

EMBRYOLOGICAL INVESTIGATIONS IN SOME SPECIES OF *IPOMAEA*

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Abstract: Embryology of *Ipomaea macrantha* R. & S., *Ipomaea hederifolia* L. and *Ipomaea pes-caprae* (L.) Sweet. has been investigated. Anther wall development is of the Dicotyledonous type and at maturity consists of the endothecium, 1-2 middle layers and a secretory tapetum. Tapetum is also formed from the connective. Microspore mother cells undergo simultaneous divisions and produce tetrahedral and decussate microspore tetrads. Pollen grains are spheroidal, echinate, polyporate and are mostly 2-celled at anthesis. Rarely 3-celled grains occur in *I. macrantha*.

Ovary is superior, bicarpellary, syncarpous and bilocular with two anatropous, unitegmic and crassinucellate ovules in each locule on axile placentation. Archegonium is single celled. Megaspore mother cell undergoes meiotic division and produce a linear tetrad of megaspores of which the chalazal one develops into an 8-nucleate embryo sac of the Polygonum type. Antipodals are ephemeral. Fertilization is porogamous. Syngamy and triple fusion occurs almost simultaneously. Endosperm is nuclear. Embryo development is of the Sherardia variation of the Solanad type. Suspensor is large and haustorial.

INTRODUCTION

Ipomaea is the largest genus of the family *Convolvulaceae* and consists of about 400 species of which most of them are lianes. The embryological studies in the family has been reviewed by Schnarf (1931), Davis (1966), Govil (1970) and Johri et al., (1992). An examination of the previous literature on the embryological studies in the family have revealed that our knowledge is rather limited and incomplete. There is very little information on certain of the embryological features like the development of the anther, ovule and the embryo. Further, diversity of opinion exists in the presence of parietal tissue in the family. Hence, it is felt that the family deserves further attention. Therefore, it is planned to work out the various embryological aspects like the development of the anther, microsporogenesis, male gametophyte, ovule, female gametophyte, fertilization, endosperm and embryo in three species of *Ipomaea* namely *I. pes-caprae* (L.) Sweet., *I. macrantha* R. & S., and *I. hederifolia* Linn.

MATERIAL AND METHODS

I. hederifolia and *I. pes-caprae* were collected in the Andhra University campus and *I. macrantha* collected at Matlapalem, East Godavari District, Andhra Pradesh, India. The materials were fixed in FAA and customary methods of dehydration, infiltration and embedding were followed. The sections were cut at 5-20 μ m in thickness and stained in Delafield's hematoxylin.

OBSERVATIONS

Microsporangium, microsporogenesis and male gametophyte: The anther is quadrisporangiate (Fig. 1A,B). In each lobe a plate of hypodermal archesporial cells gets differentiated (Fig. 1C) and they undergo periclinal divisions to form primary parietal layer towards the outside and primary sporogenous layer towards the inner side (Fig. 1D). The latter eventually functions directly as the microspore mother cells. Subsequent periclinal divisions in the primary parietal layer results in the formation of 3-5 layers of wall cells namely the fibrous endothecium, 1-2 middle layers and the tapetum. One middle layer is seen in *I. hederifolia* and *I. macrantha* (Fig. 1F) whereas two middle are noticed in *I. pes-caprae* (Fig. 1G). The innermost layer of the anther wall is the tapetum. It is uniseriate and is of the glandular type. The tapetal cells are initially uninucleate but later becomes 2-3 nucleate (Fig. 1E). Tapetum is also differentiated from the ground parenchyma of the connective and form an uninterrupted layer completely surrounding the sporogenous tissue (Fig. 1G). This part of the tapetum can be designated as the connective tapetum. Hence the tapetum is dual in origin. The cells of the C-tapetum slightly differ morphologically with the cells of the parietal tapetum by their greater radial elongation. Hence the tapetum is also dimorphic in nature.

