# EMBRYOLOGICAL STUDIES IN MENISPERMACEAE

## I. *Tiliacora racemosa* Coleb.

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### Introduction

Menispermaceae, a large family comprising 65 genera and 350 species (Willis, 1948), distributed throughout the warmer parts of the world, is one of the embryologically little-investigated families of Ranales. Hooker (1897) divided the family into four tribes: Tinosporeae, Cocculeae, Cissampelideae and Pachygoneae. Our knowledge of the embryology of the family is limited to an account of the development of the pollen and embryo sac in *Cocculus villosus* DC and *Tinospora cordifolia* Miers. (Joshi and Rao, 1935; Joshi, 1937, 1939) belonging to the tribes Cocculeae and Tinosporeae respectively. The anther tapetum in these plants is of secretory type and of sporogenous origin according to Joshi and Rao (1935). The ovule in *Cocculus* is bitegmic but in *Tinospora* it is unitegmic and Joshi (1939) considers that it represents two integuments. A nucellar cap is developed at the micropylar end owing to periclinal divisions of the nucellar epidermis. The embryo sac conforms to the Polygonum type. There is practically no information on fertilization, endosperm, embryo and seed development in any member of the family.

The present paper deals with the embryology of *Tiliacora racemosa* Coleb., a woody climbing shrub with glabrous leaves, small yellow flowers and reddish fruits, belonging to the tribe Cocculeae. The plant occurs throughout tropical India.

### Material and Methods

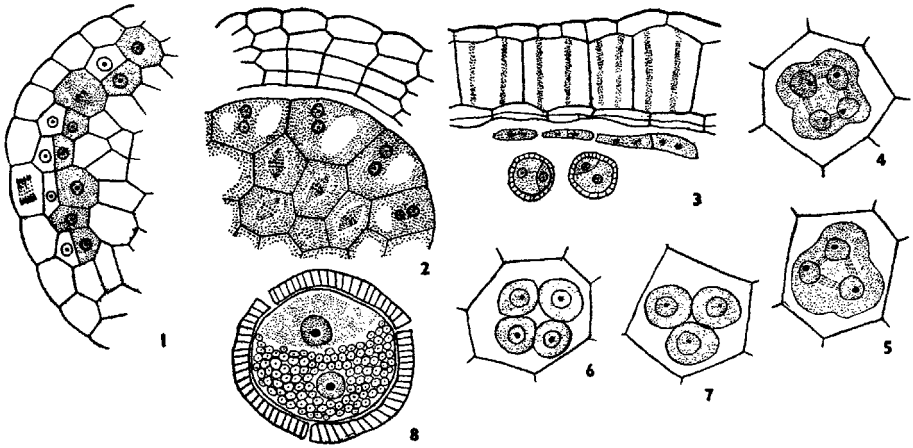
The material was collected at Yanam and Waltair and fixed in formalin-acetic-alcohol. Customary methods of dehydration and embedding were employed. Sections were cut from 6–10 $\mu$ in thickness and stained in Delafield's haematoxylin and safranin and fast green.

### Microsporogenesis

The anthers are tetralocular. The primary archesporium consists of a row of hypodermal cells. A periclinal division gives rise to an outer layer of primary parietal cells and an inner layer of primary sporogenous cells (Fig. 1). The wall of the fully developed anther is five-layered, including the epidermis. The innermost layer is the tapetum which is of the secretory type. Each cell of the tapetum is two nucleate (Fig. 2). The tapetum is biseriate at certain places. The subepidermal wall layer develops into the fibrous endothecium which is organized only after the pollen grains are fully formed (Fig. 3). The middle layers become crushed and gradually degenerate in the mature anther. The tapetum is also absorbed as the pollen grains mature.

The primary sporogenous cells undergo mitotic divisions and form a small mass of spore mother cells. All the spore mother cells undergo meiotic divisions and give rise to microspore tetrads. No degeneration of spore mother cells such as

recorded by Joshi and Rao (1935) in *Cocculus* and *Tinospora* has been observed. Divisions of the pollen mother cells are of the simultaneous type and cytokinesis takes place by furrowing (Figs. 4 and 5), as in *Cocculus* and *Tinospora*. During the division of the spore mother cells the cytoplasm shrinks from the walls resulting in the formation of a space in the peripheral region of the mother cell (Figs. 4 and 5). The tetrads are tetrahedral or isobilateral (Figs. 6 and 7). The pollen grains are shed at the two-celled stage (Fig. 8). The mature pollen grains are filled with starch grains. The exine shows three germ pores and bar-like thickenings which give a reticulate appearance in the surface view of the pollen grain. The mature pollen grains are small in size and measure about 10 $\mu$ in diameter.



