MEGASPORE FORMATION AND EMBRYO-SAC OF *ARGEMONE MEXICANA*, LINN.

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With one plate and one figure in the text.

Introduction.

Argemone mexicana Linn. is one of the first American plants to be naturalised in India. The exact date of its entrance into our country is unknown, but that it was certainly here, even in the beginning of the 19th century, is proved by a reference to the works of Roxburgh (14) and Wight and Arnott (25). At the present time it is one of the commonest weeds all over the country.

Last year, the writer collected some plants of *Argemone mexicana* from a place in Punjab near Hoshiarpur bearing flowers quite different from the normal. Their external morphology was described sometimes ago (10). Later on, it was decided to study their anatomy too, the results of which it is hoped will be published shortly. For a proper appreciation of the structure of these abnormal flowers, however, it was thought necessary to work out the structure of the normal flowers also. Consequently, parts of two normal flowers were fixed and embedded in February last and later on microtomed. Some of these slides also yielded several good sections of the ovules, showing the formation of megaspores and the embryo-sac. On consulting Schnarf (15) and Schürhoff (16), it was found that nobody has so far studied the megasporogenesis or any other phase of the life history of this species and as it was found to show some important differences from the structure normal for the flowering plants, it was considered worth while to publish the results.

The Material.

The material of *Argemone mexicana* used in the present investigation was collected at Benares from the Hindu University grounds. It consisted of two ovaries, one from an unopened bud and the other from a flower which was in full bloom. It was trimmed from the sides to remove the various prickles and the two ends to facilitate the penetration of the fixative, which was a mixture of formalin, glacial

acetic acid and 60 per cent alcohol in the proportion of 5 : 5 : 100. Sections were cut from one half of each ovary in a transverse manner and from the other half longitudinally. A combination of Safranin and Gentian Violet was used in staining.

Megaspore Formation.

The development of the megaspores was studied from sections provided by the younger ovary. This is illustrated in figures 2, 3, 4 and 5. In this ovary the megaspores were already formed, so that the earlier stages were not seen, but so far as figure 3 can give any indication, it appears that there is only a single hypodermal archesporial cell which divides by a transverse wall to form the primary wall cell and the megaspore mother cell. The wall cell appears to divide once anticlinally and once or twice periclinally to form a portion of the wall, 2 to 3 cells thick, which separates the sporogenous tissue from the epidermis of the nucellus. The megaspore mother cell, as usual, divides first by a periclinal wall into two cells, one upper and one lower. In the majority of flowering plants, both monocotyledons and dicotyledons, both these cells divide again in the same manner by periclinal walls to form a linear row of 4 megaspores. In the case of *Argemone*, however, their behavior is different. Here the upper cell divides first by an anticlinal wall to form two megaspores arranged in a line transverse to the long axis of the ovule. The division in the lower cell begins later than in the upper cell. This can be clearly seen from figures 2 and 4. Further the division wall here is periclinal, i.e., in a plane at right angles to the division wall of the upper cell and gives rise to two megaspores arranged longitudinally. The tetrad thus formed is not of a linear type, but "T-shaped", to use the terminology of Palm (11), who was the first person to give a complete classification of the different types of megaspore formation in the flowering plants.

Sometimes in longitudinal sections of the ovule, the megaspore mother cell appears to give rise only to 3 megaspores (figures 4 and 5). This is, however, only due to the plane of the section. If longitudinal sections of the ovule are cut in a plane at right angles to that of figures 2 and 3, i.e., in a more or less tangential longitudinal plane, only one of the two upper megaspores is seen at a time and it appears that there are only 3 megaspores.

The morphology of the family Papaveraceæ, to which *Argemone mexicana* belongs, has, on account of the relations of this family with the cruciferæ and the peculiar structure of the flower of two subfamilies Hypecoideæ and Fumarioideæ, been fairly extensively studied. Schnarf (15) cites more than 20 contributions dealing with the

