MORPHOLOGICAL STUDIES IN THE SAPOTACEÆ

1. Embryology of Bassia latifolia, Roxb., and related genera

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Introduction

Although Sapotaceæ are recognised as a primitive family of the Sympetetalæ and possess several remarkable features, the morphological investigation of them has been very little. Warming¹ has carried out investigations on the vascular anatomy of the ovules of Palaquium, Mimusops, and Sideroxylon. Saunders² has studied the floral morphology of the genera Chrysophyllum, Reptonia and Labatia, particularly with regard to the vascular strands of the stamens. Neither Schirhoff³ nor Schnarf⁴ mention about any morphological work on the family. Schnarf⁴ merely states that Ebenales has been very incompletely investigated, whole families being left out of consideration. Tishler,⁵ in his suggestive article on "Some Problems of Cyto-taxonomy and Cytoecology," has given a list of tropical and subtropical families of plants including Sapotaceæ whose chromosome number, cytology and morphology have not been investigated.

In the present study the development of the ovule and the embryo-sac have been traced in Bassia latifolia, Roxb., Mimusops elengi, Linn., Mimusops hexandra, Roxb., and Achara Sapota, Linn., in which some stages in the embryogeny have also been described. Further studies on the cytology and embryogeny of the above plants are in progress and will be published elsewhere.

Observations

Sapotaceæ are a tropical family of woody shrubs and trees, many of which are of conspicuous economic importance, furnishing a variety of useful products like edible fruits, oil seeds, guttapercha, balata and timber (Willis⁶). The plants usually have latex, of viscous consistency. B. latifolia is a large deciduous tree cultivated all over India. The tree is valued for its flowers, fruits, seeds and timber and is of considerable economic value. The fleshy cream-coloured corolla is edible and contains much sugar; on fermentation and distillation this yields a kind of toddy utilised on the western coasts (Mayuranathan⁷), and also as a raw material for the manufacture of power alcohol on a large scale especially in Bihar and Orissa. The seeds of this
plant have fleshy cotyledons and yield a fixed oil which is used for edible purposes and frequently as an adulterant of ghee. *M. elengi* is a handsome and elegant tree cultivated in the gardens for its edible fruits and fragrant flowers. Its bark is well known for its astringent property and a decoction prepared from it is used as a gargle. A fatty oil is distilled from its seeds in Tanjore (Chopra⁹). *M. hexandra* is a large shrub yielding an edible, reddish yellow fruit. Abnormalities like the fusion of flowers and other teratological features have been observed in this plant. *A. sapota*, popularly known as Sapodilla tree produces the highly prized edible fruit known as the Sapodilla plum. The trunk bark of *A. sapota* is a tonic and febrifuge (Tavera⁹). The family is notable on account of the hard woods known as Iron-Woods (Kraemer¹⁰). Since the latex of some of these plants is very viscid it is used by the natives of the East Indies to catch small birds by smearing the latex on long poles and fences. Abundance of brittle hairs on the floral parts is a constant feature of these plants. In the plants studied above both flowers and latex emit a peculiar smell which attract flies of all kinds. It is noteworthy that the plants of the neighbouring family (Ebenaceae) do not possess latex. The presence of tannin renders the unripe fruits very bitter. McNair¹¹ inclines to the view that the presence of tannin in these tropical plants acts as protection against injury from animals and fungi.

As far as the progress in the economy of the floral parts is considered, Sapotaceae, according to Wernham,¹² occupies the highest position among the Ebenales. Here, sympetaly is associated with an epipetalous androecium, showing varying stages of abortion of the essential parts. In the number of stamens there is a gradual reduction. *B. latifolia* has twenty fertile stamens in two whorls, while in *Mimusops* only eight stamens of one whorl are fertile, the other eight being reduced to staminodes. In *A. sapota* six perfect stamens occur along with six staminodes. The superior ovary is multilocular with a large solitary ovule in each locule, the total number of ovules being eight or ten. In the plants studied most of the ovules degenerate so that only one or two finally develop into seeds.

