THE EMBRYO-SAC OF *EUPHORBIA HETEROPHYLLA* L.—A REINVESTIGATION

By P. Maheshwari

(Dacca University, Dacca)

Received November 18, 1941

I. Introduction and Previous Work

From the point of view of floral morphology and embryology, the family Euphorbiaceæ has been of great interest for a long time. No less than 8 different types of embryo-sacs have been reported which are shown diagrammatically in Fig. 1. Most of the plants exhibit the *Normal*-type with a monosporic, 8-nucleate gametophyte. Modilewski[1] (1909, 1910, 1911) found a tetrasporic, 16-nucleate type in *Euphorbia procera* and *E. palustris*, which is now designated as the *Penæa*-form and has since been reported in *Acalypha australis* (Tateishi, 1927). In *Mallotus japonicus* (Ventura, 1934) also, 16 nuclei are present but they are organised differently—an egg apparatus, two polar nuclei and eleven antipodal cells (*Drusa*-form). Arnoldi (1912) thought that he had found 4-nucleate embryo-sacs developing from a single megaspore (*Oenothera*-type) in *Glochidion*, *Codiæum* and *Ceramanthus* (*Phyllanthus*). This claim has been disproved in the case of the last two genera by Lundberg (1931) and Maheshwari and Chowdry (1936) respectively and it seems that *Glochidion* will also yield similar results on reinvestigation. It is certain that Arnoldi missed the antipodal cells in his preparations and was thus led to a wrong interpretation (for a detailed discussion see Maheshwari, 1937).

---

[1] In his "Introduction" Sanchez (1938) quotes Modilewski's work (1910) on *Euphorbia procera* as follows:—

> "In this species, instead of only one surviving megaspore dividing to form the normal 8-nucleate embryo-sac, all the four megaspores divide simultaneously without exception so that each of them after two successive divisions gives rise to a 4-nucleate embryo-sac. At this stage, one of the 4-nucleate embryo-sacs divides twice to form a mature 16-nucleate embryo-sac, while the other three degenerate."

This is obviously a misunderstanding of the original in German. What Modilewski really says, is that there are several megaspore-mother cells, all of which may undergo reduction division and become 4-nucleate. Only one divides further, however, and forms a 16-nucleate embryo-sac. He summarises the situation as follows (p. 417):—

> "Eine von den vierkernigen Embryosackmutterzellen entwickelt sich zu einem reifen sechzehnkernigen Embryosack, während die übrigen degenerieren."