development and life history alone of the various members; and although nothing has been so far published about the morphology of the genus *Argemone*, we have a fair knowledge about the genera *Eschholtzia*, *Hypecoum*, *Chelidonium*, *Sanguinaria*, *Glaucium*, *Papaver*, *Dicentra*, *Corydalis* and *Fumaria* due to the works of Shaw (17), Huss (9), Hegelmaier (5), Surface (20), Vesque (22), Hofmiester (7), Warming (24), Guignard (4), Tischler (21), etc. A summary of all the work is given both by Schürhoff (16) and Schnarf (15), but a 'T-shaped' tetrad of megaspores has not been described in any of these genera. In this respect *Argemone* appears to be different from all of them and resembles such plants as *Butomus* described by Ward (23), *Jeffersonia* described by Andrews (1), *Potamogeton* by Holferty (8), *Diospyros* by Yasui (26), *Trillium* investigated by Heatley (5) and Spangler (18) and *Thismia* and *Gasteria* studied by Pfeiffer (12, 13) and Stiffler (19) respectively. There appears to be, however, no particular phylogenetic significance in this type of megaspore tetrad as it is found in families so wide apart as the Butomaceæ, Berberidaceæ, Potamogetonaceæ, Ebenaceæ, Liliaceæ, Burmanniaceæ and Papaveraceæ and its presence in any one plant is no guide to its affinities. Its importance lies in its serving as a link between the tetrahedral tetrad in which the pollen grains are usually arranged and the linear tetrad which is the usual form of megaspore tetrad and bridging the gap between these two extreme types.*

Of the 4 megaspores formed in *Argemone mexicana*, as is normally the case in flowering plants, the three upper ones soon degenerate after their formation and the lower one alone functions and develops into the embryo-sac (figures 2, 3 and 4). In one case, however, a deviation was noticed from this rule. Here (figure 5) it was the second megaspore from below which had remained functional, while the lowermost along with the two upper ones had degenerated. Such variations are known to occur in other plants also and a description of these, as were known in their time, is given by Coulter and Chamberlain (3) on pages 84 to 86; and as they put it, these serve to emphasize the megaspore character of all the four cells of the tetrad.

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* The following other genera of plants are mentioned by Schnarf (Embryology der Angiospermen, Berlin, 1927; pp. 97–98), in which a 'T-shaped' arrangement of the megaspores has been recorded:

Urtica, *Cynomorium*, *Allionia*, *Brasenia*, *Cabomba*, *Drimys*, *Asimina*, *Adonis*, *Ranunculus Myosurus*, *Sarracenia*, *Garcinia*, *Moringa*, *Saxifraga*, *Neurada*, *Hydrostachys*, several Cistaceæ, Malvaceæ, and Tiliaceæ, *Daphne*, *Cortusa*, *Cynoglossum*, *Erythraea*, *Gentiana*, *Villarsia*, *Asclepias*, *Valeriana*, *Triglochin*, *Ruppia*, *Tricyrtis*, *Yucca*, several Amaryllidaceæ, *Burmannia*, *Heteranthera*, *Typha* and *Platanthera*.

Embryo-Sac.

The structure of the embryo-sac was studied from sections provided by the older ovary. Owing to its large size it could not be possible to get perfect sections, showing all the parts in the same. For this reason a reconstruction was made from a number of serial sections and it is reproduced in the accompanying text-figure (fig. 1). This figure represents a longitudinal section of the ovule and show

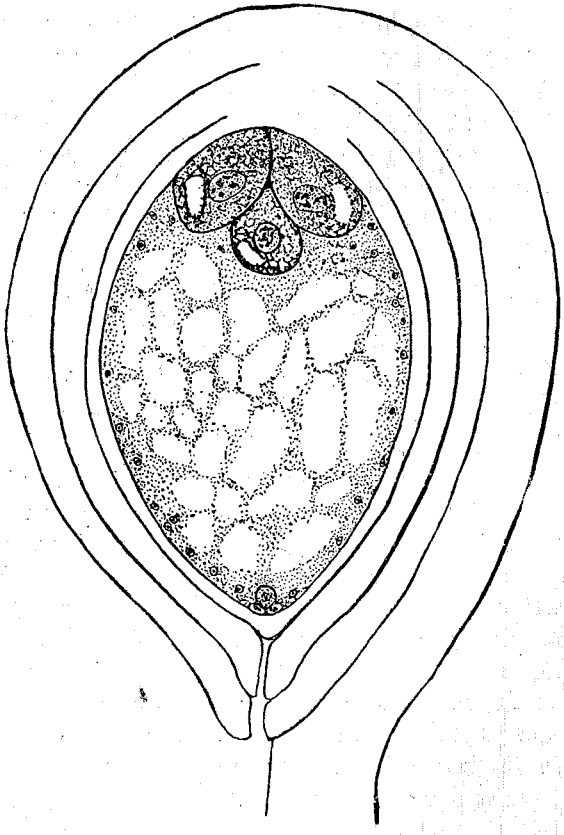

FIG. 1. *Argemone mexicana.* Longitudinal section of the ovule—a reconstruction from a number of serial sections, showing the great disparity in the size of the egg-apparatus and the antipodals. The endosperm formation has set in and the synergids have degenerated. × 100.

