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Embryology of *Indigofera* (Fabaceae)

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ABSTRACT : Embryology of *Indigofera cordifolia* and *I. linifolia* has been studied. The anthers are tetrasporangiate. The anther wall comprises of an epidermis, middle layer and tapetum. Anther tapetum is of the glandular type and the cells are uniseriate and uninucleate. Endothecium develops fibrous thickenings when the pollen grains are at 2-celled stage. Meiosis is normal and successive cytokinesis leads to the formation of tetrahedral and decussate microspore tetrads. Pollen grains are triporate and 2-celled at the time of shedding. The ovules are campylotropous, bitegmic and crassinucellate. Meiosis in megaspore mother cell leads to the formation of linear tetrad of megaspores. The chalazal megaspore of the tetrad is functional and develops into monosporic Polygonum type of embryo sac. Antipodals are three in number and are ephemeral. Endosperm development is of the Nuclear type and the embryo development conforms to the *Trifolium* variation of the Onagrad type of Johansen and Period I, series B2 and Megarche type VI of Soueges.

KEY WORDS : **Embryology, *Indigofera*, Fabaceae.**

INTRODUCTION

The genus *Indigofera* belongs to tribe Indigofereae of the subfamily Faboideae of Fabaceae. The tribe comprises of four genera and 711 species (Polhill and Raven, 1981). Embryological studies in the tribe Indigofereae include those of Rau (1953, 1954), Untawale and Deshpande (1968), Deshpande and Untwale (1971), Makde (1971), Oomman (1971), Aziz *et al.* (1972) and Cameron and Prakash (1990). A perusal of the literature reveals that most of those studies are confined to the genera *Indigofera* and *Cyamopsis*. Genus *Indigofera* is a very large genus of about 700 species (Polhill and Raven,1981). All aspects of embryology has been studied in only one species i.e. *Indigofera ennaephylla*. In other species scant attention has been paid to the development of anther, female gametophyte and embryo. Hence embryology of two species of *Indigofera* i.e., *Indigofera cordifolia* Roth and *I. linifolia* (L.f.) Retz. has been undertaken.

MATERIALS AND METHODS

The material for the present study was collected from Sri Krishnadevaraya University campus, India during the months of July-September. Buds, flowers and fruits at different

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stages of development were fixed in formalin - acetic acid - alcohol (FAA). Dehydration and infiltration were done in tertiary Butyl Alcohol and embedded in paraffin wax. Sections were cut at a thickness of 4-8 μm and were stained in Delafield's haematoxylin. They were mounted in Canada balsam. Voucher specimens have been deposited in the Herbarium of the Department of Botany, Sri Krishnadevaraya University, Anantapur, India.

RESULTS

Microsporangium, Microsporogenesis and Male gametophyte:

Anthers are tetrasporangiate (Figs. 1 A and 2 A). In the young stage in transverse section (T.s.), the anther shows an oval-shaped mass of meristematic cells surrounded by an epidermis. When a four-lobed contour is formed, a hypodermal layer of 3-5 cells gets differentiated as the male archesporium by their large size, dense cytoplasm and conspicuous nuclei (Fig. 2 B). The cells of male archesporium by undergoing periclinal divisions give rise to the primary parietal layer towards periphery and the primary sporogenous layer towards inside (Figs. 1 B and 2 C). The primary parietal layer undergoes periclinal division and produces an outer and an inner parietal layers (Figs. 1 B, C and 2 D, E). The inner parietal layer directly functions as the tapetum and outer parietal layer divides periclinally resulting in an outer endothecium immediately beneath the epidermis and the middle layer just above the tapetum (Figs. 1 D, E and 2 F-H).

The epidermal cells undergo only anticlinal divisions to cope up with the developing anther. Later on these calls expand lengthwise in mature anther. The hypodermal layer develops fibrous thickenings at the time of formation of 2-celled pollen grains and functions as fibrous endothecium (Figs. 1 F and 2 I). The middle layer gets crushed and degenerated during the meiotic stage. Tapetum on the connective side develops from the cells of connective tissue. Anther tapetum is of the glandular type. The tapetal cells are uniseriate and uninucleate. Tapetal cells get absorbed by the time the pollen grains reach the maturity.

The primary sporogenous cells in *Indigofera cordifola* function directly as pollen mother cells (Fig. 1 E). In *I. linifolia* they undergo divisions only transversely resulting in a single row of pollen mother cells (Figs. 2 G, H). The pollen mother cells undergo meiosis and simultaneous cytokinesis (Figs. 1 G, H and 2 J-N) resulting in microspore tetrads. The microspore tetrads are commonly tetrahedral and occasionally decussate (Figs. 1 I, J and 2 O, P). Microspores separate off from the tetrads and develop exine (Figs. 1 K and 2 Q). The single nucleus in the pollen grain moves towards one pole (Fig. 1 K) where it divides asymmetrically to form a large vegetative cell and a small lenticular generative cell (Figs. 1 L and 2 R). The generative cell gets pinched off from the wall and moves into the center of vegetative cell (Figs. 1 M and 2 S, T). Mature pollen grains at the time of shedding are 2-celled with three germ pores (Figs. 1 M and 2 T).

