# MICROSPOROGENESIS, MACROSPOROGENESIS, AND DEVELOPMENT OF THE MACROGAMETOPHYTE AND SEEDS OF *DUBOISIA LEICHHARDTII* (F.v.M.) AND *D. MYOPOROIDES* (R.Br.)

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*Summary*

In *Duboisia Leichhardtii* and *D. myoporoides* macrosporogenesis and the development of the embryo-sac are similar to the descriptions reported for other genera of the family Solanaceae.

The haploid number of chromosomes in both species is 30.

A generative and vegetative nucleus is formed in each microspore which later becomes filled with starch grains and uninucleate as a result of degeneration of the vegetative nucleus. At maturity the pollen grains are devoid of starch and are uninucleate. Division of the generative nucleus to form two male nuclei presumably occurs just prior to the discharge of the pollen tube.

Double fertilization occurs and the fusion of one male nucleus with the fusion nucleus takes place before fertilization of the egg.

Embryogeny is comparable to published descriptions for other genera of the Solanaceae but the development of the endosperm is different.

A primary endosperm of large thin-walled cells is present until the stage when the ovules have almost reached mature seed size and the embryo is at about the 14-celled stage. Meristematic activity commencing in cells in the vicinity of the embryo then results in the formation of a dense compact "secondary" endosperm. Cells of the secondary endosperm become filled with oil.

Degeneration of whole ovules may take place but "empty" seed develop from ovules which contain no embryo and in which secondary endosperm has not formed.

## I. INTRODUCTION

During the course of experimental work with *Duboisia myoporoides* and *D. Leichhardtii* considerable difficulty has been experienced in germinating seed. As previously reported (Loftus Hills and Kelenyi 1946) "most seed samples although they appeared normal contained a variable proportion of empty or partly filled seed. The number of full seeds in a series of samples derived from individual trees of both species ranged from nil to 80 per cent. and averaged 13 per cent. The fluctuations did not appear to be related to the geographical origin of the samples or to the year in which the seed was collected. The phenomenon may be due to self sterility, lack of effective pollination or some other cause and an explanation is now being sought."

As part of the enquiry into the reason for the "emptiness" of such a high proportion of *Duboisia* seeds, an examination was made of the morphological development of the ovule and seed of both species. Material was collected

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from young trees in experimental plots at Canberra during 1946-47 and 1948-49, fixed in formalin acetic alcohol, embedded in paraffin wax, and microtomed. Mature seed of *D. Leichhardtii* collected in 1946-47 was 85-90 per cent. sound and only 10-15 per cent. empty. In 1948-49 there was an even lower proportion of empty seed in *D. Leichhardtii* though in *D. myoporoides* almost 80 per cent. was empty.

Germination of the morphologically sound seed in both species was, however, most unsatisfactory and it became apparent that a propagation problem unrelated to seed emptiness existed. Investigations in progress aim to discover the conditions which the sound seed requires for proper germination.

The observations which were made during the course of these studies on the microsporogenesis and the development of the macrogametophyte and the ovule are now presented, because no account of the life history of any of the three species of *Duboisia* has been reported previously.

In most respects development is comparable to that in other genera of the Solanaceae. The main difference is in the development of the endosperm which in *Duboisia* is of a type which so far as the author is aware has not been previously described. The emptiness of seed is probably caused primarily by lack of fertilization. The bulk of the material examined was of *D. Leichhardtii*. This species has therefore been made the main subject of description, observation on *D. myoporoides* being made in a comparative manner.

## II. Macrosporogenesis

A single integument is developed in the young ovule and an hypodermal archesporial cell functions directly as the megaspore mother cell. The nucellus consists of a single layer of cells enclosing the megaspore mother cell and projects beyond the integument at this stage (Plate 1, Fig. 1).