**Materials and Methods**

The materials for this investigation were obtained from plants growing in the Government Botanical Gardens, Bangalore. Bouin's fluid was used for fixation. Sectioning of the materials was found to be difficult because of two factors. The ovary, even in the early stages, is densely clothed externally by a felt of brittle hairs, the removal of which is not an easy process. Secondly, in the older stages, the tissues of the ovary become impregnated
by tannin, added to the sclerenchimatisation of the parts. These difficulties could not be overcome by any means and only after repeated attempts a few satisfactory stages could be obtained. The usual processes of dehydration and infiltration were followed and sections were cut from 10 μ to 16 μ in thickness, and stained in iron-alum-hæmatoxylin.

**Ovary and Ovule Development**

The ovary is syncarpous, and contains groups of darkly staining tannin cells which occur to a greater extent in *B. latifolia* (Fig. 1) than in the other species studied. The placentation is axile. Each locule of the ovary encloses a solitary anatropous ovule, and with the micropyle pointing towards the base of the ovary. The integument is single and massive. The nucellus consists of a single layer of large cells and disorganises early.

A hypodermal cell at the tip of the developing nucellus is early differentiated as the primary archesporial cell. Simultaneously with its differentiation, the ovule begins to curve. This cell elongates and shows deeply staining homogeneous cytoplasm. Its nucleus is large and lies at the micropylar end of the cell which becomes wedge-shaped. A characteristically large nucleolus, surrounded by a clear area, is enclosed by the nuclear membrane.

The archesporial cell functions directly as the megaspore mother-cell without cutting off a parietal cell. The nucellus at this stage consists of large cells which, however, become crushed and disorganised later in the course of development of the embryo-sac. Considerable time elapses between the formation of the primary sporogenous cell and the onset of meiosis. During this period the integument continues to grow and envelop the nucellus leaving a narrow micropylar canal. This canal varies in its length, being longest in *M. hexandra*, and shortest in *B. latifolia*.

**Tetrad Formation**

When the nucleus of the enlarging megaspore mother-cell shows signs of approaching meiosis its chromatin-linen network resolves itself into an irregular mass of threads which soon contract into a synizetic knot (Figs. 2 and 30). Division figure of metaphase was observed in *B. latifolia* (Fig. 9). The first division is transverse, the micropylar cell being smaller than the chalazal cell. The lower dyad cell elongates and becomes more than twice as long as the micropylar cell (Figs. 3, 10, 21 and 31). It is in the next division, in the formation of the tetrad, that the plants deviate from the normal course. Two types of tetrads have been observed, the first being the usual linear tetrad and the second, a variation of the T-shaped tetrad. Both types occur in *Achras sapota*, while only the latter type is present in the three other species (Figs. 4, 6, 13 and 32).
Fig. 1. *Bassia latifolia.*—Longitudinal section of a bud. ×26. Figs. 2–6. *Achras sapota.*—Fig. 2. Archesporeal cell. ×300. Fig. 3. Dyad. ×300. Fig. 4. Tetrad. ×300. Fig. 5. 2-Nucleate embryo-sac with the disorganised megaspores forming a dark cap over it. ×300. Fig. 6. Linear tetrad. ×300.

The lowermost functional megaspore increases greatly in size and the three outer cells of the tetrad become compressed, and disintegrate. The nucleus of the enlarging megaspore divides once and the daughter nuclei migrate to the opposite poles of the cell. A large vacuole develops between them. Each of the two nuclei now divides, and the embryo-sac attains greater size, the chalazal end penetrating into the chalazal region of the ovule and the micropylar end of the sac coming into contact with the innermost layer of the integument, in consequence of the disorganisation of the nucellar tissue. An increased vacuolation accompanies the rapid growth of the embryo-sac. In *M. elengi* and *B. latifolia* the integumental cells bordering the embryo-sac become filled with tannin and the number of tannin-filled cells increases as the embryo-sac grows further. There is no regularly organised tapetal jacket in any of the plants studied (Figs. 14, 15, 34, 35, 36 and 39).

