STUDIES IN THE EMBRYOLOGY OF SOME VERBENACEÆ

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The earliest investigations on the embryology of Verbenaceæ are by Hofmeister (1858) and Treub (1884), both of whom worked on the development of the endosperm and haustoria in *Verbena officinalis*. Warming (1873) and later Jönsson (1879–80) also worked on the same species. Karsten (1891) suggested that the embryo-sac development in *Avicennia officinalis* is of the 'Allium type'. As early as 1896, Koorders worked on the morphology, physiology and embryology of *Tectona grandis*. Later, Kanda (1920) studied several other species of *Verbena* and reported a 'Helobial type' of endosperm development, but this observation has been criticised by Dahlgren (1923) and Schnarf (1925) working on *Verbena officinalis*, both of whom reported the 'Cellular type' of endosperm development. Schnarf (1931) also worked on the genus *Vitex*. Schwencke (1931) reported that the development and the sequence of wall-formation of endosperm in *Verbena* is variable. Junell (1934) has made a comprehensive study of the family. This has been summarised by Misra (1939) as follows:—

"Nucellus conforms to the typical sympetalous type. There is one integument. In *Avicennia* this does not cover the whole nucellus. The archesporuim in the ovule is hypodermal in origin and may consist of one or more cells. Normally one of these cells develops into the megaspore-mother cell without cutting any parietal cell. The embryo-sac development corresponds to the 'Normal type', but in the genus *Avicennia* perhaps it may agree with the 'Scilla type'. The shape of the mature embryo-sac is variable in different genera. The egg-apparatus consists of an egg and two hooked synergids. In *Verbenoideæ*, *Pityrodia* and *Callicarpa* the polar nuclei do not fuse before fertilisation, but in *Clerodendron* and *Cornutia* they fuse before fertilisation. The antipodals are three in the beginning, but they may often divide afterwards and increase in number. They, however, never increase in size and generally degenerate early".

Patermann (1935) has made a detailed study of the haploid generation in a large number of genera and species of the family. His observations may be summarised as follows: One integument, the innermost layer of which may give rise to a tapetal tissue. The archesporium is hypodermal and unicellular (2-celled in *Premna integrifolia*) and directly functions as the megaspore-mother cell. Reduction division results in a linear tetrad of megaspores, generally the chalazal megaspore alone functions to give rise to a 'Normal type' of embryo-sac.

The three antipodals degenerate quickly. Endosperm haustorium is variable in development.

In India, Misra (1937) was the first to publish a note on the "Antipodals of Verbenaceæ" based on his observations on *Clerodendron phlomidis*. This was followed by a detailed "Contribution to the Embryology of the Verbenaceæ" by the same author in 1939. He found a "Normal type" of development of the embryo-sac and the ephemeral nature of the antipodals in *Caryopteris wallichiana*. The antipodals of *C. phlomidis*, however, multiply to form about 20 cells. The latest work on the embryology of Verbenaceæ is that of Tatachar (1940) on *Lantana indica* and *Stachytarpheta indica*. He found a 'Normal type' of embryo-sac development. Both chalazal and micropylar haustoria were found to be present.

Materials and Methods

The present investigation is based on *Lippia nodiflora* Rich., *Tectona grandis* Linn. and *Vitex negundo* Linn. The materials of these were collected from different localities near Calcutta, and fixed between 8 a.m. to 2 p.m. in July and August, 1947. They were first treated with Carony's fluid and then fixed in Nawaschin's fluid. A suction pump was used to facilitate penetration of the fixing fluid. The bracts, calyx and the corolla (except in small buds) were removed before fixation. The materials were dehydrated in alcohol, cleared in xylol and embedded in paraffin in the usual way. Considerable difficulty was experienced in cutting the sections due to the presence of glands and hairs on the floral envelopes as also on account of the hard endocrap of the seed. Sections were cut 10–16 μ thick depending on the stage required for study. Sections were generally stained in Heidenhain's Iron-Alum-Hæmatoxylin. A few slides were also stained in Newton's Iodine Gentian-Violet.

Observations

I. *The development of the flower.*—The organogeny of the flower follows the same pattern in all the three species. The sepals are the first members to appear on the flower primordium (Figs. 1, 37 and 64). Another whorl of appendage soon becomes apparent above this and as it develops it separates into two distinct whorls, the outer differentiating as the corolla and the inner as the androecium (Figs. 2, 3, 38, 39, 65 and 66). Thus the petals and stamens originate from common primordia. The primordia of the two carpels next arise near the centre of the flower (Figs. 3, 40, 66 and 67). As they grow, they arch inwards, meet and fuse together to give rise to the style and stigma. Later the ovules develop inside the ovary. In *Tectona grandis* the central axis elongates to give rise to the lateral placentæ bearing the four pendulous ovules (Figs. 41 and 42). In the other two plants the placentæ arise from the base of the carpels (Figs. 4, 5, 67 and 68). In *Lippia nodiflora*, the two ovules grow on the two sides of the placental cushion in diametrically opposite directions (Figs. 4 and 5). In *Vitex negundo* the four ovules are borne on two such tissues in each ovary.

In *Lippia nodiflora*, the cells of the central portion of the style elongate and become free from each other at an early stage of development and give rise to large intercellular spaces. Later on these cells seem to degenerate and give rise to a large air space in this region.

