EMBRYOLOGICAL STUDIES IN THE LEGUMINOSÆ

II. A Contribution to the Embryology of *Mimosa hamata*

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THE present communication forms the second paper in a series of studies undertaken by the author on the embryology of Mimosaceæ. The first paper (Dnyansagar, 1949) dealt with the embryology of *Leucæna glauca* in which a number of interesting features, such as (1) the separation of microspore mother-cells, eventually leading to the organisation of simple pollen-grains, (2) sub-hypodermal origin of the archesporium in the ovule, (3) masssive nucellus, (4) extensive development of the parietal tissue in the ovules, (5) the inner integument developing first and the outer integument starting later but overgrowing the inner, (6) the formation of the micropyle only by the outer integument, (7) the occurrence of degenerations in both the microsporangia and the embryo-sac and (8) the massive type of the proembryo, were observed. The study of *Mimosa hamata* has revealed that while in certain characters, it resembles *Leucæna glauca*, in others, it radically differs. It shows the massive nucellus, the extensive parietal tissue, the earlier origin of the inner integument and the massive type of the proembryo as in *Leucæna glauca*, but in the formation of the compound grains, the hypodermal origin of the archesporium in the ovule and absence of any type of degenerations, it is distinctly different. In regard to the formation of the micropyle, it is similar in a way because it is formed mostly by the outer integument.

A review of the previous work on the embryology of the Mimosaceæ has already been given in the first paper (Dnyansagar, 1949). To this may be added a recent paper on the embryology of *Acacia farnesiana* by Narasimhachar (1948).

MATERIAL AND METHODS

The material was fixed in the formlin-acetic-alcohol and Navaschin's fluid. Sections were cut 8–12 μ thick and were stained chiefly with Harris' hæmatoxylin (Johansen, 1940). For smears, Belling's aceto-carmine and its modification, as suggested by Tiwary (1945), were used.

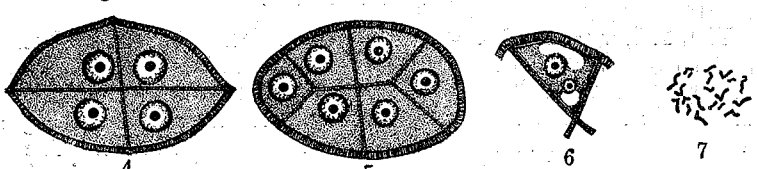
THE INFLORESCENCE AND THE FLOWER

The inflorescence of *Mimosa hamata* is a globose head with male and bisexual flowers. The latter are usually situated higher up in the head. Flowers are pink and range from 60–70 per inflorescence, but

on an average the total number of fruits formed in an inflorescence never exceeds half a dozen. Most of the flowers of an inflorescence drop off without forming of any fruit as reported in *Neptunia oleracea* by Singh and Shivapuri (1935). The floral parts arise in acropetal succession and conform to $K_{(4)}$, C_4, $A\,4+4$, G_1. They are cyclic in their arrangement as in *Leucæna glauca* (Dnyansagar, 1949).

MICROSPOROGENESIS

The archesporial cells are hypodermal in origin and can be distinguished at a very early period. In this respect *Mimosa hamata* is different from *Albizzia lebbek* (Maheshwari, 1931), *Neptunia oleracea* (Singh and Shivapuri, 1935) and *Leucæna glauca* (Dnyansagar, 1949), where primary archesporium differentiates rather late. The primary archesporial cells in an anther appear in four groups and subsequently give rise to inner primary sporogenous and outer primary parietal layers. The parietal tissue is eventually composed of a tapetal layer, one or two middle layers and the enodothecium (Fig. 1). The tapetum is of the secretory type and its cells remain uninucleate throughout their life as observed previously in all investigated species of Mimosaceæ. The fate of the parietal tissue is the same as in *Leucæna glauca* (Dnyansagar, 1949). A transverse section of an anther shows usually 16–48 microspore mother-cells within a microsporangium (Fig. 1), while a longitudinal section shows usually 64–128 microspore mother-



FIGS. 4–7. *Mimosa hamata*.—Fig. 4. A compound grain consisting of 4 microspores. Fig. 5. Another compound grain consisting of 6 microspores. Fig. 6. An individual 2-celled pollen-grain at the time of shedding. Fig. 7. Metaphase, first meiotic division, showing 20 chromosomes. (All drawings, ×2,400).

cells arranged in several rows (Fig. 2). Thus a large number of microspore mother-cells are produced per microsporangium in *Mimosa hamata*. This is indeed noteworthy for a member of the Mimosaceæ In *Albizzia lebbek* (Maheshwari, 1931) and *Acacia Baileyana* (Newman, 1934), only 4 microspore mother-cells have been reported per microsporangium. In *Neputina oleracea*, Singh and Shivapuri (1935) have recorded 20 as the maximum number of microspore mother-cells in a microsporangium, while in *Acacia farnesiana* (Narasimhachar, 1948), only 2–4 microspore mother-cells are produced per microsporangium.

