A COMPARISON OF EMBRYO SACS AND HAUSTORIA IN SELECTED SPECIES OF IMPATIENS

BY

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A THESIS

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I. THE PROBLEM

The object of this problem was to study the ovule development of selected species of Impatiens. Special emphasis was placed on the occurrence, origin, and nature of the haustoria in these species.

II. INTRODUCTION

The genus, Impatiens, is of embryological interest for two reasons. The conflicting statements concerning the types of embryo sacs in certain of the species must be reconciled (Maheshwari, 1955). The synergid, embryo sac and endosperm haustoria present interesting research material which may aid in the clarification of this family's phylogenetic position.

III. MEMBERS OF THE FAMILY AND THEIR GEOGRAPHICAL DISTRIBUTION

Genera and Species

The Balsaminaceae contains two genera, Impatiens and Hydrocera. Engler and Prantl (1889) and Lawrence (1951) place this family in the order Sapindales, while Hutchinson (1926) and Rendle (1952) place it in the Geraniales. Engler and Prantl reported that the genus Impatiens contained 220 species. However, Lawrence holds that there are over 420 species. Hydrocera is a monotypic genus having one species H. triflora (I. triflora L. Tytonia natans G. Don) (Engler and Prantl, 1889).

Geographical Distribution

Europe

A few species of Impatiens have been reported in northern Europe. I. parviflora D.C. Prod. is prevalent in Norway (Nordhagen, 1944). This and other species extend south to Denmark (Kloos, 1947) and the Netherlands (Jorgensen, 1926).

Asia

The greatest numbers of Impatiens species occur in Asia and Africa. I. fragicolor Marquand, I. nyimana Marquand (Marquand, 1929) and I. glandulifera Royle (Fernald, 1950) were reported growing in the Eastern Himalayas and Tibet. I. aliciae Fischer, I. leptura Hook., and I. cothurnoides Fischer (Fischer, 1931; 1934; 1935) are found in South India and Assam; and I. parkinsonii Fischer (Fischer, 1926) grows in Burma.

Species of Impatiens are distributed throughout Siam, the Malaya Peninsula, and Java and include I. foxworthyi Henderson (Henderson, 1927), I. patula Craib, I. kerriae Craib (Craib, 1926), I. benthami Steenis and I. platypetala Lindl. (Steenis, 1948).

Africa

The range of Impatiens stretches from French West Africa where I. bennae Jacques-Felix is encountered (Jacques-Felix, 1949) south to the Belgian Congo where Robyns found I. wittei Schulze in 1947 and into Angola where Schulze has recently discovered I. exelli Schulze (Schulze, 1955). I. Sultani Hook. and I. Walleriana Hook. are indigenous to tropical Africa and especially Zanzibar (Index Kewensis).

America

In America, species of Impatiens are found from Newfoundland to the Central American Republics (Smith, 1897). I. capensis Meerb., I. pallida Nutt., I. glandulifera Royle., and I. balsamina Linn., are the most prominent species in North America. I. capensis and I. pallida are native to this continent. They are found from south-western Newfoundland to Saskatchewan, south to Nova Scotia, the New England States, Georgia, Tennessee, Montana and Kansas (Fernald, 1950). I. glandulifera, introduced from Asia, stretches through Nova Scotia, south to New Brunswick, north to north-eastern Quebec and Ontario (Fernald, 1950). I. balsamina originally introduced from India has been cultivated in America for many years. Occasionally it may escape cultivation and become a weed (Engler and Prantl, 1889).

IV. DESCRIPTION OF EACH OF THE SELECTED SPECIES OF IMPATIENS

American Species

I. capensis Meerb.

I. capensis, spotted Touch-me-not (Johnson, 1931), was formerly known as I. biflora Walt. or I. fulva Nutt. It is an herbaceous annual growing in shaded areas in acid or subacid swamps (Fernald, 1950) or in woods where the ground has been covered with water for part of the time (Marie-Victorin, 1947). The plants are from 50-100 cm. in height.

The stems are succulent and often streaked with red and the leaves are alternate, simple, ovate and estipulate. No glands are present on the petioles.

The flowers are zygomorphic and borne several on axillary peduncles. Both cleistogamous and chasmogamous flowers may be found.

The chasmogamous flowers are the conspicuous ones and are orange with brown spots. Each flower has four sepals. The anterior sepal is notched (two sepals joined) while the posterior one is longer than broad (two sepals joined), and its spur is bent back parallel to it. There are two petals, each 2-lobed. The lateral petal is large and deeply spotted with brown while the upper petal is much smaller. There are five stamens. The scales which are attached to the filaments are connivent over the stigma which is sessile and 5-toothed (Fernald,, 1950).

The ovary is 5-laculed containing several anatropous ovules with axile placentation. The fruit is a fleshy capsule. It springs open when ripe, the five valves becoming elastically coiled and shooting

out the seeds. Each seed contains a straight embryo and lacks endosperm.

Asian Species

I. glandulifera Royle (I. roylei Walp. or I. glanduligera Lindl).

This plant is a tall coarse-growing herb, sometimes up to 180 cm. in height. I. glandulifera is most commonly found growing on waste ground or in ditches. The stems are succulent and usually streaked with red. The leaves are opposite, ovate or ovate-lanceolate, with sharply serrate margins. Pairs of glands appear along the petiole.

The flowers are purple and borne in racemes on axillary peduncles near the apex of the plant. Of the three sepals, two are ovate with prominent midribs, the third forms a large pouch with a short spur at its base. The upper petal is like the standard of a papilionaceous flower while the two laterals are irregular in shape and form a lip in front of the flower (Hutchinson, 1955). No cleistogamous flowers are present (Bennett, 1880). The five stamens overlap the ovary which is 5-loculed and contains several anatropous ovules with axile placentation. The fruits and seeds are like those of I. capensis.

