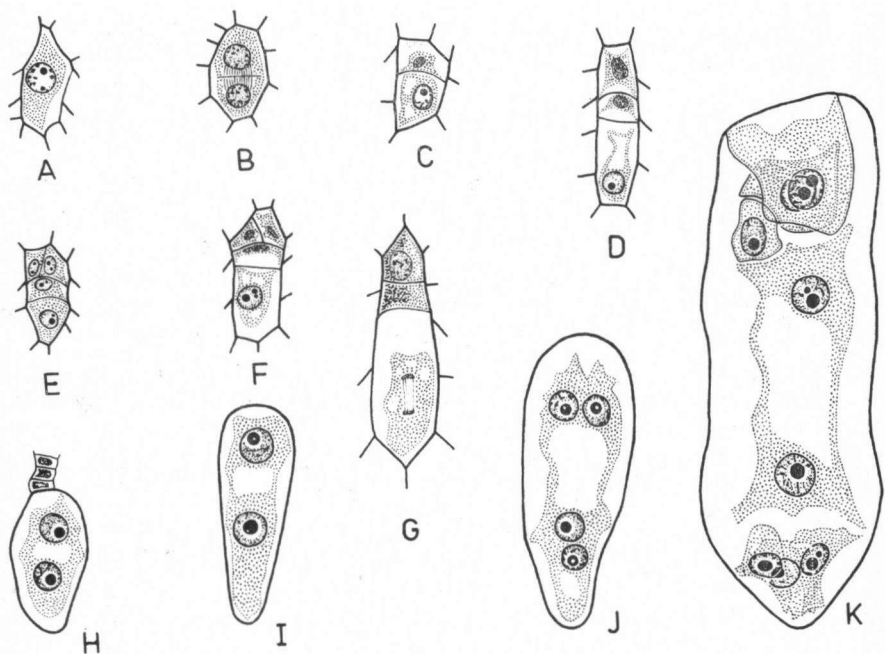

Fig. 1A-K – Megasporogenesis and female gametophyte.   A, B: Megaspore mother cells.
C: Dyad; the upper cell has degenerated.   D: "Triad" showing two degenerated cells.   E:
T-shaped tetrad.   F, G: Functional megaspore.   H: Two-nucleate embryo sac with remnants
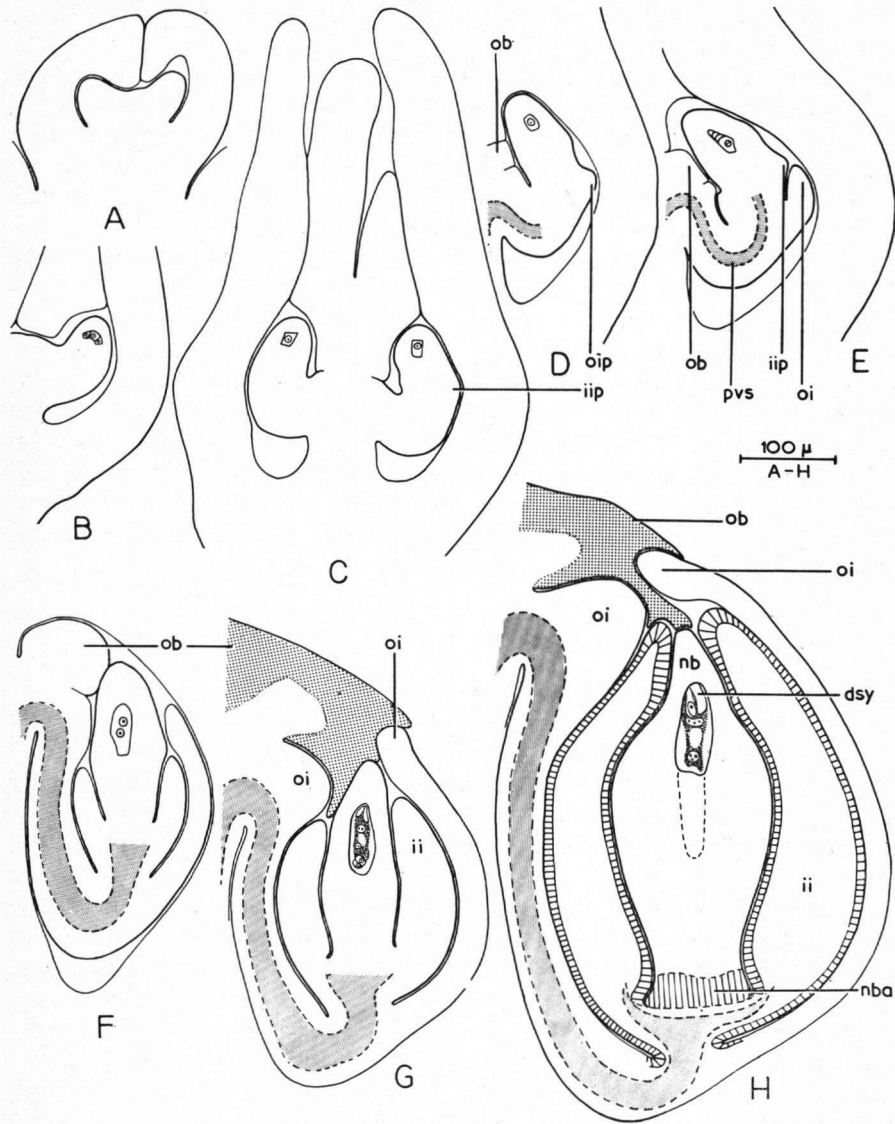of megaspores.   I, J: Two and 4-nucleate gametophytes.   K: Mature embryo sac.