Ovary and Ovule:

The ovary is mono-carpellary and unilocular with ovules on marginal placentation. Ovule is campylotropous, bitegmic and crassinucellate (Figs. 3 E, F and 4 F). The ovule arises as a papillate outgrowth. It gets curved during megaspore mother cell stage and by the embryo sac stage it attains its characteristic campylotropous form (Figs. 3 A, B, E and 4 B, C, F).The

integrument initiation begins at the archesporial cell stage (Figs. 3 A, B) and by the megaspore tetrad stage the nucellus almost gets covered by the integuments(Fig. 4 B). The micropyle is formed by both the integuments. Both the integuments are two cell layered, but in the micropylar region the outer integument is more than two cells thick. In *Indigofera linifolia* soon after fertilization cells surrounding the embryo sac become enlarged, radially elongated and acquire dense cytoplasm (Fig. 4 F, G).

Female archesporium is hypodermal and single-celled. The archesporial cell undergoes a periclinal division forming an outer parietal cell and an inner megaspore mother cell (Fig. 3 A). Parietal cell undergoes periclinal divisions and anticlinal divisions forming parietal tissue.

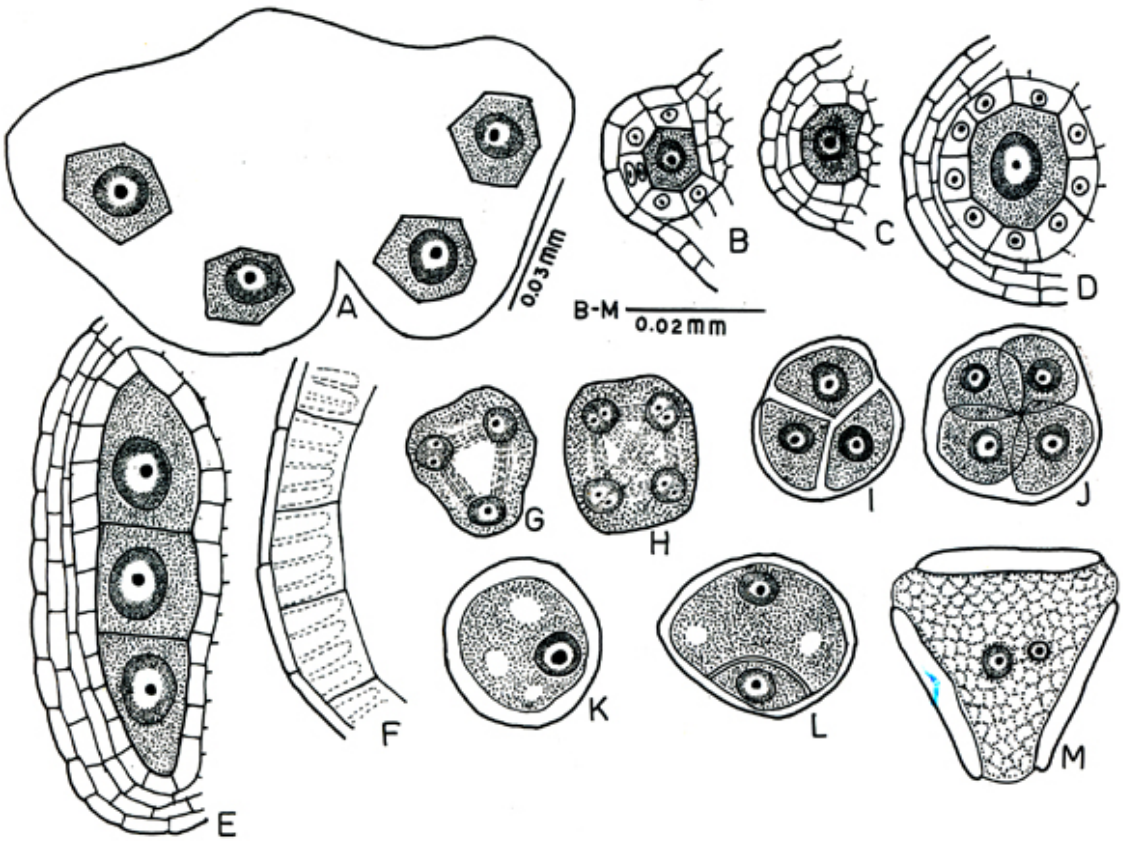


Fig. 1 A-M. *Indigofera cordifolia*. A: T.s. of tetra-sporangiate anther with pollen mother cells; B: T.s. of anther lobe showing epidermis, primary parietal layer and sporogenous cell. Note primary parietal layer is dividing; C: T.s. of anther lobe showing epidermis, outer and inner parietal layers and sporogenous cell; D: T.s. of anther lobe showing epidermis, endothecium, middle layer, tapetum and pollen mother cell; E: L.s. of anther lobe showing epidermis, endothecium, middle layer, tapetum and pollen mother cells; F: Epidermis and fibrous endothecium; G & H: Pollen mother cells in meiosis; I: Tetrahedral microspore tetrad; J: Decussate microspore tetrads; K: Uuinucleate pollen grain; L & M: 2-celled pollen grains.