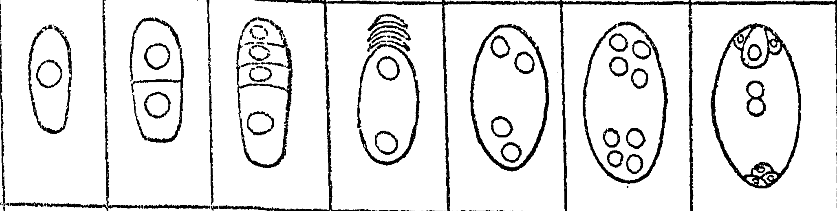
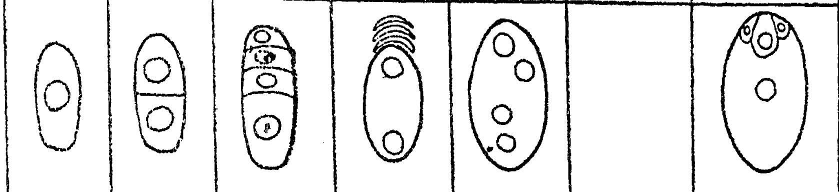
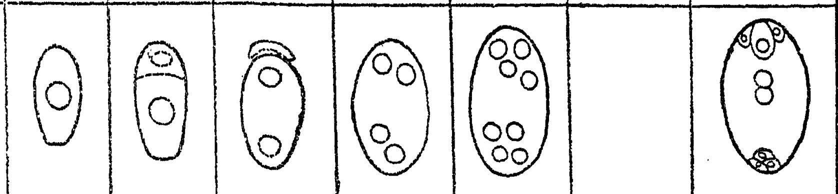
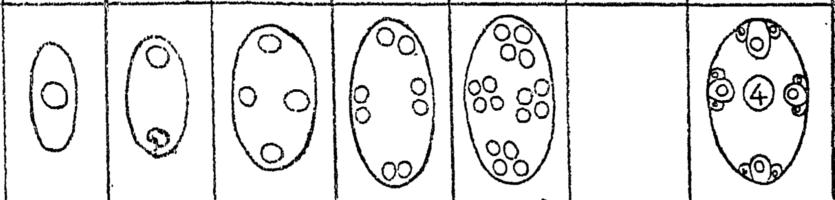
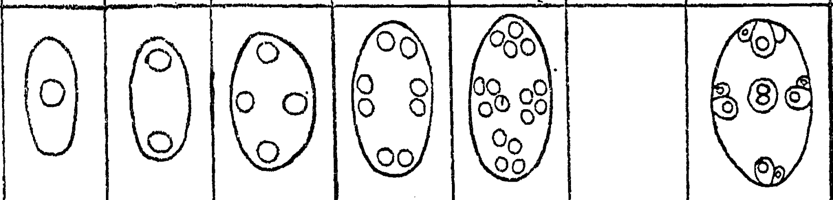
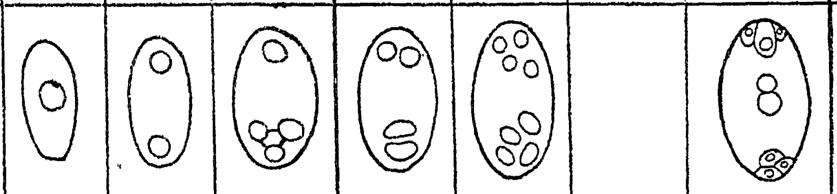
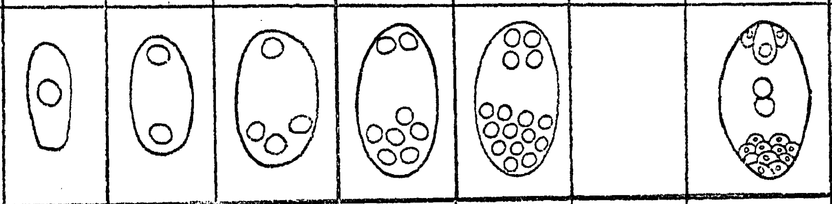
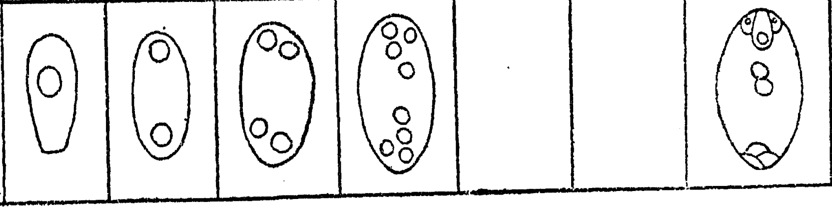
Type	Megaspore mother cell	I Division	II Division	III Division	IV Division	V Division	Mature embryo sac	Remarks
Normal-type								Occurs in by far the largest number of investigated genera and species.
Oenothera-type								Reported in *Codiæum*. *Ceramanthus* and *Glochidion* by Arnoldi (1912). First two cases already disproved (see Maheshwari, 1937); the third is also extremely doubtful.
Allium-type								Reported in:—*Euphorbia mauritanica* (Ventura, 1933).
Penaea-form								Reported in :— *Eurphorbia procera* (Modilewski, 1909) *E. palustris* (Modilewski, 1911)
Acalypha indica-form								*Acalypha indica* (See Maheshwari and Johri, 1940)
Fritillaria-type								Probably occurs in *Euphorbia dulcis* (Carano, 1926)
Drusa-form								Reported in *Mallotus Japonicus* (Ventura, 1934)
Adoxa-type								Reported in *Euphorbia heterophylla* (Sanchez, 1938). Dealt with in the present paper and shown to be incorrect.