the structure of the ovule when endosperm formation had already set in. Sections showing a stage earlier than this were not available, except a 2-nucleate embryo-sac which was provided by the younger

ovary and is sketched in figure 3, but it seems to be fairly certain from the study of the egg-apparatus and the antipodals that the normal '8-nucleate type' of embryo-sac is formed.

The chief feature of the embryo-sac is the great difference in the size of the cells of the egg-apparatus, namely the egg cell and synergids and the antipodals. The latter are about 8 to 10 times larger than the former. The egg-cell is just about 25 *microns* in diameter. The synergids are even smaller than this. They soon get disorganised and do not appear to take any part in the process of fertilisation. The antipodals, on the other hand, at this stage are about 200 *microns* in diameter and form the most conspicuous part of the embryo-sac. Their detailed structure is shown in fig. 6. At this time, disorganisation has already set in, as can be clearly seen from the appearance of the nuclear wall. The protoplasm is of an alveolar nature and in the nucleus, its wall is breaking down. The nucleolus has broken up into a number of darkly staining masses which seem to be in a process of gradual dissolution. In some of these nucleolar masses small shining bodies could be seen. Their exact nature has not been studied by the writer but these appear to be of the nature of dissolution products.

Another feature of the antipodals is the presence of large vacuoles inside them. These are clearly shown in Fig. 6 which represents a transverse section of the antipodals. The vacuoles seem to occupy a more or less definite position. In a transverse section these are found towards the outside of the nucleus of every antipodal cell and on the side away from the centre of the embryo-sac. In longitudinal sections of the ovule, the antipodal cells show a vacuole toward their anterior end facing the micropyle. It thus appears that every antipodal cell has got a vocuole which starts from the micropylar anterior end of the cell and extends peripherally on to the side away from centre of the embryosac.

In the above structure of the antipodals, *Argemone mexicana* shows perfect resemblance with the other Papaveraceæ whose structure is known. Schürhoff (16) says that the "Papaveraceæ are characterised by large antipodals. These are uninucleate, in groups of three and possess a vacuole in the anterior plasma." The embryosac of *Argemone* agrees in every way withthe above characterisation. Here, however, we know further that the vacuoles, which are certainly present in the anterior part of the plasma of the antipodals, extend into their sides also.

Huss (9), who studied the antipodals of *Fumaria*, *Corydalis* and *Papaver*, found that the antipodals are not very big in the young condition but these enlarge to a very great extent by the time these

begin to degenerate. The antipodals of *Argemone* studied by the writer were in early stages of degeneration. He did not see any young antipodals and it is not possible to say, whether the antipodals in this plant are very large in size from the very beginning or these attain this large size only at the time of their degeneration. The degeneration stages of the antipodals of *Argemone* show great resemblance with similar stages figured by Huss in his material.

Another feature appearing to be peculiar to the embryosac of *Argemone mexicana* is the very early and quick development of the endosperm. The formation of endosperm had set in all ovules of the older ovary examined by the writer whether there was a trace of the penetration of the pollen tubes or not and fertilisation had taken place or not. In figure 1, for instance, the egg has not yet divided when a good deal of endosperm has been formed. This leads one to suspect that here also the formation of the endosperm may be beginning even before fertilisation, as reported by Coulter (2) for *Ranunculus*. The very near position of the two families in every recent system of classification of flowering plants lends a still further colour to this suspicion But, whether this is true or not, this much is certain, that a great deal of endosperm is formed before the first division of the oospore and the beginning of embryo-formation.

The pollen-tube was seen to enter through the micropyle in all the cases observed.

A few early stages in the formation of embryo were seen in this ovary, but these were too few to enable one to give any description.

Summary.

1. The megaspores of *Argemone mexicana* form a 'T-shaped' tetrad.

2. The embryosac is characterised by very large antipodals which are 8 to 10 times as large as the egg-cell. They have got large vacuoles running from their anterior ends on to the outer sides. The synergids are even smaller than the egg-cell and degenerate very soon. A great deal of endosperm is formed before the first division of the oospore.