II. *Development and structure of the glands.*—The floral envelopes and the bracts, especially the latter, are covered externally by unicellular and multicellular hairs and glands of various kinds.

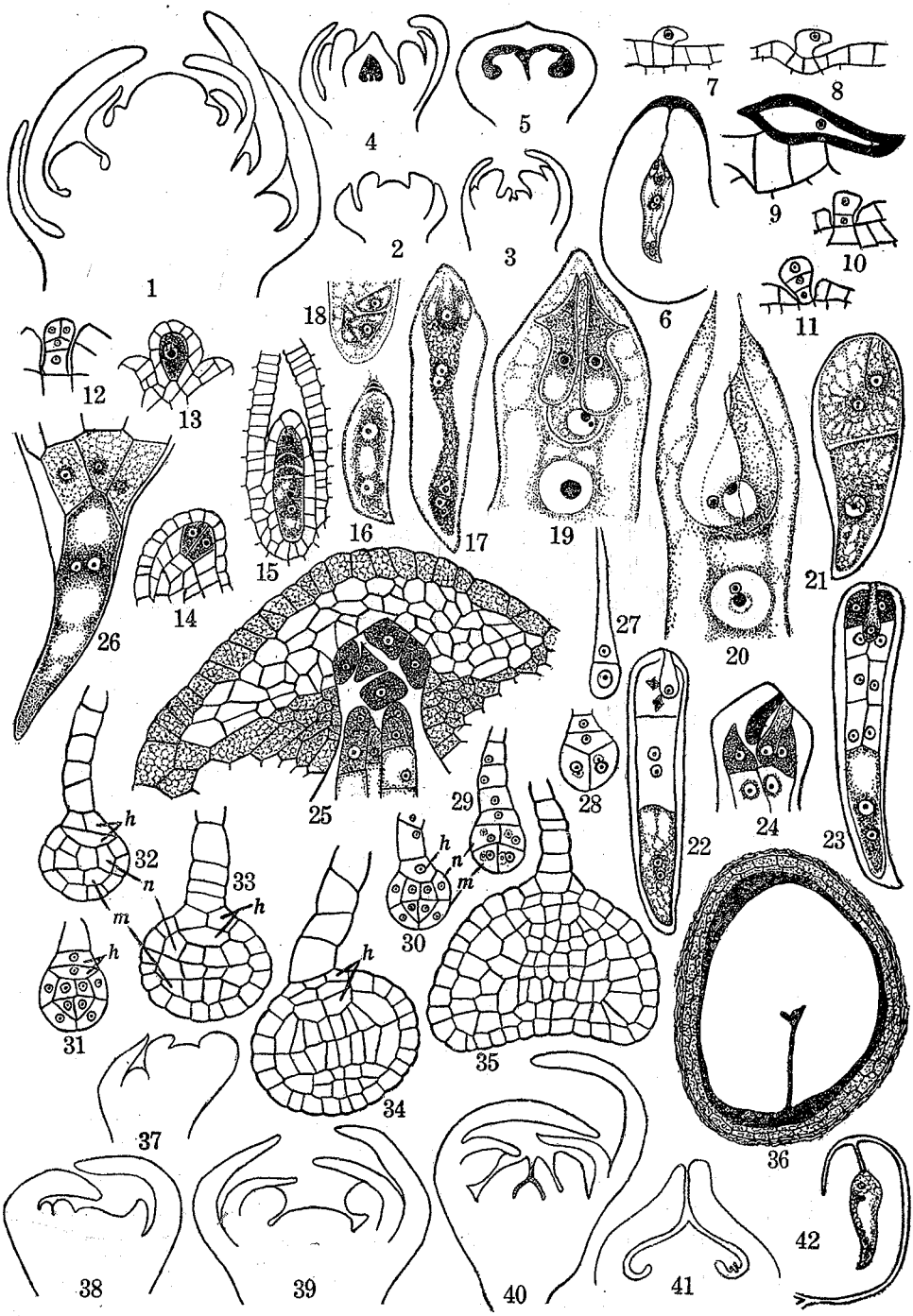
All the glands are derived from the epidermal cells of the organs. Most of them are globular and composed of four or more cells with a long or short stalk. In *Lippia nodiflora* the stalk is absent or composed of one cell only; in *Vitex negundo* it is always 1-celled, but in *Tectona grandis* it is mostly multicellular.

Generally an epidermal cell protrudes from the surface and divides by a transverse wall (Figs. 7, 43, 69 and 70). The outer cell divides again by a transverse wall (Figs. 44 and 71). The apical cell functions as the primary gland cell and the other as the primary stalk cell. The latter sometimes also takes part in the formation of the gland as has been observed on the petals of *Vitex negundo* (Fig. 72). The stalk may become 2–5-celled in *Tectona grandis* (Figs. 48–50). The apical cell next divides vertically twice in succession to form a globular structure (Figs. 45–47 and 72–74). In *Tectona grandis* the gland cells may undergo more transverse and longitudinal divisions to make the gland 2- or 3-tiered, each tier consisting of eight cells (Figs. 48, 50 and 51). In other cases the four cells of the apical tier may undergo only vertical divisions to make the tier 8-celled (Figs. 49). The upper cell of the stalk may also function as a gland cell (Figs. 45, 47 and 49). In *Lippia nodiflora* and sometimes in *Vitex negundo*, the primary gland cell undergoes first a transverse division and then the new apical cell alone divides twice by vertical walls at right angles to each other to form a 5-celled gland in 2-tiers (Figs. 10–12 and 75). The cell of the lower tier of the gland may also divide vertically to make a 8-celled gland in two tiers, as in *Vitex negundo* (Fig. 76).

