EMBRYOLOGICAL STUDIES IN THE LEGUMINOSÆ

III. A Contribution to the Embryology of Pithecolobium saman
Benth. Syn. Enterolobium saman Prain

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INTRODUCTION

For some time past, local members of the Mimosaceæ have been under investigation with the object of studying their embryology. The work on two plants has already been completed (Dnyansagar, 1949, 1950). The present contribution is the third in the series. In Leucaena glauca and Mimosa hamata, the plants already worked out by the author (Dnyansagar, 1949, 1950), a number of common features, such as a single-layered tapetum of the secretory type, a massive nucellus, extensive parietal tissue during megasporogenesis, the inner integument developing first and the outer overgrowing it, hooked synergid with filiform apparatus, the division of the primary endosperm nucleus before the first segmentation of the oospore and lastly, a massive proembryo without any suspensor, were observed. These, it is interesting to record, have also been found in Pithecolobium saman. In addition, the present plant resembles Mimosa hamata in hypodermal origin of the archesporium and Leucaena glauca in regard to the formation of the micropyle by the outer integument alone. But it differs from both the other plants in the comparatively lesser number of microspore mother-cells and the formation of pollinia.

The relevant literature has been quoted previously and it is hardly necessary to repeat the same.

MATERIAL AND METHODS

Material was collected from the plants growing in Nagpur and the Botanic Gardens, Poona. The number of fruits formed in the case of trees growing in Poona was several times more than in the case of the trees growing in Nagpur; so most of the fruit-collections were made from the trees growing in Poona. The material was fixed in the formalin-acetic-alcohol and Randolph's modification of Navaschin's fluid (Johansen, 1940). Sections were cut 8–12 µ thick and were stained chiefly with iron-alum haematoxylin and differentiated in an aqueous solution of picric acid (Maheshwari, 188
1933). Earlich's haematoxylin (Johansen, 1940) was also used. Both the stains were found to be satisfactory. For root tips, iron-alum haematoxylin and Feulgen's stain were used. For pollen preparation, methyl green-glycerene jelly (Wodehouse, 1935) and anilin-oil-gentian violet method (Wodehouse, 1935) were employed.

**Chromosome Number**

Chromosome counts were made from polar views of the metaphase in the cells of the root-tip. The somatic or 2 n-chromosome number was determined to be 18 (Fig. 2). Darlington and Janaki Ammal (1945) have given the list of somatic or diploid number of chromosomes as 24, 26, 48 and 52 in the Mimosaceae. The number 18 is, therefore, an addition to the list.

**The Inflorescence and the Flower**

*Pithecolobium saman*, popularly known as Rain tree, is a big tree of rapid growth with large spreading branches. It looks beautiful with its numerous rose-coloured heads of flowers with long stamens against the dark green leaflets. The inflorescence is an axillary glose head with bisexual flowers. The latter are rose-coloured and range from 15-25 per inflorescence. In each head, the terminal or central flower is distinct from the others as in *Albizia lebbek* (Maheshwari, 1931). It is larger in size and swollen at the base due to the presence of a nectary (Fig. 1 a) which does not develop in the lateral flowers (Fig. 2 b). Occasionally, two carpels, separate from each other, are found in a flower.

The flowering period commences from April and lasts upto July. Most of the inflorescences drop off especially during summer and only a few are left to form fruits which begin to mature in January. The number of fruits in each inflorescence is very small being only one or two. It is usually from the lateral flowers that the fruits are formed as in *Albizia lebbek* (Maheshwari, 1931).

The floral parts arise in acropetal succession and are cyclic in their arrangement as in *Leucaena glauca* (Dnyansagar, 1949) and *Mimosa hamata* (Dnyansagar, 1950). The floral formula is K\(_{(5)}\), C\(_{(5)}\), A\(\infty\), G-l. The androecium is monoadelphous and the filaments are long and pink.