African Species

I. Walleriana Hook.

I. Walleriana is a succulent herb from 60-120 cm. in height. The stem is streaked with red especially near the apex. No glands are present on the petioles.

The leaves are alternate, simple and ovate-oblanceolate with serrate margins and estipulate.

The flowers are subumbellate and borne on axillary peduncles. They are zygomorphic and fuchsia-coloured. Each flower has three sepals, the two lateral ones are small and pushed forward, while the posterior one is large and forms a long thin spur. The anterior petal is large, with two lateral and two posterior petals in pairs on each side. Each of the five stamens has a short broad filament and anther which coheres to and covers the pistil. The pistil is sessile and 5-toothed. The 5-loculed ovary contains several anatropous ovules with axile placentation. The fruits and seeds resemble those of I. capensis and I. glandulifera but are much smaller.

I. Sultani Hook.var. Beauty of Klettgau (red-flowered).

I. Sultani is an herbaceous plant from 60-100 cm. high. The stems are succulent and streaked with red. There are pairs of stalked glands along the petioles. The leaves are estipulate, simple, and lanceolate with serrate margins. The lower leaves are alternate, but the upper ones are whorled (Bailey, 1922).

Cleistogamous flowers are not present. The resupinate chasmogamous flowers are red and borne two to an axillary peduncle. Of the three sepals, the two lateral ones are small and curve toward the anterior, while the posterior one is large and forms a short slender spur. Each flower has five petals. The anterior one is large while the laterals and posteriors are in pairs on each side of the flower. The stamens, five in number, have short broad filaments and anthers which are connate over the pistil. The pistil is very short and 5-toothed.

The ovary is 5-loculed and contains several anatropous ovules with axile placentation. The fruits and seeds are like those of I. Walleriana.

I. Sultani Hook. var. _____ (pink-flowered)

Flower colour is the only significant difference between this and the preceding variety. This variety of I. Sultani has a bright pink flower while the flowers of I. Sultani var. Beauty of Klettgau are red. It is believed by Ottley (1918) that the original I. Sultani was red and that the pink varieties are sports or hybrids.

V. REVIEW OF THE LITERATURE.

Embryology.

Monosporic 8-nucleate Embryo Sac.

The monosporic, bisporic and tetrasporic types of embryo sac development are well known (Johansen, 1945; and Maheshwari, 1941, 1945, 1948, 1950, and 1955). It should be recalled that apart from the number of megaspores which take part in the development of the sac, the further classification depends on the number of nuclear divisions involved and the total number of nuclei present in the mature sac.

Impatiens follows the monosporic Polygonum Type of development. In this type one of the cells of the sporogenous tissue in the developing ovule enlarges to form an archesporial cell. This cell gives rise to the megaspore mother cell either by further enlargement or by division. Four megaspores result from meiosis of the megaspore mother cell. Three of these megaspores degenerate. The remaining functional megaspore divides giving rise to two nuclei, one at either end of the elongating sac. The second division gives rise to a pair of nuclei at either end of the sac while the third division results in the formation of a micropylar quartet of nuclei and a chalazal quartet. This 8-nucleate sac differentiates in such a way that a 3-celled egg apparatus (an egg and two synergids) and an upper polar nucleus are situated at the micropylar end of the sac and three antipodal cells and a lower polar nucleus lie at the chalazal end of the sac.

Previous Embryological Studies on Impatiens.

Brunotte (1900), Longo (1909), Raitt (1916), Ottley (1918), Caroll (1919), Lebon (1929), Dahlgren (1934) and Steffen (1948, 1951) have worked on various phases in the embryology of different species of Impatiens.

The ovule of I. Sultani (Ottley, 1918) possesses one hypodermal archesporial cell which is also the megaspore mother cell. Dahlgren (1934) found the same situation in I. glandulifera. Steffen (1948) reported on the many celled archesporium of the Balsaminaceae. Double archesporia occurred in 1% of the cases in I. glandulifera and 0.5% of the cases in I. matheola. Most of these double archesporia are subepidermal cells. In 3.3% of the cases the double archesporium developed to the prophase of the archespore, in 1.3% development was up to the tetrad and in 0.12% normally differentiated sacs were produced.

The nucellus of the species of Impatiens studied (Raitt, 1916; Ottley, 1918; Caroll, 1919) was found to be a single layer of cells at the sides and the micropylar end of the sac; below the embryo sac it was composed of five rows of elongated tracheid -like cells which disappeared as the embryo sac enlarged. All authors were in agreement that there are two integuments. Ottley (1918) stated that the outer integument arises from the inner integument in I. Sultani. However, Raitt (1916) and Longo (1909), in I. pallida and I. capensis noted that the outer integument varies in origin and extent.

Ottley (1918), Dahlgren (1934) and Steffen (1951) reported in I. Sultani and I. glandulifera that the archesporial cell enlarges to

become the megaspore mother cell without cutting off a tapetal cell.

Ottley (1918) stated that the embryo sac of I. Sultani is bisporic in origin. At one point, however, she mentioned the origin of the sac as that of Lilium, while in another case she considered the origin as bisporic. Steffen (1951), Caroll (1919), and Raitt (1916) observed that the sacs of I. glandulifera, I. capensis and I. pallida are monosporic arising from the chalazal megaspore of a normal tetrad. Dahlgren (1934) accepts the view that I. glandulifera is monosporic.

A normal 8-nucleate embryo sac is formed in all the species studied. The egg is located below the two synergids with filiform apparati. Ottley (1918) reported a change in the situation of the synergid nuclei according to their age. Steffen (1951) noted the enlargement and persistence of one or both synergids. The two polar nuclei lie near the egg and they do not fuse until the sperm nucleus appears beside them. The antipodal cells are ephemeral in all cases. The embryo sac of I. Sultani (Ottley, 1918) absorbs the nucellar epidermis and by means of an antipodal haustorium destroys all the nucellus between the sac and the chalaza.