Most of the glands in *Lippia nodiflora* are unicellular. The functional epidermal cell in this case, as it grows, curves and lies parallel to the surface of the epidermal cells. Ultimately it gives rise to a very large cell without cutting any stalk cell. The mature gland is broad at the centre and tapers at both ends, being acute at one end and acuminate at the other (Fig. 9).

The glands are filled up with a yellowish-red granular matter. The cells are all thick-walled and uni-nucleate, but in the later stages of development the nucleus becomes unrecognisable due to the thick precipitation of the cell contents. Thus the cells appear as a homogeneous deeply coloured mass.

III. *The ovule and integuments.*—The ovule initial arises as a small papillate protuberance from the placenta. It begins to curve by unilateral growth before the differentiation of the archesporial cell (Figs. 4 and 5). When the curvature is nearly 90 degrees, the primary archesporium differentiates. The integumentary initial appears still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FIGS. 1–42

FIGS. 1–36. *Lippia nodiflora.*—Fig. 1. Longitudinal section of an inflorescence. Figs. 2–6. Development of the floral organs and ovules. Figs. 7–12. Development and structure of the glands. Fig. 13. Differentiation of the hypodermal one-celled archesporium. Fig. 14. Section of the nucellus with a two-celled archesporium. Fig. 15. A linear tetrad showing functional chalazal and degenerating micropylar megaspores. Fig. 16. Two-nucleate embryo-sac. Fig. 17. Mature embryo-sac. Fig. 18. Organisation of the antipodals. Fig. 19. Micropylar end of the embryo-sac showing the secondary nucleus and hooked synergids. Fig. 20. Double fertilisation. Figs. 21–23. Development of the endosperm. Figs. 24–25. Development of the micropylar haustorium. Fig. 26. The chalazal haustorium and the connecting cells. Figs. 27–35. Stages in the development of the embryo. Fig. 36. Structure of the mature seed. Figs. 1–6, 36, ×60; Figs. 7–12, 21–23, ×240; Figs. 13–17, 24–35, ×350; Figs. 18–20, ×800.

FIGS. 37–42. *Tectona grandis.*—Development of the flower and ovule. (For further explanation see text.) Figs. 37–39, ×30; Fig. 40, ×20; Figs. 41–42, ×60.

later. Ultimately the ovule becomes anatropous by further curving (Figs. 5, 6, 41 and 42).

As characteristic of the Sympetalæ, the integument is one in number. Soon it becomes massive, completely covers the thin nucellus and forms a long micropyle (Figs. 6, 42 and 68). The complete enclosure of the nucellus by the integument takes place during the reduction division of the megaspore-mother cell. In *Lippia nodiflora*, the integument is composed of 6–8 layers of cells, in *Tectona grandis* of 7–8 layers, and in *Vitex negundo* it is 7–10-layered.

IV. *The tapetum.*—The innermost layer of cells of the single integument forms the tapetal jacket around the embryo-sac. The cells of this layer remain undivided and uninucleate, but become rectangular in shape and elongated in the radial direction (Figs. 15, 54 and 81). These cells are rich in cytoplasm and form a specialised part of the integument.

After the degeneration of the single layer of nucellar cells, the female gametophyte lies directly against the tapetal jacket. In *Lippia nodiflora* and *Tectona grandis* the innermost cells of the lower part of the integument do not organise as tapetal cells and so the chalazal end of the embryo-sac remains covered by the undifferentiated cells of the innermost layer of the integument, while in *Vitex negundo* the tapetum covers only the middle portion of the embryo-sac. Later, in the post-fertilisation stages these tapetal cells become disorganised due to the activity of the endosperm tissue.

V. *Development of the megaspores.*—The hypodermal archesporial cell originates before the appearance of the integumentary initial (Fig. 14). The nucellus is thin and composed of only one layer of cells around the archesporial cell (Figs. 13, 52 and 77). The number of archesporial cell is generally one, but in rare cases in *Lippia nodiflora* two archesporial cells have been found to occur side by side (Fig. 14).

The archesporial cell never cuts off a parietal cell, but functions directly as the megaspore-mother cell as seen in other plants of the family previously investigated. The megaspore-mother cell enlarges considerably, especially in the radial direction. Then it undergoes

reduction division and a linear tetrad of megaspores is formed (Figs. 15, 53 and 78). The chalazal megaspore functions to give rise to the *Normal type* of embryo-sac, while the other three megaspores degenerate (Figs. 15 and 78). In *Lippia nodiflora*, the micropylar megaspore is the last to degenerate (Fig. 15).