The process of meiosis in *Mimosa hamata* takes place in the normal way. The microspores aggregate in groups of 4–8 leading to the formation of compound grains of varying sizes (Fig. 3). Wodehouse in his treatise on pollen-grains (1935) states that the most outstanding character of the pollen-grains of the Mimosaceæ is their tendency to form compound grains. The present observation thus falls in line

with Wodehouse's statement on the subject. In the previously investigated species, the conditions vary in different plants. In *Neptunia oleracea* (Singh and Shivapuri, 1935), *Prosopis glandulosa* (Wodehouse, 1935) and *Leucœna glauca* (Dnyansagar, 1949), the pollen-grains are simple. In *Albizzia lebbek* (Newman, 1934) and *Acacia farnesiana* (Narasimhachar, 1948), the entire mass of pollen-grains in a sporangium constitutes a pollinium.

The individual mature pollen-grains are usually wedge-shaped in outline and are 8–10 μ in diameter (Fig. 6) and the group as a whole is 16–22 μ in diameter (Figs. 4 and 5). Each grain is convex on the outside and tapers inwards towards the centre of the group or is truncated depending upon its position in the group. The nucleus of the pollen grains divides once and they become bicelled at the time of shedding (Fig. 6). They are shed as compound grains.

Chromosome number.—Chromosome number has been counted from polar views of the metaphase in the first meiotic division of the microspore mother-cell and haploid number in this species is 20 (Fig. 7). Senn (1938) states that the *n*-chromosome number distribution in Mimosaceæ is 12, 13, 24 and 26. Darlington and Janaki-Ammal (1945) have given the number as 12 and 13. In *Leucœna glauca* (Dynansagar, 1949), it is 18. Hence *n* 20 is a new chromosome number for the family.

Megasporogenesis

The solitary carpel contains 6–12 ovules. These are borne in two alternating rows on the marginal placenta and arise as small papillæ at the time of the microspore mother-cell stage in the anthers. The ovules begin to curve upwards towards the stylar end on coming close to the opposite wall of the ovary and become anatropous. The same condition is found in a number of other Leguminous species including *Albizzia lebbek* (Maheshwari, 1931), *Neptunia oleracea* (Singh and Shivapuri, 1935) and *Leucœna glauca* (Dnyansagar, 1949).

The nucellus of the ovule is massive from the beginning. At the tetrad stage there are about 4–5 layers of nucellar cells above the tetrad, 3–5 layers on the sides and 3–5 layers below (Fig. 9). The primordia of integuments appear after the differentiation of the archesporium. The inner integument develops first and the outer follows a little later. Both the integuments are in the beginning composed of two layers of cells, but their later growth is very unequal. The outer integument grows faster than the inner and at the same time becomes several layered, while the inner one remains comparatively much thinner and consists of three layers at the most. The micropyle is formed mostly by the outer integument, the inner integument contributing to its formation for a short length only (Fig. 11). Pantulu (1945) also has recorded a similar condition in several species of *Cassia*, but in some other Leguminosæ, the construction of the micropyle is different. In *Albizzia lebbek* (Maheshwari, 1931), *Neptunia oleracea* (Singh and Shivapuri, 1935) and *Leucœna glauca* (Dnyansagar, 1949), even though the inner integument appears first, the outer integument alone forms the micropyle. In *Acacia farnesiana* (Narsimhachar, 1938), the inner integument

covers only the lower half of the mature embryo-sac, while the outer integument is in level with the apex of the nucellus.