Megasporogenesis and Female gametophyte:

Before undergoing the first meiotic division, the megaspore mother cell becomes four times as long as broad (Fig. 3 B), the meiotic division results in the formation of a linear megaspore tetrad (Fig. 4 A, B).

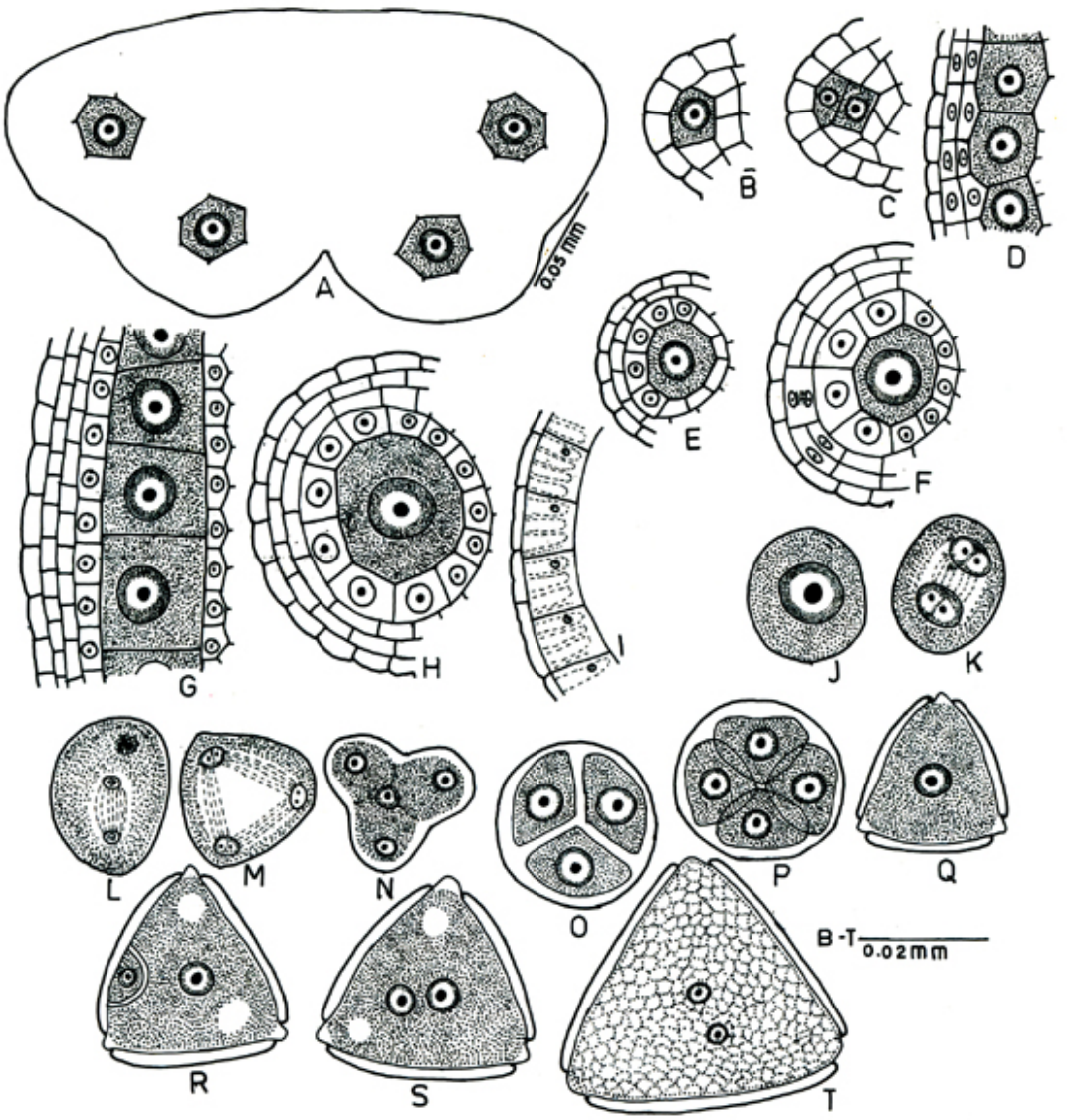


Fig. 2 A-T. *Indigofera linifolia*. A: T.s. of tetra-sporangiate anther with pollen mother cells; B: T.s. of anther lobe with male archesporium; C: T.s of anther lobe with parietal cell and sporogenous cell; D: L.s. part of anther lobe showing epidermis, outer and inner parietal layers and sporogenous cells; E: T.s. of anther lobe showing outer and inner parietal layer and sporogenous cell. Note outer parietal layer is dividing; G: L.s. a part of anther lobe showing endothecium, middle layer and pollen mother cells; H: T.s. of anther lobe showing epidermis, endothecium, middle layer, tapetum and pollen mother cell; I: Epidermis and fibrous endothecium: J-N: Pollen mother cells in meiosis; O: Tetrahedral microspore tetrad; P: Decussate microspore tetrad; Q: Uninucleate pollen grain; R-T: 2-celled pollen grains.