The primary sporogenous cells directly function as the microspore mother cells without undergoing any divisions and they can be distinguished by their larger size, conspicuous nuclei and dense cytoplasm (Fig. 1G). Meiotic divisions in the microspore mother cells are simultaneous and results in the formation of tetrahedral tetrad (Fig. 1H). Rarely decussate tetrads are also seen (Fig. 1I). The microspores are first pyramidal in shape because of the contact with the other microspores in the tetrad but later becomes spherical. In the young microspores the cytoplasm is dense and the nucleus is centrally located. The microspore nucleus divides and the two daughter nuclei are separated by a poorly defined membrane. This 2-celled stage is the condition found at the shedding time (Fig. 1K). Rarely 3-celled pollen grains are observed in *I. macrantha* (Fig. 1L). Thus the pollen grains are 2-celled at shedding in *I. pes-caprae* and *I. hederifolia* while they are 2 or 3-celled in *I. macrantha*. The mature pollen grains are spheroidal, echinate and pantoporate (Fig. 1J-L) and they are largest (134-156 μ) in *I. hederifolia* (Fig. 1J).

Megasporangium, megasporogenesis and the female gametophyte: Ovary is superior, bicarpellary syncarpous and bilocular with two erect, unitegmic, anatropous and crassinucellate ovules in each locule. The ovular primordium arises as a conical protruberance from the placenta. This gradually increases in size and form the nucellus of the ovule. The archesporium is differentiated hypodermally (Fig. 2B) and it divides producing a primary parietal cell towards outside and primary sporogenous cell towards inside. The former divides anticlinally and forms a few wall cells. The primary sporogenous cell functions directly as the megaspore mother cell (Fig. 2C). Concomitantly, just below the level of the primary sporogenous cell some of the hypodermal cells near the periphery of the primordium undergo periclinal divisions. This initiates the development of an integumentary primordium. It grows very fast and this differential growth on the sides of the ovule results in the curvature of the primordium which results in anatropous condition (Fig. 2A). Later, the parietal layer further divides both anticlinally and periclinally and give rise an extensive parietal tissue as a result of which the megaspore mother cell becomes deep seated (Fig. 2D).

The megaspore mother cell undergoes the meiosis and results in the formation of a linear tetrad of megaspores (Fig. 2E). The chalazal one is functional and develops into an 8-nucleate embryo sac of the polygonum type (Fig. 2F,G). The embryo sac at maturity is broader at micropylar end and somewhat narrower at the chalazal end (Fig. 2G, I) in *I. pescaprae* and *I. hederifolia* while it is spindle shaped in *I. macrantha* (Fig. 2H). The egg apparatus consist of

an egg cell which is centrally located and two pear shaped synergids on either side. The two polars generally lie near the egg apparatus and fuse before fertilization. The antipodals are three in number, ephemeral and are uninucleate.

Fertilization: It is porogamous. After entering into the embryo sac, the pollen tube attacks one of the synergids and destroys it (Fig. 2J). The other synergid persists till fertilization. Syngamy and triple fusion occurs almost simultaneously.

Endosperm: The fertilized egg undergoes a period of rest before dividing while the primary endosperm nucleus divides almost immediately. It undergoes a number of free nuclear divisions and they were not followed by the cell plate formation (Fig. 2K). The free nuclei take up peripheral position due to the formation of a large central vacuole. The cell plate formation commences from the periphery and gradually extends towards the centre. The cells of the endosperm are polygonal in shape and are uninucleate. At maturity most of the endosperm will be consumed by the growing embryo and only traces of the endosperm are visible during the advanced stages of the embryo development.