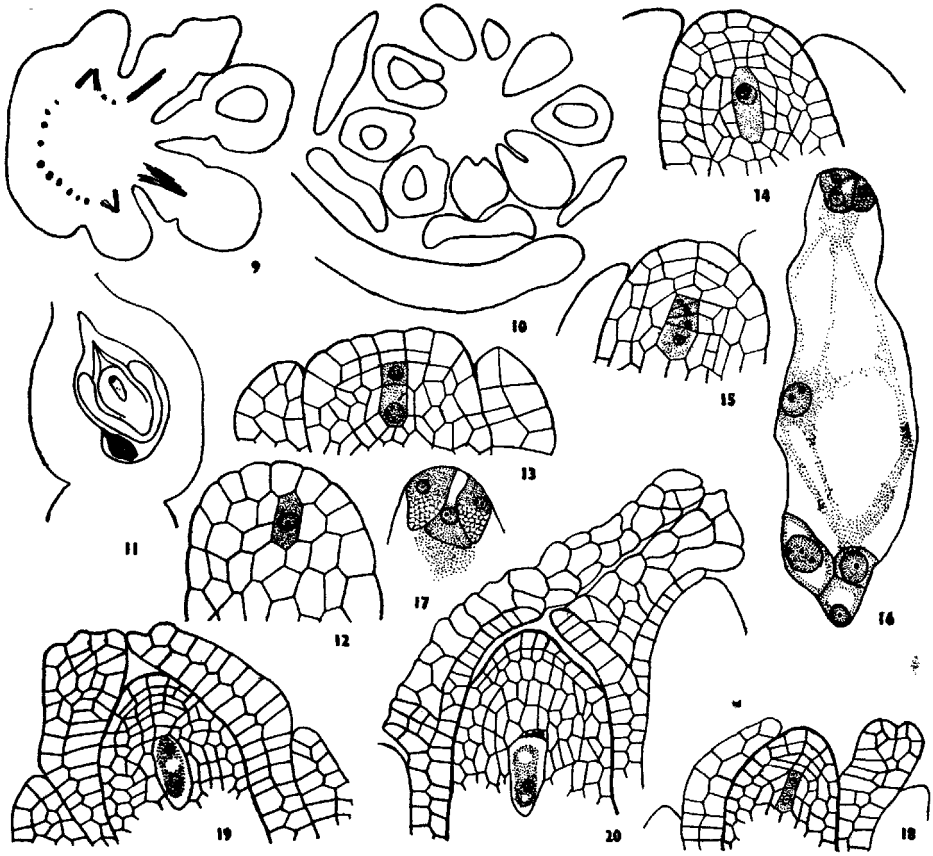

TEXT-FIG. 1

Figs. 1–8: Fig. 1. L.s. portion of anther lobe showing primary archesporium. Most of the cells have divided periclinally, cuting off the parietal cells (×582). Fig. 2. T.s. portion of anther lobe showing divisions in the spore mother cells (×872). Fig. 3. T.s. mature anther wall showing fibrous endothecium and remnants of the middle layers and tapetum (×582). Figs. 4 and 5. Division in the pollen mother cells showing furrowing (×872). Figs. 6 and 7. Microspore tetrads (×872). Fig. 8. Mature two-celled pollen grain (×1424).

## THE OVULE

The gynaecium usually consists of nine free carpels arranged spirally on the receptacle. The spiral arrangement is brought out in serial microtome sections of a flower (Figs. 9 and 10) in which the different carpels are seen to arise at different levels from the receptacle. Each carpel has two ovules borne on a marginal placenta. In *Cocculus* there are only four carpels of which one degenerates early. In *Tinospora* there may rarely be up to six carpels of which one degenerates early. Joshi (1937) is of the opinion that the gynaecium of Menispermaceae might have arisen from an ancestral condition with numerous spirally arranged carpels. On the basis of this view the gynaecium of *Tiliacora* seems to be less evolved than that of *Cocculus* and *Tinospora*. In *Tiliacora*, as in *Cocculus* and *Tinospora* (Joshi, 1937 and 1939), only the upper ovule reaches maturity while the lower one degenerates early (Fig. 11). Usually this happens at the megaspore mother cell stage but very rarely development may proceed to the eight nucleate embryo sac stage. In the degenerating ovule the integuments develop rather feebly although the nucellus may become massive. In the course of development, the upper ovule becomes curved upwards and the lower downwards (Fig. 11), as in *Cocculus* (Joshi, 1937). The two ovules continue to grow straight in the early stages till they touch the carpel walls. Joshi