The upper three micropylar megaspores degenerate and the chalazal one becomes functional (Fig. 4 A, B). The functional megaspore undergoes a mitotic division resulting in two nuclei which move to the opposite poles due to the formation of a large vacuole in the center (Fig. 3 C). These two nuclei undergo two more mitotic divisions resulting in the formation an 8-nucleate, 7-celled embryo sac. (Figs. 3 D, F and 4 C-E). Thus the development of the embryo sac is of the monosporic Polygonum type (Figs. 3 E, F and 4 E).

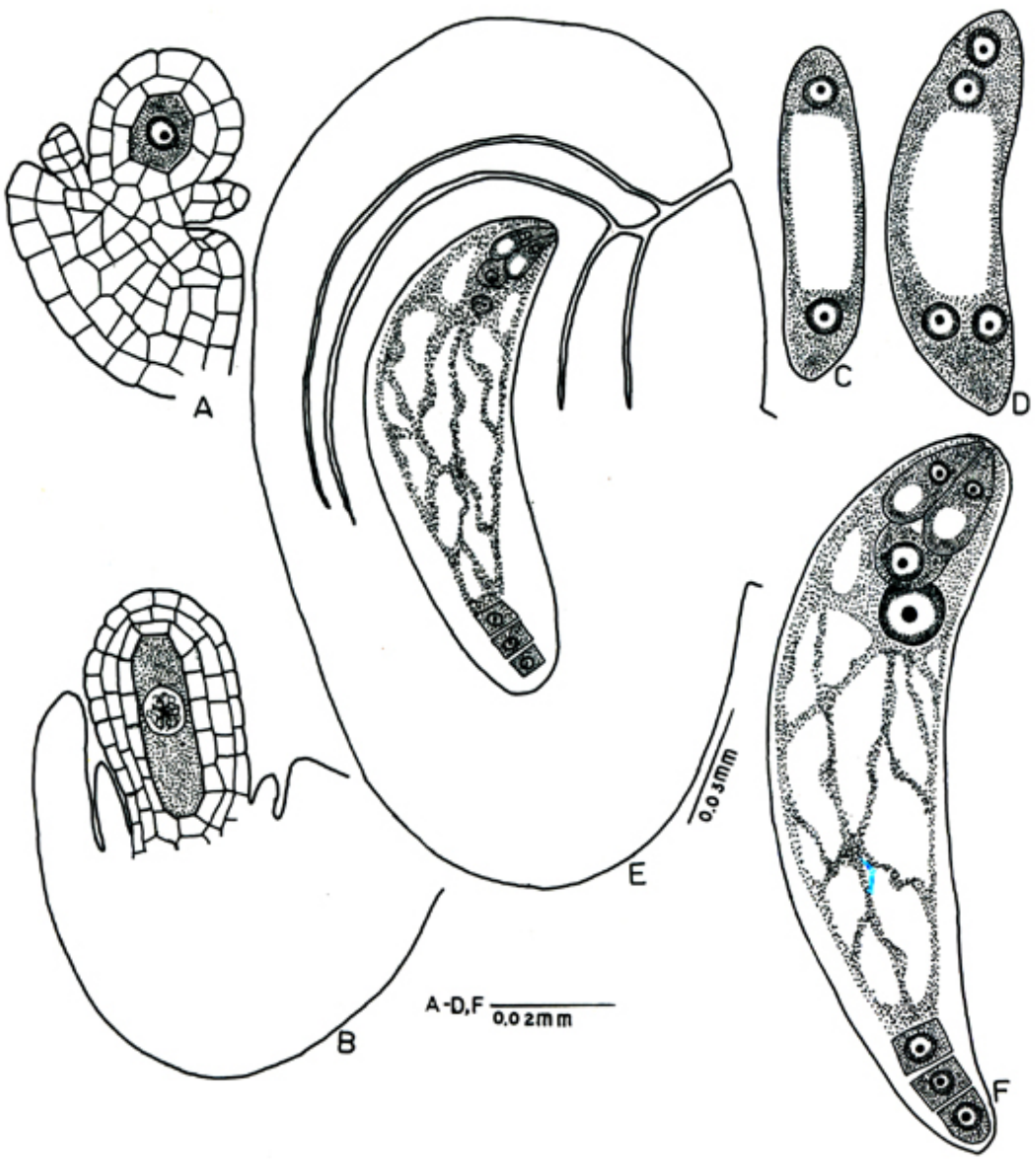


Fig. 3 A-F. *Indigofera cordifolia*. A: Ovule showing megaspore mother cell; B: Ovule showing megspore mother cell in meiosis; C: 2-nucleate embryo sac; D: 4-nucleate embryo sac; E: Ovule showing organised embryo sac; F: Organised embryo sac.