Fig. 1. Diagram showing the different types of embryo-sac development reported in the Euphorbiaceæ

Carano (1926) noted that in *Euphorbia dulcis* three of the four nuclei, formed after the first two divisions, pass down to the chalazal end of embryo-sac and during the next division the spindles of these 3 chalazal nuclei fuse together resulting in a secondary 4-nucleate stage. This by a further division gives rise to an 8-nucleate embryo-sac organised in the normal fashion. A comparison with Bambacioni's figures of *Fritillaria persica* (1928) seems to indicate that the development is identical.

Yet another variation was noted by Ventura (1933), who reports an *Allium*-type of embryo-sac in *E. mauritanica*, and D'Amato[2] (1939), in a work dealing with several spp. of *Euphorbia*, reports that in most cases the *Normal*-type was observed, but some spp. occasionally or regularly show the *Allium*-type.

More recently Maheshwari and Johri (1940) have published a preliminary note on the embryo-sac of *Acalypha indica*, in which 16 nuclei, formed by 2 divisions of the megaspores, are sometimes organised as in *Euphorbia virgata* and *Acalypha australis*, but more frequently form four pairs of 2 cells each leaving 8 nuclei to fuse in the centre. Several other irregularities have been noted and described in the full paper which is in the press.[3]

The embryo-sac of *Euphorbia heterophylla*, which forms the subject of the present paper, was first investigated by Modilewski (1910), who reported a *Normal*-type of embryo-sac in this and several other species of the same genus. His description of this species being very brief and unillustrated, Sanchez (1938) reinvestigated it, and found an *Adoxa*-type of embryo-sac. From his figures and descriptions, however, this appeared so doubtful that a reinvestigation was undertaken.

2. *Observations*

The material was collected from some plants growing in the Government Nursery, Dacca, and in the writer's private garden. Nawaschin's fluid and formalin-acetic-alcohol were used for fixation. The sections were cut at 10 μ and stained in iron-hæmatoxylin.

A description of the ovary and ovules has already been given by Sanchez and my observations agree with his. The growth of the integuments is very tardy but the nucellus is well developed. The inner integument starts first at about the time the megaspore-mother cell is already well formed, but is soon surpassed by the outer.

[2] The full paper of this author was not available and hence I am unable to discuss his observations in detail.

[3] This was to have been published a couple of years ago in the *Beih. bot. Cbl.*, but no information about it is now available due to the war.

With regard to the early development of the embryo-sac Sanchez writes as follows:

"Following the synezetic contraction the macrospore-mother cell proceeds to usual heterotypic division, resulting in the formation of two daughter nuclei. These nuclei move and lie opposite each other at the ends of the enlarged macrospore-mother cell (Fig. 3 B)[4] one toward the micropylar end and the other toward the chalazal end. A large vacuole is formed between them while the two daughter nuclei become surrounded with dense protoplasm. From this stage the macrospore-mother cell grows rapidly especially in width (Fig. 3 B).

The two daughter nuclei divide simultaneously in the usual manner into 4-nuclei. These nuclei are not separated by walls, but remain in pairs separated by the large central vacuole. All the four macrospores become functional because they all participate in the formation of the female gametophyte or embryo-sac. Thus none of them degenerated or disintegrated as observed in the normal type of embryo-sac development, where only one surviving macrospore becomes functional.....The third division seems to take place immediately after the four nuclei are fully developed, for the 4-nucleate embryo-sac stages are comparatively few."

A critical study of the figures presented by Sanchez, the more important of which are reproduced here (Fig. 2) reveal a great gap between the megaspore-mother cell and the 2-nucleate stage (compare also his diagrams of the ovules at this stage, Fig. 2 *a–c*). The beginning of vacuolation even before the reduction divisions are over is most unlikely. As pointed out by Rutgers (1923), Fagerlind (1938) and Maheshwari (1941), polarity in the embryo-sac begins only after the formation of the megaspores. There is no case where it is known to commence immediately after the first reduction division as supposed by Sanchez.