Raitt (1916) and Caroll (1919) reported that the pollen tube branches on entering the sac in I. pallida and I. capensis. Steffen (1951) observed two pollen tubes entering the embryo sac of I. glandulifera and he recorded dispermy in 0.3% of the cases. Raitt (1916) and Caroll (1919) may have interpreted two pollen tubes as a branching pollen tube.

The early authors, Brunotte (1900), Longo (1909), Raitt (1916),

Ottley (1918) and Caroll (1919) observed that I. amphorata, I. pallida, I. Sultani and I. capensis have nuclear endosperm. However, I. Sultani (Lebon, 1929) and I. glandulifera (Dahlgren, 1934; Steffen, 1951) have cellular endosperm.

Caroll (1919) in his study of I. capensis did not report endosperm haustoria. In. I. pallida, Raitt (1916) observed that the endosperm produces an haustorial protuberance into the micropyle. Several nuclei migrate into this haustorium. Lebon (1929) traced the origin of the chalazal haustorium in I. Sultani. Here, the endosperm nucleus divides transversely to form a small micropylar cell and a large chalazal cell. While the micropylar cell divides transversely, the chalazal cell also divides transversely to produce two cells and the lower one penetrates into the chalazal tissue. Longo (1909) observed micropylar and chalazal haustoria in I. amphorata which arise from the nucellar tissue. Because the micropylar and chalazal haustoria of I. Sultani arise from the endosperm, Ottley (1918) believed the same to be true of I. amphorata. The micropylar haustorium of I. Sultani is extensive entering the outer integument and penetrating the funiculus where it branches. When first formed, the micropylar haustorium is multinucleate but later most of the nuclei degenerate. The chalazal haustorium is a simple uninucleate protuberance which degenerates soon after the division of the zygote. The origin and development of the endosperm and haustoria in I. glandulifera have been clearly discussed by Dahlgren (1934). The primary endosperm nucleus divides into two chambers. Free nuclear divisions occur in the large chalazal chamber. The small

micropylar chamber forms three cells. The uppermost of these develops a large haustorium which branches into the funicular tissue. The second cell gives rise to the endosperm which surrounds the young embryo. After several free nuclear divisions have occurred in the third cell, it fuses with the large basal chamber.

Haustoria

Definition

Crété (1951) gives two definitions for haustoria. The first states that an haustorium is a cell or group of cells which represent by their typical formations in related families and genera, an identity of origin, location and destination. The second defines an haustorium as an element or group of elements which has undergone a notable increase in size and whose nuclei have become greatly hypertrophied.

For the purposes of the present study a combination of these two definitions seems indicated. Namely, an haustorium is a cytoplasmic projection of a cell or group of cells which may or may not contain one or more hypertrophied nuclei and which has a specific origin, nature, and extent.

Types

Haustoria may be of six different types depending on their origin.

Megaspore Haustoria

One or more megaspores of a tetrad may produce lateral

tubes which grow upward into the nucellar tissue. While this is not a common phenomenon it has been reported in Rosularia and Sedum (Mauritzon, 1933), Laurus (Bambacioni-Mezzetti, 1935), some members of the Rubiaceae (Fagerlind, 1937) and Potentilla (Rutishauser, 1945).

Synergid Haustoria

In most cases, the synergids degenerate and disappear before or soon after fertilization occurs. However, a few cases have been reported in which one or both synergids persist after fertilization and sometimes show increased activity.

Dahlgren (1924) and Schurhoff (1926) found in the genus Calendula that a large synergid haustorium is developed. Dahlgren (1924) has reported a similar synergid haustorium in Ursinea anthemoides which grows toward the micropylar end of the ovule and reaches nearly to the funiculus.

Cooper (1942a) noted the existence of synergid haustoria in Lobelia cardinalis, but Subramanyam (1951) reported these to be endosperm haustoria.

Many investigators have observed the persistence of synergids for long periods after fertilization. Rodolico (1930) noted that the synergids are very prominent in Buphthalmum salicifolium and Paetow (1931) found this to be true also in Dysoxylem ramifolia. Fedortschuk (1931) observed the persistence of one synergid in Cuscuta monogyna until the embryo is well advanced. Mauritzon (1936) showed that in Berberis vulgaris, B. cretica, B. empetrifolia and Mahonia

aquifolium one synergid persists and probably assumes an haustorial character. Persistent synergids have been observed in Angelonia grandiflora (Srinivasan, 1940) and Cuscuta hyalina (Tiagi, 1951a). Johri and Tiagi (1952) noted that one of the synergids in Cuscuta reflexa enlarges after fertilization and can be recognized until the proembryo has elongated and the stem tip has differentiated.

Antipodal Haustoria

Antipodal cells, like synergids, are usually short lived, but they may remain after fertilization and increase in size and number (Maheshwari, 1950).

Sometimes one or more antipodals give rise to haustoria which penetrate the nucellar tissue. Chamberlain (1895) reported that the lower antipodal cell of Aster Novae-Angliae differs in its size, consistency of cytoplasm, appearance of nuclei and effect on the surrounding tissues. He writes of "this antipodal growth" destroying the adjacent nucellar tissues and sometimes equalling half the length of the original sac. However, it is not clear from his account whether "this antipodal growth" refers to the enlarged antipodal cell or to an elongation of the sac itself.

Coulter and Chamberlain (1903) described the enlargement of the basal antipodal cell and its penetration into the chalazal tissue as a common feature of the Compositae.

Two antipodal cells of Grindelia squarrosa (Howe, 1926) persist after fertilization. One or both of them grow laterally into

the integument and extend nearly to the surface of the ovule.