expressed the view that due to the pressure exerted by the opposing carpel walls at this stage the ovules change the direction of their growth and the upper becomes bent upwards and the lower downwards. The fact that the ovules become flattened at this stage furnishes a proof for this view. A similar condition is also seen in *Tiliacora* as suggested by the flattening of the ovule. The ovule is bitegmic and crassinucellate. It is curved but the curvature does not affect the embryo sac till the eight nucleate embryo sac stage. After fertilization, however, the curvature extends also to the embryo sac. Thus according to Maheshwari's (1950) definition, the ovule is campylotropous in the early stages and becomes amphitropous after




TEXT-FIG. II

Figs. 9–20: Figs. 9 and 10. T.s. flower showing serial microtome sections of gynaecium to illustrate the spiral arrangement of carpels (×25). Figs. 11. L.s. carpel showing upper functional ovule and lower degenerated ovule (×35). Fig. 12. L.s. ovule primordium showing single celled archesporium (×485). Fig. 13. L.s. ovule showing parietal cell cut off by the archesporial cell and periclinally divided nucellar epidermis (×339). Fig. 14. L.s. nucellus showing megaspore mother cell (×291). Fig. 15. L.s. nucellus showing linear tetrad of megaspores (×339). Fig. 16. A fully developed embryo sac; one of the antipodal nuclei has 3 nucleoli (×291). Fig. 17. Egg-apparatus showing synergids devoid of hooks (×485). Fig. 18. L.s. ovule showing growth of the inner integument in the micropylar region and first division of the megaspore mother cell (×215). Fig. 19. L.s. ovule showing growth of the inner integument in the micropylar region and two nucleate embryo sac ×215). Fig. 20. L.s. ovule showing advanced stage in the outgrowth of the inner integument and functional megaspore (×50).

fertilization. The micropyle is straight. The inner integument is two cells thick to start with and the outer several cells thick. A little later in the development of the ovule the inner integument becomes many cells thick at the micropylar region (Figs. 18, 19, 20 and 21), as will be described later. There is a small air space between the inner integument and the nucellus in the micropylar region (Figs. 19, 20 and 21). In the early stages the integuments are closely pressed against each other and also against the nucellus. In *Cocculus* both the integuments are two to three cells thick and in the mature ovule the inner becomes many layered in the micropylar region. There is an air space between the inner integument and the nucellus in the micropylar region. In *Tinospora* the single integument is as thick as both the integuments of *Cocculus* put together. The closely appressed condition of the integuments of *Tiliacora* lends support to Joshi's (1939) view that the single integument of *Tinospora* might have arisen from a two-integument condition.

## Megasporogenesis and Embryo Sac

The single hypodermal archesporial cell makes its appearance before the integuments are differentiated in the young ovule (Fig. 12). It undergoes a periclinal division to form an outer primary parietal cell and an inner megaspore mother cell (Fig. 13). The primary parietal cell, by a series of anticlinal and periclinal divisions, gives rise to 3-4 layers of parietal tissue. The nucellar epidermis undergoes a periclinal division and becomes two-layered in the apical region (Fig. 13). In *Cocculus* the nucellus has an epidermal cap of 2–4 layers thickness (Joshi, 1937). In *Tiliacora* the megaspore mother cell becomes elongated and its nucleus lies towards the micropylar end (Fig. 14). It then undergoes two meiotic divisions and gives rise to a linear tetrad (Fig. 15). The chalazal megaspore functions, and forms an embryo sac of the Polygonum type (Fig. 16), while the rest of the three megaspores degenerate (Fig. 20). The egg apparatus is small relative to the size of the embryo sac. The antipodals are ephemeral and some show two to three nucleoli (Fig. 16). In *Cocculus* and *Tinospora* the antipodals are persistent and the synergids have small hooks; in *Tinospora* the synergids may sometimes show an egg-like appearance. In *Tiliacora* the nuclei in the synergids are towards the micropylar end and numerous small vacuoles are found at the lower end; hooks are absent (Fig. 17). In the egg the nucleus lies towards the lower end while there is a large vacuole at the micropylar end. The two polar nuclei fuse near the middle of the embryo sac. In the full grown ovule the parietal tissue is composed of five to six layers of which the outer two are epidermal derivatives.