Fig. 2A-H – Development of ovule (*dsy*, degenerated synergid; *ii*, inner integument; *iip*, primordium of inner integument; *nb*, nucellar beak; *nba*, nucellar base; *ob*, obturator; *oi*, outer integument; *oip*, primordium of outer integument; *pvs*, provascular strand). A, B: L.s. ovary with ovular primordia. C-F: Initiation and ontogeny of integuments in young ovules. G, H: L.s. ovules at mature embryo sac and post-fertilization stages.

pattern. The two peripheral layers (l_1 and l_2) are primarily maintained by anticlinal cell divisions.

The first specialization in the ovular primordium is the differentiation of one or more subdermal archesporial cells, which divide to form primary sporogenous and primary parietal cells (*fig. 3A*). The central sporogenous cell is generally more prominent, and functions as megaspore mother cell (*fig. 3B, C*). The parietal cells and their derivatives undergo repeated periclinal (sometimes anticlinal) divisions giving rise to a multi-layered tissue.

The primordium of the inner integument becomes visible as a small annular swelling (around the entire circumference) in the middle region of the young ovule (*figs. 2C, 3B, C*). This is immediately followed by the initiation of the primordium of the outer integument (*figs. 2D, 3C*). Differentiation of these primordia is caused by the mitotic activity of 1 or 2 subdermal cells, pushing up the dermatogen. The dermal cells in these areas also divide periclinally.

Simultaneously, the megaspore mother cell enlarges appreciably (*figs. 1A, 3B*). As a result of reduction divisions a linear tetrad is organized, though T-shaped tetrads also frequently occur (*figs. 1B, C, E, F, 3C, D*). On rare occasions the micropylar dyad cell degenerates, or fails to divide, so that after the division of the lower dyad cell a "triad" is produced (*fig. 1D*).

4. ADVANCED OVULAR ONTOGENY

Female Gametophyte: – The chalazal megaspore functions and produces 2-, 4- and 8-nucleate gametophytes (*figs. 1G-K, 3E*). The egg apparatus, polar nuclei, and antipodal cells are organized in the usual manner. The antipodal cells are ephemeral, and disorganize at the time of double fertilization. By the time the primary endosperm nucleus divides, both synergids have already degenerated (*figs. 2H, 3F*).

Nucellus and Obturator: – The initial apical growth of the nucellus is caused by mitotic activity in the subdermatogen. Later periclinal divisions occur only in the epidermal cells of the nucellar flanks (*fig. 3D, E*) so that, at fertilization, the nucellar beak is composed of a multilayered dermal tissue at its flanks, and a 1-layered dermatogen at its tip (*fig. 3F*). The subdermal cells of the nucellar beak are loosely knit together and later on disintegrate into a mucilaginous mass. The basal region of the nucellus consists of a compact mass of radially arranged, thin-walled cells with dense cytoplasm. This tissue develops into a conspicuous podium which persists in the mature seed (*figs. 2H, 4A, C, 5*). The funicular vascular bundle ramifies in the chalazal region to form peripheral strands which extend into the base of the inner integument. The chalazal tissue situated between the vascular supply and the podium is characterized by its somewhat flattened and darkly staining cells. In contrast to *E. milii* (see BOR & BOUMAN 1974), this tissue does not develop into a hypostase, however.