The division of the four nuclei which results in the eight-nucleate embryo-sac is not marked by anything unusual. In *M. hexandra* the eight-nucleate embryo-sac shows, just before the organisation of the mature embryo-sac, four large nuclei at each pole. The chalazal region of the embryo-sac is narrowed to a finger-like projection in which the four nuclei are contained.
(Fig. 26). But in *B. latifolia* (Fig. 16) three of the nuclei at the antipodal end are much smaller than the other nuclei and degenerate early so that the organised embryo-sac shows only the egg apparatus and polars (Figs. 17, 27, 38 and 40). The antipodals degenerate early in all the four species: The polar nuclei fuse just beneath the egg to form the secondary nucleus. The large secondary nucleus maintains the same position until fertilisation (Figs. 17 and 28).

The synergids are hooked and beaked. In *B. latifolia* the synergids form a very prominent part of the egg apparatus being much elongated and having
narrow apices which follow the contour of the micropylar region of the embryo-sac (Figs. 17 and 18). The presence of a filiform apparatus in the synergids was not observed. The egg-cell is also considerably large and its broad basal portion extends beyond the bases of the synergids. A large vacuole develops in its apical region and the egg-nucleus remains embedded in the dense cytoplasm of the basal portion.

Figs. 14-19. *Bassia latifolia*

Fig. 14. 2-Nucleate embryo-sac. ×300. Fig. 15. 4-Nucleate embryo-sac. ×300. Fig. 16. 8-Nucleate embryo-sac before organization. ×300. Note the three small antipodal nuclei. Fig. 17. Mature embryo-sac surrounded by the tannin-filled integumental cells, and hypostase. ×300. Fig. 18. Section of a portion of the ovary showing the ovule and the tannin cells. The hypostase is prominently developed. ×130. Fig. 19. Embryo-sac after fertilisation showing the fertilised egg, the secondary nucleus and the remains of the pollen tube. ×300.
In *B. latifolia* and *M. elengi* the embryo-sac has on either sides numerous darkly staining tannin-filled integumental cells. In both, the chalazal extremity of the embryo-sac is connected to a well-defined hypostase which leads to the vascular trace of the ovule (Figs. 18 and 38). In *A. sapota* the hypostase is not so clearly defined as in the two species mentioned above. On the other hand, in *M. hexandra*, the chalazal cells, near the embryo-sac,
get loosened and become enlarged. The size of their nuclei also becomes more prominent after fertilization so that these chalazal cells may be supposed to have a nutritional significance (Figs. 26, 27 and 28).

**Fertilization**

The pollen tube enters the ovule by way of the micropyle. Double fertilisation has been seen in *M. hexandra* and *A. sapota*. Both the synergids

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**Figs. 30-38. Mimusops elengi**

**Fig. 30.** Archesporial cell in synizesis. ×300. **Fig. 31.** Dyad. ×300. **Fig. 32.** Tetrad ×300. **Fig. 33.** The enlarging chalazal megaspore with the disintegrating micropylar megaspores. ×300. **Fig. 34.** 2-Nucleate embryo-sac with the disorganised megaspores forming a cap ×300. **Fig. 35.** The same at a later stage. ×300. **Fig. 36.** 4-Nucleate embryo-sac showing the tannin cells and hypostase ×300. **Fig. 37.** Spindle formation in the 4-nucleate embryo-sac × 300. **Fig. 38.** Mature embryo-sac surrounded by tannin cells. × 300.
are usually destroyed. The nucleoli of the fusing male and female nuclei are often distinguishable for a considerable time (Figs. 29 and 41). Most of the ovules develop into mature embryo-sacs and then degenerate. Many others do so even in the earlier stages. Perhaps this explains the reason for the development of only two or three ovules into the seed stage. The remains of the pollen tube are visible for a considerable time after fertilisation.