The first division of the nucleus of the megaspore mother cell is meiotic and at metaphase the spindle is parallel with the length of the cell (Plate 1, Fig. 2). The integument now slightly projects beyond the nucellus. A cell wall is formed between the two daughter nuclei (Plate 1, Fig. 3) and these cells divide simultaneously to form a linear tetrad of four megaspores. The integument meanwhile continues to grow rapidly and "embeds" the nucellus and by the time the four megaspores have been formed a micropyle of some length exists.

The three megaspores nearest the micropyle soon degenerate whilst the chalazal megaspore increases in size and becomes functional (Plate 1, Figs. 4 and 5). Macrosporogenesis in *Duboisia* is therefore similar to that in other described species of Solanaceae (e.g. Cooper 1931; Rees-Leonard 1935; Smith 1935; Cochran 1938).

## III. Development of Macrogametophyte (Embryo-Sac)

The functional megaspore increases in size considerably before the first division of its nucleus and becomes roughly egg-shaped with a narrow projection at the chalazal end. The nucleus divides and one of the daughter nuclei passes

to the micropylar and one to the chalazal end. Each again divides to give two nuclei at the chalazal end and two at the micropylar (Plate 1, Fig. 6). During this time the nucellus degenerates and disappears entirely at the micropylar end though persisting for some time at the chalazal end of the sac. The innermost layer of cells of the integument assumes a palisade-like arrangement. This "tapetum" is just becoming apparent at the stage represented in Plate 1, Figure 6, and is very definite by the time the embryo-sac is mature, as shown in Plate 1, Figure 7.

Development of the embryo-sac proceeds in a normal manner to an 8-nucleate stage. A normal egg apparatus is developed and the two polar nuclei come together usually at the micropylar end of the sac where they fuse. The three antipodal nuclei are most ephemeral and could rarely be found by the time the egg apparatus had been organized and the polar nuclei moved together.

The embryo-sac with the egg and synergids at the micropylar end and the two polar nuclei about to fuse is shown in Plate 1, Figures 7 and 8. These photographs are of successive sections of the same embryo-sac. In Plate 1, Figure 7, the two polar nuclei are shown, while in Plate 1, Figure 8, the egg and two synergids are visible together with the cytoplasm surrounding the polar nuclei just below and to the left of the egg. Not properly visible in the photograph but discernible in the section are two antipodal nuclei at the chalazal end of the sac. The egg apparatus of a mature embryo-sac is shown in Plate 1, Figure 9. It is a diagrammatically normal type. The nucleus of each pear-shaped synergid lies at its narrow end above a vacuole. The egg cell is large with the nucleus lying within the side farthest removed from the micropyle. The large fusion nucleus occupies a position just below and to one side of the egg. In *D. myoporoides* the egg apparatus presents a slightly different appearance in that the large fusion nucleus is more often found placed against the wall of the sac and to one side of the egg rather than below it and the synergids are fuller, not so narrow, and do not stain so darkly.

Development of the embryo-sac is thus of quite normal solanaceous type and in detail approaches very closely to that of *Solanum* as described by Rees-Leonard (1935).

## IV. Microsporogenesis

Differentiation of sporogenous cells in the anther occurs at a very early stage. Development of the anther was not followed in detail but appears to be essentially similar in the early stages to that described for *Capsicum* by Cochran (1938). At the time the pollen mother cells have formed, the wall in the anther consists of an epidermis, an undifferentiated endothecium, one or two layers of darkly-staining tangentially elongated cells (referred to as the middle layer or outer tapetum), and a tapetum of binucleate cells. The cells of the tapetum enlarge and separate as the pollen mother cells separate and become round prior to meiosis. At meiosis the haploid number of chromosomes is observed to be 30 (Plate 3, Fig. 4). The diploid number has been confirmed

at 60 in both species in mitotic root-tip divisions. Meiosis occurs in the microspore mother cells before the megaspore mother cell, and when the latter divides tetrads or free pollen are present in the anthers.

The tapetum commences to disorganize about the time of tetrad division of the microspores. The cells of the middle layer also degenerate and appear to commence to do so before the tapetal cells. By the time the functional megaspore has developed (as in Plate 1, Fig. 1) the tapetum is staining lightly and the endothecial cells elongating radially.

