CONTRIBUTION TO THE MORPHOLOGY OF MOLLUGO NUDICAULIS, LAMK.

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1. Introduction.

A search through the available literature shows that little attention has been paid to the family Asioaceae. The following is a brief summary of the work done on the embryology of representatives of this family:

Pollen.—In Mesembrianthemum pseudotruncatellum (Schmid, 1925) the pollen grains are three-nucleate at the time of shedding and have three germ pores.

Ovule.—In Mesembrianthemum pseudotruncatellum (Schmid, 1925), M. crystallinum (Woodcock, 1930), M. linguiforme and Tetragonia expansa (Huber, 1924) the ovules are campylotropous and have three integuments. There is a single hypodermal archesporial cell which cuts off a wall cell. The cells of the nucellar epidermis also divide except those lying just above the embryo sac which simply stretch out radially.

Megaspore formation and embryo sac.—The megaspore mother cell divides to form a tetrad of four megaspores of which the chalazal produces an eight-nucleate embryo sac in Mesembrianthemum floribundum (Jönsson, 1879), M. pomederianum (Dahlgren, 1916), M. linguiforme (Huber, 1924) and Tetragonia expansa (Dahlgren, 1916; Huber, 1924).

In Mesembrianthemum corymbosum and M. ecklonis, Guignard (1882) reported that there is a tetrad of three cells, a condition arising due to the failure of division in the upper dyad cell.

In Mesembrianthemum pseudotruncatellum (Schmid, 1925) no tetrad is formed at all and the embryo sac development is of the 'Lilium type'.

Endosperm.—The nuclear type of endosperm has been found in Mesembrianthemum pomederianum (Dahlgren, 1916), M. linguiforme (Huber, 1924), M. pseudotruncatellum (Schmid, 1925), M. crystallinum (Woodcock, 1930) and Tetragonia expansa (Dahlgren, 1916; Huber, 1924).

Embryo.—The first division wall in the fertilised egg is transverse and a long suspensor is formed. The mature embryo is curved like a horse-shoe as in other Centrospermales.
2 Material and Methods.

The material was collected from Bichpuri about seven miles south-west of Agra. Some imbedded material fixed in formalin-acetic-alcohol was very kindly handed over to me by Mr. B. L. Gupta to whom I am indebted for it. Formalin-acetic-alcohol, corrosive sublimate-formalin-acetic-acid-alcohol, chrom-acetic acid (chromic acid 1 gm., acetic acid 3 c.c. and water 100 c.c.) and Nawaschin's fluid were used for fixing. The last fixative gave the best results. Sections were cut 5—12 microns thick, stained in Heidenhain's Iron-alum Hæmatoxylin and differentiated in an aqueous solution of picric acid (Maheshwari, 1933) which gave very satisfactory results. Some slides were also stained with crystal violet and iodine (Newton's method) but the results obtained were not so encouraging.

3 Investigation.

Flower.—The inflorescence is a raceme. The scapes are 3—8 inches long, wiry and trichotomously branched above. The flowers are axillary, small, greenish and have long and slender pedicles. Each flower has five sepals which are persistent and about one-tenth of an inch in length. The petals are absent. There are 3—5 stamens. The trilocular ovary is formed by the fusion of three carpels and there are two rows of ovules in each loculus on an axile placenta.

Microsporogenesis.—The earliest stage that I found shows a longitudinal row of sporogenous cells with a layer of wall cells cut off on the outside (Fig. 11). The primary parietal layer divides to form two layers (Fig. 12). Of these the one adjacent to the epidermis is the endothecium while the inner divides again producing the middle layer and the tapetum (Fig. 13). The endothecium develops the usual thickenings at maturity. The middle layer degenerates early and practically disappears by the time the reduction divisions in the pollen mother cells are over. In the beginning the tapetal layer consists of large uninucleate cells (Fig. 13) but later on the tapetal nuclei divide mitotically and the cells become binucleate (Fig. 14) and occasionally trinucleate.

In the meantime, the sporogenous cells divide to form a large number of microspore mother cells which have a large nucleus with one or two prominent nucleoli and are filled with dense cytoplasm (Figs. 13 and 14). The two divisions in the mother cells are simultaneous and the arrangement of the microspores is tetrahedral (Fig. 15) but rarely it may be isobilateral (Fig. 16). After separation the young microspores round up and enlarge, and the usual exine and intine get differentiated. The microspore nucleus divides and a large tube cell and a small crescent-shaped generative cell are
formed (Fig. 17). Later the nucleus of the generative cell also divides and forms two male nuclei (Fig. 18). At this stage practically no structure can be made out in the male nuclei and the tube nucleus begins to show signs of degeneration. There are usually three and rarely four germ pores in each pollen grain.

Pollen grains have also been found to germinate inside the anther and the pollen tubes ranged in length up to 26 microns.