VI. *Development of the embryo-sac.*—The functioning megaspore enlarges and the nucleus situated at the middle of the cell divides. In *Vitex negundo* this division may take place at a very early stage, even before the degeneration of any one of the remaining three micropylar megaspores (Figs. 80). The daughter nuclei then move towards the two poles and there they divide twice in succession to form an 8-nucleate embryo-sac (Figs. 17, 56 and 81). Meanwhile the embryo-sac increases considerably in size and crushes the one-layered surrounding nucellar tissue and comes to lie directly against the tapetal jacket. The latter differentiates very early when the functional megaspore begins to grow in size (Figs. 15 and 54). The cells of the nucellar cap also degenerate when the embryo-sac is 1- or 2-nucleate (Fig. 54). In *Vitex negundo*, however, this occurs later. The rate of enlargement of the embryo-sac with respect to the various developmental stages varies in the three plants. A comparative account of the size of the embryo-sac at different stages is presented in the following table:

TABLE I

The comparative enlargement of the embryo-sac at different stages of development (size in microns)

Name of the plants	1-nucleate stage	2-nucleate stage	4-nucleate stage	8-nucleate stage	Mature embryo-sac
Lippia nodiflora Rich.	32	52	80	..	112
Tectona grandis Linn.	70	120	170	184	320
Vitex negundo, Linn.	27	..	..	212	..

VII. *Organisation of the embryo-sac.*—In *Lippia nodiflora*, the mature embryo-sac is somewhat fusiform in shape, being tapering at both the ends. The micropylar half, however, is broader than the chalazal (Fig. 17) one. The synergids are prominently hooked and possess prolonged acute beak-like tips as noted by Misra in *Clerodendron phlomidis* and *Caryopteris wallichiana* (Fig. 19). They are elongated and pear-shaped bodies with vacuoles at the chalazal end. The nucleus is small in size and situated above the vacuole. The egg is flask-shaped and shows a large vacuole towards its micropylar end (Fig. 17). Its nucleus is slightly larger than the nuclei of the synergids.

The two polar nuclei migrate towards the centre of the embryo-sac, come to lie side by side and finally fuse together near the middle of the embryo-sac long before fertilisation (Fig. 19).

The three antipodal cells are at first small in size and triangular or rectangular in shape (Figs. 17 and 18). They are arranged to form a pyramidal structure, two lying at the chalazal end and the other above them. Later, the antipodals, specially the chalazal two, increase in size and become elongated. After the complete organisation of the embryo-sac the antipodals begin to degenerate and before fertilisation the disorganisation is complete.

In *Tectona grandis*, the micropylar end of the embryo-sac is much broader than the chalazal half, which is very narrow (Fig. 57). The egg apparatus is much bigger than that in *Lippia nodiflora*, but its organisation is similar. The synergids are elongated and very big in size. They are neither hooked nor have beak-like apices, and the chalazal end is not smooth and oval as seen in *Lippia nodiflora*, but is very much acute (Fig. 57). A very big vacuole is present at the chalazal end and the small nucleus is situated over it. The egg is almost covered by them, only the chalazal end being visible. The nucleus of the egg is embedded in scanty cytoplasm and is slightly bigger than that of the synergids. A prominent vacuole is present at the micropylar end of the egg. The behaviour of the polar nuclei is similar to that of *Lippia nodiflora*. The secondary nucleus lies in the upper half of the embryo-sac (Fig. 57). The antipodal cells degenerate very early and it is difficult to trace them in the mature embryo-sac. Their arrangement in this species is similar to that of the previous one, but the form is different. The two chalazal cells are much more elongated, the nucleus lying at the micropylar end of the cells (Fig. 58). The upper one degenerates first.

An abnormal case in relation to the organisation of the embryo-sac was discovered where two of the four micropylar nuclei in an 8-nucleate embryo-sac were found fusing together to form the secondary nucleus. The four chalazal nuclei remained in their original position (Fig. 56).

In *Vitex negundo*, properly organised embryo-sac could not be obtained. Only in a single instance two of the four chalazal nuclei were found lying side by side about the middle of the embryo-sac. It seems from their position that they are about to fuse together. The outer two chalazal nuclei were lying close to each other near the chalazal end of the embryo-sac. The four nuclei of the embryo-sac at the micropylar end also lie very close to each other (Fig. 82).

Though the female gametophyte of the plant usually fails to grow to maturity, yet the ovaries of some of the flowers in an inflorescence enlarge. Microscopic preparations show the presence of some living organisms within these ovaries. They are seen developing in the various parts of the ovary, such as the ovule, in the ovarian cavity and the tissues of the ovary wall (Fig. 84). In one instance the somatic cells of the orgaism were found in divisional stages. The organisms appear to be the larvæ of some insect. As a result of this attack the tissues of the ovary become hypertrophied. Sections of these ovaries show the presence of a narrow canal leading to the exterior. From this it appears that the insect has deposited its eggs through this canal

and the larvæ develop by feeding on the ovarian tissues. The later stages, however, have not been observed. Figs. 83 and 84 give an idea of the comparative size of the normal and hypertrophied ovaries of the plant.

VIII. *Fertilisation.*—Stages of fertilisation have been observed in *Lippia nodiflora* only. At this time the secondary nucleus lies near the egg apparatus and the egg becomes enlarged. The pollen tube enters the embryo-sac by way of the micropyle and destroys one of the synergids during its penetration into the embryo-sac. The two male nuclei approach the egg and the secondary nucelus respectively, and double fertilisation occurs in a normal way (Fig. 20). The other synergid also degenerates soon after fertilisation. In *Tectona grandis*, however, the synergids persist for some time in the post-fertilisation stages.

IX. *Development of the endosperm and endosperm haustoria.*—Most of the embryo-sacs of *Tectona grandis* degenerate in the process of development, especially in the later and post-fertilisation stages (Fig. 60).