The nucleus of the microspore divides into a generative and vegetative nucleus, the latter being circumscribed by the formation of a cell wall (Plate 3, Fig. 5). The microspores later become packed with starch grains and the vegetative nucleus degenerates (Plate 3, Fig. 6). Disorganization of the tapetum continues and it has disappeared by the time the megaspore is binucleate.

The starch gradually disappears in the pollen grains as they mature and the connective tissue between the locules of the anther breaks down. At anthesis the pollen grains are uninucleate (rarely binucleate), devoid of starch, and have a well-defined intine and exine and three germ pores (Plate 3, Fig. 7), and there is only one sac in the anther.

Upon germination* the generative nucleus may pass into the pollen tube almost immediately or the pollen tube may grow for quite a considerable length whilst the nucleus remains in the grain (Plate 3, Figs. 8 and 9). The division of the generative nucleus into the two male gametes was not observed in artificial culture and presumably does not take place until the pollen tube reaches the embryo-sac.

## V. Fertilization

The pollen tube enters the embryo-sac through the micropyle. At the time of entry the tip of the tube is swollen and stains very deeply (Plate 1, Fig. 10). The two male nuclei may sometimes be distinguished before the tip bursts. Even after discharge the deeply-staining contents of the tube considerably obscure one's view. The synergids are disorganized upon the entry of the tube. One male nucleus fuses with the fusion nucleus and the second male nucleus with the egg. The fusion of the male nucleus with the fusion nucleus always occurs first and the endosperm nucleus has passed to the chalazal end of the sac and may have divided before fertilization of the egg occurs. In Plate 1, Figures 11 and 12, photographs of two successive sections show one male nucleus about to fuse with the fusion nucleus whilst the other is just discharging from the pollen tip. For some time after the fusion of the egg and the male nucleus the zygote nucleus may remain binucleolate.

* Germination of pollen grains was effected by dusting on to a flattened drop of 15 per cent. sucrose solution to which 0.5 per cent. agar had been added on a slide and placing in a moist chamber. When germination had taken place the preparation was fixed in Alcohol-Acetic (3 pts. to 1 pt.), cleared in absolute alcohol, stained in 1 per cent. lacmoid in 50 per cent. aqueous glacial acetic, rinsed in absolute alcohol, drained, and a drop of euparal and a cover slip added. In this way very satisfactory permanent preparations could be made.

Fertilization in *Duboisia* differs mostly from the observations made by Cooper (1931) for *Lycopersicum*, by Cochran (1938) for *Capsicum*, and by Clarke (1940) for *Solanum*, in that the fusion of the egg and the second male nucleus is delayed.

## VI. Development of Embryo, Endosperm, and Seed

After fertilization the endosperm nucleus migrates to the chalazal end of the sac and undergoes a number of divisions before the first division of the zygote. Several densely cytoplasmic endosperm cells "settle" at the bottom of the sac and form a base upon which a "free" nuclear endosperm develops. The zygote becomes pear-shaped and the stalk end fixes the young embryo to the integument at the micropylar end of the sac (Plate 2, Fig. 1).

The development of the embryo appears to be quite normal and follows generally the course described for other solanaceous plants. *Duboisia* also agrees with other accounts in that the first division of the zygote does not take place until some considerable time after fertilization and until after a varying amount of endosperm tissue has been formed. Here, however, agreement ceases and the development of the endosperm in *Duboisia* differs most markedly from other species of this family which have been described. Endosperm development is nothing like that figured by Smith (1935) for the tomato or Cochran (1938) for pepper.