The synergids are pear-shaped and without hooks in *Irdigofera cordifolia* (Fig. 3 E, F) while they are pear-shaped and hooked in *Irdigofera linifolia* (Fig. 4 E). Generally the two polar nuclei fuse before the fertilization and form the secondary nucleus. Secondary nucleus is generally situated near the egg. Antipodals are three in number and they are ephemeral. Starch grains are seen in the embryo sac of *Irdigofera cordifolia*.

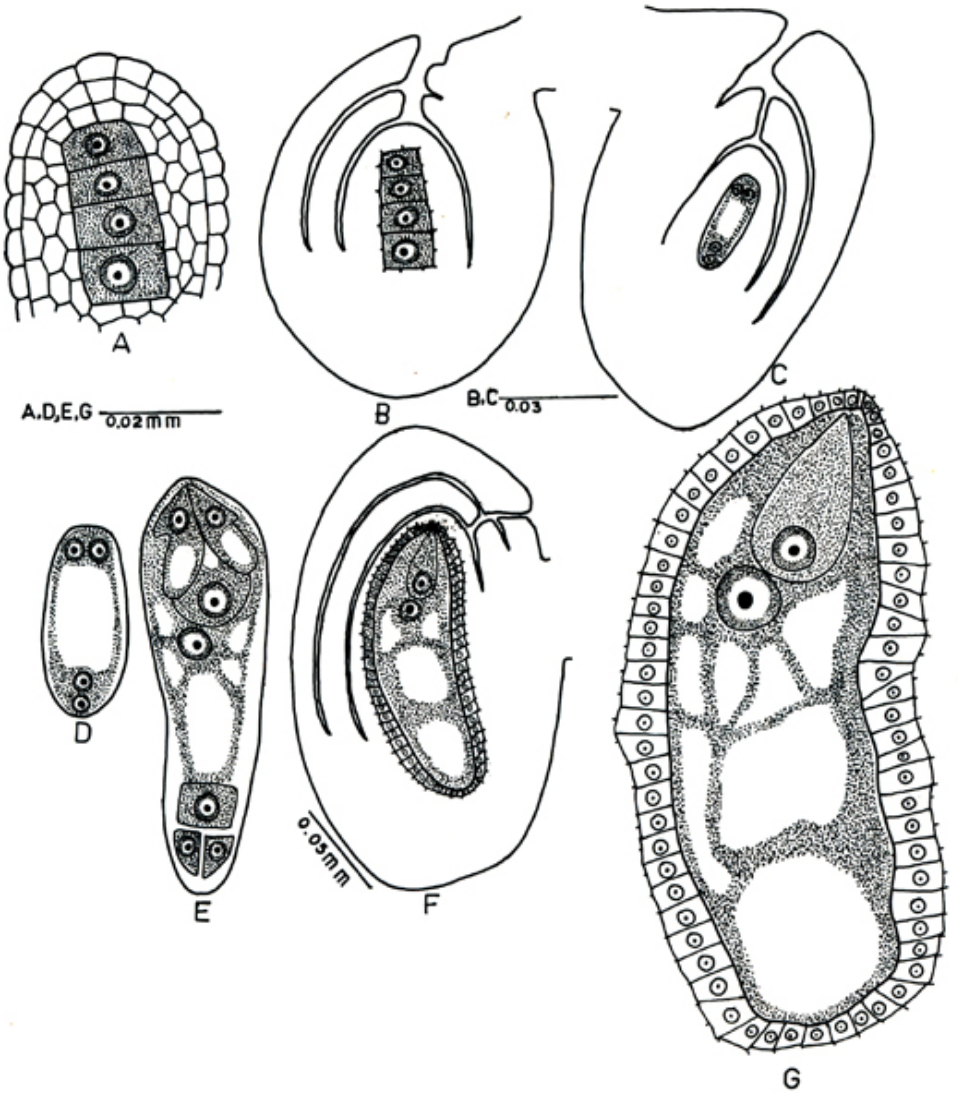


Fig. 4 A-G. *Indigofera linifolia*. A: Linear megaspore tetrad; B: Ovule along with linear megaspore tetrad; C: Ovule with 4-nucleate embryo sac; D: 4-Nucleate embryo sac; E: Organised embryo sac; F: Ovule with zygote and primary endosperm nucleus. Note darkly stained uncellar cells; G: Zygote and primary endosperm nucleus with darkly stained nucellar cells.