The primary archesporial cell in *Mimosa hamata* differentiates in the hypodermal region of the nucellus even before the appearance of the integument primordia. Fig. 8 shows that the primary archesporial cell has cut off an outer parietal cell and an inner megaspore mother-cell. The former has divided by an anticlinal wall into two cells. An hypodermal origin of the archesporium has been observed in *Albizzia lebbek* (Maheshwari, 1931), *Neptunia oleracea* (Singh and Shivapuri, 1935), *Mimosa pudica* (Narasimhachar, 1945), *Acacia farnesiana* (Narasimhachar, 1948) and in several species of *Cassia* (Pantulu, 1945), while in *Pachyrhizus angulatus, Cajanus indicus, Dolichos lablab* (Roy, 1933), *Cassia tomentosa* (Saxton, 1907) *Cassia tora* (Datta, 1933) and *Leucæna glauca* (Dnyansagar, 1949), a sub-hypodermal origin has been described.

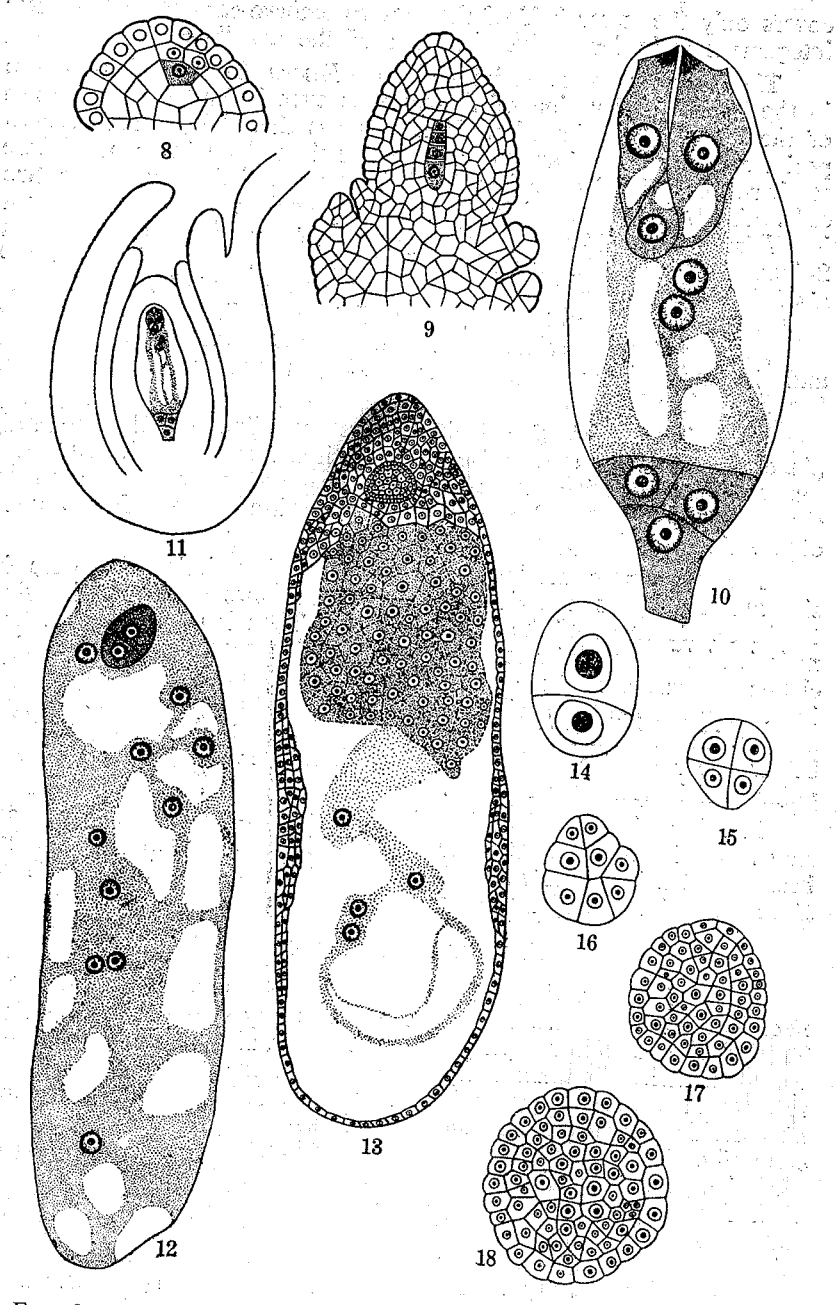
The primary parietal cell divides first anticlinally and then in all planes and forms an extensive parietal tissue. Pantulu (1945) states that such an extensive development of the parietal tissue seems to be characteristic of the Cæsalpiniaceæ and Mimosaceæ. The present observations lend support to this view.