In Putoria (Fagerlind, 1936) and Galicum (Fagerlind, 1937) the basal antipodal cell gives rise to an aggressive haustorium. In Artemisia (Diettert, 1938) the basal antipodal cell penetrates the chalazal tissue.

Rao (1942) studied the antipodal cells of four genera of the Santalaceae. The antipodals of Thesium disappear immediately after fertilization, those of Osyris become organized and persist until endosperm formation, and those of Santalum enlarge but disappear when the endosperm haustorium reaches the antipodal end of the sac. In Scleropyrum the antipodal nuclei are never organized as cells. As the antipodal embryo sac haustorium elongates, the antipodal nuclei divide and migrate into it.

The antipodal cells of Chrysothamnus nauseosus speciosus (Snow, 1948) often divide so that eight or ten cells are formed. The basal one elongates rapidly to form an haustorium.

Haustorial antipodal cells have also been observed by Rao (1955) in Chrysalidocarpus lutescens. Sachar (1955) reported the presence of very large antipodal cells in Argemone mexicana. These cells with large hypertrophied nuclei increase greatly in size after fertilization. The enlarged antipodals produce the same effect on the surrounding tissues as haustoria, although no haustorial projections are formed.

Suspensor Haustoria

In general, in angiosperms, the function of the suspensor

is confined to pushing the embryo deeper into the endosperm. There are instances, however, where the suspensor cells give rise to prominent haustoria which penetrate the cells of the endosperm and in some cases reach the surrounding tissues of the ovule.

Thus, in some members of the Orchidaceae, the basal cell of the proembryo forms a filament of cells which frequently becomes very long and twisted and grows out as far as the placenta (Treub, 1879: Stenar, 1937, 1940; Swamy, 1941).

In three members of the Fumariaceae (Souèges, 1946.) three-fourths of the zygote is given over to the production of an haustorial suspensor.

Within the Leguminosae there are many variations in the suspensor. Some members of the family have no suspensor, sub-family Mimosaceae; others have a rudimentary suspensor, Trifolium; and others have large spherical haustorial suspensors, Pisum, Melilotus, Vicia, Crotalaria (Guignard, 1882; Cooper, 1938; Souèges, 1948; Rau, 1950).

The suspensor haustoria of the Crassulaceae are abundantly branched and so extensive that they often appear outside the ovule (Mauritzon, 1933).

The Geraniales show a wide variety of suspensor modifications. Erythroxylum has no suspensor haustorium, while Radiola and Biophytum have quite well developed ones (Mauritzon, 1934a). Walker (1947) has reported long massive haustorial processes in Tropaeolum majus.

Stolt (1928) and Souèges (1940) observed the suspensor haustoria in Myriophyllum. The large basal cell of the embryo divides

longitudinally forming two daughter cells which become the suspensor haustoria and show a very marked resemblance to synergids.

Suspensor haustoria have also been noted in five members of the Convolvulaceae (Raghava Rao, 1940) and also in members of the Rubiaceae (Hofmeister, 1858; Lloyd, 1902; Souèges, 1925).

Embryo sac Haustoria

Angiosperms may show growth at the ends of their embryo sacs in the form of haustorial projections.

The drawings made by Guignard (1885) of embryo sacs of certain members of the Santalaceae illustrate that chalazal and micropylar haustoria are formed. However Guignard does not mention them as such in the context of the paper. In Dendrophthora (York, 1915) and other members of the Loranthaceae (Rauch, 1936; Singh, 1950) the integuments are absent and the sacs elongate considerably toward the chalazal and the micropylar ends.

In Phaseolus vulgaris (Weinstein, 1926), Thesium intermedium (Modilewski, 1928a) and Melilotus (Cooper, 1933) the micropylar end of the nucellus breaks down and the embryo sac elongates greatly, the basal portion becoming tubular and embedding itself deeply in the nucellus, and the apical portion extending so as to lie in direct contact with the cells lining the micropylar canal. The embryo sacs of Rosularia pallida and Sedum sempervivoides develop persistent haustoria (Mauritzon, 1933). In Utricularia (Kausik, 1938a), Vandellia and Torenia (Krishna Iyengar, 1940a, 1941) the nucellar tissue breaks down very early and the embryo

sac protrudes out of the ovule while the chalazal part of the sac comes in direct contact with the placenta. Bell (1957) has reported the presence of micropylar and chalazal embryo sac haustoria in the blueberry.

In some plants the chalazal end of the embryo sac pushes through the nucellar tissues just before or during fertilization. This type of haustorium, called a caecum, has been observed in Myzodendron punctulatum (Johnson, 1890), Allium(Modilewski, 1928b), Santalum album (Srinivasa Iyengar, 1937), Lobelia (Kausik, 1938b; Subramanyam, 1949), Oxybaphus nyctagineus (Cooper, 1949), Cephalostigma schimperi (Kausik and Subramanyam, 1947), Cassia (Rau, 1951a), and Opilia amentacea (Shamanna, 1955).

The embryo sac haustorium arises laterally instead of from the poles of the sac in Physostegia (Sharp, 1911), Veltheimia (Stiffler, 1925), Anthericum (Schnarf, 1928), Paradisea (Stenar, 1928) and Comandra umbellata (Ram, 1957).

Endosperm Haustoria.

Haustoria developed from endosperm tissue are those which have been most frequently observed.

Nuclear Endosperm Haustoria

In some members of the Proteaceae (Kausik, 1938a,b; 1942) most of the endosperm nuclei are distributed in the upper region of the embryo sac. In Macadamia ternifolia, cells form in this region while the lower part of the sac develops into a lobed and branched free

nuclear haustorium. However, in Grevillea robusta the endosperm is organized into three distinct regions. The first consists of small regularly arranged cells, the second consists of large irregularly arranged cells, and the third is a coiled tubular free nuclear haustorium referred to as a vermiform appendage.