The placental obturator is initiated at the beginning of meiosis 1 (*figs. 2D, 3C*). The dermal cells of the obturator multiply primarily by anticlinal divisions,
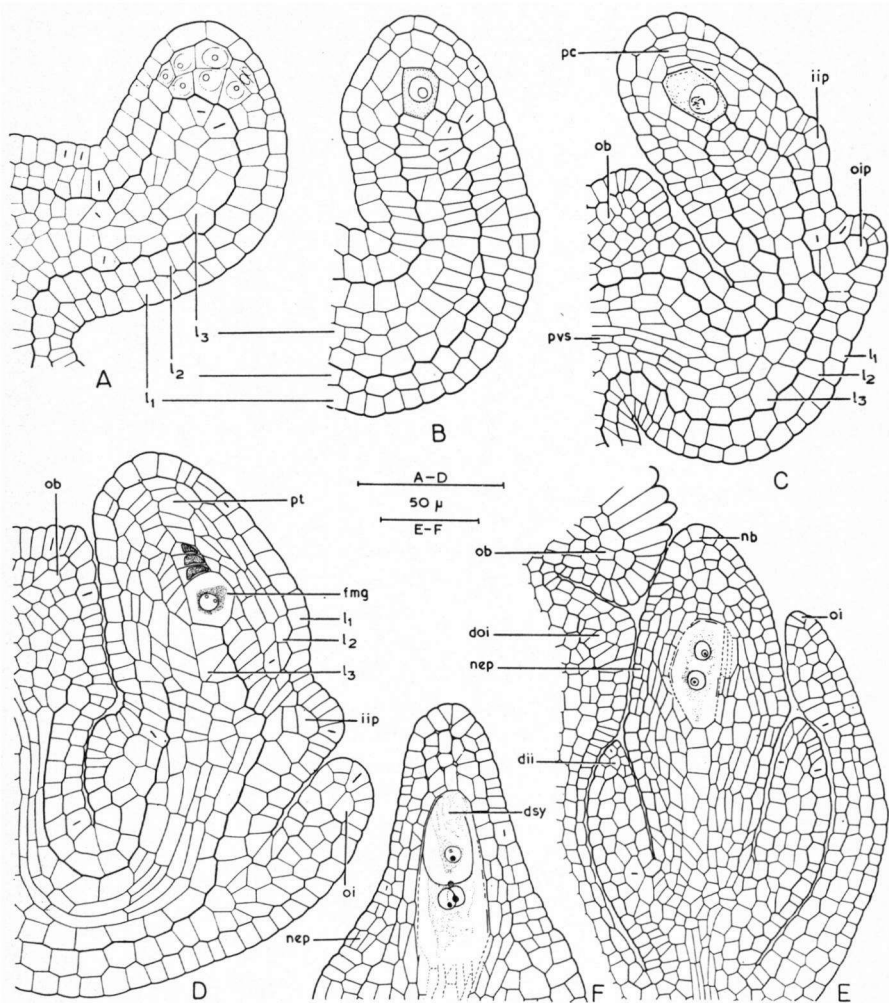
Fig. 3A-F – Development of ovule (*dii*, dermal tip of inner integument; *doi*, dermal tip of outer integument; *dsy*, degenerated synergids; *fmg*, functional megaspore; *iip*, primordium of inner integument; l_1, dermatogen; l_2, subdermatogen; l_3, central core; *nb*, nucellar beak; *nep*, nucellar epidermis; *ob*, obturator; *oi*, outer integument; *oip*, primordium of outer integument; *pc* parietal cells; *pt*, parietal tissue; *pvs*, provascular strand).   A: L.s. ovule with multiple archesporium.   B: Same, initiation of inner integument.   C: Initiation of outer integument and obturator.   D: Linear tetrad of megaspores.   E: Two-nucleate embryo sac. F: Nucellar beak at double-fertilization stage. Three zones are separated by a thicker line: dermatogen, subdermatogen, and central core are distinguishable in *figures A-D*.

and elongate radially. Ultimately, they become transformed into trichomatic cells containing one or more nuclei. Occasionally periclinal divisions can be observed which give rise to multi-cellular hairs (*fig. 6A*).

Inner Integument: – Immediately after its initiation, the outer integument tends to grow around the nucellus, but the inner one remains primordial for a longer period. The elongation of the ovule, during this period, causes a stretching of the base of the primordium (*fig. 3C, D*). Eventually, the inner integument envelops the nucellus (*fig. 3E*). The subdermal cells, which initially divide only periclinally, begin to divide in various planes causing the growth of the integument in thickness. After fertilization, both the inner and outer epidermal layers differentiate into conspicuous layers of closely-packed cells containing darkly staining nuclei. In the apical portion of the inner epidermis (where the inner integument surrounds the nucellar beak and micropyle) several periclinal divisions take place. As a result, the apical region of the inner integument consists of a group of small cells (with dense cytoplasm) which are dermal in origin, and can be recognized in the young seed (*fig. 4B*).