BENARES,
20th August 1932.

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Description of the Plate.

All the figures are of *Argemone mexicana* and were drawn with the help of a camera lucida.

Fig. 2. A tetrad of 4 megaspores, the two upper arranged transversely, the two lower longitudinally. The two upper-most have completely degenerated, while the second from below is in the process of degeneration. The lowermost is the functional megaspore. × 1300.

Fig. 3. A part of the longitudinal section of the ovule, showing the upper part of the nucellus and a part of the inner integument. The megaspore tetrad here is older than the one shown in fig. 2. All the three upper spores have completely degenerated while the nucleus of the lowermost functional megaspore has divided to form a 2-nucleate embryosac. × 1300.

Fig. 4. A view of the megaspore tetrad in a tangential longitudinal section of the ovule. Here only one upper spore is seen. The second spore from below has not yet degenerated. × 1300.

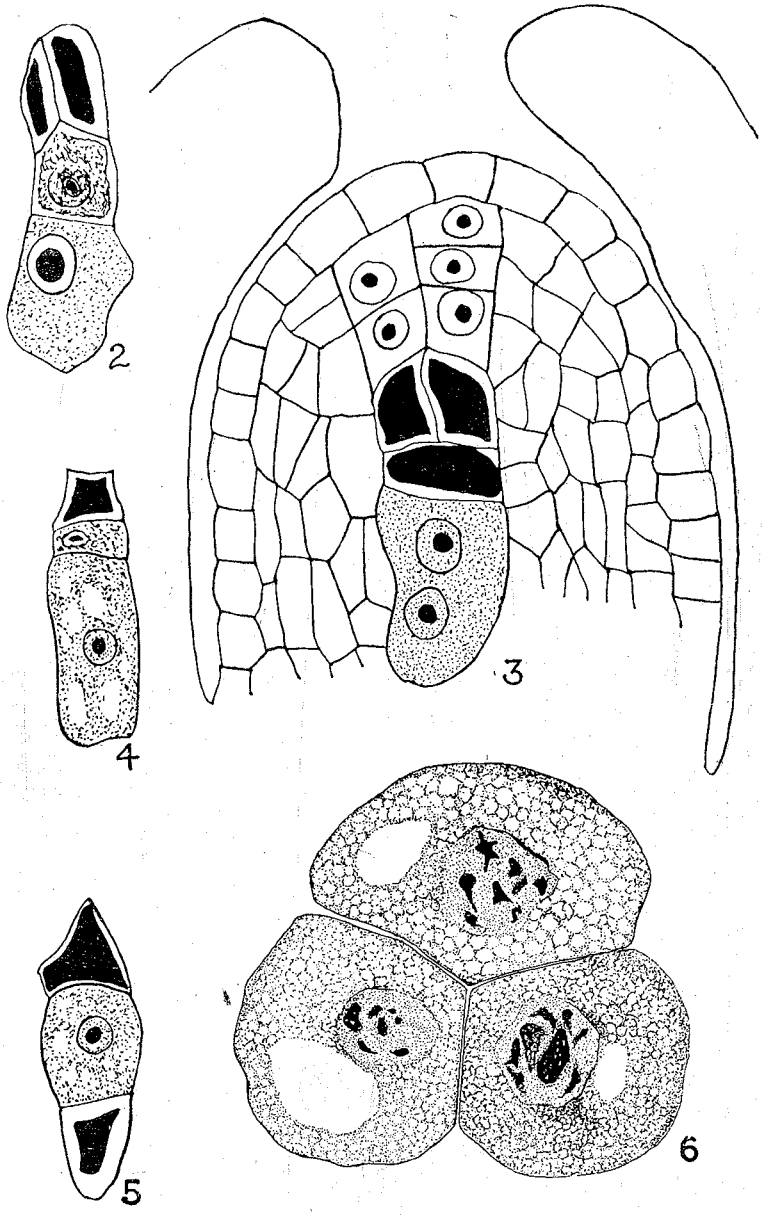

PLATE I. J. I. B. S. XII : 2.

Fig. 5. Same as figure 4. Here, however, the lower-most spore has degenerated and the second spore from below is the functional one. × 1300.

Fig. 6. A transverse section of the three antipodals. These are just beginning to degenerate. The wall of the nucleus is becoming faint, the nucleous has broken up into a number of darkly staining masses which are being gradually dissolved. Each antipodal cell shows a vacuole towards the outside. × 400.