## Fertilization

The short style is solid and is provided with transmitting tissue consisting of two layers of richly protoplasmic cells formed by the inner epidermis of the adjoining carpellary margins. The pollen grains germinate on the stigmatic surface which is situated on one side of the style. The pollen tube makes its way through the transmitting tissue in an intercellular manner. Fertilization is porogamous.

As mentioned before the inner integument is at first only two-layered. Some of its cells in the micropylar region begin to divide in various planes by the time the megaspore mother cell undergoes the first division (Fig. 18). The divisions continue to take place (Figs. 19 and 20), until about the time of fertilization, and result in the formation of a filamentous structure which projects towards the base of the style (Fig. 21). The divisions of the integumentary cells do not take place at the same rate in all the ovules. In one ovule the protrusion of the integument was already far advanced at the uninucleate embryo sac stage (Fig. 20) while in another ovule it was still in the initial stages even at the two nucleate embryo sac

stage (Fig. 19). Its cells are flared up, irregular in shape and filled with rich protoplasmic contents. It is suggested that this protrusion of the inner integument serves as an obturator although the passage of the pollen tube through this structure has not been observed.

Several kinds of obturators differing in their morphology have been described in plants belonging to diverse families. They include outgrowths from the placenta, from the base of the stylar canal or even from the integument (Maheshwari, 1950). Fagerlind (1944) described in *Myriocarpa* and *Leukosyke* the development of an obturator from the inner integument. The structure described in *Tiliacora* resembles the integumentary obturator of *Myriocarpa* although it is not so extensive. To the writer's knowledge the presence of such a structure has not so far been reported in any member of the Ranales.

Triple fusion takes place much earlier than syngamy. The secondary endosperm nucleus is large in size with a big nucleolus.