Members of the Rutaceae possess nuclear endosperm which forms a J-shaped tubular haustorium (Mauritzon, 1935a).

The chalazal part of the free nuclear endosperm in some of the Leguminosae (Dnyansagar, 1954a, b) becomes a tubular haustorium which remains as late as the appearance of the cotyledons.

In Rothia trifoliata, Glycine javonica, Teramnus labiales and Cassia (Anantaswamy Rau, 1951a, 1951b, 1951c, 1953) a few endosperm nuclei are situated at the chalazal end of the embryo sac. Here a tubular free nuclear haustorium develops. This condition is also found in the Cucurbitaceae (Chopra, 1955) and in Chrysalidocarpus lutescens (Rao, 1955).

Cellular Endosperm Haustoria.

The haustoria formed by cellular endosperm may arise at the chalazal end of the sac, or at the micropylar end, or at both ends. The occurrence, origin, and nature of these haustoria have been used to set up a phylogenetic system within or among certain families such as the Diapensaceae and the Empetraceae (Samuelsson, 1913), the Scrophulariaceae (Schmid, 1906; Glišić, 1936-1937; Krishna Iyengar, 1940b), the families within the Gamopetaleae (Crété, 1951), and the Orobanchaceae, the

Scrophulariaceae and the Bignoniaceae (Crété, 1955).

The evolutionary tendency in the Scrophularineae (Krishna Iyengar, 1940b) and in other families is a reduction in the number of cells and nuclei composing the haustoria. A summary and comparison of the haustoria types in cellular endosperm based on this evolutionary tendency is presented in tabular form. (Table 1)

TABLE I. HAUSTORIA TYPES IN CELLULAR ENDOSPERM

	PLANT	HAUSTORIA	COMMENTS	AUTHOR
MICROPYLE	Lantana in dica	M^{x}	2 cells uninucleate	Tatachar, 1940.
	Hydrocera triflora	M	1 cell, multinucleate	Venkateswarlu, Atchutaremamurti, 1955a.
CHALAZA	Phryma leptostachya	C^{ex}	4 cell, uninucleate	Cooper, 1941
	Verbena	C	1 cell, uninucleate	Kanda, 1920
	Santalum album	C	1 cell, uninucleate	Srinivasa Iyengar, 1937.
	Peltandra virginica	C	1 cell, uninucleate	Goldberg, 1941.
	Wolffia	C	1 cell, uninucleate	Maheshwari, 1954.
	Striga lutea	C	1 cell, uninucleate	Michell, 1915.
	Comandra umbellata	C	1 uninucleate primary haustorium 4 uninucleate secondary haustoria	Ram, 1957.
MICROPYLE AND CHALAZA	Ehretia laevis	M C	4 cells, uninucleate 4 cells, uninucleate	Johri, Vasil. 1956
	Isoplexis caneriensis	M C	4 cells, uninucleate 4 cells, uninucleate	Krishna Iyengar, 1939.
	Celsia coromandeliana	M C	4 cells, uninucleate 4 cells, uninucleate	Krishna Iyengar, 1939.
	Caltalpa	M C C	4 cells, uninucleate secondary haustoria 4 cells, uninucleate	Mauritzon 1935b.
	Torenia	M C	4 cells, uninucleate 2 cells, uninucleate	Krishna Iyengar, 1941.
	Sopubia trifida	M C	4 cells, uninucleate 2 cells, uninucleate	Krishna Iyengar, 1940c.

TABLE I. HAUSTORIA TYPES IN CELLULAR ENDOSPERM

	PLANT	HAUSTORIA	COMMENTS	AUTHOR
	Ilysanthes hyscopioides	M	4 cells, uninucleate	Krishna Iyengar, 1940b.
		C	2 cells, uninucleate	
	Bonnaya tenuifolia	M	4 cells, uninucleate	Krishna Iyengar, 1940b.
		C	2 cells, uninucleate	
	Sopubia delphinifolia	M	4 cells, uninucleate	Krishna Iyengar, 1937.
		C	2 cells, uninucleate	
	Alonsoa	M	4 cells, uninucleate	Krishna Iyengar, 1937.
		C	2 cells, uninucleate	
MICROPYLE AND CHALAZA	Scrophularia Marylandica	M	4 cells, uninucleate	Schertz, 1919
		C	4 cells, uninucleate	
	Coldenia procumbens	M	4 cells, uninucleate	Venkateswarlu Atchutarmamurti, 1955b.
		C	1 cell,, binucleate	
	Pedicularis palustris	M	2 cells, binucleate	Steffen, 1956.
		C	1 cell, binucleate	
	Pentstemon secundiflorus	M	2 cells, uninucleate	Evans, 1919.
		C	4 cells, uninucleate	
	Trapella	M	2 cells, uninucleate	Oliver, 1888.
		C	2 cells, uninucleate	
	Lobelia nicotianaefolia	M	2 cells, uninucleate	Kausik, 1938b.
		C	2 cells, uninucleate	
	Lobelia trialata	M	2 cells, uninucleate	Kausik, Subramanyam, 1945.
		C	2 cells, uninucleate	
	Lobelia pyramidalis	M	2 cells, uninucleate	Subramanyam, 1949.
		C	2 cells, uninucleate	
	Lobelia cardinalis	M	2 cells, uninucleate	Subramanyam, 1951.
		C	2 cells, uninucleate	
	Capalostigma schimperi	M	2 cells, uninucleate	Kausik, Subramanyam, 1947.
		C	2 cells, uninuclate	
	Blueberry	M	2 cells, uninucleate	Bell, 1957.
		C	2 cells, uninucleate	