**Microsporogenesis**

The wall of the young anther beneath the epidermis is made up of the endothecial layer, one or two middle layers and a unilayered tapetum (Fig. 3). The tapetum is of the secretory type and its cells remain uninucleate throughout as is the case in all the investigated species of the Mimosaceae.
Figs. 1-8. Fig. 1 A. Longitudinal section of basal part of terminal flower showing nectary, $n$ at the base. B. Longitudinal section of basal part of lateral flower with no nectary, $\times 2.5$. Fig. 2. Polar view of metaphase in mitotic division in a cell of root tip showing 18 chromosomes, $\times 950$. Fig. 3. Transverse section through part of an anther showing microspore mother-cells, mmc, tapetum of uninucleate cells, $t$, middle layers, $m$, endothecium, end. and epidermis epi., $\times 500$. Fig. 4. Transverse section of part of an anther showing microspores surrounded by uni-layered tapetum, $\times 225$. Fig. 5. Surface
view of 32-celled pollinium showing 16 peripheral and 16 central grains, the latter being in 8 superposed pairs. The pollinium consists of 8 sectors, each sector, s, being made up of 2 peripheral, conjoint and polyhedral grains, cp and 2 central, superposed and wedge-shaped grains, sw, × 225. Fig. 6. Individual pollen grain showing exine, ex, intine, in, 3 germ-pores, gp and nucleus, n. × 1,000. Fig. 7. Pollinium showing pollen tubes, pt emerging from its grains, × 100. Fig. 8. Part of pollen tube showing generative cell, g and tube-cell, t, × 1,000.

The wall of the mature anther consists of the epidermis and the endothecium which is now prominent with fibrous thickened bands. At this stage, the tapetum and the middle layers can no longer be seen having been used up during sporogenesis. In each microsporangium, usually 8 microspore mother-cells are produced, these after undergoing the reduction divisions, produce 32 microspores which adhere together to form a pollinium (Fig. 4 and Plate VII, Fig. 1) as in Albizia lebbek (Maheshwari, 1931), Acacia Baille- yana (Newman, 1934) and Acacia farnesiana (Narasimhachar, 1948).

A mature pollinium is 120-35 μ in diameter. It consists of generally 32 grains and occasionally 28 grains. In the normal 32-celled pollinium which takes up a somewhat flattened elliptical or spherical shape, 16 grains are arranged centrally and 16 at the periphery, those in the centre being in two superposed groups of 8 each. The 16 peripheral grains are all in a plane at right angles to and bisecting the central group. The peripheral grains are so placed that their 16 contacts with each other are alternately opposite and midway between 8 contacts of the central group. As a result of such arrangement, the group as a whole has become flattened with the intersecting lines between the individual grains crossing each other at right angles.

Fig. 5 shows a normal elliptical 32-celled pollinium consisting of 16 peripheral grains and 16 central grains in two superposed groups of 8 each. It is made up of 8 tetrahedral tetrads each occupying a sector; in each sector, two grains are at the periphery and these are conjoint and polyhedral (cp) and two are at the centre, these being superposed and wedge-shaped (ws) as indicated in one such sector, s.

The individual grains are 25-30 μ in diameter. In each grain, the exine on the exposed surface is thick while that on the inner side is thin and delicate, allowing the grain to be suitably shaped by the pressure against the neighbouring cells. Underneath the intine, there are three small areas placed at three corners opposite to the thickened exine (Fig. 6). These are the germ pores.

The pollinia are shed as such. Each grain has vacuolated cytoplasm and contains a single nucleus in a young pollinium. It becomes bi-celled at the time of its shedding.
GERMINATION OF THE POLLEN-GRAINS

The germination of the grains has been studied by examination of both fixed carpels and living material in culture. A large number of styles were dissected after maceration in 40% acetic acid at 45°C. Pollinia were found to be lodged in the stigma.

60–70 pollinia were dusted on 15%, 20% and 30% cane-sugar solutions to which neutral red was added for vital staining. A few pollen-grains in two or three pollinia germinated and sent out the tubes. Brough (1927) used this method of culture on the surface employing cane-sugar solutions of various concentrations successfully with Dampiera stricta. Newman (1934), however, failed to get positive results by this method with Acacia Baileyana.