## Endosperm

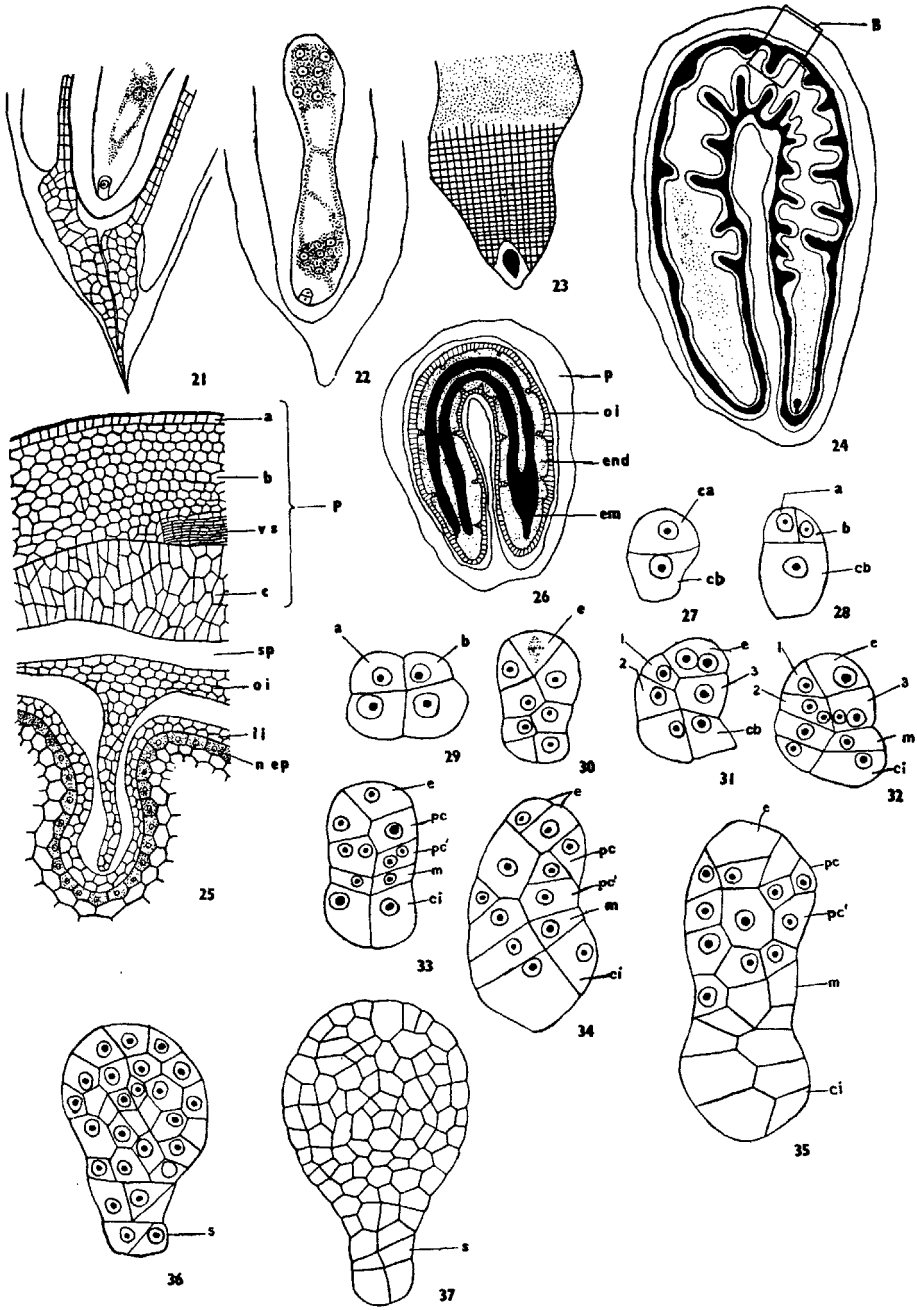
The endosperm primordium divides earlier than the fertilized egg. By the time the zygote undergoes the first division there are already 10–12 endosperm nuclei in the embryo sac (Fig. 22). The endosperm remains nuclear till a late stage. Some of the endosperm nuclei show 2-3 nucleoli. At first the endosperm nuclei lie along the periphery of the embryo sac leaving a central vacuole but as they increase in number they occupy the entire space of the embryo sac. Wall formation commences at the micropylar end at about the time the embryo becomes globular (Fig. 23). In certain members of the related families like Magnoliaceae and Anonaceae (Earle, 1938; Corner, 1949) the endosperm grows enormously and becomes cellular even by the time the fertilized egg completes the first division. In *Tiliacora* the endosperm gradually grows at the expense of the nucellus and ultimately consumes it. Prior to the absorption of the nucellus and during the period of growth of the endosperm the cells of the nucellar epidermis become glandular. In the Anonaceae Corner (1949) observed that the cells of this layer are filled with oil globules. These are not observed in *Tiliacora*.

At about the time the proembryo is 3 or 4 celled the outer integument begins to give out small protuberances towards the inside. The protuberances gradually elongate and extend from the periphery towards the middle of the nucellus. The inner integument is seen as a thin membrane surrounding the nucellus. The infoldings of the outer integument are plate like in form and are arranged in a transverse manner, more or less parallel to one another. They become deeper and deeper (Fig. 24) and render the nucellus ruminate. As the infoldings of the outer integument elongate the inner integument is pushed to the inside and stretched up. It then gradually degenerates and its remnants can be seen till a late stage. In the later stages the nucellus is replaced by the expanding endosperm. In the different genera of Anonaceae the ruminations are variously formed. In some both the integuments may take part or in others only the outer or the inner (Corner, 1949). In *Degeneria*, a member of Degeneriaceae, ruminations which are not so well developed, are formed by the outer integument (Swamy, 1949) while in Myristicaceae (Mauritzon, 1939) it is the inner integument which gives rise to the ruminations.

It will thus be seen that in *Tiliacora* as in members of Anonaceae (Corner, 1949), the ruminations are initially a feature of the nucellus and it is only later that the endosperm grows into the nucellus and gradually replaces it.

## Embryo

The interval between fertilization and the first division of the zygote is very short as compared with the enormous postponement of embryo development in




TEXT-FIG. III

Figs. 21–37: Fig. 21. L.s. micropylar region of the ovule showing fully formed outgrowth of the inner integument, fertilized egg and endosperm primordium (×104). Fig. 22. L.s. ovule showing a few endosperm nuclei and two-celled proembryo (×30). Fig. 23. Cell wall formation in the endosperm and globular embryo (×24). Fig. 24. L.s. fruit showing ruminated nucellus and endosperm (×18). Fig. 25. Sector marked 'B' in Fig. 24 showing:

other families of Ranales like Anonaceae and Magnoliaceae. The first division of the zygote takes place six weeks after pollination in *Magnolia grandiflora* (Earle, 1938). In several of the Anonaceae, the zygote divides several weeks after fertilization and by that time the seed is full grown (Corner, 1949). In *Tiliacora*, however, the first division of the zygote takes place soon after fertilization.