The sequence of development of the endosperm and chalazal haustorium in *Lippia nodiflora* and *Tectona grandis* is similar. The primary endosperm nucleus which lies near the fertilised egg divides and the accompanying development of a thin membranous transverse wall gives rise to a primary micropylar and a primary chalazal chamber (Figs. 21 and 60). Next the nucleus of the smaller chalazal chamber divides to form a 2-nucleate cell, which functions directly as the chalazal haustorium (Figs. 22, 23, 61 and 62). In no case a longitudinal or transverse wall was seen in this haustorial cell to separate the two nuclei. In most instances the cell remains binucleate all along and becomes very big and elongates in the direction of chalaza. It contains dense cytoplasm which takes a very deep stain. It is somewhat triangular in shape with its apex pointing towards the base of the ovule. The nuclei are situated centrally and a big vacuole appears in the basal part of the cell. Sometimes due to the division of one or both the nuclei the cell becomes 3- or 4-nucleate (Fig. 63). The nuclei, especially in *Tectona grandis*, often contain more than one nucleoli. When the haustorium reaches its full dimensions, its apex pierces into the conducting tissue of the ovule. The surrounding cells become rich in cytoplasm and begin to stain deeply, but those in direct contact with the apical part of the haustorium become somewhat empty due to the activity of the latter.

The nucleus of the micropylar chamber divides and a longitudinal wall is formed in between the two resulting nuclei (Fig. 61). Then, both the cells divide by successive transverse walls and ultimately five tiers of cells (including the chalazal haustorium) are formed (Figs. 23 and 62).

The development of the micropylar haustorium has been studied only in *Lippia nodiflora*, where it differentiates from the cells of the uppermost one of the five tiers. The cells are very rich in cytoplasm

and take a very deep stain (Figs. 23 and 24). They round up and ultimately get free from the cells of the next tier of endosperm cells. The cells of the next tier and of a few tiers below function similarly and behave as haustoria. Thus, ultimately, many of the endopserm cells take part in the formation of the micropylar haustorium. The cells are comparatively smaller than those composing the endosperm tissue and are uninulceate. They are very rich in cytoplasm and take a dark stain. Due to their activity, the cells of the integument at the micropylar end become empty, bounded only by the cell walls (Fig. 25). The epidermal and specialised tapetal cells, however, are not affected by the haustorium.

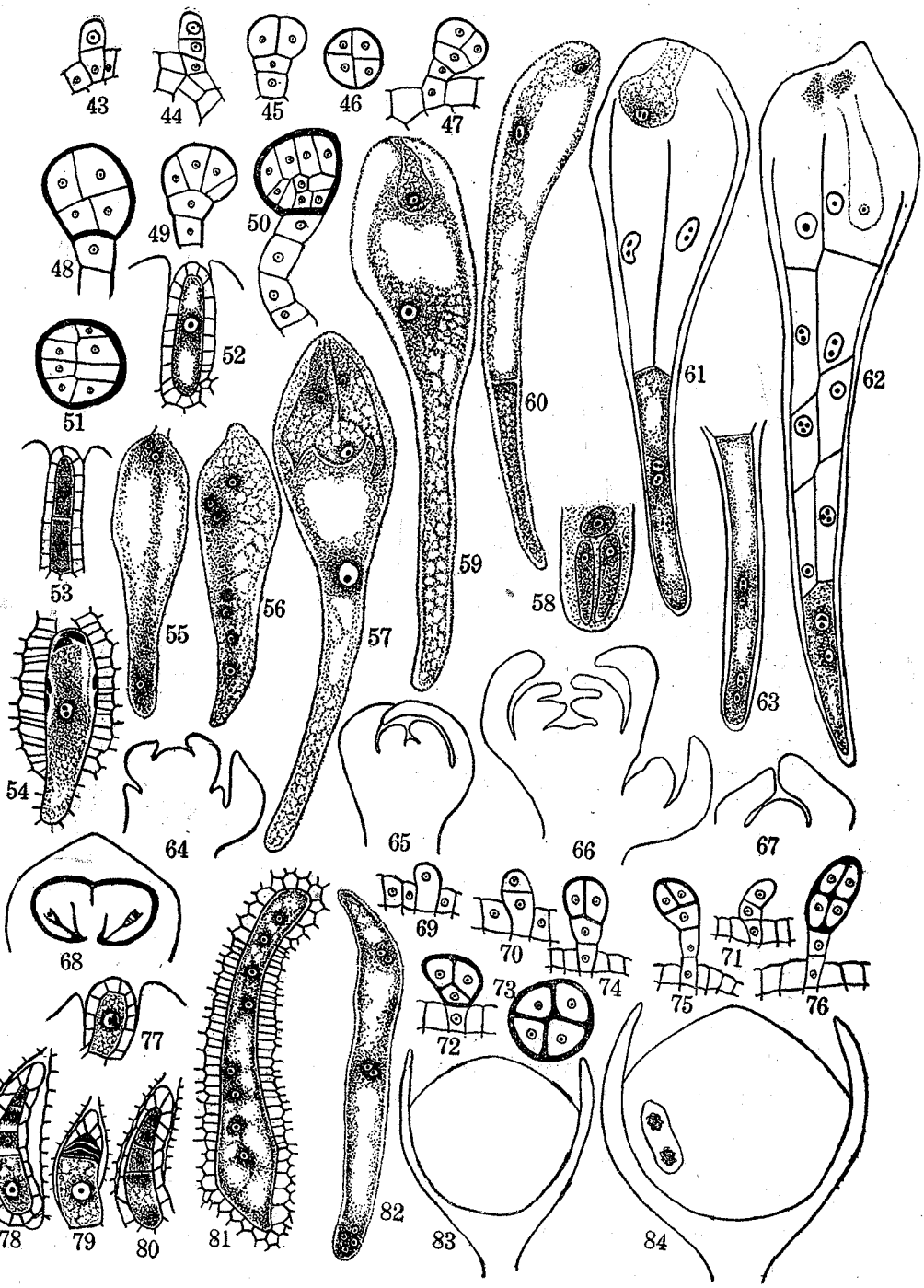
The tissue of the micropylar haustorium, as it grows, proceeds towards the micropylar end of the ovule and thus forms a short, narrow neck-like structure in between the endosperm tiers and the functional haustorial tissue. Such a development has also been found by Junell (1934) in *Amethystea cœrulea.*