Pollinia were also dusted on slides with a thin film of sugar-agar using 1% agar and 15%, 20% and 30% concentrations of cane-sugar. The medium with the sugar-agar ratio 20:1 gave satisfactory results. 35% to 45% grains in a pollinium germinated. The tubes attained a length of 0.10 mm. to 0.15 mm. The pollen-tubes were formed from the inner sides of the grains and emerged between the individual grains (Fig. 7) as in Acacia Baileyana (Newman, 1934) and other species of Acacia (Wodehouse, 1935).

It appears that the wall of the pollen-tube is a continuation of the intine. The generative cell precedes the tube cell into the tube (Fig. 8) as in Acacia Baileyana (Newman, 1934). The tube nucleus subsequently disorganises. A spindle of dividing generative nucleus parallel to the shorter axis of the tube was seen in the case of a tube grown on sugar solution.

MEGASPOROGENESIS

6–15 Ovules are borne in two alternating rows on the ventral suture of the ovary. These are seen as small papillae at the time, microspore mother-cells have been formed in the anthers. The young ovules are at first quite straight, standing almost at right angles to the ventral suture but they begin to curve upwards towards the stylar end on coming close to the dorsal wall of the ovary and assume an anatropous form. This gradual assumption of anatropy has been reported in Albizia lebbek (Maheshwari, 1931), Neptunia oleracea (Singh and Shivapuri, 1935), Leucaena glauca (Dnyansagar, 1949), Mimosa hamata (Dnyansagar, 1950) and several other leguminous plants.

Each ovule shows a massive nucellus right from the start, as in Albizia lebbek (Maheshwari, 1931), Neptunia oleracea (Singh and Shivapuri, 1935), Cassia (Pantalu, 1945), Leucaena glauca (Dnyansagar, 1949) and Mimosa
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*hamata* (Dnyansagar, 1950). But at the time of formation of the tetrad of megaspores, there are 5–6 layers of the nucellar cells above the tetrad, 5–6 layers on the sides and 3–5 layers below (Fig. 10).

The two integuments begin to appear after the archesporium has been differentiated, the inner one appearing first and the outer one almost immediately afterwards. Both the integuments, at this stage, consist of two layers of cells each (Fig. 10), as in *Leucaena glauca* (Dnyansagar, 1949) and *Mimosa hamata* (Dnyansagar, 1950). Later, the outer integument begins to grow faster and ultimately alone forms the micropyle (Fig. 13), as in *Albizia lebbek* (Maheshwari, 1931), *Neptunia oleracea* (Singh and Shivapuri, 1935) and *Leucaena glauca* (Dnyansagar, 1949); it now consists of several layers of cells in the region surrounding the micropyle and gradually thins out towards the base. The further development of the inner integument after its initiation is perhaps unlike anything so far recorded in the previously investigated species of the Mimosaceae. It reaches at the most, the middle of the nucellus when the embryo-sac is fully mature and the micropyle completely organised at the top (Fig. 13). And what is still more interesting is that this integument starts developing again after fertilisation and ultimately reaches the upper level of the nucellus when the endosperm is just beginning to be formed (Plate I, Fig. 2).