Embryogeny: The division of the zygote occurs invariably after that of the primary endosperm nucleus. The first division of the zygote is transverse giving rise to a terminal cell *ca* and a basal cell *cb* (Fig. 2L). The latter undergoes another transverse division resulting in two cells which can be designated as *m* and *ci* (Fig. 2M). The division of the apical cell *ca* is belated. However, it also divides transversely giving rise to two cells namely *l* and *l'*. Thus a 4-celled linear proembryo is formed (Fig. 2N). Later, the cell *l* divides transversely and produce two cells namely *l₁* and *l₂* (Fig. 2O). As cell divisions and cell enlargement takes place, the developing embryo elongates and grows deeper into the embryo sac. Further divisions take place in all planes and a globular embryo is formed (Fig. 2P-R). In the globular embryo, the cells of the embryo proper are smaller in size and richer in cytoplasm than the cells of the suspensor. The suspensor is massive and is formed from the derivatives of the basal cell. This type of embryo development can therefore be classified as *Sherardia* variation of the *Solanad* type as outlined by Johansen (1950) and Maheshwari (1950). It belongs to the Grand Period I, megarche type V, Series *C₂* and subseries *a₁* according to the Soue'ges system. In *I. hederifolia* the suspensor consists of a mass of uniformly large cells (Fig. 2Q S). In *I. macrantha*, the suspensor has no definite shape and consists of many large cells. The cells at the base are larger in size while these towards the embryo proper are relatively smaller (Fig. 2R). The suspensor becomes very large due to rapid cellular growth and is haustorial in function.

DISCUSSION

In the present study the anther wall development conforms to that of the dicotyledonous type and in this respect it agrees with the other species of *Ipomaea* investigated so far. The tapetum is of the secretory type and is uniserial with uninucleate cells to begin with. Later due to mitotic divisions the number of nuclei in the tapetal cells may increase up to 2-3 per cell. Rao (1940) also noted the secretory tapetum and multinucleate tapetal cells in *I. learii* and *I. staphylina*. But he noted amitotic divisions in the tapetal nuclei. Chan and Hillson (1971) in *I. reptans*, Govil (1976) in *I. purpurea* also reported mitotic divisions and 2-3 nucleate tapetal cells. Chen and Hillson (1971) reported a very interesting type of anther tapetum in *I. reptans*. According to them "shortly after meiosis is completed, the cells of the tapetum begin to lose contact with one another. Their walls break down but the