The micropylar haustorium does not persist for a long time. As soon as the embryo begins to grow, these haustorial cells become functionless and degenerate. On the other hand, the chalazal haustorium persists for a considerable length of time and degenerates only when the endosperm has become quite massive and the embryo has grown considerably. It is interesting to note that neither the tapetal cells nor the epidermal cells of the ovule are destroyed by the activity of the micropylar hautsorium (Fig. 25).

The further development of the endosperm tissue may now be considered. The endosperm becomes massive within a short time. In the advanced stages the lowermost tier of the endosperm tissue next to the chalazal haustorium becomes active and its cells begin to stain more deeply than the other endosperm cells. These cells are smaller and have presumably a conducting function (Fig. 26). This has also been found in *Lantana indica* by Tatachar (1940).

As the endosperm tissue develops, it fills the cavity of the embryo-sac. In the later stages when both the haustoria are disorganised, the two outermost layers of endosperm cells become very active. The cells are smaller than the rest, but are very rich in ctyoplasm. They draw their nutrition from the surrounding integumental tissue to nourish the developing embryo. Consequently, the surrounding cells become empty, leaving only the thickened cell walls, which later on become compressed. These two layers persist in the mature seed. At this stage the cells are very rich in starch grains and take deep stain (Fig. 36). The presence of a layer of endosperm cells surrounding the embryo in the mature seed has also been observed in the Scrophulariaceæ by Srinivasan (1940).

X. *Development of the embryo and seed.*—The development of the embryo has only been studied in *Lippia nodiflora.* The fertilised egg rests for a considerable time before development. In fact it divides for the first time when a fair amount of endosperm tissue is formed and the micropylar and chalazal haustoria are organised. It elongates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FIGS. 43-84

FIGS. 43–63. *Tectona grandis*.—Figs. 43–51. Development and structure of the glands. Figs. 46 and 51 show transverse sections, while the rest represent longitudinal sections. Fig. 52. Megaspore-mother cell. Fig. 53. Homotypic division of the megaspore-mother cell. Figs. 54–56. Development of the embryo-sac. Fig. 57. Mature embryo-sac. Fig. 58. Organisation of the antipodals. Fig. 59. The fertilised egg and the primary endosperm nucleus. Fig. 60. Degenerating embryo-sac with two-celled endosperm. Figs. 61–62. Development of endosperm and endosperm-haustorium. Fig. 63. A four-nucleate chalazal haustorium. Figs. 43–54, 63, ×350; Figs. 55–57, 59–62, ×235; Fig. 58, ×800.

FIGS. 64–84. *Vitex nigundo*.—Figs. 64–68. Development of the floral organs and ovules. Figs. 69–76. Development and structure of the glands. (Fig. 73 shows a transverse section). Fig. 77. First division prophase in megaspore-mother cell. Figs. 78–80. Linear tetrads. Fig. 81. Eight-nucleate embryo-sac. Fig. 82. An abnormal embryo-sac. Figs. 83–84. Comparative size of the affected and unaffected ovaries. Figs. 69–80, ×350; Figs. 81–82, ×235; Figs. 64–65 and 67, ×60; Figs. 66 and 68, ×45; Figs. 83–84, ×20. For further explanation, see text.

considerably before divison forming a tubular structure with the nucelus at its apex. It penetrates through the endosperm cells in the micropylar region and becomes deeply embedded in the endosperm tissue (Fig. 23). The same behaviour of the growing oospore has also been recorded in *Lantana indica* and *Stachytarpheta indica* (Tatachar, 1940) and other Verbenaceous plants (Schnarf, 1931) and in the related families Labiatæ and Scrophulariaceæ (Ganguli, 1948; Iyenger, 1939, 1940 *a*, 1940 *b*, 1940 *c*, 1941; and Srinivasan, 1940).

Even after the intrusion of the elongating oospore into the endosperm tissue, the latter does not divide at once, but penetrates still deeper. The first division is by a transverse wall (Fig. 27). Further transverse walls appear in the daughter cells and thus a three to six cells long proembryo is formed. This type of development of the proembryo conforms to Johansen's "Solanad type". Next, the apical cell of the proembryo divides vertically twice in succession and a quadrant is formed (Fig. 28). Unlike *Leonurus sibiricus* and *Anisomeles indica* (Ganguli, 1948), the next divisions are transverse in all the four cells of the quadrant and thus a 2-tiered octant is derived (Fig. 29). This type of development has been observed previously also in *Lantana indica* and *Stachytarpheta indica* (Tatachar, 1940). The two quadrant tiers of the octant may be designated as *m* and *n*; *m* being the apical one. Next both the tiers divide periclinally and thus the dermatogen layer is differentiated simultaneously in both the tiers (Figs. 30 and 31).