Indeed it was this discrepancy in the drawings of Sanchez that suggested this reinvestigation.

My observations show that the megaspore-mother cell stage is identical with that figured by Sanchez (Figs. 3 and 4). The first reduction division is followed by wall formation and the second division also proceeds quite normally in the lower dyad but is frequently delayed in the upper. A row of four cells is formed in most cases but sometimes the division is incomplete in the upper dyad and only 3 cells are then observed (Figs. 5–8).

[4] This is reproduced here as Fig. 2 *e*.

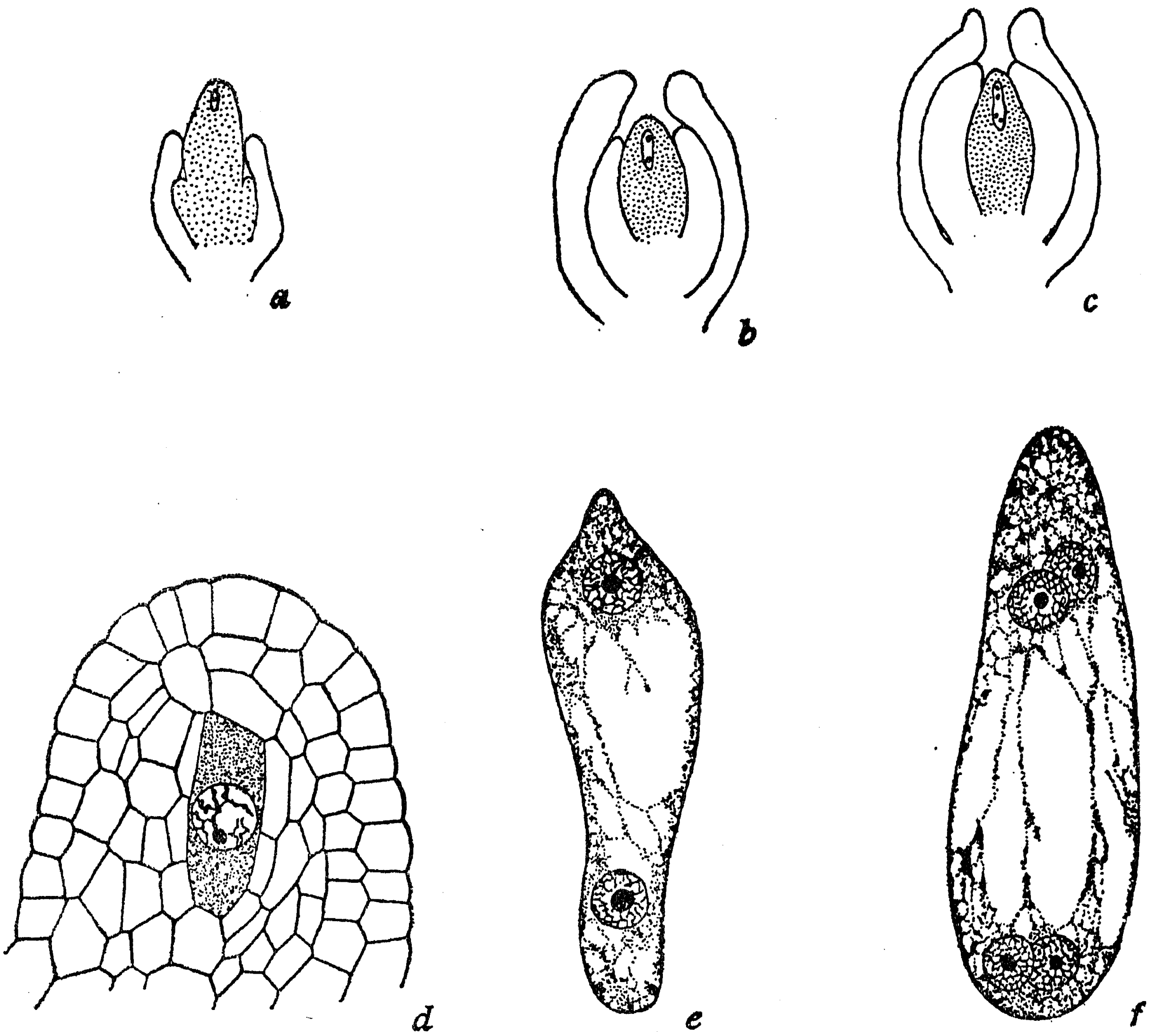


FIG. 2. Drawings of megaspore-mother cell and 2-nucleate and 4-nucleate embryo-sacs (copied from Sanchez, 1938). Figs. *a*, *b* and *c* show diagrams of ovules at the stages shown in *d*, *e* and *f*.

The lowest cell of the tetrad enlarges and functions. I was able to see the 3 *degenerating megaspores quite distinctly in several preparations and can therefore state definitely that the development is not of the* Adoxa-*type but of the* Normal-*type.*

Vacuolation begins first only after megaspore formation has been completed and the chalazal cell has enlarged appreciably. The subsequent stages showing 2, 4 and 8 nuclei are passed through normally and are identical with those figured by Sanchez. It is therefore unnecessary to duplicate them here.

3. *Discussion*

The above observations remind one of exactly similar errors committed by some other authors. Perhaps, *Typha latifolia* presents a more or less identical case. Schaffner (1897) studied the embryo-sac of this plant and mentions having taken extreme care in tracing out the development step by step. A row of megaspores was " never seen " and the development was stated to be of the *Adoxa*-type. Nevertheless, Dahlgren (1918), twenty years later, proved that the 4 cells are formed as usual and it is the chalazal megaspore which functions to give rise to the embryo-sac.