During early post-fertilization stages, the major part of the inner integument shows large and vacuolated cells rich in amyloplasts. The cells of the inner epidermis elongate radially, and fibrous thickenings on their walls become discernible, in the young seed at the globular embryo-stage. At the chalazal end these cells enlarge considerably, and divide once or repeatedly, thus widening the base of the integument (*fig. 4A*). The proximal cells of the inner epidermis, adjoining the podium, undergo several periclinal divisions and contain dense cytoplasm. They have an appearance similar to the cells of the nucellar podium, and seem to fuse with them during the later stages of seed development (*fig. 5*). The cells of the outer epidermis also extend radially to an appreciable extent, and become obliquely oriented. Sclerification takes place in these, and also in the epidermal cells surrounding the tip of the nucellar beak and the endostome (*fig. 4D*).

Outer Integument: – The initiation of this integument can be traced back to a single subdermal initial which undergoes repeated periclinal divisions. The longitudinal row of cells thus formed pushes up the dermatogen (*fig. 3C*). The outer integument becomes 4-layered as a result of further divisions in the subdermal layer. In the apical region the inner epidermal cells begin to divide periclinally (*fig. 3E*). At the 4-nucleate stage of the embryo sac the outer integument encloses the nucellus completely. It also forms a conspicuous "bulge" (mainly of dermal derivation) on the funicular side (*fig. 2F, G*). After fertilization the apex of the outer integument bends over the ovular tip and, finally, closes the endostome (*figs. 2H, 4A*). Concomitantly, an inconspicuous caruncle develops around the micropyle, on the ventral side of the young seed. This is derived from the distal portion of the outer integument, and is predominantly of dermal origin. The peripheral cells of the caruncle elongate radially, and are covered with a cuticle (*fig. 4C, E*).
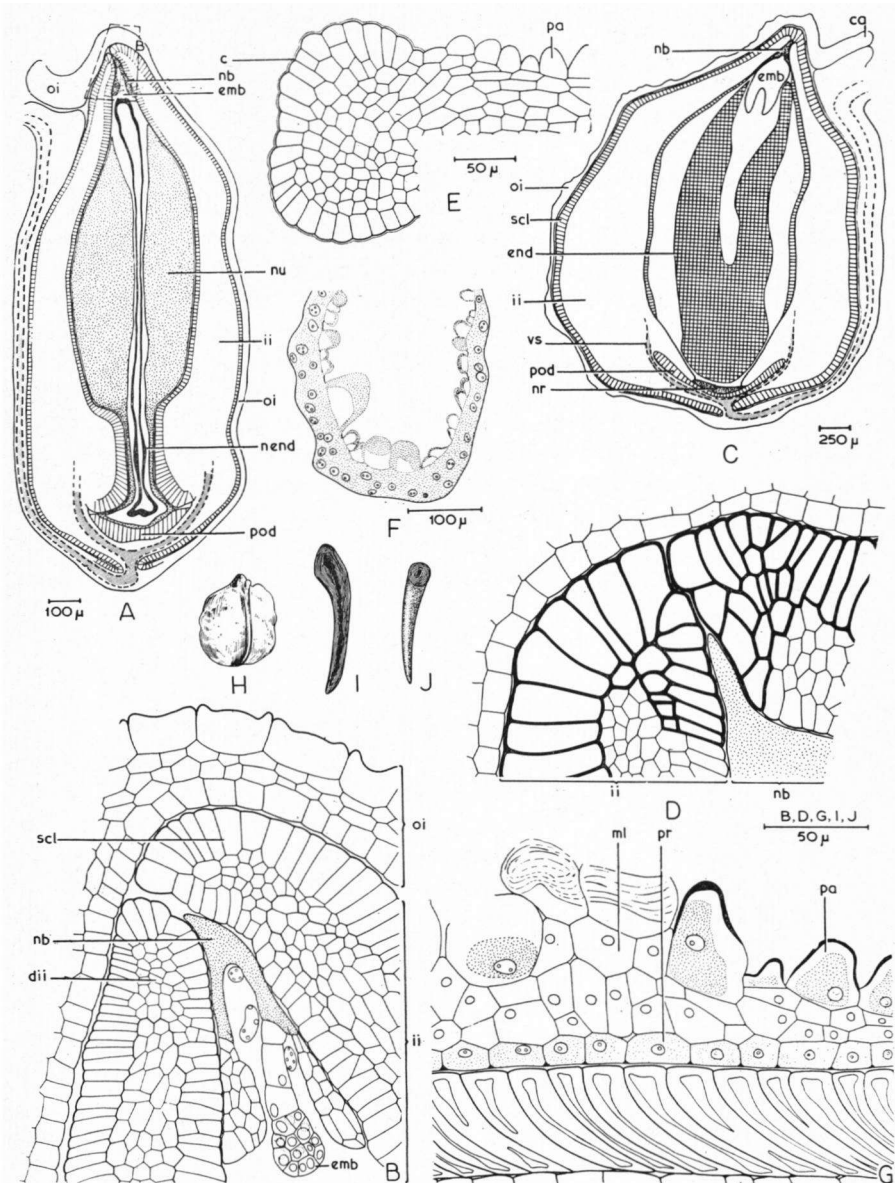
Fig. 4A-J – Development of seed (*c*, cuticle; *ca*, caruncle; *dii*, dermal rim of ii; *emb*, embryo; *end*, endosperm; *ii*, inner integument; *ml*, middle layer; *nb*, nucellar beak; *nend*, nuclear endosperm; *nr*, nucellar remnants; *nu*, nucellus; *oi*, outer integument; *pa*, papilliform cells; *pod*, podium; *pr*, prismatic layer; *scl*, sclerotic layer; *vs*, vascular supply). A: L.s. young seed at globular proembryo stage. B: Enlargement of portion B marked in *A*. C: L.s. seed, dicotyledonous embryo stage. D: Micropylar area of almost ripe seed in l.s. E: L.s. caruncle. F: Chalazal part of nuclear endosperm enlarged to show enucleate cytoplasmic vesicles. G: Part of mature seed coat, l.s. H: Seed in surface view. ×6. I, J: Sclereids from seed coat.