The archesporium is hypodermal and consists of a single cell. It differentiates even before the appearance of the integument primordia. Such an early differentiation is a common occurrence in the Leguminosae. The archesporial cell cuts off a parietal cell (Fig. 9) which divides both anticlinally and particularly forming an extensive parietal tissue as a result of which the megaspore mother-cell becomes deep-seated. Such an extensive development of the parietal tissue seems to be characteristic of the Cæsalpiniaceæ and Mimosaceae, as has been stated by Pantalu (1945). About 5–6 epidermal cells at the micropylar end divide periclinally and form a distinct epidermal cap over the parietal tissue (Fig. 10). Such a multiple epidermal cap has been recorded by Guignard (1881) as a common feature in the Leguminosæ. It may further be mentioned that some epidermal cells on lateral sides of the ovule, become involved in the process and ultimately a very much extensive cap is seen at the time of the mature embryo-sac (Fig. 13). The megaspore mother-cell, after its differentiation, enlarges considerably and in doing so, it crushes the surrounding nucellar cells. After a period of rest, it undergoes two reduction divisions and a linear tetrad of megaspores is formed. The lowermost develops into the normal type of the embryo-sac after three successive free nuclear divisions (Figs. 11 and 12).
Figs. 9-17
Figs. 9-17. Fig. 9. Longitudinal section of nucellus showing parietal cell, \( p \) which has divided anticlinally and megasporangium mother-cell, \( \text{MMC} \), \( \times 1,000 \). Fig. 10. Longitudinal section of ovule showing deep-seated linear tetrad of megasporangia and primordia of two integuments, \( o_t \) and \( ii \), \( \times 450 \). Fig. 11. 4-Nucleate stage of embryo-sac, \( \times 1,000 \). Fig. 12. Mature embryo-sac showing \((a)\) egg apparatus consisting of an egg, \( e \) and two synergids, \( s \). Fig. 12a. Micropylar part of embryo-sac showing egg-apparatus. One synergid, \( s \) with filiform apparatus, \( f \) and egg, \( e \) are seen in this section. 12b, chalazal part of embryo-sac showing three antipodal cells. Fig. 13. Longitudinal section of ovule with its inner and outer integuments, \( ii \) and \( o_t \) respectively, micropyle, \( m \) and epidermal cap, \( epc \), \( \times 225 \). Fig. 14. Embryo-sac showing undivided oospore, \( we \) and free endosperm nuclei, \( end \), \( \times 225 \). Fig. 15. Upper part of embryo-sac showing transversely divided oospore, \( e \) and remnants of two disorganised synergids, \( s \), \( \times 500 \). Fig. 16. Massive proembryo, \( \times 225 \). Fig. 17. Advanced pro-embryo showing tip of radicle, \( red \) and two cotyledons, \( cot \times 100 \).

The two synergids are hooked and have each a filiform apparatus at the apex (Fig. 12a). Dahlgren reviewed the literature on hooked synergids in 1938. He states that among the Mimosaceae they have been reported in Albizzia lebbek, Acacia Baileyana and Neptunia oleracea. To this list, may be added Acacia farnesiana (Narasimhachar, 1948), Leucaena glauca (Dnyansagar, 1949), Mimosa hamata (Dnyansagar, 1950) and Pithecolobium saman. Among the Papilionaceae and Caesalpiniaceae, these have been reported only in Medicago sativa (Cooper, 1935) and Cassia (Pantalu, 1945) respectively. The antipodals are definite cells (Fig. 12b). The two polar nuclei meet more or less near the centre.

Starch grains are deposited in the embryo-sac at the 8-nucleate stage. The presence of starch in the embryo-sac seems to be of wide and variable occurrence. Dahlgren (1939) has reviewed the literature on the presence of starch grains in the embryo-sacs. According to him, in general, the production of starch grains seems to attain its maximum immediately before fertilisation. The present observation agrees with his statement. Among the Mimosaceae, these have been reported to occur from the 8-nucleate stage of the sac onwards in Albizzia lebbek (Maheshwari, 1931), Neptunia oleracea (Singh and Shivapuri, 1935), Acacia farnesiana (Narasimhachar, 1948) and Mimosa hamata (Dnyansagar, 1950). In Acacia Baileyana and Acacia discolour (Newman, 1934), starch appears in the early stages of the germination of the megaspore. Among the Papilionaceae, it is found by Reeves (1930), Cooper (1935), Cooper, Brink and Albrecht (1937) in Medicago sativa from the 2-nucleate stage and it is abundant in mature embryo-sacs.