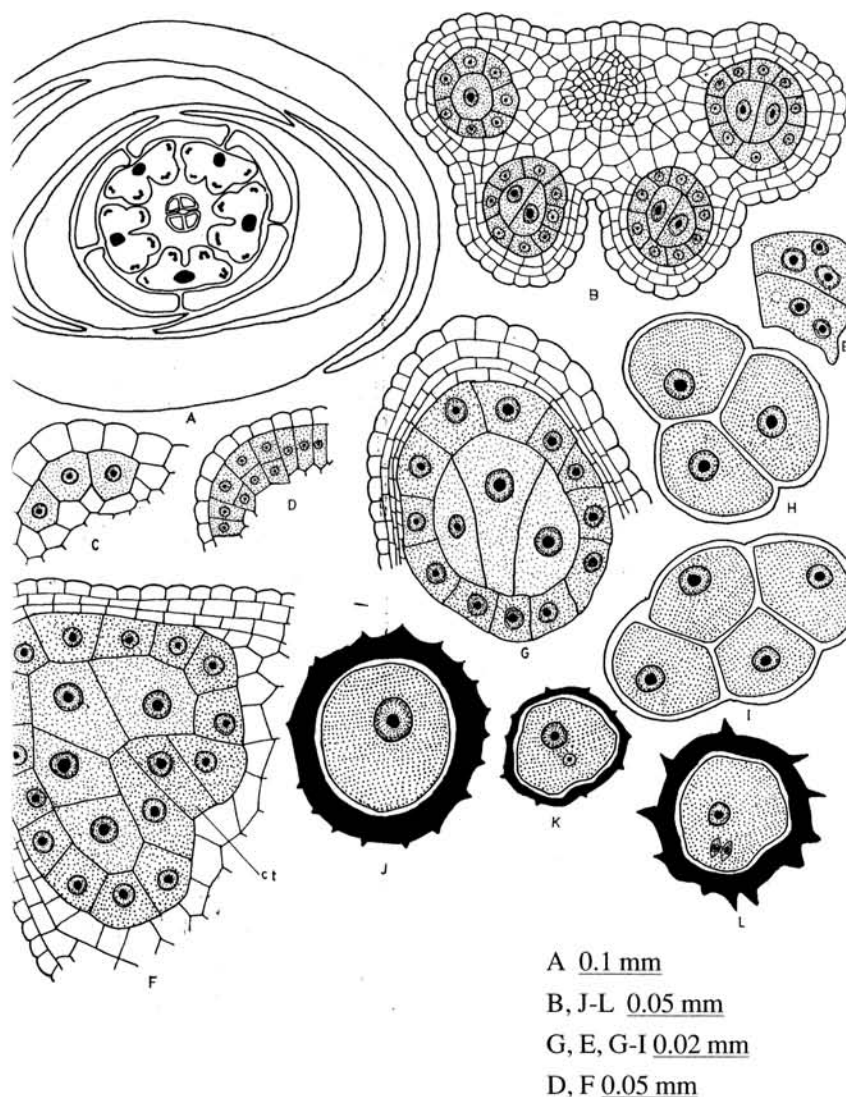


FIGURE-1A-L.

Fig. 1. A, D, F, I, K, L. : *Ipomaea macrantha* R. & S.

Fig. 1. B, C, E, J, : *Ipomaea hederifolia* Linn.

Fig. 1. G, H. : *Ipomaea Pes-Caprae* (L.) Sweet.

FIGURE I.

A. T.s. of Flower showing 5 tetrasporangiate anthers and a bicarpellary, syncarpous and bilocular ovary.

B. T.s. of anther showing wall layers and pollen mother cells.

C. T.s. of anther lobe showing archesporial cells.

D. T.s. of anther showing the formation of primary sporogenous and primary parietal layers.

E. Tapetal cells with 2-3 nuclei respectively.

F. T.s. anther lobe showing the anther wall, tapetum and sporogenous cells. Note the protruding connective tapetum.

G. T.s. anther lobe showing wall layers, tapetum and pollen mother cells.

H. Tetrahedral microspore tetrad.

I. Decussate microspore tetrad.

J-L. Pollen grains.

ct, Connective tapetum.