Seed and Seed Coat: – As the seed matures disorganization and resorption proceeds in the central part of the nucellus which finally disappears completely. Both the podium and the nucellar beak persist, however, although the latter shrinks considerably during subsequent stages. The suspensor of the embryo is embedded in the remnants of the nucellar beak (*figs. 4B, 6B*).

An intensive resorption also takes place in the middle layers of the inner integument. In the mature seed they persist as a thin membrane covered by a sclerotic outer layer. In contradistinction to *E. milii* the macrosclereids are more uniform in size, have a smooth surface and are slightly truncate (*fig. 4I, J*).

The cells of the outer integument in the mature seed (*fig. 4G*) are represented by: (*a*) an epidermal layer (consisting of papillose cells and slime cells); (*b*) a middle layer; and (*c*) a prismatic layer (see BOR & BOUMAN 1974).

The endosperm is nuclear. At the globular proembryo stage enucleate cytoplasmic vesicles develop which become prominent at the chalazal end with an accumulation of multi-nucleolate nuclei in the dense cytoplasm (*fig. 4F*). The walls of the endosperm cells appear slightly thickened, and only a few layers persist in the mature carunculate seed (*fig. 4H*).

5. DISCUSSION

A comparison of the growth habit, anatomy and morphology of *Euphorbia geniculata* and *E. milii* reveals a considerable heterogeneity (for the organogeny and the structure of the cyathium, see SCHMIDT 1907, MICHAELIS 1924, SATTLER 1973; for the various sections of the genus *Euphorbia*, compare, e.g., PAX & HOFFMAN 1931 who refer the two species to different sections: *Euphorbium* and *Poinsettia*), but the structure and ontogeny of the ovules are remarkably similar (especially during the pre-fertilization stages). The ovular primordia are composed of three organized zones: a central core sheathed by two external layers, the subdermatogen and the dermatogen. This zonation remains discernible in young ovules but becomes less obvious in older stages. The inner integument arises first as an annular bulge around the ovule; the initiation of the outer one follows almost immediately. S. P. SINGH (1959), who states that in *E. geniculata* the outer integument arises first, was probably misled because the outer integument exhibits an intense mitotic activity directly after its genesis. Such reports have also been published for other species of *Euphorbia* (POISSON 1878, SCHWEIGER 1905, THATHACHAR 1953) and need to be checked.

Contrary to the condition in *E. milii*, the inner epidermis of the inner integument differentiates into a conspicuous layer. Its cells elongate radially, and develop fibrous thickenings in the young seed stage. This condition was observed by LANDES (1946) in *E. dentata*, and by MAKDE & MUKHERJEE (1970) in *E. heterophylla*.