The first formed endosperm cells are densely cytoplasmic – about five or six in number – and form a compact mass at the chalazal end of the sac. Upon this "base" other endosperm cells are developed stretching into the sac and a number of "free" nuclei formed which settle around the periphery of the sac. Prior to the formation of cell walls, there are well-defined cytoplasmic units at the chalazal end of the sac. (These units were called cells above.) Further from the chalazal end the cytoplasmic units become less well defined until in the centre of the sac or at the micropylar end there is the appearance of a number of nuclear units in one cytoplasmic unit, i.e. free nuclei. Because of the very early division into cytoplasmic units at the chalazal end of the sac, it is difficult to say when cell walls are first formed, but they are developed before the first division of the zygote (Plate 2, Fig. 3).

The ovule grows very rapidly by the development of the integument and the cells of the endosperm tissue become larger. The ovule develops to almost double the size shown in Plate 2, Figure 3, before the first division of the zygote. The enlargement of the ovule and the cells of the endosperm continues until at the stage when the embryo is 4-5-celled an appearance in section is presented as shown in Plate 1, Figure 4. The embryo is developed at the micropylar end of the ovule, the centre of which appears as a cavity. Actually this cavity is filled with endosperm tissue which now consists of very large cells with walls of extreme delicacy. The cells of the outer layer of the integument continually enlarge and later develop to form the seed testa.

Plate 2, Figures 5-7, shows the development of the embryo to the stage when about 12-14 cells are seen in longitudinal section. About this stage

meristematic activity recommences in the endosperm cells in the vicinity of the embryo and smaller celled secondary endosperm tissue starts to form (Plate 2, Figs. 7 and 8). This development of a compact endosperm tissue starting at the micropylar end of the sac continues until the whole sac is filled. In Plate 2, Figure 9, an intermediate stage is shown. The embryo is larger than that shown in Plate 2, Figure 8, and the new endosperm tissue fills most of the sac. By the time the embryo has reached the stage shown in Plate 2, Figure 10, the sac is filled with a dense endosperm tissue. Plate 2, Figures 11 and 12, and Plate 3, Figures 1-2, show further stages in development which result in a well-formed curved embryo.

The developing endosperm tissue absorbs the inner tissues of the integument, and as the seed matures its cells become filled with oil. The cells of the outer layer of the integument have continued to enlarge during the growth of the ovule. Their inner and radial walls become very much thickened. Upon maturation of the seed the outer walls break down and the thickened radial walls form the walls of the pits upon the surface.

## VII. Degeneration of the Ovules and Empty Seed

Degeneration of some of the ovules may occur at almost any stage of development but most commonly after the formation of the megaspore mother cell and before maturation of the embryo-sac. The first signs of degeneration occur in the nuclei, then the sac shrinks and stains very deeply. Following the disorganization of the sac the whole ovule degenerates and aborts.

Those ovules which develop into mature but "empty" seeds never contain an embryo nor any secondary endosperm tissue. However, primary endosperm tissue is formed and this tissue is composed of smaller and not such thin walled cells as when an embryo has been formed. In Plate 3, Figure 3, a longitudinal section through an almost mature empty seed is shown. The endosperm tissue is practically devoid of any stored metabolites, and when the seed fully matures and dries, this tissue breaks down leaving a large cavity within the testa.

It is improbable that in the development of such seed the egg was ever fertilized and that the lack of an embryo is due to degeneration. No instances of embryonal degeneration have been observed. It could not be determined whether fertilization of the fusion nucleus occurs and endosperm formation is normal or whether the fusion nucleus develops to form the endosperm tissue without fertilization. In view of the rather different nature of the tissue of the primary endosperm ovules without embryos it would seem that the latter alternative is the most probable. Secondary endosperm does not develop and it may be taken that the presence of an embryo is necessary to stimulate the formation of this tissue.

## VIII. Acknowledgment

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## Explanation of Plates 1-3

Photomicrographs of *Duboisia*.

### Plate 1

Fig. 1.—Median L.S. young ovule showing megaspore mother cell at "M." x 276.

Fig. 2.—L.S. ovule with megaspore mother cell showing early telophase of first (meiotic) division. x 517.