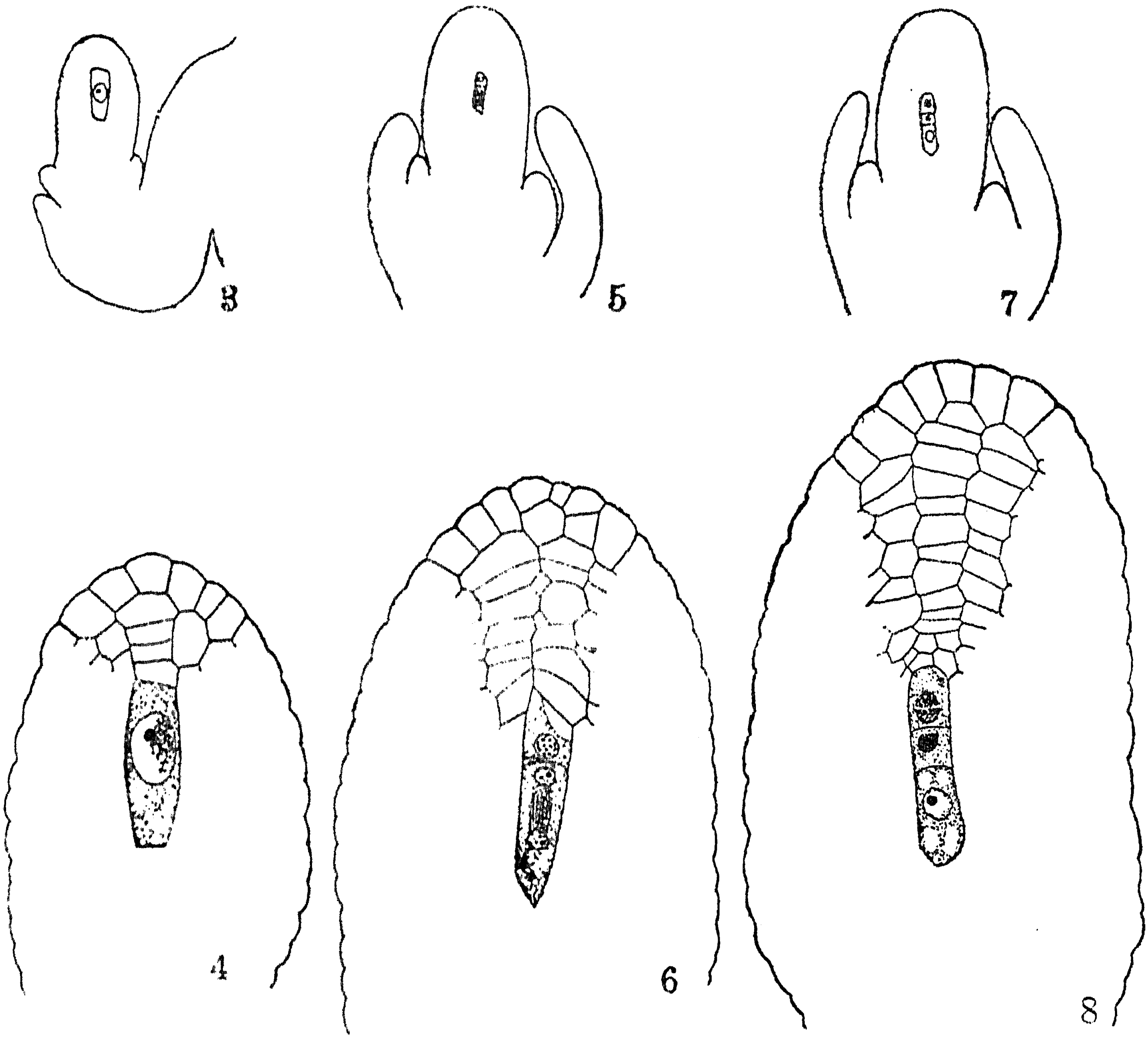


FIG. 3. L.S. ovule at megaspore-mother cell stage. × 300. FIG. 4. Nucellus with megaspore-mother cell. × 1,050. FIGS. 5, 7. L.S. ovule with stages in megasporogenesis. × 300. FIGS. 6, 8. Nucelli of ovules in Figs. 5 and 7 respectively. × 1,050.

It is a great pity that several authors have not drawn any detailed drawings but merely given diagrams showing the number of nuclei. It is impossible to discuss these cases, but other errors of interpretation, where at least the illustrations were executed with sufficient care, have often been rectified without much difficulty. The chief traps for workers on embryo-sac development are either in connection with megasporogenesis or the organisation of the mature embryo-sac. With regard to the former it may be stated that as a rule *vacuolation and polarity in the embryo-sac follow the formation of megaspores and are never seen before the reduction divisions are over.* Thus, the megaspore-mother cell and dyad stages are free from any conspicuous vacuoles. The early tetrad stage also does not show appreciable vacuolation, which starts first only after the four megaspores (or megaspore nuclei) have been formed and the next stage is about to commence. This rule applies to all embryo-sacs, whether monosporic, bisporic or tetrasporic. Thus, the difference between the four-nucleate stage of a monosporic embryo-sac and a tetrasporic one is very well marked (Fig. 9). The former shows