The outer integument invariably consists of 4 layers (cf. S. P. SINGH 1959, his fig. 5). In the apical region the inner epidermal cells divide periclinally, thus forming a conspicuous distal rim, dermal in origin. The rudimentary caruncle originates as an outgrowth of this rim around the exostome (BAILLON 1876 –
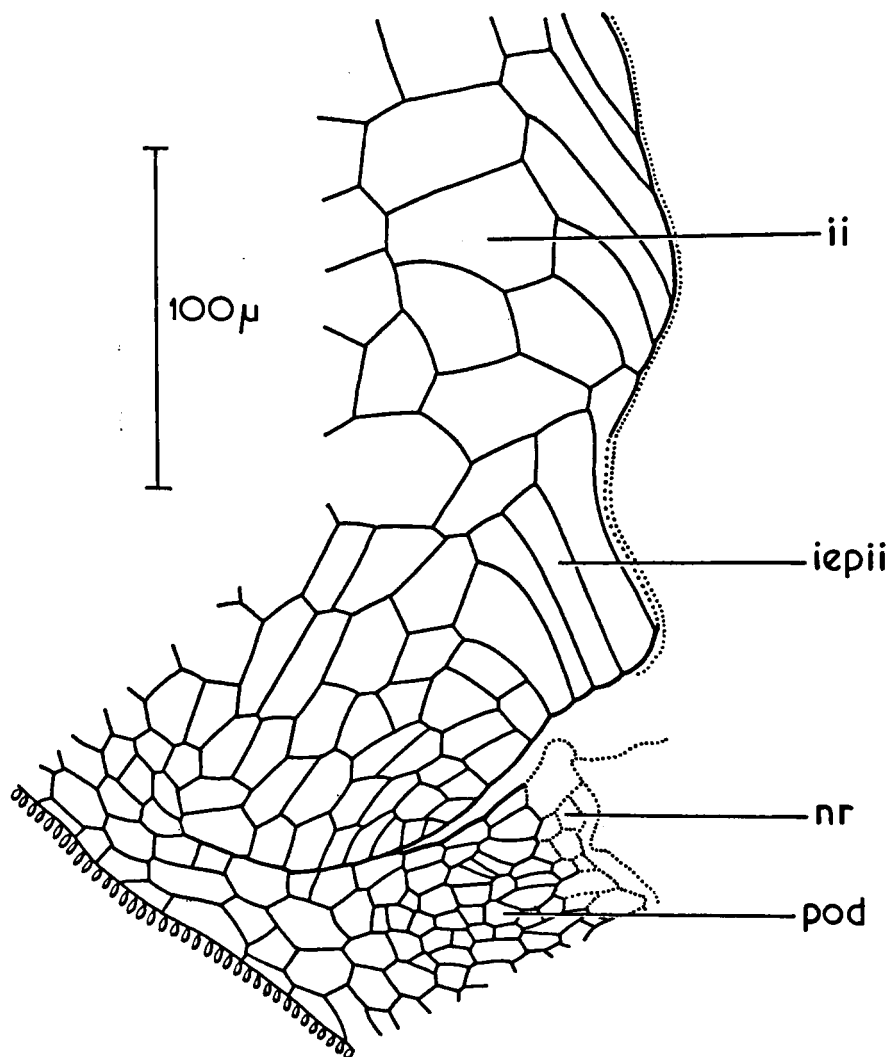
Fig. 5 – Part of the chalazal region of young seed (*iepii*, inner epidermis of inner integument; *ii*, inner integument; *nr*, nucellar remnants; *pod*, podium).

"*arille micropylaire*"; PFEIFFER 1891 – "*Exostomarillen*").

The initial growth of the nucellus in both species is caused by mitotic activity of the subdermatogen. Subsequently, periclinal divisions, mainly in the epidermal cells of the nucellar flanks, contribute to the formation of the nucellar beak. THATHACHAR (1953) observed that in *E. hypericifolia* "... *no periclinal divisions were noticed in any epidermal cell while the anticlinal divisions were common*
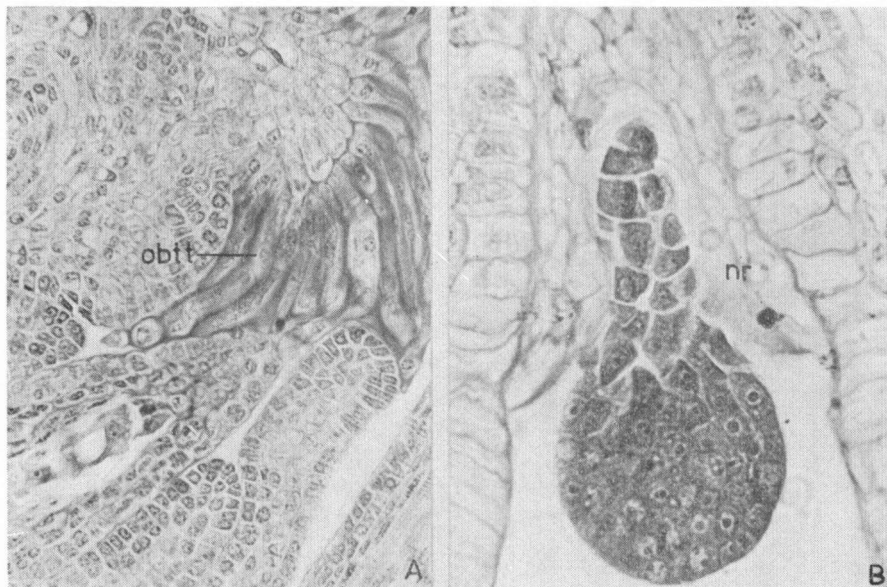
Fig. 6A, B – A: Micropylar region of ovule at mature embryo sac stage (*obtt*, multicellular obturator trichome).   B: Globular embryo (*nr*, nucellar remnants).