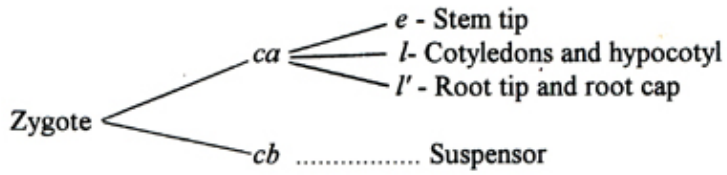
Fertilization:

The actual course of pollen tube is not traced. But it appears to enter through the micropyle into one of the synergids. Syngamy and triple fusion occur simultaneously and result in the formation of zygote and primary endosperm nucleus (Fig. 4 F, G).

Development of Endosperm and Embryo:

Endosperm development is of the Nuclear type. The primary endosperm nucleus divides prior to the division of zygote (Fig. 5 A). Repeated free nuclear divisions produce large number of nuclei. Along with free nuclear divisions the embryo sac increases in its size considerably. A big vacuole appears in the endosperm and all free nuclei are pushed towards the periphery (Fig. 5 A). A large number of endosperm nuclei along with cytoplasm become aggregated around the embryo (Fig. 6 A-C). The free nuclear endosperm persists for considerable time (Fig. 6 C) with a broad haustorium. Wall formation in the endosperm takes place at the globular stage of embryo and when the embryo is cordate, almost all the endosperm becomes cellular including the haustorium (Figs.5 B and 6 B). The developing embryo consumes the entire endosperm and no trace of the endosperm is left in the mature seed.

The zygote undergoes a transverse division resulting in two super-imposed cells, the apical cell *ca* and the basal all *cb*. The later divides by a transverse wall into *m* and *ci* (Fig. 5 C). The terminal cell *ca* divides by a vertically oblique wall and gives rise to two dissimilar juxtaposed cells. Thus the 4-celled proembryo (Fig. 5 C) conforms to the category B2 of the tetrads in the system of Soueges (1948). Another oblique division in one of the larger derivative cell of *ca* differentiates into the epiphysis *e* (Fig. 5 D). The derivatives of *cb* contribute to the suspensor (Figs. 5 C-F, 6 E-H). The derivatives of the epihyseal initial *e* gives rise to the stem tip. The two cells derived from *ca* divide further to give rise to a quadrant which is generally disposed in a tetrahedral manner. The next division in tier *ca* is transverse resulting in two tiers *l* and *l'* (Figs. 5 D and 6 E). Tier *l* gives rise to the cotyledons and the hypocotyl, while *l'* gives rise to the root tip and root cap (Figs. 5 C-F and 6 E-H). The following schematic representation shows the destination of the various tiers of proembryo.



Thus the development of embryo conforms to the *Trifolium* variation of Onagard type of Johansen (1950) and Period I, Series B2 and Megarche type VI of Soueges.

DISCUSSION

In the tribe Indigofereae very little is known regarding the microsporogenesis and male gametophyte. Makde (1971) reported 2 rows of male archesporum in *Indigofera pulchella*

while Oomman (1971) observed a single plate of male archesporial cells in *Indigofera tinctoria*. In the present study in both the species of *Indigofera* male archesporium is single-layered.