The megaspore mother-cell after its differentiation increases in size and undergoes a period of rest. It becomes deep seated owing to the development of the parietal tissue. Then it undergoes the two meiotic divisions and forms a linear tetrad of megaspores (Fig. 9). The chalazal megaspore of the tetrad develops into the embryo-sac according to the normal type (Fig. 10). The two synergids are hooked and are provided with a filiform apparatus at the apex. The egg protrudes below the synergids. The antipodals form three definite cells. The polar nuclei meet near the egg.

Starch grains are deposited in the embryo-sac at the 8-nucleate stage. A similar type of embryo-sac studded with starch grains has been reported in *Albizzia lebbek* (Maheshwari, 1941) and *Acacia farnesiana* (Narasimhachar, 1948).

Endosperm and Embryo

The primary endosperm nucleus undergoes a number of free nuclear divisions before the oospore shows any sign of segmentation. These divisions follow rapidly until the sac is almost filled with endosperm nuclei. Simultaneously, the embryo-sac enlarges with the result that vacuoles appear here and there (Fig. 12). The oospore now divides traversely into an upper larger cell and a lower smaller cell (Fig. 12). With increasing vacuolation, the endosperm nuclei are driven to the periphery where they continue to divide as before. Wall formation sets in centripetally and a distinct parietal layer of endosperm tissue now begins to form from the micropylar end. By this time the oospore has divided by a longitudinal wall giving rise to a 4-celled proembryo. Fig. 13 which representing a later stage shows the advancing cellular endosperm in the micropylar region of the sac, while as yet there are only a few nuclei below. This stage of



FIGS. 8–18. *Mimosa hamata*.—Fig. 8. L.S. of nucellus showing parietal layer of two cells and the megaspore mother-cell. Fig. 9. L.S. of an ovule showing a

linear tetrad of megaspores and primordia of two integuments. Fig. 10. Mature embryo-sac. Fig. 11. L.S. of an ovule at the mature embryo-sac stage. Fig. 12. Embryo-sac showing the first division of the oospore and formation of free nuclei of the endosperm. Fig. 13. Embryo-sac showing a several-celled spherical proembryo, parietal cellular endosperm, advancing cellular endosperm towards the micropylar region and free nuclei towards chalaza. Fig. 14. First transverse division of the oospore. Fig. 15. Four-celled proembryo. Fig. 16. Transverse division of both epibasal cells and oblique divisions of the right hand cells of the middle and lower layers. Figs. 17 and 18. Spherical massive proembryo. Figs. 8, 16 and 18, ×430; Fig. 9, ×400; Figs. 10, 13–15, ×860; Fig. 11, ×86; Figs. 12 and 17, ×345.

development was seen when the proembryo had become quite massive and several celled (Fig. 19).

As described above, the first division of the oospore is transverse and the second one longitudinal (Figs. 14 and 15). It appears that the two epibasal cells then divide by transverse walls so that three rows of two cells each, are formed and subsequently, the right hand cells of the middle and lower rows divide obliquely (Fig. 16). Further divisions occur in all planes and a massive spherical proembryo is formed (Figs. 17 and 18). There is no differentiation of a suspensor.

The development of the endosperm and the embryo has been studied only in a few species of the Mimosaceæ and the points worth recalling are these. In *Acacia farnesiana* (Narasimhachar, 1948), even at as late a stage as the development of the cotyledons in the embryo, the chalazal end of the embryo-sac contained free endosperm nuclei, though in the micropylar part the cellular endosperm had developed. In *Mimosa pudica* (Narasimhachar, 1946), the endosperm shows wall formation only towards the micropylar end. The massive type of the proembryo as observed here, has also been described in other species of the *Mimosaceæ*, *e.g.*, *Acacia Baileyana* (Newman, 1934), *Mimosa pudica* (Narasimhachar, 1946) and *Acacia farnesiana* (Narasimhachar, 1948).

Summary

The inflorescence is a globose head and consists of 60–70 flowers, but the number of fruits that are formed per inflorescence never exceeds half a dozen.

The primary archesporium in an anther differentiates at a very early period. The tapetum is of the secretory type and consists of uninucleate cells.

A very large number of microspore mother-cells is produced per microsporangium and these give rise to a large number of compound pollen-grains within a single microsporangium.