TABLE I. HAUSTORIA TYPES IN CELLULAR ENDOSPERM

	PLANT	HAUSTORIA	COMMENTS	AUTHOR
	Veronica	M	2 cells, uninucleate	Weib, 1932.
		C	1 cell, binucleate	
	Orobanche uniflora	M	2 cells, uninucleate	Cassera, 1935.
		C	1 cell, binucleate	
	Stachytarpheta indica	M	2 cells, uninucleate	Tatacher, 1940
		C	1 cell, binucleate	
	Didymocarpus tomentosa	M	2 cells, uninucleate	Thathachar, 1942.
		C	1 cell, binucleate	
MICROPYLE AND CHALAZA	Rehmannia angulata	M	2 cells, uninucleate	Krishna Iyengar, 1942.
		C	1 cell, binucleate	
	Plantago lanceolata	M	2 cells, uninucleate	Cooper, 1942b.
		C	1 cell, binucleate	
	Striga orobanchoides	M	2 cells, uninucleate	Tiagi, 1956.
		C	1 cell, binucleate	
	S. euphrasioides	M	2 cells, uninucleate	Tiagi, 1956.
		C	1 cell, binucleate	
	Rhyncoglossum obliquum	M	2 cells, uninucleate	Thathacher, 1943.
		C	1 cell, uninucleate	
	Bignonia megapatomica	M	1 cell, tetranucleate	Swamy, 1941.
		C	2 cells, uninucleate	
	Orthosiphon stamineus	M	1 cell, tetranucleate	Murthi, 1947.
		C	1 cell, binucleate	
	Orobanche aegyptiaca	M	1 cell, binucleate	Tiagi, 1951b.
		C	1 cell, binucleate	
	Utricularia coerulea	M	1 cell, binucleate	Kausik, 1938a.
		C	1 cell, binucleate	
	Acanthaceae	M	1 cell, binucleate	Mauritzon, 1934b.
	Ruellia	C	1 cell, tetranucleate	
	Blechum			
	Asteracantha			
	Hemigraphis			
	Brillantaesia			

TABLE I. HAUSTORIA TYPES IN CELLULAR ENDOSPERM

	PLANT	HAUSTORIA	COMMENTS	AUTHOR
MICROPYLE AND CHALAZA	Acanthaceae	M	1 cell, binucleate	Mauritzon, 1934b.
	Beloperone	C	1 cell, binucleate	
	Justicia			
	Adhatoda			
	Cerathemum			
	Jacobinia			
	Schaveria			
	Vandellia	M	1 cell, binucleate	Krishna
	hirsuta	C	1 cell, tetranucleate	Iyengar, 1940a.
	V. scabra	M	1 cell, binucleate	Krishna
		C	1 cell, teteranucleate	Iyengar, 1940a
	Dipteracanthus	M	1 cell, binucleate	Maheshwari,
	Patulus	C	1 cell, tetranucleate	Negi, 1955.
	Impatiens	M	1 cell, uninucleate	Dahlgren, 1934.
	glandulifera	C	1 cell, multinucleate	
	Salvia	M	1 cell, uninucleate	Carlson,
		C	1 cell, binucleate	Stuart, 1936.
	Orobanche	M	1 cell, uninucleate	Tiagi, 1951b.
	cernua	C	1 cell, binucleate	
	Incarvillea	M	1 cell, uninucleate	Mauritzon, 1935b.
		C	1 cell, uninucleate	

x Micropylar

xx Chalazal

Helobial Endosperm Haustoria.

Monochoria (Ono, 1928; Juliano, 1931) whose endosperm is of the helobial type possesses endosperm haustoria. After the micropylar and chalazal endosperm chambers are formed, two tubular outgrowths are produced from the micropylar chamber and these grow into the chalazal tissue. Later these two haustoria fuse with the main body of the endosperm.

VI. MATERIALS AND METHODS.

A collection of buds, open flowers and fruits of *I. capensis* was made in August, 1957 at Petite Rivière, Nova Scotia. The materials for the study of *I. glandulifera*, *I. Walleriana* and both varieties of *I. Sultani* were collected in the McGill University Greenhouses.

The petals of the open flowers of all species were removed and the buds and fruits were slit with a razor blade to ensure rapid penetration of the fixing fluid. The material was immediately placed in bottles containing the fixative (absolute alcohol and glacial acetic acid 1:1) and pumped for two minutes with an Anco-Hyvac Vacuum Pump.

Fisher Tissuemat with an A.S.T.M. Melting Point 54-56° C. and 56-60° C. was used as an embedding medium. Kraft P.C. containers served as embedding boats. These plastic containers were employed instead of paper boats because they could be re-used indefinitely. The buds of all species were cut at 8 micra, while open flowers and fruits were cut at 12 micra.

The sections were mordanted in 3% iron alum for three hours, stained in 0.5% Heidenhain's Iron Hematoxylin for twelve hours and destained with a weak solution of iron alum.

The use of eosin as a counter stain with Heidenhain's Iron Hematoxylin proved beneficial in bringing out the structural details of both synergid and endosperm haustoria.

A Spencer Research Microscope with achromatic lenses was used throughout the study.

Kodak Super Pancho Press Type B film was used in photomicrography and Kodalith Ortho 2 film in photographing the drawings. Magnifications of the drawings and photomicrographs were calculated by comparing the measurements of the diameters of nuclei and the length and breadth of cells in the photographs with the actual measurements of these structures.

VII. RESULTS.

Embryology.

I. capensis.

In I. capensis several anatropous ovules develop in each of the five carpels. Embryo sac development is of the Polygonum Type. The archesporial cell differentiates just below the nucellar epidermis and by the time the ovule reaches a length of 41.9 micra it is 10.7 micra in diameter. At this time the nucellus consists of richly protoplasmic rectangular cells with large nuclei enveloped by the nucellar epidermis. The inner integument originates about 7.1 micra below the archesporial cell (Plate 1, Figure 1).