**Ovule.**—There are two anatropous ovules in each loculus of the ovary. As in other members of the family, both the integuments are two cells thick except the inner which is thicker at the end forming the micropyle (Fig. 1). The cells of the nucellar epidermis divide as can be seen in Figs. 1, 23 and 24, and a nucellar cap is formed as in the members of the allied family Phytolaccaceae, viz., *Petiveria alliacea*, *Phytolacca octandra*, *Villamilla peruviana*, *Rivinia brasiliensis*, *R. humilis* (Mauritzon, 1934) and *Phytolacca decandra* (Lewis, 1905).

**Megasporogenesis.**—Usually there is a single hypodermal archesporial cell (Fig. 19) which divides periclinally into a primary wall cell and the megaspore mother cell (Fig. 22). Sometimes two and three sporogenous cells have also been found (Figs. 20 and 21) but only one of them functions. It is at this stage that the two integuments make their appearance.

The megaspore mother cell enlarges considerably and after the first reduction division (Fig. 23) is over a wall is laid down between the two daughter nuclei (Fig. 24). There is a well-defined interphase and the two daughter nuclei become well organised. The lower of the two daughter cells is slightly larger than the upper (Fig. 24). After the second reduction division the wall laid down in the upper dyad cell is either longitudinal or oblique (Fig. 25). The chalazal megaspore functions. The two topmost megaspores formed by the division in the upper dyad cell are the first to degenerate. Some doubtful cases showing only three megaspores were also noticed. In *Rivinia humilis*, *R. brasiliensis*, *Phytolacca octandra*, *Petiveria alliacea* and *Villamilla peruviana* (Mauritzon, 1934) also, there are only three cells. The upper cell often has two nuclei and it is only rarely that a wall is laid down between them and when it does happen a T-shaped tetrad is
formed. In *Mesembrianthemum pseudotruncatellum* (Schmid, 1925) no tetrad is formed at all and the embryo sac development is of the 'Lilium type'. This, however, needs confirmation as other species of this genus are normal.

The chalazal megaspore enlarges (Fig. 25) and gives rise to a two-nucleate embryo sac (Fig. 26). The two nuclei divide to form four (Fig. 27) which again divide to produce an eight-nucleate embryo sac of the usual organisation (Fig. 28). The polar nuclei fuse before fertilisation in the middle of the embryo sac and the fusion nucleus moves upwards and comes to lie near the egg (Fig. 31). It is variable in shape and may be spindle-shaped (Fig. 30), oval (Fig. 31) or with an uneven outline. The antipodal cells are very ephemeral and degenerate long before the fusion of the polar nuclei (Figs. 28 and 29). At this stage the chalazal end of the embryo sac begins to push downwards into the tissue of the nucellus.

Mature embryo sacs of *Mesembrianthemum linguiforme* (Huber, 1924), *M. pseudotruncatellum* (Schmid, 1925), several species of *Mesembrianthemum* investigated by D'Hubert in 1896, *Tetragonia expansa* (Huber, 1924) and *Aizoon canariensis* (Dahlgren, 1927) are reported to contain starch grains but in *Mollugo nudicaulis*, I have not been able to detect any, either in young or in mature embryo sacs.

**Pollination and Fertilisation.**—Self-pollination seems to occur here as pollen tubes were seen in the ovules of an ovary belonging to an unopened flower and pollen grains with pollen tubes were found both inside the anther and on the stigma of the same flower. Self-pollination is reported in *Mollugo verticillata* (Hagerup, 1932) also. The pollen tube enters through the micropyle. Actual double fertilisation has not been observed but in one case I saw a pollen tube containing the two male nuclei lying by the side of the egg, and several cases showing the remains of pollen tubes in embryo sacs containing young embryos have been seen. Fig. 31 shows a fertilised egg, the primary endosperm nucleus and the remains of a pollen tube with the degenerated tube nucleus.

**Endosperm.**—The presence of several endosperm nuclei at the two-celled embryo stage suggests that probably the primary endosperm nucleus divides first. The endosperm is free nuclear (Fig. 10) and grows rapidly through mitotic divisions. The embryo sac is lined with a thin layer of protoplasm except at the chalazal end where it is very dense (Fig. 10). A similar condition has also been reported by Lewis (1905) in *Phytolacca decandra* and by Mauritzon (1934) in *Rivinia*
brasiensis, R. humilis, Petiveria alliacea, Phytolacca octandra and Villamilla peruviana. The embryo sac continues to push its way down into the tissue of the nucellus until it reaches almost to the chalazal end of the seed. It forms an extensive horse-shoe shaped structure with the embryo growing inside it (Fig. 10). Wall formation in the endosperm starts at the micropylar end at the time of differentiation of the two cotyledons and then extends to the chalazal region.