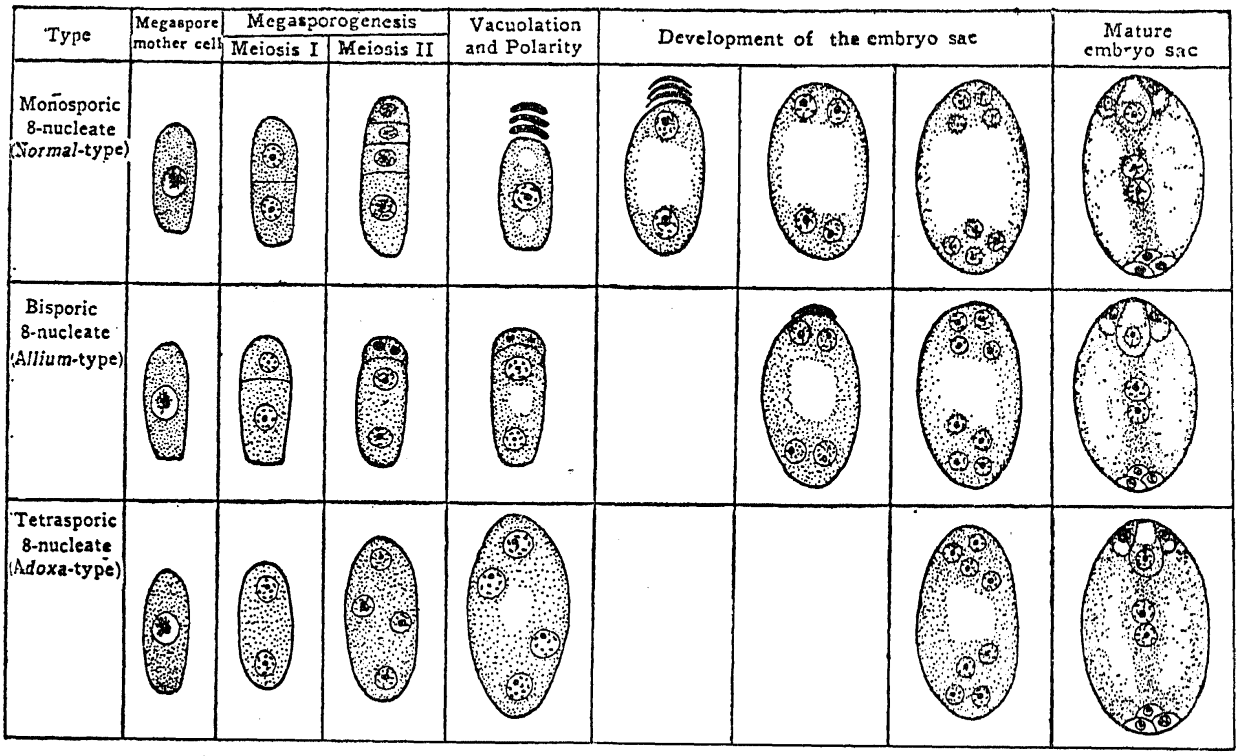


FIG. 9. Diagram to show the stage at which vacuolation begins in the development of monosporic, trisporic and tetrasporic embryo-sacs

a large central vacuole with the nuclei and cytoplasm limited to the periphery, while the latter begins to show a central vacuolation only at a later stage, just preparatory to the next division, and even then this is far less appreciable than in the monosporic and bisporic embryo-sacs. Note also the difference between the 2-nucleate stage of a monosporic and a tetrasporic embryo-sac (Fig. 9). The well-marked polarity and vacuolation in Figs. 3 *b* and 4 *a* of Sanchez (reproduced here as Figs. 2 *e* and 2 *f*) entirely go against the interpretation that they are pre-reduction stages.

I should like to state here that I do not consider vacuolation and polarity to be an infallible means of judging whether the embryo-sac is monosporic or tetrasporic, but I agree with Rutgers (1923) that it serves as a good and fairly reliable guide, which merits proper consideration.

The other error, concerning the stage immediately preceding the organisation of the mature embryo-sac, is also well exemplified in the Euphorbiaceæ. Arnoldi (1921) mistook the normal 8-nucleate embryo-sacs of *Codiæum* and *Phyllanthus* for 4-nucleate because of the early fusion of the polar nuclei and the disappearance of the antipodals. Sometimes the antipodal cells (or nuclei) may be in a narrow pouch that is easily missed in thin sections. In still other cases they become laterally placed due to a downward extension of the main body of the embryo-sac (see Kajale, 1940, and the literature quoted therein).

4. *Summary*

A reinvestigation of the embryo-sac of *Euphorbia heterophylla* shows that the development is not of the *Adoxa*-type as reported by Sanchez (1938) but of the *Normal*-type. The megaspore-mother cell gives rise to a tetrad of megaspores of which the chalazal cell functions to produce a normal 8-nucleate embryo-sac.

It is pointed out that as a rule vacuolation and polarity are not seen in embryo-sacs until after the reduction divisions are over. They form such reliable guides that all cases, where the reported observations are not in accordance with the above, deserve a fresh study.