The differentiation of the other histogenic layers now begins. The dermatogen cells divide further only by the formation of the anticlinal walls. The next divisions in the axial cells of the apical tier (*m*) are longitudinal and those in the other tier (*n*), are both transverse and longitudinal (Figs. 32 and 33). Thus a layer of periblem cells differentiates in between the dermatogen layer and the innermost two rows of cells, *i.e.*, the plerome. Simultaneously the tier '*n*' becomes 2-tiered. The periblem cells next divide by anticlinal and periclinal walls. The plerome cells also divide in both directions to form the central tissue (Fig. 34).

The cells of the tier *m* divide actively and give rise to the stem tip and the two cotylendons in the mature embryo. The stem tip appears

at a later stage of development of the embryo. It is very small and originates from the central region of the apical tier *m*. The cotyledonary initials arise from the peripheral region of the apical tiers and grow very vigorously to give rise to the two big cotyledons. The penultimate tier also grows actively and the hypocotyl and radicle of the embryo are derived from these cells.

The development of the hypophysis may now be considered. The penultimate cell of the proembryo functions directly as the hypophysis (*p*). It divides transversely after the differentiation of the dermatogen layer, in the tiers derived from the apical cell of the proembryo (Fig. 31). Further divisions in these two cells take place by successive vertical walls at right angles to each other and thus two quadrant tiers are formed (Figs. 32 and 33). The upper tier completes the periblem of the root apex, while the lower one divides again tangentially (Figs. 34 and 35). The upper cells derived from these tangential divisions add to the dermatogen of the root apex, while the lowermost cells form the root cap. Thus the hypophysial cell also contributes considerably to the embryo proper as has been found in some Labiatæ (Ganguli, 1948).

The suspensor is composed of about 6 cells. In the later stages of development these cells gradually become inconspicuous and finally degenerate after the development of the cotyledons. Their main function is to push the embryonal mass deep into the endosperm tissue. The suspensor is always uniseriate and never becomes massive.

The mature embryo is globular in form. The cotyledons are not very long, but they are comparatively thick. The cells of the mature embryo are full of starch grains (Fig. 36).

The mature seed is surrounded by two layers of endosperm cells as mentioned previously. These endosperm cells are very rich in starch grains. The cells of the integument become empty in the course of development of the seed, leaving only the cell walls which persist in mature seed. These become inconspicous and compressed at maturity due to the pressure of the inner growing tissues (Fig. 36).

Discussion

As indicated previously, the development of the different floral whorls takes place in the following sequence: sepals, stamens, petals and carpels. It should be noted, however, that the same primordium gives rise to the stamen and petal, of which the former differentiates earlier. Similar observations have been made by Misra (1939) working on *Stachytarpheta indica*, Ganguli (1948) in *Leonurus sibiricus* and *Anisomeles indica* and Iyengar (1940 *c*) in *Sopubia trifida*. But Kanda (1920) had described a different sequence of floral development in Verbenaceæ. He states that the stamens are the first to appear on the floral primoridum and are followed by sepals, petals and carpels successively. Considering all other investigations, Kanda's observations appear to be very doubtful.

A single hypodermal archesporial cell directly functions as the megaspore-mother cell, as is characteristic of the Bicarpellatæ. In

one instance in *Lippia nodiflora*, however, two archesporial cells have been observed. Junell (1934) in *Pityrodia bartlingii* and Patermann (1935) in *Premna integrifolia* have also recorded two archesporial cells.

Junell (1934) observed a tendency of more than one megaspore to develop further in *Lantana camara* and *L. involucrata* and in *Bouchea incrassata* and *Patræa volubilis*. *Citharexylum ilicifolium* is another exceptional case where more than one megaspore develop (Patermann, 1935). In *Avicennia officinalis* it has been observed that though only one of the four megaspores is functional, the remaining three do not degenerate, but persist till fertilisation (Patermann, 1935). In the present investigation it has been found that the chalazal megaspore alone functions, and the embryo-sac shows a normal type of development as found in all other plants of this family. Only in *Avicennia officinalis*, Karsten (1891) reports an "Allium type" of development, but Maheshwari (1937) doubts the validity of the statement and thinks that this requires re-investigation.

The synergids are hooked and with beak-like apex in *Lippia nodiflora* as found by Misra (1939) in *Clerondendron phlomidis* and *Caryopteris wallichiana* and by Junell (1934) in several other plants.

According to Schnarf (1925) the polar nuclei fuse long before fertilisation and the secondary nucleus lies in the middle of the embryo-sac, but Patermann (1935) observes that they fuse together near the egg cell only when the pollen tube has entered the embryo-sac. The present observations support the statement of Schnarf (1925).