Embryo — The first division of the oospore is transverse (Fig. 2). Figs. 3 and 4 show further stages in the development of the filamentous pro-embryo and up to this stage all the divisions are transverse. The terminal cell divides by a longitudinal wall (Fig. 5). A few more transverse and longitudinal divisions follow (Figs. 6 and 7) and the embryo becomes globular (Fig. 8). Fig. 9 shows a more advanced stage where the two cotyledons have just been marked out.
In the mature seed the external layer of the outer integument and the internal layer of the inner integument become greatly thickened and hardened. A similar condition has been reported by Mauritzon (1934) for *Rivinia humilis*, *R. brasiliensis*, *Phytolacca octandra*, *Peltiera alliacea* and *Villamilia peruviana*. The outer layer of the outer integument becomes hard in *Mesembrianthemum linguiforme* (Huber, 1924), *M. crystallinum* (Woodcock, 1930) and *Phytolacca americana* (Woodcock, 1924).

4. Summary.

1. The early development of the anther does not present any feature of special interest and as usual the microspore mother cells are surrounded by a bi-nucleate tapetum, an ephemeral middle layer and an endothecium. The arrangement of microspores is tetrahedral or rarely isobilateral. The pollen grains are three-nucleate at the time of shedding. The tube nucleus shows signs of degeneration quite early. Pollen grains have also been found to germinate inside the anther.

2. The ovules are anatropous and have two integuments, each of which is two cells thick except the inner which is thicker at the top.

3. The cells of the nucellar epidermis divide to form a nucellar cap. The mature embryo sac lies four to five cells deep.

4. Usually the nucellus has a single hypodermal archesporial cell which divides to form the primary wall cell and the megaspore mother cell. Occasionally two or three megaspore mother cells are also present.

5. The first and the second divisions of the megaspore mother cell are followed by wall formation and a T-shaped tetrad of megaspores is formed of which the chalazal functions. In some doubtful cases only three megaspores were seen.

6. The mature embryo sac is eight-nucleate with the usual organisation. The antipodal cells are very ephemeral.

7. The endosperm nucleus divides prior to the division of the egg nucleus and undergoes several free nuclear divisions.

8. The first division of the fertilised egg is transverse. A long suspensor is formed and the embryo is dicotyledonous.

In the end I regard it my pleasant duty to express my sincere thanks to Dr. P. Maheshwari for his valuable suggestions and keen interest during the course of investigation. I am also indebted to my friend Mr. B. M. Johri for the help he rendered in several ways.
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EXPLANATION OF TEXT-FIGURES.

TEXT-FIG. 1.—Longitudinal section of the ovule with a two-nucleate embryo sac. × 396.
TEXT-FIgs. 2-10.—Development of the embryo.

Fig. 2. A two-celled embryo; Fig. 3. A three-celled embryo; the nucleus of the central cell is dividing; Fig. 4. A filamentous pro-embryo of five cells; Figs. 5-8. Further stages in the development of the embryo; Fig. 9. Older embryo with two cotyledons just appearing; Figs. 2-9. × 527; Fig. 10. Embryo sac with a young embryo and the endosperm; the endosperm nuclei at the chalazal end have been closely aggregated in a dense mass of cytoplasm. × 240.

EXPLANATION OF FIGURES.

PLATE XXXVIII.

Fig. 11.—Longitudinal section of an anther-lobe showing the sporogenous tissue and primary parietal layer. × 800.
Fig. 12.—Same, older stage showing two wall layers. × 800.
Fig. 13.—Same, still older stage showing three wall layers; the innermost is the tapetum and surrounds the microspore mother cells. × 800.
Fig. 14.—Same, showing the binucleate tapetum, degenerated middle layer and microspore mother cells. × 800.
Fig. 15.—Tetrahedral tetrad of microspores. × 800.
Fig. 16.—Isobilateral tetrad of microspores. × 800.
Fig. 17.—Pollen grain showing tube and generative cells. × 800.
Fig. 18.—Mature pollen grain showing two spindle-shaped male nuclei and the tube nucleus. × 800.
Fig. 19.—Longitudinal section of the nucellus showing the hypodermal archesporial cell. × 800.
Fig. 20.—Two sporogenous cells lying one above the other. × 800.
Fig. 21.—A row of three sporogenous cells. × 800.
Fig. 22.—Megaspore mother cell. × 800.
Fig. 23.—Megaspore mother cell in metaphase of first reduction division. × 800.
Fig. 24.—Megaspore mother cell divided into two daughter cells. × 800.
Fig. 25.—Tetrad of megaspores; the upper two have degenerated and the chalazal has enlarged. × 800.
Fig. 26.—Two-nucleate embryo sac. × 800.
Fig. 27.—Four-nucleate embryo sac. × 800.
Fig. 28.—Eight-nucleate embryo sac. × 800.
Fig. 29.—Older embryo sac showing the two polar nuclei in contact; the antipodals have degenerated. × 800.
Fig. 30.—Mature embryo sac showing the spindle shaped fusion nucleus. × 800.
Fig. 31.—Upper part of a mature embryo sac showing the fertilised egg and primary endosperm nucleus. The remains of the pollen tube with the degenerated tube nucleus are seen towards one side of the egg. × 800.