**Endosperm**

In the post-fertilisation stages of the embryo-sac, the primary endosperm nucleus is the most prominent and the largest nucleus in the embryo-sac. It lies very near the fertilized egg (Figs. 17, 28 and 41). It undergoes a period of rest after which it divides. The division of the primary endosperm nucleus is rather late, occurring just after the first transverse division of the fertilised egg (Fig. 42). Soon after, a number of free-nuclear divisions take place with most of the endosperm nuclei massed at the antipodal region. Wall formation commences from the periphery of the embryo-sac, which now attains greater dimensions (Fig. 43). As the embryo develops further the cellular endosperm forms a sheath around it.

**Embryo (A. sapota)**

After a prolonged period of rest, the fertilized egg grows out into a slender suspensor. The first division of the fertilized egg takes place earlier than the division of the primary endosperm nucleus (Fig. 42). After the first transverse wall is laid the primary endosperm nucleus divides into a number of free nuclei which surround the pro-embryo. Two more transverse walls are laid in the pro-embryo (Fig. 43). Due to the hardening of the ovary wall in later stages, which offers difficulty in sectioning several further stages in the development of the embryo could not be obtained. In much later stages, when the ovule attained a greater size, the ovules were separated from the ovary, fixed and sectioned. These sections show a spherical embryo attached to a massive multi-cellular suspensor with the endosperm nuclei scattered around. The suspensor is very peculiar consisting of cells of varying size (Figs. 44 and 45). Most of the cells of the suspensor are bloated and seem to be densely packed with nutritive material. Later when the embryo develops cotyledonary bulges, the suspensor degenerates. Further stages show the enlarging embryo and the rapid cellular development of the endosperm. A section of the seed shows an embryo surrounded by the endosperm. The radicle is very short and fits into the narrow groove of the endosperm sheath at its top (Fig. 46).
Fig. 39. 2-Nucleate embryo-sac. $\times 300$. Fig. 40. Mature embryo-sac. $\times 300$. Fig. 41. Fertilisation. The male nuclei are clearly visible. $\times 300$. Fig. 42. 2-Celled pro-embryo with undivided primary endosperm nucleus. $\times 300$. Fig. 43. 3-Celled pro-embryo. The endosperm nuclei are massed at the antipodal region. $\times 300$. Fig. 44. Spherical embryo attached to the massive suspensor. Fig. 45. Embryo with the cotyledonary bulges and a portion of the suspensor. $\times 36$. Fig. 46. Section of a seed showing the testa, endosperm and embryo.

**Discussion**

Ebenales is regarded by Coulter and Chamberlain\(^\text{13}\) as a “Particularly puzzling alliance whose affinities are uncertain”. It is being realised now-a-days that the study of plants in all their aspects, including their embryology and vascular anatomy of their floral structures is absolutely necessary for setting up a Natural System of Classification.
Sapotaceae belongs to a transition group of families and may be expected to show a combination of both primitive and advanced characters. There is no doubt that more of specialisation is encountered here than in the neighbouring family Ebenaceae in the stabilisation of sympetalaly, the occurrence of a single integument and the suppression of the parietal tissue.

As stated before two types of tetrads have been noticed. The formation of a linear tetrad along with a variation of the T-type was seen in A. sapota. Different types of tetrad formation have been observed in Diospyros kaki by Yasui. In this species the occurrence of three types of tetrads, viz., a T-type, a linear type and the type seen in the Sapotaceae have all been recorded. In the other three species studied here only the last type, viz., a variation of the T-type has been observed. This fact reveals that a process of transition in the formation of the embryo-sac is taking place in these plants. In his studies on the Apocynaceae, Andersson makes a special mention of the presence of oblique spindles and oblique walls in the upper dyad cell of Apocynum cannabinum and the lower dyad cell of Nerium oleander respectively. The tetrad seen in the Sapotaceae is a combination of the two cases observed by Andersson. In a masterly review of the types of embryo-sacs in the Angiosperms, Maheshwari has described the different types of tetrad formation. But the types of tetrads occurring in Ebenales seem to have been missed.