The first division of the fertilized egg is transverse resulting in the formation of the apical cell *ca* and the basal cell *cb* (Fig. 27). The apical cell divides by a vertical wall into two daughter cells of unequal size, *a* and *b* (Fig. 28). This is followed by an oblique-vertical division of the basal cell resulting in two juxtaposed cells (Fig. 29). The four-celled proembryo thus consists of two superposed tiers of two cells each.

The bigger of the daughter cells, *a*, divides by an oblique wall and gives rise to a triangular cell *e* which functions as the epiphyseal initial (Figs. 30, 31 and 32) and another daughter cell numbered 3 in Figs. 31 and 32. The second daughter cell functions as a subepiphyseal cell. The smaller daughter cell *b* derived from *ca* divides by an obliquely vertical wall and gives rise to two daughter cells numbered 1 and 2 in Figs. 31 and 32. These two also function as subepiphyseal cells. Thus as a result of 3 divisions in *ca*, there are formed 4 daughter cells of which one functions as the epiphyseal initial and the rest as subepiphyseal cells. The epiphyseal initial divides transversely (Figs. 30 and 35) giving rise to two superposed cells the derivatives of which form the stem apex.

The subepiphyseal cells divide transversely (Figs. 32 and 33) and form two superposed tiers, *pc* and *pc'*. Further divisions in these tiers are irregular (Figs. 34 and 35). Tier *pc* contributes to the central cylinder of the stem and the cotyledons, while *pc'* gives rise to the hypocotyledonary region.

The two daughter cells of the basal cell undergo transverse division resulting in the formation of two superposed tiers of cells, the upper *m* and the lower *ci* (Figs. 30, 32 and 33). Further divisions in the tier *m* are rather irregular (Figs. 34, 35 and 36) and contribute to the formation of the hypophyseal region. The lower tier *ci* gives rise to the suspensor. The suspensor is usually short consisting of two tiers of two cells each (Fig. 37). Occasionally the cells of the suspensor may enlarge in size to give rise to a foot-like structure (Fig. 35).

The mature embryo is dicotyledonous and curved and is imbedded in the massive endosperm (Fig. 26). The ratio between the size of the embryo and that of the seed is far greater in *Tiliacora* than in most members of Ranalian families like Anonaceae and Magnoliaceae (Corner, 1949; Earle, 1938). The cotyledons are very much elongated.

The derivatives of the apical cell of the two-celled proembryo take a major part in the formation of the embryo proper while *ci* gives rise to the suspensor and the hypophyseal region is formed by *m*. So the embryo development conforms to the Onagrad Type of Johansen (1950) and keys out to the Trifolium Variation which is characterized by the formation of an epiphysis.

It was formerly regarded that the development of the embryo in many of the Ranales is irregular and that it resembles the condition in monocotyledons (Earle, 1938). However, it has recently been shown by Johansen (1950) on the basis of published figures and descriptions that the embryo development in some members of Ranalian families like Ranunculaceae, Magnoliaceae and Berberidaceae conforms to the Myosurus Variation, Onagrad Type. Recently Swamy (1949) described the

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*p*—pericarp, *a*—epidermis, *b*—outer zone of parenchymatous cells, *vs*—vascular strand, *c*—inner zone of elongated cells, *sp*—space between pericarp and outer integument, *oi*—outer integument, *ii*—inner integument, *nep*—nucellar epidermis (×60). Fig. 26. L.s. mature fruit showing mature dicotyledonous embryo imbedded in the ruminate endosperm. *p*—pericarp, *oi*—outer integument, *end*—endosperm, *em*—embryo (×5). Figs. 27–37. Various stages in the development of the embryo. 1, 2, 3 in Figs. 31 and 32 represent subepiphyseal cells (Figs. 27–35: ×582; Fig. 36: ×406; Fig. 37: ×290).

development of the embryo in *Degeneria*. He found that, although there is an apparent irregularity in the development, the method of tissue differentiation in the undifferentiated mass of cells is regular. However, he did not assign it to any type. As the derivatives of the apical cell alone seem to form the embryo proper, the development of the embryo in *Degeneria* probably conforms to the Onagrad Type. The Trifolium Variation has not so far been reported in any other Ranales.