Fig. 3.—L.S. ovule with megaspore mother cell in late telophase of first division showing deposition of dividing wall. x 564.

Fig. 4.—L.S. ovule. Three micropylar megaspores have degenerated. The chalazal megaspore shows more clearly in the next section. x 238.

Fig. 5.—L.S. ovule showing functional megaspore and two of the three degenerated micropylar megaspores. x 255.

Fig. 6.—L.S. ovule showing developing megaspore at the 4-nucleate stage. Two nuclei are visible at the chalazal end but only one appears in this section at the micropylar end of the megaspore. x 255.

Fig. 7.—L.S. embryo-sac showing the two polar nuclei about to fuse. They are situated at the micropylar end of the sac. x 276.

Fig. 8.—L.S. embryo-sac. This section is next in the series to that shown in Figure 7. The two synergids and egg at "E" can be seen. Just to the right of the egg the surrounding cytoplasm of the polar nuclei is visible. x 276.

Fig. 9.—L.S. micropylar end of embryo-sac showing mature egg apparatus in detail. The fusion nucleus at "F" and the egg at "E." There is a large and conspicuous vacuole below the nucleus of each synergid (S). x 429.

Fig. 10.—L.S. of micropylar end of embryo-sac showing entry of pollen tube at "P." Egg nucleus at "E." Fusion nucleus at "F." x 429.

Figs. 11 and 12.—L.S. micropylar end of embryo-sac after discharge of pollen tube. These two figures are photographs of successive sections. In Figure 11 the egg nucleus is at "E" and one male nucleus at "M." Just below the male nucleus is the edge of the fusion nucleus. In Figure 12 the fusion nucleus (F) is cut medianly and the second male nucleus is at "M." Pollen tube at "P." x 429.

### Plate 2

Fig. 1.—L.S. embryo-sac after fertilization. Single celled embryo at "EM" and the two synergids. The endosperm nucleus has migrated to chalazal end of sac and divided (EN). x 108.

Fig. 2.—L.S. embryo-sac showing single celled embryo at "EM" and developing endosperm at "EN." x 163.

Fig. 3.—L.S. ovule with single celled embryo at "EM" and cellular primary endosperm tissue. x 83.

Fig. 4.—L.S. ovule which has almost reached mature seed size. Few celled embryo at "EM." Endosperm tissue of large very delicate walled cells not visible in photograph. x 31.

Fig. 5.—More detailed view of micropylar end of ovule shown in Figure 4. x 83.

Fig. 6.—Detailed view of embryo at comparable stage to that shown in preceding photograph. Several of the thin-walled and large cells of the primary endosperm are visible. x 211.

Fig. 7.—Micropylar end of ovule in which endosperm nuclei in the vicinity of the embryo have divided to form a more dense compact tissue (S.EN). x 103.

Fig. 8.—Detailed view of embryo at about 14-15-celled stage with meristematic endosperm cells surrounding. x 251.

Fig. 9.—Showing secondary endosperm tissue (S.EN) filling up the embryo-sac and embryo at further stage of development. x 108.

Figs. 10-12.—Further stages in the development of the embryo. When the embryo has reached the stage shown in Figure 10 the secondary endosperm tissue has almost completely filled the sac and digestion of this tissue by the developing endosperm is definite. Figures 10 and 11 x 108; Figure 12 x 34.

PLATE 3

Fig. 1.—L.S. of mature seed from which the testa has been removed showing suspensor of embryo and radicle end of the hypocotyledonary axis in detail. The cells of the dense endosperm tissue are filled with oil drops. x 33.

Fig. 2.—L.S. of mature seed from which testa has been removed. No epicotyl has been formed. x 33.

Fig. 3.—L.S. of an "empty" seed. No embryo nor secondary endosperm has been developed. The thin-walled cells of the primary endosperm contain practically no stored metabolites. x 48.