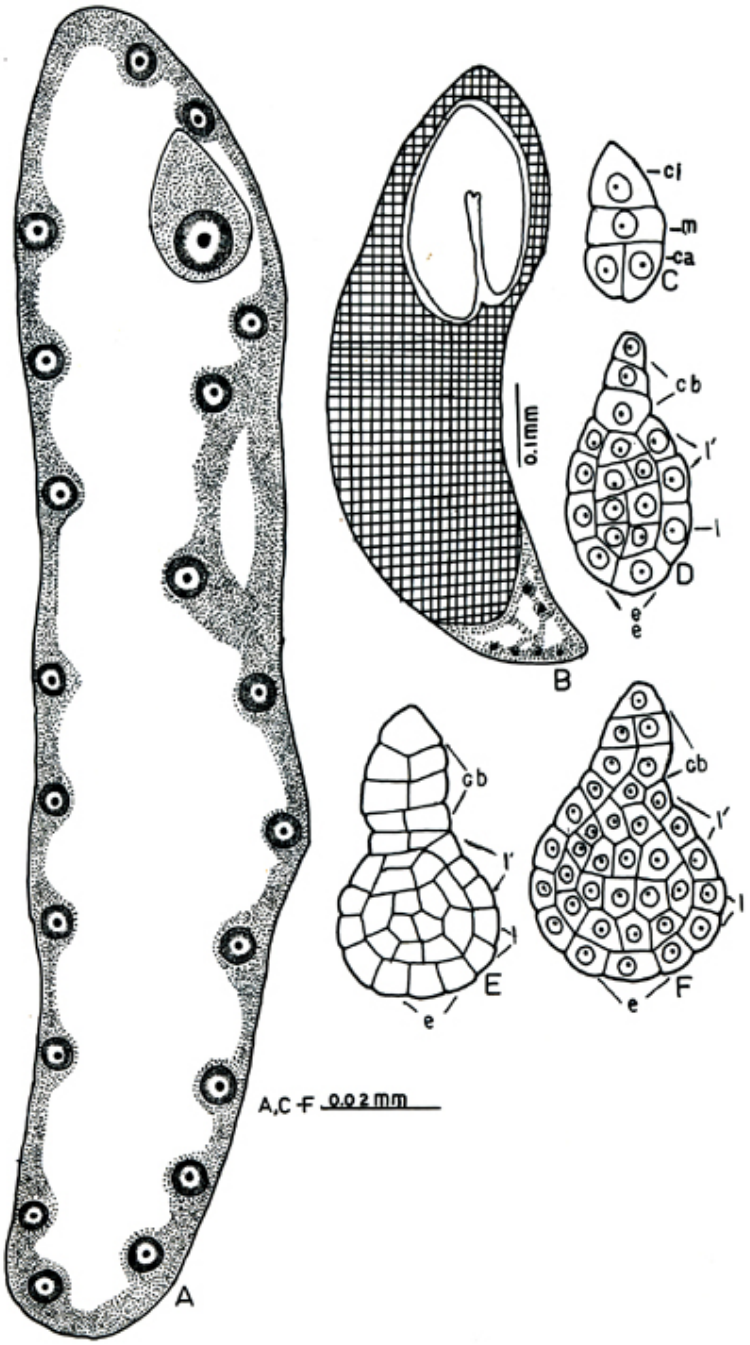
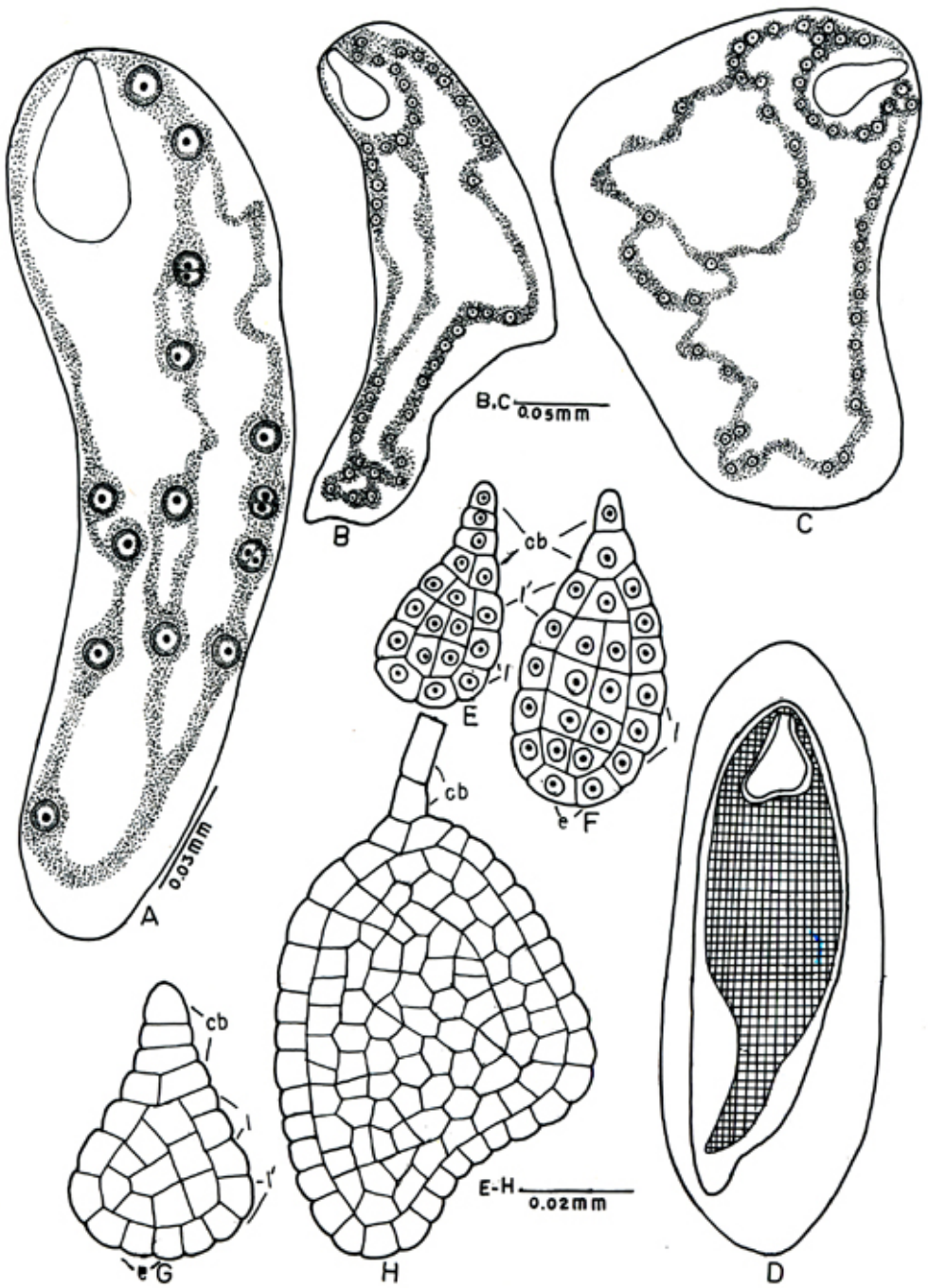


Fig. 5 A-F. *Indigofera cordifolia*. A & B: Stages in the development of endosperm; C-F: Stages in the development of embryo.