The megaspore mother cell arises directly from the archesporial cell, and is approximately 23.4 micra when meiosis begins (Plate 1, Figure 2). By the time the megaspore mother cell reaches pachytene of meiosis 1 (Plate 1, Figure 3), the outer integument is developing from the nucellus below the inner integument. Plate 1, Figure 4 shows a megaspore mother cell in the late prophase of meiosis. At this stage the inner integument has enclosed the nucellus and the outer integument is approximately 30 micra in length.

The megaspore mother cell divides to form a dyad. The micropylar cell of the dyad divides first (Plate 1, Figure 6) and the two resulting cells begin to degenerate immediately. One of the cells resulting from the division of the chalazal cell of the dyad (the micropylar) degenerates, leaving the other to become the functional megaspore (Plate 11, Figure 7). Both T-shaped and linear tetrads are

found (Plate II, Figure 7, 8 and 9).

The functional megaspore divides to form the 2-nucleate stage of the developing sac (Plate II, Figure 10). Two subsequent synchronous divisions of these nuclei occur resulting in the 8-nucleate embryo sac (Plate II, Figure 12).

The mature sac organizes promptly with the egg apparatus at the micropylar end. The egg is small and lies below the two synergids. The latter are somewhat elongated cells (15.2 micra long) with rounded tips. The fourth nucleus at the micropylar end of the sac becomes a polar nucleus. At the chalazal end, three antipodal cells are formed but degenerate promptly. The polar nucleus from this group and the polar nucleus from the micropylar group move to a position near the center of the sac (Plate II, Figure 12). The nucellar cells become absorbed while the sac increases in length from 27 to 100 micra.

As the polar nuclei move together the egg enlarges and extends toward the middle of the sac and the surrounding cytoplasm becomes denser. The synergids increase rapidly in length averaging between 30 and 80 micra. A large vacuole is formed at the tip of each synergid and the nucleus lies above the vacuole. The ends are club-shaped (Plate III, Figure 13). At first the synergids lie side by side, but as they lengthen, they overlap each other (Plate III, Figure 14 and 15). The portion of the synergid which extends into the micropylar canal is much thinner than the remainder and the micropylar end tapers to a point. As the synergids develop into the micropylar canal a vacuole appears between the nucleus and the extending tip so that some cytoplasm is

concentrated at this upper end. Synergids have been observed extending as far as five-eighths of the length of the micropylar canal. The nucellar tissue in the micropylar canal region is absorbed. The contents of the integumentary cells next to the micropylar canal show signs of depletion (Plate III, Figures 14 and 15). However, the synergids are always unicellular and in no case show any branching into the surrounding tissue. At this stage the micropylar end of the sac has broadened considerably while the length of the sac is now approximately 200 micra (Plate III, Figure 16).

One or both of the synergids disappear when the pollen tube enters the sac. However, in some cases both synergids persist after the entrance of the pollen tube. A small vacuole can be seen at the tip of the pollen tube and the sperm nuclei and tube nucleus are situated above it (Plate IV, Figure 18). At the time of entry of the pollen tube and its progress toward the egg and the polar nuclei, the synergids lose some of their intense staining capacity, and their nuclei become hypertrophied (Plate IV, Figures 17, 18, and 19). Frequently more than one pollen tube enters the embryo sac (Plate IV, Figure 20).

Plate V, Figure 21 shows the fusion of one sperm nucleus with the egg and the other with the polar nuclei.

Apart from this normal type of fertilization, two sperm nuclei have been observed lying near the egg which would seem to be explained by the entry of more than one pollen tube (Plate V, Figure 22).

After fertilization the endosperm nucleus divides (Plate V, Figure 23). The first division of the endosperm nucleus is followed

by wall formation, giving rise to a cellular type of endosperm (Maheshwari, 1950). The sac is divided into a small micropylar chamber and a large chalazal chamber (Plate V, Figure 24). Divisions take place in both chambers. The divisions in the micropylar chamber are followed by wall formation, while those in the chalazal chamber are free nuclear. After two or three cellular divisions in the endosperm, the zygote divides transversely (Plate V, Figure 25). The embryo is of the Asterad Type (Maheshwari, 1950).

As soon as cells are formed in the micropylar chamber, two cells migrate toward the micropylar end of the sac on either side of the dividing zygote. This gives rise to two uninucleate endospermal projections one on each side of the zygote.

Haustoria varying in width from 1.42 micra to 2.81 micra arise from these cells and extend into the micropylar canal. These projections adhere closely to the sides of the canal and branches from them invade the cells of the integument for a short distance. At the same time nuclear divisions take place in the haustoria so that they become multinucleate (Plate VI, Figure 26). The haustorial projections fuse and are still present when differentiation begins in the embryo (Plate VI, Figure 27).

Although a great deal of endosperm is formed, all except a peripheral cellular layer is absorbed by the time the cotyledons have differentiated (Plate VI, Figure 29).

I. glandulifera.

The archesporial cell gives rise directly to the megaspore mother cell. The megaspore mother cell is surrounded by a layer of nucellar cells at the sides and at the micropylar end. At the base of the megaspore mother cell the nucellar cells are narrow. The megaspore mother cell is approximately 47.6 micra in diameter, and by the time meiosis sets in, it is nearly surrounded by the inner integument. The outer integument is also well advanced (Plate VII, Figure 30).

Frequently two archesporial cells occur. In this case the micropylar archesporial cell divides once almost at right angles to the axis of the embryo sac and both of the daughter cells degenerate. The chalazal archesporial cell then proceeds through normal stages and gives rise to a liniar tetrad of megaspores. The chalazal megaspore becomes the functional megaspore and divides to form the 2-nucleate embryo sac. The three micropylar megaspores degenerate (Plate VII, Figure 31).