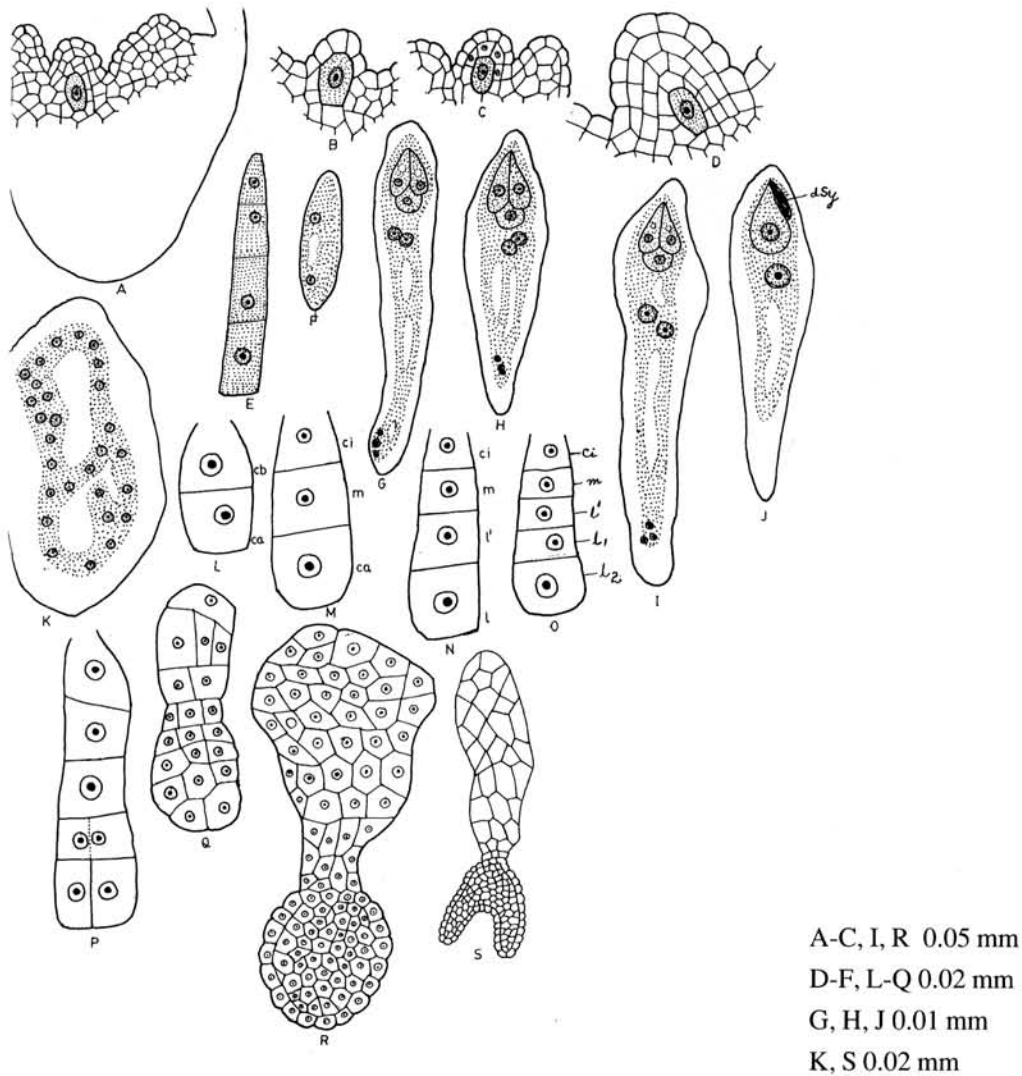


FIG. 2 A-S.

Fig. 2. A-D, H, J, K, R : *Ippmaea marcantha* R. & S.

Fig. 2. E, F, I, L, P, Q, S : *Ipomaea hederifolia* Linn.

Fig. 2. G, M-O. : *Ipomaea Pes-Caprea* (L.) Sweet.

FIG. 2

A. Unitegmic, anatropous ovuls showing the parietal tissue and the megasporemother cell.

B. Ovule showing the archesporial cell.

C. Ovule showing a few parietal cells and sporogenous cell. Note the integumentary primordia.

D. L.s. part of ovule showing the parietal tissue and a megaspore mother cell.

E. Megaspore tetrad.

F. 2-nucleate embryo sac.

G-I. Mature embryo sacs, Note the degenerating antipodals.

J. Embryo sac showing zygots and the degenerating synergid and primary endosperm nucleus.

K. Embryo sac showing nuclear endosperm.

L-S. Embryogeny.

dsy: degenerating synergid.

protoplasts, which remain alive, coalesce to form masses of multinucleate tapetal plasmodia that may either stay in a parietal position or protrude into the neighbourhood of the microspores. As the microspores develop, these plasmodia are gradually absorbed. At the time of dehiscence they have completely disintegrated". They did not assign the tapetum to any particular type. i.e. glandular or plasmodial. Hence, the plant deserves a reinvestigation. In the present study the tapetum is found to be dual in origin and dimorphic in nature (Periasamy and Swamy, 1966; Raghavan and Ratnaparkhi, 1973).

Erdtman (1952) classified the pollen grains of *Convolvulaceae* into two categories: 1) Ipomaea type and 2) Other types. In the Ipomaea type the grains are polyporate, large, crassinucellous and spiniferous. The mature pollen grains in the present study are in line with the earlier observations made in the family. The pollen grains are 2-celled at the time of shedding in the members studied at present. In *I. macrantha* they are rarely 3-celled. Rao (1940) in *I. learii* and *I. staphylina* also reported that the pollen are generally 2-celled and rarely 3-celled at the shedding stage. Similarly 2-celled pollen grains at the time of liberation were also observed in *I. reptans* (Chan and Hillson, 1971). However, Federshuk (1931), Smith (1934), Finn (1937), Tiagi (1951), Johri and Tiagi (1952), Vij and Madhu (1980) reported 3-celled pollen grains in the members studied by them.