Development of the Embryo-sac

The lowermost megaspore develops into the mature embryo-sac and the other three megaspores degenerate as in the Ebenaceae. It is noteworthy that even before the egg-apparatus and the polars are organised the three nuclei at the antipodal end disappear as in Diospyros Virginiana (Hague). The early degeneration of the antipodals has been noted by Mauritzon, and Joshi and Venkateshwarlu in the Lythraceae. Both Mauritzon and Maheshwari incline to the view that such a type of embryo-sac forms an intermediate stage in the derivation of the Oenothera-type of embryo-sac in which the antipodals are entirely absent.

The synergids form the prominent feature of the embryo-sac, especially in B. latifolia. Large synergids have been observed by Anantaswamy Rao and Andersson in some of the Apocynaceae.

The presence of a well-formed and regularly arranged tapetal jacket around the embryo-sac is lacking in A. sapota and M. hexandra. In M. hexandra, the enlargement of the cells towards the chalazal extremity of the embryo-sac is interesting. In B. latifolia and M. elengi, on the other hand, there is a feeble attempt towards the formation of a nutritive jacket of
cells around the embryo-sac. The accumulation of tannin in these cells may be considered as a reserve material capable of further utilization. Mcnair also inclines to the same view regarding the presence of tannin in cells around the embryo-sac. The absence of a definite nutritive jacket of cells around the embryo-sac in the Sympetalae have been reported in some families like the Apocynaceae (Anantaswamy Rao), in the Convolvulaceae (Raghava Rao) and in an advanced group like the Labiatae (Junell and Narasimha Murthy). Therefore the significance of a tapetal tissue in deciding the status of a family is rather doubtful.

The hypostase tissue, common to a large number of the higher Sympetalae, is well developed in B. latifolia and M. elengi. Finally, a comparative study of the embryo-sac formation in these plants, may be supposed to show a rather primitive type of organisation.

*Embryo*

The development of the embryo has been traced in A. sapota. Some of the stages in the formation of the suspensor and embryo could not be obtained, as already stated, due to the difficulty encountered in sectioning the material. One of the stages shows a three-celled pro-embryo surrounded by the endosperm nuclei. The next stage obtained was that of a multicellular and massive suspensor with a spherical embryo attached to it. The cells of the suspensor are not uniform but show diversity in their size and form. Such a suspensor, which is expanded in the middle and tapering at the ends, may be compared to the suspensors observed in the Leguminosae. Cases of massive suspensors among the Sympetalae have been reported in a few families. Raghava Rao has recorded the presence of large suspensors in the Convolvulaceae. A massive suspensor similar to that of A. sapota has been observed by Hague in Diospyros Virginiana. Further investigation of the Ebenales may throw more light on the presence of similar suspensors in several families.

With regard to the functions of such a massive suspensor, the first is evidently to push the growing embryo into the centre of the nutritive endosperm, and secondly, to act as a storehouse of nutrition for the embryo as is suggested by its swollen appearance. Because of the nature of its irregular divisions and its similarity to the suspensors of the Leguminosae it may be regarded as primitive.

In concluding this investigation it may be stated that the species studied show several distinct features which no doubt represent transitional characters. The most striking feature is the formation of the suspensor in A. sapota. In
view of the fact that the family shows uncertain affinities, a complete study of as many genera as are available would form a valuable contribution to our knowledge of the position of the Family in a Natural System of Classification.

Summary

1. Development of the embryo-sac in four species, viz., B. latifolia, Roxb., Minusops elengi, Linn., M. hexandra, Roxb., and A. sapota, Linn., has been studied. Further some stages in the Embryogeny of A. sapota have been traced.

2. The ovules are anatropous and have a single massive integument. The nucellus consists of a single layer of cells.

3. The archesporial cell does not cut off a parietal cell, but functions directly as the megaspore mother-cell.

4. Two types of tetrads have been noticed, the lowermost megaspore developing into the mature embryo-sac.

5. A definite nutritive jacket for the embryo-sac is absent in all the four species. The hypostase is prominently developed in B. latifolia and M. elengi.

6. The antipodals degenerate early.

7. Fertilisation is porogamous. Double fertilization has been observed in M. hexandra and A. sapota.

8. The primary endosperm nucleus divides later than the fertilized egg.

9. The developing embryo in A. sapota has a massive suspensor.

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