Flg. 6 A-H. *Indigofera linifolia*. A-D: Stages in the development of endosperm; E-H: Stages in the development of embryo.

Davis (1966) and Prakash (1987) reported that anther wall development in Faboideae is of the Dicotyledonous type. Such a type has also been met within the present study in two species of *Indigofera.* However Oomman (1971) reported that anther wall development in *Indigofera tinctoria* and *Rhynchosia capitata* is of the Basic type. Similarly Despande (1977) also reported Basic type of anther wall development in *Tephrosia* and *Uraria*. In the whole of the Faboideae these are the only reports where a Basic type has been reported and it appears doubtful. Anther wall in *Indigofera tinctoria* (Oomman, 1971) is five cell-layered while in *Indigofera pulchella* (Makde, 1971), it is six cell-layered. In the present study of *Indigofera* the anther wall, including the epidermis, is four cell-layered.

Pollen grains are generally shed at 2-celled stage in majority of Faboideae studied including *Indigofera cordifolia* and *I. linifolia* (present study). Ovules in the subfamily Faboideae are highly heterogeneous. The present study shows campylotropus ovules. Campylotropous ovules have been reported in the majority of the genera so far studied. In *Indigofera pulchella* (Makde, 1971) and *Indigofera tinctoria* (Oomman, 1971) campylotropous ovules have been reported. In *Indigofera oblongifolia* Aziz *et al* (1972) reported that ovules are hemianatropous.

Female archesporium in the two species of the present investigation is hypodermal. Although sub-hypodermal origin are also common, Aziz *et al* (1972) also reported sub-hypodermal archesporium in *Indigofera oblongifolia.* In the tribe Indigoferaeae both single-celled and multicelled archesporium is seen. Single-celled female archesporium is seen in *Indigofera oblongifolia* (Aziz *et al.*, 1972), *Indigofera cordifolia* and *I. linifolia* (present study). It is 2-celled in *Indigofera pulchella* (Makde, 1971) and *I. tinctoria* (Oomman, 1971).

Giant antipodals have been recorded in *Indigofera australis* (Cameron and Prakash, 1990), *I. pulchella* (Makde, 1971) and *I. tinctoria* (Oomman, 1971). Commenting on this Cameron and Prakash (1990) said that giant antipodals may be a generic character of the genus *Indigofera*. However, in *Indigofera oblongifolia* (Aziz *et al.*, 1972), *I. cordifolia* and *I. linifolia* (present study) antipodals are normal and ephemeral. Hence although it is useful identification feature at species level, giant antipodals is not a generic feature.

All the members of the tribe Indigofereae are in conformity in the development of endosperm. It is of the Nuclear type. In all the members of Indigofereae studied so far, the entire endosperm including the haustorium becomes cellular.

In the tribe Indigofereae embryo development has been studied in only two species. Rau (1954) and Green *et al* (1964) reported that embryo development in *Cyamopsis psoraliodies* follows Period I, Megarche type VI, Series B. Whereas Deshpande and Untawale (1971) reported Megarche type V of embryo development in *Indigofera enneaphylla*. In the present study in *Indigofera* spp. embryo development is found to be Megrache type VI. An extensive review of the literature reveals that in subfamily Faboideae Megarche-type V has been reported only in *Indigofera enneaphylla* (Desphande and Untawale, 1971) and this observation appears to be erroneous.

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木藍屬(豆科)植物之胚胎學研究

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摘　　要

本文研究心臟葉木藍（*Indigofera cordifolia*）和細葉木藍（*I. linifolia*）的胚胎學。此兩種植物的花藥都具有四個小孢子囊，花藥壁是由表皮、中間層和營養層所構成，而營養層爲分泌型，是由單核的單層細胞組成。當花粉粒在兩個細胞時期，藥室內壁行纖維狀加厚。小孢子母細胞減數分裂與質分裂後形成四面體型與交叉型的小孢子四分體。花粉粒具3孔，釋出時爲兩個細胞時期。胚珠爲彎生型，具雙珠被與厚珠心。大孢子母細胞減數分劣後形成線型的大孢子四分體。近合點的大孢子具功能，且發育爲單孢子蓼型的胚囊，有三個暫時性的反足細胞。胚乳發育呈核型，而胚胎的發育則爲 Johansen 提出的柳葉菜型的椒草屬（*Trifolium*）變型，和 Souéges 的 Megarche-type VI型，第一階段，B2係列。

關鍵詞：胚胎學、木藍屬、豆科。

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