Junell (1934) noted that the antipodals of this family frequently divide and thus their number increases. Misra (1937) also found in *Clerodendron phlomidis* that the three antipodals multiply to form about 20 cells; Tatachar (1940) reported that the nuclei of the antipodals of *Lantana indica* undergo repeated mitotic divisions and ultimately each antipodal cell becomes 3-6-nucleate. Later, they fuse together, become elongated and vacuolated, and function as a chalazal haustorium. On the other hand, Patermann (1935) found in the species of Verbenaceæ he studied, small and quickly degenerating antipodals arranged together in the form of a pyramid. Similar results have been obtained in course of the present investigation in the plants studied. Thus it appears that the divisions of the antipodal cells to form a cell complex is not a characteristic feature of Verbenaceæ.

The endosperm is of the Cellular type as seen in other members of the family by previous workers. Kanda's statement regarding the development of Nuclear type of endosperm in *Verbena anguistifolia* has been disputed by Schwencke (1931) and Schnarf (1925). In the related family Labiatæ (Ganguli, 1948) also, the cellular type of endosperm prevails.

Development of endosperm-haustoria appears to be a characteristic feature of the family Verbenaceæ as it has been found in almost all the species studied so far. Generally the haustorium develops at the chalazal end of the embryo-sac, but in some species it may be present

at both the ends. Variation regarding the structure and development of the haustoria in various members of the family has been observed.

In *Verbena officinalis* (Schwencke, 1931), a binucleate chalazal haustorium alone is present. Such a binucleate chalazal haustorium has also been found in *Verbena anguistifolia*, *Lantana trifida* and *Avicennia officinalis* (Patermann, 1935). This haustorium is developed directly from the lower compartment formed after the first transverse division of the primary endosperm nucleus. This type of development of the chalazal haustorium has also been found in some Labiatæ (Ganguli, 1948) and Scrophulariaceæ (Iyenger, 1939, 1940 *a*, 1940 *b*, 1940 *c* and 1941).

The second type of haustorial development occurs at both ends of the embryo-sac. In *Verbena canadensis* and *Canadea aubletia* (Patermann, 1935), the endosprerm haustoria at both ends are binucleate. In *Canadea aubletia*, a cell complex at the chalazal end is seen surrounding the lower portion of the haustorium.

The micropylar endosperm-haustorium of *Lantana indica* (Tatachar, 1940) is simple and composed of two uni-nucleate cells. The chalazal haustorium of this plant is not derived from the endosperm, but from the antipodal cells. The multi-nucleate antipodal cells fuse together to form the prominent haustorium. In *Duranta plumieri* (Patermann, 1935), the chalazal haustorium is 4-nucleate, but the micropylar one is 2-nuclete.

Tatachar (1940) has described the chalazal haustorium of *Stachytarpheta indica* as unicellular and binucleate, but according to Patermann 1935) the haustorium of this plant and also that of *Stachytarpheta cayennensis* is definitely 4-nucleate. On the other hand, Patermann (1935) described the micropylar haustorium in these two plants as a binucleate cell. According to Tatachar (1940) it is composed of 4-uninucleate cells in *Stachytarpheta indica*. Junell (1934) also reported a 4-celled micropylar haustorium in *Stachytarpheta dichotoma* and a multi-cellular haustorium in *S. anguistifolia*. Thus it appears that there is considerable variation in the development of the haustorium in the same species developing under different conditions.

In the present investigation the chalazal haustorium is found to be binucleate and it is derived from the primary chalazal cell resulting from the first division of the primary endosperm nucleus. The structure, function and mode of development of this haustorium is like that of *Verbena officinalis* as summarised by Patermann (1935). In *Lippia*, Junell (1944) records the occurrence of an unicellular 4-nucleate chalazal haustorium, but the present investigation shows that the haustorial cell is 2-nucleate, though in rare cases a 3- or 4-nucleate chalazal haustorium is observed. Koorders (1896) and Junell (1934) both worked on *Tectona grandis*. It is interesting to note, however, that none of them mentioned the presence of a chalazal haustorium in the plant, which the present investigation has definitely demonstrated to be present, the development of the haustorium following the same course as in *Lippia nodiflora*. Koorders (1896) and Junell (1934) have, however,

described a feebly developed micropylar haustorium in *Tectona grandis*. This point could not be verified on account of the difficulty of sectioning the material.

SUMMARY

The present investigation deals with the development of the flower and female gametophyte in *Lippia nodiflora*, *Vitex negundo* and *Tectona grandis*, development of endosperm and endosperm-haustoria in *Lippia nodiflora* and *Tectona grandis*, and development of embryo in *Lippia nodiflora*.

The sequence of floral development is sepals, stamens, petals and carpels. The development of the various kinds of glands on the floral envelopes has been studied.

The ovule has a single integument and a thin nucellus. There is single hypodermal archesporial cell which directly functions as the megaspore-mother cell. In *Lippia nodiflora*, a 2-celled archesporium occurs rarely.

The development of the embryo-sac is of the normal type. The synergids of *Lippia nodiflora* are hooked and possess a beak-like apex; those of *Tectona grandis* are acute at the base. The antipodals are small and ephemeral.

Endosperm is of the Cellular type. The chalazal endosperm haustorium in both the plants is unicellular, 2-nucleate and develops directly from the chalazal chamber after the first division of the primary cell. The micropylar endosperm haustorium in *Lippia nodiflora* is multicellular.

The suspensor pushes the embryonal cell deep into the endosperm. The embryo is of the "Solanad Type". It grows only after the development of the endosperm and endosperm haustoria. The mature embryo is surrounded by two layers of endosperm cells.

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