5. *Acknowledgements*

In conclusion, I wish to express my gratefulness to my wife and sister for the assistance they gave in the preparation and staining of the slides and to my colleague and friend Mr. Reayat Khan for going through the typescript.

LITERATURE CITED

Arnoldi, W. .. "Zur Embryologie einiger Euphorbiaceen," *Trav. Mus. bot. Acad.*, St. Pétersbourg, 1912, **9**, 136–54.

Bambacioni, V. .. "Ricerche sulla ecologia e sulla embriologia di *Fritillaria persica* L.," *Ann. Bot.* (Roma), 1928, **18**, 7–37.

Carano, E. .. "Ulteriori osservazioni su *Euphorbia dulcis* L. in rapporto col suo comportamonto apomittico," *ibid.*, 1926, **17**, 50–79.

Dahlgren, K. V. O. .. "Die jüngeren Entwicklungsstadien der Samenanlagen von *Typha latifolia* L.," *Svensk. bot. Tidskr.*, 1918, **12**, 207–11.

D'Amato, F. .. "Ricerche embryologiche e caryologiche sul genere *Euphorbia*," *Nuovo Gior. Bot. Ital.*, 1939, **46**, 470–509.

Fagerlind, F. .. "Wo kommen tetrasporische durch drei Teilungsschritte vollentwickelte Embryosäcke unter den Angiospermen vor?" *Bot. Notiser*, 1938, 461–98.

Kajale, L. B. .. "A contribution to the embryology of the Amarantaceæ," *Proc. Nat. Inst. Sci.*, India, 1940, **6**, 597–625.

Lundberg, F. .. "Bemerkungen über die Embryosackentwiklung bei *Codiæum*," *Bot. Notiser*, 1931, 346–49.

Maheshwari, P. .. "A critical review of the types of embryo-sacs in Angiosperms," *New Phytol.*, 1937, **36**, 359–417.

——— .. "Recent work on the types of embryo-sacs in Angiosperms—A critical review," *Jour. Ind. Bot. Soc.*, 1941, **20**, 229–61.

——— and Chowdry, O. R. .. "A note on the development of the embryo-sac in *Phyllanthus niruri* Linn.," *Curr. Sci.*, 1937, **5**, 535–36.

——— and Johri, B. M. .. "A note on the embryo-sac of *Acalypha indica* L.," *ibid.*, 1940, **9**, 322–23.

Modilewski, J. .. "Zur Embryobildung von *Euphorbia procera*," *Ber. dtsch. bot. Ges.*, 1909, **27**, 21–26.

——— .. "Weitere Beiträge zur Embryobildung einiger Euphorbiaceen", *ibid.*, 1910, **28**, 413–18.

——— .. "Über die abnormale Embryosackentwicklung bei *Euphorbia palustris* L. and anderen Euphorbiaceen," *ibid.*, 1911, **29**, 430–36.

Rutgers, F. L. .. "Embryo-sac and embryo of *Moringa oleifera*. The female gametophyte of Angiosperms," *Ann. Jard. bot. Buitenzorg*, 1923, **33**, 1–66.

Sanchez, S. T. .. "Embryo-sac development in *Euphorbia heterophylla* Linn.," *Univ. Philippines Nat. & Appl. Sci. Bull.*, 1938, **6**, 59–75.

Schaffner, J. H. .. "The development of the stamens and carpels of *Typha latifolia*," *Bot. Gaz.*, 1897, **24**, 93–102.

Tateishi, S. .. "On the development of the embryo-sac and fertilisation of *Acalypha australis* L. (Preliminary note)," *Bot. Mag. Tokyo*, 1927, **51**, 477–85.

Ventura, M. .. "Sviluppo del gametofito femminile di *Euphorbia mauritanica* L.," *Ann. Bot.* (Roma), 1933, **20**, 267–73.

——— .. "Sulla poliembrionia di *Mallotus japonicus* Muell. Arg.," *ibid.*, 1934, **20**, 568–78.

1751-41 Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent, and Published by The Indian Academy of Sciences, Bangalore.