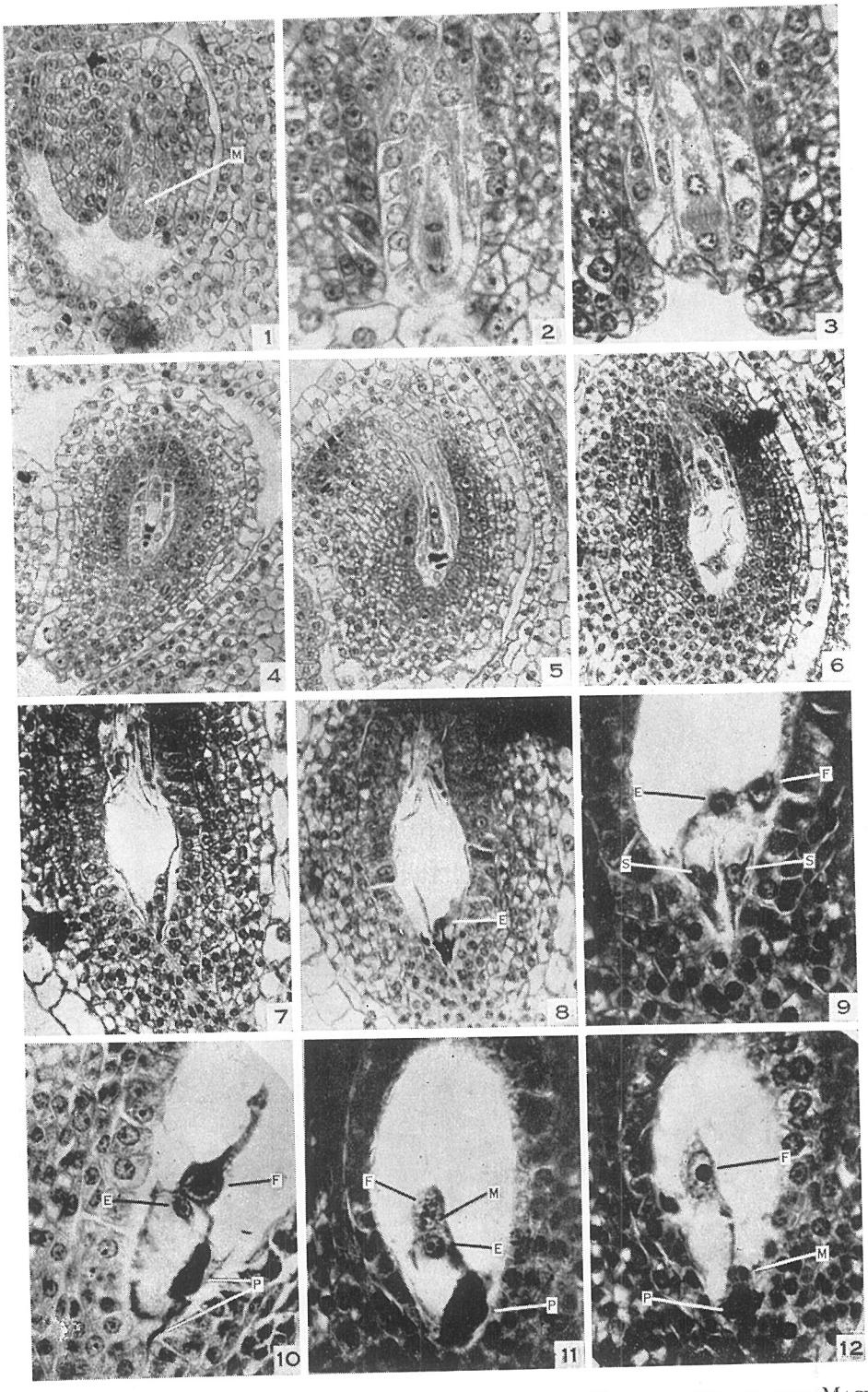
Fig. 4.—The metaphase plate of the second division of a pollen mother cell in which 30 chromosomes may be counted. x 1213.