## Fruit and seed development

After fertilization the ovule increases in size. The funicle elongates enormously as a result of which the ovule becomes greatly curved. In this it resembles *Cocculus* and differs from *Tinospora* in which the elongation of the funicle is not so well marked and the ovule undergoes only a slight curvature.

The nucellar cells begin to divide and the nucellus occupies the entire space of the ovule except for a small region at the micropyle, a condition found in many Anonaceae (Corner, 1949). Finally in the mature seed the nucellus is completely replaced by the endosperm.

The fruit is a drupe. In the early stages the carpel wall is made up of a number of layers of parenchymatous cells traversed by vascular strands. As the fruit enlarges in size, during the development of embryo and endosperm, the ovary wall becomes differentiated into an epidermis of radially elongated cells (*a*), an outer zone consisting of 6 or 7 layers of polygonal parenchymatous cells (*b*) and an inner zone of elongated cells (Fig. 25). In the mature fruit the outer layer becomes fleshy.

The outer integument which is made up of 4-5 layers of cells forms the outer seed coat. It shows infoldings protruding into the nucellus and later into the endosperm (Fig. 25). The inner integument, which remains two cells thick and thin walled, becomes gradually disorganized, and only its remnants are found in the mature seed.

## Discussion

So far only three genera of the Menispermaceae, namely, *Cocculus*, *Tinospora* and *Tiliacora* (the last forming the subject of the present study) have been investigated embryologically. Secretory type of tapetum, triporate pollen grains, degeneration of one of the ovules, crassinucellate ovule, formation of a nucellar cap and Polygonum type of embryo sac are features common to all three. Out of these *Tiliacora* and *Cocculus*, both of which belong to the tribe Cocculeae, show the following features in common: the nature and number of integuments and their spatial relations, the formation of the micropyle by the inner integument alone, the elongation of the funicle after fertilization and the straight nature of the micropyle.

The presence of an obturator-like outgrowth of the inner integument, ephemeral nature of the antipodals and the hookless synergids are features in which *Tiliacora* differs from both *Cocculus* and *Tinospora*.

Details about fertilization, embryo, endosperm and seed coat development are available only in *Tiliacora racemosa* (present report) and comparison of these phases of life history in the various genera of the family must await further studies in the family.

## Summary

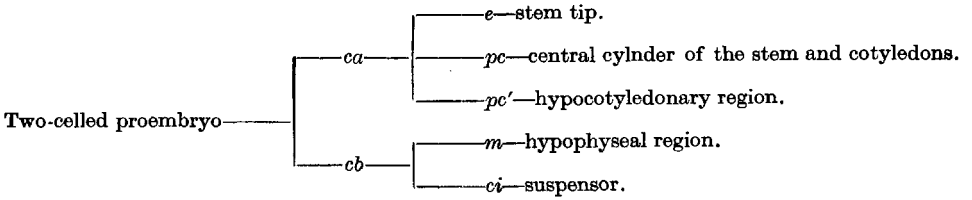
The anther wall is five-layered. The tapetum is of secretory type. Endothecium is fibrous. Cytokinesis is by furrowing. Pollen tetrads are tetrahedral or isobilateral. Pollen grains are triporate and two-celled at the time of shedding.

The ovule is bitegmic, amphitropous-campylotropous and crassinucellate and shows a nucellar cap. The micropyle is formed by the inner integument alone, which at the time of fertilization gives rise to an obturator like outgrowth. The megaspore mother cell forms a

linear tetrad of which the chalazal megaspore functions. The embryo sac is of the Polygonum type. The antipodals are ephemeral and synergids are devoid of hooks. Polar nuclei fuse before fertilization. Fertilization is porogamous.

The endosperm is free nuclear. Wall formation starts from the micropylar end at the globular stage of the embryo. First the nucellus and later the endosperm become ruminate on account of infoldings of the outer integument in post-fertilization stages.

Embryo development conforms to the Onagrad type and keys out to the Trifolium Variation of Johansen (1950). The following scheme summarises the derivation of the various organs of the mature embryo from the proembryonic cells:




The fruit wall is made up of an epidermis, an outer fleshy zone of 6 or 7 layers of parenchymatous cells and an inner zone of elongated cells.

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