**Endosperm and Embryo**

The primary endosperm nucleus divides rapidly by many free nuclear divisions before the first segmentation of the oospore as in Acacia farnesiana (Narasimhachar, 1948), Leucaena glauca (Dnyansagar, 1949) and Mimosa...
hamata (Dnyansagar, 1950). Due to these divisions, the sac becomes filled with endosperm nuclei and at the same time, it enlarges resulting in the appearance of vacuoles here and there (Fig. 14). Cell formation commences at the micropylar end and proceeds along the wall of the sac, resulting in the formation of a distinct parietal layer all round. It then advances from the micropylar side downwards till just a part is left on the chalazal side in which only free endosperm nuclei are present, the walls being not laid even as late as the appearance of the cotyledons in the embryo (Plate VII, Fig. 3). Similar condition was observed by Narasimhachar (1948) in Acacia farnesiana. No trace of the endosperm was observed in the mature seed of the Pithecolobium saman.

The first division of the oospore is transverse (Fig. 15, Plate VII, Fig. 2), as in Acacia Baileyana (Newman, 1934), Acacia farnesiana (Narasimhachar, 1948), Leucaena glauca (Dnyansagar, 1949) and Mimosa hamata (Dnyansagar, 1950). In the preparation illustrating this division, the disorganised synergids were seen lying in their respective positions one on either side, even the remains of the filiform apparatus being slightly visible in one of the synergids. It could not be determined whether the second wall was transverse or longitudinal. There, however, seems to be no doubt that the further divisions occur in all planes and a massive proembryo is formed (Fig. 16). It is usually spherical, sometimes it assumes various forms of pear shape. It has no suspensor. Such a massive type of the proembryo has been described in several species of the Mimosaceae, e.g., Acacia Baileyana (Newman, 1934), Acacia farnesiana (Narasimhachar, 1948), Leucaena glauca (Dnyansagar, 1949) and Mimosa hamata (Dnyansagar, 1950). Fig. 17 shows the embryo consisting of young radicle and two cotyledons between which there is the region whence the plumule will arise.

**Summary**

1. *Pithecolobium saman* Benth. is a large tree of rapid growth with large spreading branches.

2. The inflorescence is a globose head and consists of 15-25 flowers. The central flower in each head differs from other flowers in the presence of a nectary which is lacking from others. The number of fruits that are formed per inflorescence is 1-2.

3. The $2n$-chromosome number is 18.

4. During microsporogenesis, 16-32 megaspores are produced per microsporangium and these form a single pollinium.
5. Pollinia are shed as such and individual pollen-grains are bi-celled at the time of shedding. The pollen-tubes are formed from the inner side of the grains and emerge from within the group between the individual grains.

6. The ovules are anatropous and have two integuments, of which the outer forms the micropyle.

7. Megasporogenesis is of the normal type. The archesporium is single-celled and hypodermal. Extensive parietal tissue is formed as a result of several periclinal and anticlinal divisions of the primary parietal cell. A linear tetrad of megaspores is formed, of which the lowermost functions.

8. The embryo-sac conforms to the 8-nucleate Normal type. The synergids are hooked and each has a filiform apparatus. The antipodals form definite cells.

9. The primary endosperm nucleus divides several times before the first segmentation of the oospore. Wall formation in the endosperm commences from the micropylar end, the chalazal end containing only free endosperm nuclei for a long period.

10. The first division in the oospore is transverse. Later, by repeated divisions, a spherical or pear-shaped proembryo is formed. This latter has no suspensor.

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

**Literature Cited**


**EXPLANATION OF PLATE**

**PLATE VII**

**FIG. 1.** Transverse section of part of anther showing pollen grains surrounded by parietal tissue, × 525.

**FIG. 2.** Upper part of the sac showing the transversely divided oospore, *e* remnants of two disorganised synergids, *s*, free endosperm nuclei, *end* and the inner integument, *i*, × 140.

**FIG. 3.** Longitudinal section of older seed showing embryo with two cotyledous and cellular endosperm which has not extended up to the chalazal end, × 46.