Fig. 5.—Pollen grains in which the nucleus has divided to form a generative and vegetative nucleus. x 507.

Fig. 6.—Pollen grains at a later stage of development when they are filled with starch grains and the vegetative nucleus is degenerating. x 253.

Fig. 7.—Mature pollen grains showing absence of starch, the three germ pores and single nucleus and tube. x 507.

Figs. 8 and 9.—Germinating pollen grains showing behaviour of the single nucleus. x 253.

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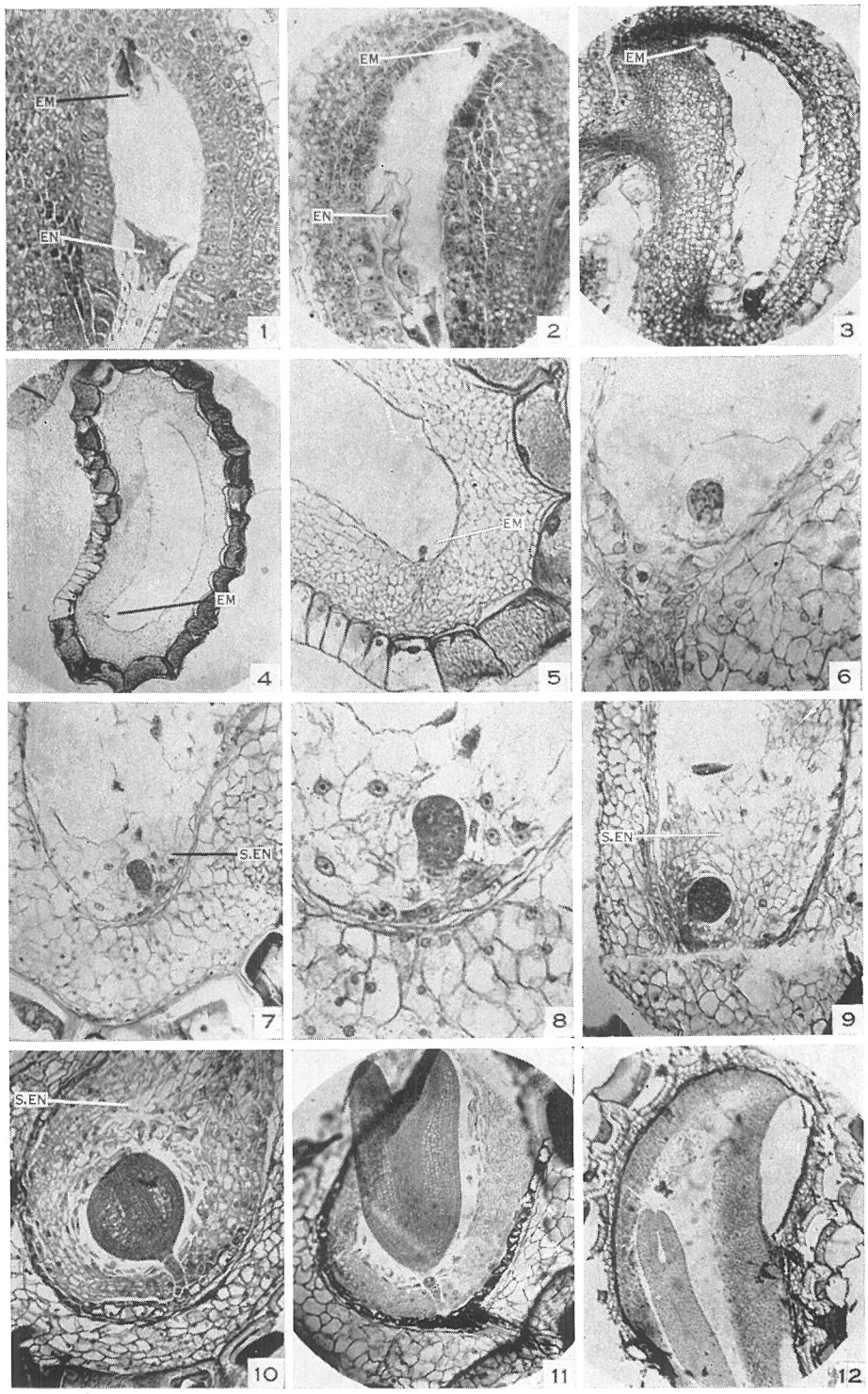