In the present study, the ovary is bicarpellary, syncarpous and bilocular with two ovules in each locule on placentation. Sometimes the ovary becomes 4-locular due to the development of the false septum, This is in accordance with the hitherto investigated members of the family. But Kuar and Singh (1970) reported a rare instance of trilocular ovary in *I. purpurea* with two ovules in each locule. The ovule is anatropous, unitegmic and crassinucellate in the *Ipomaea* species studied at present as in the majority of the members of the family studied so far. However, anatropous, unitegmic and tenuinucellate ovules are recorded in *I. staphylina* (Rao, 1940).

Archivesporium is single celled (present study) as in majority of the members investigated. Two or more celled archivesporium was reported in *I. trifida* (Kenyan, 1928). Most of the gamopetalous families are characterised by tenuinucellate ovules. However, the *Convolvulaceae* is controversial in this respect. Formation of definite parietal cells have been reported in *I. learii* (Rao, 1940); *I. pulchella*, *I. horsefalliae*, *I. obscura*, *I. sepiaria* (Rao, 1944), *I. trifida* (Kenyan, 1928), *I. hispida* (Kaur, 1981). However, Rao (1940) did not report parietal cells in *I. staphylina*. Svensson (1925) holds the view that the parietal cells reported are not true parietal cells but they are spidermal in origin. Rao (1940) is of the opinion that many of the *Convolvulaceae* taxa reported lacking the parietal tissue appear to be doubtful and need a reinvestigation.

Development of the female gametophyte is of the Polygonum type in several members studied so far. However, Johri & Nand (1934) and Johri and Tiagi (1952) in *Cuscuta reflexa*; Govil and Lavania (1980) in *C. eurpoea* and *C. calycina* reported bisporic Allium type of embryo sac development. Rao (1944) reported an abnormal embryo sac consisting of 6 nuclei at the chalazal end and 2 nuclei at the micropylar region. Kaur (1981) in *I. hispida* reported a rare case of an embryo sac with reversed polarity. Jos (1963) recorded twin embryo sacs in an ovule in *I. pestigridis*.

The embryogeny in *Convolvulaceae* appears to be heterogenous. *Ipomaea* species studied at present shows Sherardia variation of the Solanad type (Johansen, 1950). Chen and Hillson (1968) also reported Solanad type of embryo development in *I. reptans*. However, Kaur and Singh (1970) reported Fumaria variation of Caryophyllad type of embryo development in *I. signata*, *I. purpurea* and *I. carnea*.

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三種牽牛花屬植物胚胎發生的檢視

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觀察大花牽牛 (*Ipomaea macrantha*)、圓葉蔦蘿 (*I. hederifolia*) 及馬鞍藤 (*I. pes-carprae*) 這三种植物的胚胎發生過程，它們的花藥壁發育都屬於雙子葉植物型，即是在成熟時為有內殼、一至二層的中間層及分泌型的營養層所組成。營養層由藥隔處逐漸形成。小孢子母細胞會同時進行減數分裂，之後產生四面體型、十字對生的四分子孢子，花粉為橢圓形、外壁具刺紋、多孔，在花期時多以兩個細胞型存在。在大花牽牛中則偶有三個細胞的。

子房上位、具有兩個心皮、為合生型、內有兩室，每室含兩個倒生、單一種皮、珠心肥厚的胚珠，為中軸胎座，孢原為單細胞。大孢子母細胞進行減數分裂後產生一個線型的四分大孢子，靠近合點的一個會發育成蓼型的七細胞八核之胚囊。反足細胞為暫存的，授精作用在珠孔端發生。雙重授精的過程幾乎是同時。胚乳為無壁式的。胚胎的發育與茄科型不同。懸柄大且有吸足的功能。