(fig. 4F)". He obviously studied only young ovules, as is also clear from his illustrations. MUKHERJEE's (1965) report that in *E. peltata* "*the parietal cell as well as the nucellar epidermis divide to form the primary and secondary parietal tissues, respectively (fig. 12)*", is also at variance with our findings. DAVIS' (1966) statement that in Euphorbiaceae "*the apical cells of the nucellar epidermis divide periclinally and give rise to a nucellar cap...*", holds true for *Acalypha* (KAJALE & MURTHY 1954, MUKHERJEE 1958), *Antidesma* (R. P. SINGH 1965), etc., but needs correction for *Euphorbia*[1]. The same applies to her report that "*in* Euphorbia geniculata... *the nucellus forms a beak which extends beyond the inner integument...*".

The basal part of the nucellus shows a remarkable, post-fertilization mitotic activity. This is accompanied by a considerable elongation of the basal cells of the inner epidermis of the inner integument. The proximal cells of this epidermal layer contribute to the formation of the podium which, however, is predominantly of nucellar origin. A similar condition can be observed in LANDES' (1946) fig. 25 of the chalazal region of a young seed of *E. dentata*. But she makes no mention of this and states: "*The inner integument is clearly separated from the nucellus*". In *E. milii* the podium develops into a more prominent structure, and

[1] In *E. hirta, E. preslii* (WENIGER 1917), *E. hirta* (KAJALE & RAO 1943), *E. thymifolia* (BANERJI & DUTT 1944), *E. nutans, E. maculata* (LANDES 1946), *E. hypericifolia* and *E. microphylla* (MUKHERJEE 1957, 1961) the nucellar beak extends beyond the exostome. Kajale & Rao report that it is formed by the actively dividing parietal and epidermal cells.

is present in addition to a hypostase (BOR & BOUMAN 1974).

The vascularisation of the ovules of *E. geniculata* exhibits an interesting variation. The vascular strand differentiates within the inner region, and extends into the base of the inner integument. In young seeds spiral tracheids can be observed in the peripheral parts of the bundle. In other species of *Euphorbia* the funicular vascular supply branches into a number of strands which terminate at the base of the hypostase (LANDES 1946, KAJALE 1954, MUKHERJEE 1965), or enter the hypostase (BOR & BOUMAN 1974).

There does not seem to be any correlation between the successive developmental stages of the ovule and the development of the female gametophyte. Frequently, the ovules may be in an advanced stage of development while the female gametophyte is still very young. The formation of enucleate cytoplasmic vesicles in the endosperm has previously been reported in *E. dulcis* (KAPIL 1961) and *E. helioscopia* (R. P. SINGH 1969), which are both plants of higher altitudes or temperate regions. *E. geniculata*, a plant of warmer regions, also shows well-developed vesicles. We are unable to prove any relation between higher altitudes or temperate conditions and vesicle formation (see D. SINGH 1964).

The seed and seed coat in *E. milii* and *E. geniculata* also show a great similarity. especially in the number of constituent layers and the structure of the epidermal cells which are papillose, pigmented, or mucilaginous. The raised portions of the subrugose testa are formed by the local enlargement of cells differentiating into macrosclereids (in the inner integument), and of the middle and epidermal layers (in the outer integument). A similar structure of the seed coat was observed in *E. peplus* (GRAM 1895–96), in *E. heterophylla* (MAKDE & MUKHERJEE 1970, although the authors do not mention the presence of mucilaginous cells), and in *E. chamaesyce* and *E. humifusa* (RAILJAN 1973).

ACKNOWLEDGEMENTS

REFERENCES

BAILLON, E. H. (1876): Sur l'origine du macis de la muscade et des arilles en général. *Adansonia* 11: 329–340.

BANERJI, I. & M. K. DUTT (1944): The development of the female gametophyte in some members of the Euphorbiaceae. *Proc. Indian Acad. Sci.* 20B: 51–60.