Each compound grain consists of 4–8 pollen-grains which are generally wedge-shaped. Even at the time of shedding, the grains are compound and each pollen-grain is usually 2-celled.

The haploid chromosome number is found to be 20.

The ovules are anatropous and have two integuments. The micropyle is formed mostly by the outer integument.

There is a single hypodermal archesporial cell in the ovules. The primary parietal cell undergoes divisions so that the megaspore mother-cell comes to lie 3–5 layers deep in the nucellus.

A linear tetrad of megaspores is formed of which the chalazal one functions.

The mature embryo-sac conforms to the normal 8-nucleate type. The antipodals form definite cells.

The primary endosperm nucleus divides several times by free nuclear divisions before the division of the oospore. When the pro-embryo becomes 4-celled, wall formation commences in the endosperm at the periphery and extends centripetally around a central vacuole. Further development of the endosperm proceeds from the micropylar end.

The first division of the oospore is transverse and the second one longitudinal. Later divisions are irregular resulting in the formation of a spherical proembryo of the massive type. There is no suspensor.

In conclusion, the author wishes to express his gratitude to Professor R. L. Nirula, under whose guidance the work was carried out, for helpful suggestions and criticism throughout the course of this investigation.

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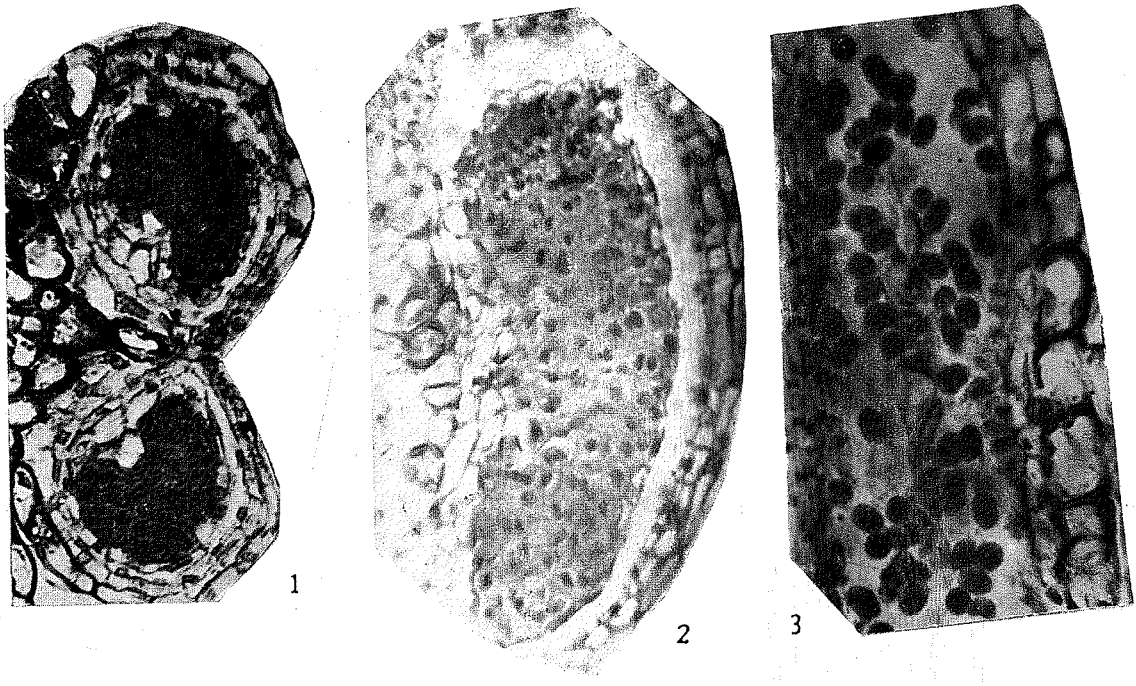

FIGS.1–3. *Mimosa hamata*.—Fig. 1. T.S. of an anther-half showing parietal tissue and a large number of microspore mother-cells in each lobe. Fig. 2. L.S. of a microsporangium showing several rows of microspore mother-cells. Fig. 3. L.S. of a mature microsporangium showing compound pollen grains. ×350.

FIG. 19. *Mimosa hamata*.—L.S. of a young seed showing several-celled spherical proembryo, parietal cellular endosperm, advancing cellular endosperm in the upper region and a few free nuclei below. ×80.

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