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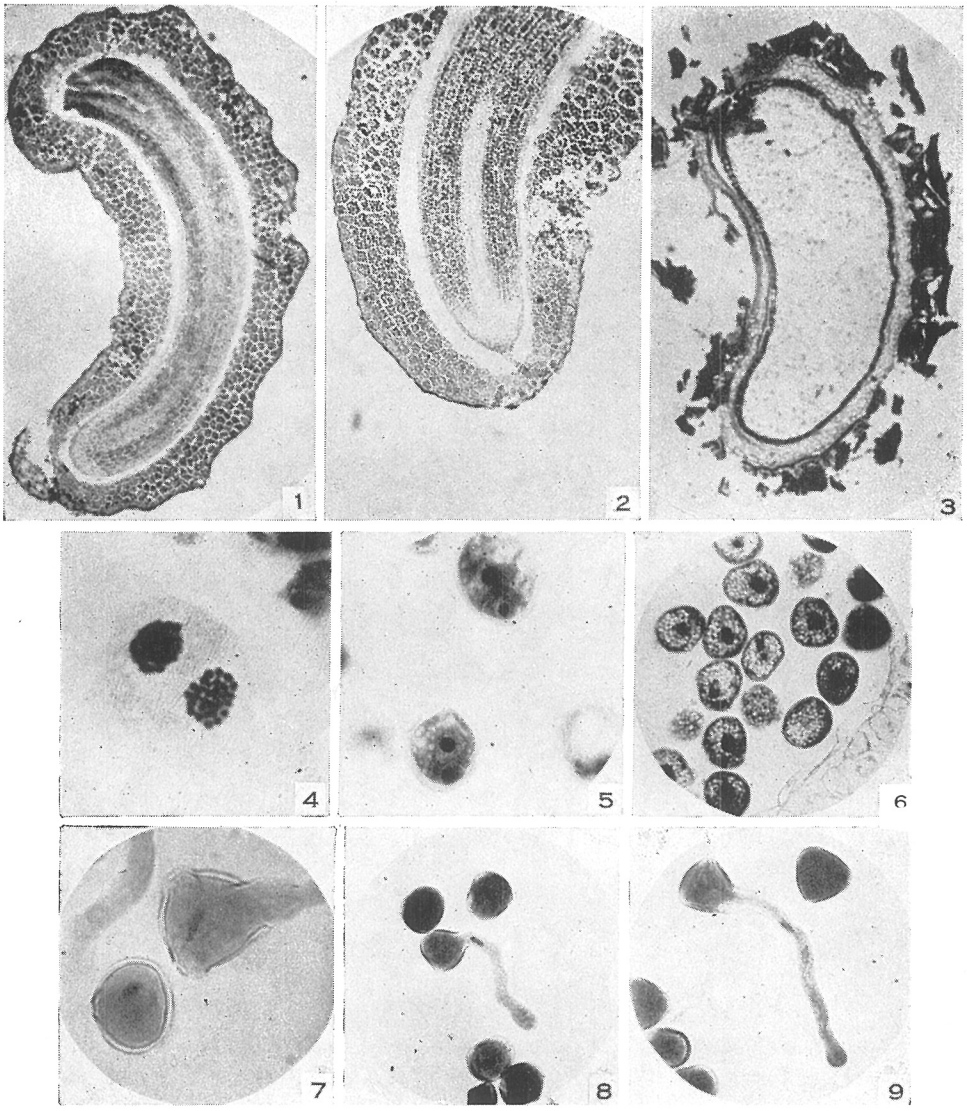


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