BOR, J. & F. BOUMAN (1974): Development of ovule and integuments in Euphorbia milii and Codiaeum variegatum. *Phytomorphology* 24: 280–296.

CHAMBERLAIN, C. J. (1901): *Methods in Plant Histology.* Chicago, U.S.A.

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

GRAM, B. (1895–96): Om frøskallens Bygning hos Euphorbiaceerne. *Bot. Tidsskr.* 20: 358–385.

KAJALE, L. B. (1954): Fertilization and the development of embryo and seed in Euphorbia hirta Linn. *Proc. natn. Inst. Sci., India* **20**: 353–360.

— & C. V. RAO (1943): Pollen and embryo sac of two Euphorbiaceae. *J. Indian bot. Soc.* **22**: 229–236.

— & MURTHY, K. S. N. (1954): The embryo sac of Acalypha ciliata Forsk. *J. Indian bot. Soc.* **33**: 417–422.

KAPIL, R. N. (1961): Some embryological aspects of Euphorbia dulcis L. *Phytomorphology* **11**: 24–36.

LANDES, M. (1946): Seed development in Acalypha rhomboidea and some other Euphorbiaceae. *Am. J. Bot.* **33**: 562–568.

MAHESHWARI, P. (1942): The embryo sac of Euphorbia heterophylla L. A reinvestigation. *Proc. Indian Acad. Sci.* **40B**: 158–166.

MAKDE, K. H. & P. K. MUKHERJEE (1970): Study of seed coat and fruit wall in Euphorbia heterophylla Linn. *Maharashtra V. M. Patrika* **5**: 42–46.

MICHAELIS, P. (1924): Blütenmorphologische Untersuchungen an den Euphorbiaceen. *Bot. Abh.* **3**: 1–150.

MUKHERJEE, P. K. (1957): Studies in the embryology of Euphorbia hypericifolia Linn. *Bull. bot. Soc. Univ. Saugar* **9**: 7–18.

— (1958): The female gametophyte of Acalypha malabarica Müll. with a brief discussion on the Penaea type of embryo sac. *J. Indian Soc.* **37**: 504–508.

— (1961): Embryology of two Euphorbiaceae. *Proc. Indian Acad. Sci.* **53B**: 217–229.

— (1965): Contribution to the embryology of Euphorbia peltata Roxb. *Proc. natn. Acad. Sci., India* **35B**: 327–337.

PAX, F. & K. HOFFMANN (1931): Euphorbiaceae. In: ENGLER, A. & K. PRANTL, *Die Natürlichen Pflanzenfamilien*. Leipzig.

PFEIFFER, A. (1891): Die Arillargebilde der Pflanzensamen *Bot. Jahrb.* **13**: 492–540.

POISSON, J. (1878): Du siège des matières colorées dans la graine. *Bull. Soc. Bot., France* **25**: 47–60.

RAILJAN, A. F. (1973): On importance of anatomical structure of the seed coat for the taxonomy of the genus Euphorbia L. *Bot. J. bot. Soc. U.S.S.R.* **58**: 239–245 (*In Russian*).

SATTLER, R. (1973): *Organogenesis of Flowers. A Photographic Textatlas*. Toronto, Canada.

SCHMIDT, H. (1907): Über die Entwicklung der Blüten und Blütenstände von Euphorbia L. und Diplocyathium n.g. *Beih. bot. Cbl.* **22** (1): 21–69.

SCHWEIGER, J. (1905): Beiträge zur Kenntnis der Samenentwicklung der Euphorbiaceen. *Flora* **94**: 339–379.

SINGH, D. (1964): Cytoplasmic nodules in the endosperm of Angiosperms. *Bull Torrey bot. Club* **91**: 86–94.

SINGH, R. P. (1962): Forms of ovules in Euphorbiaceae. In: MAHESHWARI, P. (ed.), *Plant Embryology – A Symposium*, p. 124–128. Council of Scientific and Industrial Research, New Delhi.

— (1965): Structure and development of seeds in Euphorbiaceae: Antidesma menasu Miquel. *Balwant Vidya. J. Agric. Sci. Res., Agra* **7**: 96–99.

— (1969): Structure and development of seeds in Euphorbia helioscopia. *Bot. Mag., Tokyo* **82**: 287–293

SINGH, S. P. (1959): Structure and development of seeds in Euphorbia geniculata Orteg. *J. Indian bot. Soc.* **38**: 103–108.

THATHACHAR, T. (1953): Morphological studies in the Euphorbiaceae. *J. Mysore Univ.* **13B**: 363–388.

WENIGER, W. (1917): Development of embryo sac and embryo in Euphorbia preslii and E. splendens. *Bot. Gaz.* **63**: 266–281.