A normal 8-nucleate (Polygonum Type) embryo sac is formed. The egg apparatus situated at the micropylar end of the sac consists of two elongated synergids (12.1 micra long) and a smaller egg below them. The polar nuclei are large (about 8 micra in diameter) and lie in the center of the sac. The three antipodal cells are situated at the chalazal end of the sac. Of these, two lower ones are somewhat longer than the upper one and have vacuoles at their tips. The whole sac is about 74.5 micra long at this time and lacks vacuoles (Plate VII, Figure 32). Slightly later it becomes vacuolate (Plate VII, Figure 33).

In the mature sac, the synergids lengthen (24.9 micra), the egg protrudes into the sac, the polar nuclei lie very close together, and the antipodals begin to degenerate. At this time a small tubular projection can be seen at the chalazal end of the sac in which the antipodals are degenerating (Plate VII, Figure 34).

This projection develops into a long branching haustorium which penetrates the nucellar tissues at the chalazal end of the sac. The synergids have vacuolate tips and hooked indentations. These extend further into the micropyle (Plate VIII, Figures 35a, 35b and 36).

In some sacs the polar nuclei fuse before the entrance of the pollen tube and the synergids with their hypertrophied nuclei continue growth becoming 80.9 micra in length (Plate VIII, Figure 37). However, in the majority of cases observed the pollen tube enters the sac before fusion of the polars (Plate VIII, Figure 38). At this time the embryo sac haustorium is branching in the chalazal nucellar cells (Plate IX, Figure 39).

It is usual for a number of pollen tubes to enter the sac and develop toward the egg and the fused polar nuclei (Plate IX, Figure 40). The synergids disappear. The polar fusion nucleus is very large reaching a diameter of 25.6 micra. At this time, the whole embryo sac extends some distance into the micropyle and the integumentary tissues of this region are nearly depleted of their contents. Just below its micropylar extension the sac widens for a short distance and then becomes narrow again. The widening is in the region of the polar fusion nucleus and the nucellar tissue in this region is absorbed. The embryo sac

haustorium is still evident at the time of fertilization (Plate IX, Figure 41).

The fertilized egg degenerates immediately after the entrance of the pollen tube which would seem to be explained by the incompatibility of the sperm nuclei and the egg. However, the chalazal haustorium continues its growth and when it reaches the vascular trace leading from the funiculus, it turns and extends into the funicular tissue (Plate X, Figure 44).

A few free nuclear divisions occur in the endosperm. The nuclei are concentrated near the micropylar region and the cytoplasm sends out cytoplasmic projections which penetrate the nucellar and integumentary cells which border the sac. The egg with the sperm lying near it does not fuse or divide (Plate X, Figure 45).

Fruits with viable seeds could not be found. (Plate X, Figure 46).

I. Walleriana

The archesporial cell of I. Walleriana is differentiated by the time the ovule has reached a length of approximately 40 micra and itself is 14.2 micra in diameter. The inner integument is already developing some 17 micra below it (Plate XI, Figure 48). Two archesporial cells in one ovule are fairly common (Plate XI, Figure 47).

The archesporial cell develops directly into the megaspore mother cell. In the prophase of meiosis 1, the megaspore mother cell is 31.2 micra long and the inner integument has developed slightly beyond the base of the megaspore mother cell. There is as yet no sign of the

outer integument (Plate XI, Figure 49).

By the time the first meiotic division occurs, the megaspore mother cell is 40.5 micra in length, the inner integument has enveloped the cell and the outer integument is observable (Plate XI, Figure 50). The megaspore mother cell divides (Plate XI, Figure 51) to form a dyad (Plate XI, Figure 52). Both cells of the dyad divide and the two cells of the micropylar pair degenerate. The upper cell of the chalazal pair degenerates while the lower one (Plate XII, Figure 53) becomes the functional megaspore (Plate XII, Figure 54).

After a very short interval, the functional megaspore divides giving rise to the 2-nucleate stage of the embryo sac (Plate XII, Figure 55). Each of these nuclei divides simultaneously to form the 4-nucleate sac (Plate XIII, Figure 56). A third synchronous division produces the 8-nucleate embryo sac (Plate XIII, Figure 57).

The organized embryo sac is 51.8 micra long and consists of an egg apparatus (two synergids and an egg) at the micropylar end, two polar nuclei in the middle of the sac, and three ephemeral antipodal cells at the chalazal end of the sac. The two synergids have large nuclei and vacuoles at the ends nearest the micropyle. The egg lies a short distance below the two synergids. One of the antipodal cells is larger than the other two (Plate XIII, Figure 58).

The chalazal end of the mature embryo sac forms an haustorium which penetrates the chalazal tissue (Plate XIII, Figure 59).

During the process of fertilization one or both of the synergids disappear. The polar nuclei do not fuse prior to the arrival of the

sperm nucleus and then all three fuse together (Plate XIII, Figure 60). At the time of fertilization the antipodal embryo sac haustorium which appeared at the onset of the maturing sac branches and penetrates deeper into the chalazal tissue (Plate XIII, Figure 61).

The endosperm nucleus divides a number of times before the division of the zygote. The micropylar chamber formed by the division of the endosperm nucleus is smaller than the chalazal chamber and the divisions here are cellular, while those in the chalazal chamber are nuclear. Cytoplasmic projections from the first two cells of the endosperm nearest the micropyle can be observed extending up the micropylar canal. One endosperm nucleus from the chalazal chamber enlarges and migrates into the chalazal embryo sac haustorium (Plate XIV, Figure 62).

The first division of the zygote is transverse (Plate XIV, Figure 63) and the embryo development is of the Asterad Type (Maheshwari, 1950). By the time the embryo is 12-celled, the micropylar haustorium (formed by the fusion of the two projections from two cells of the endosperm) protrudes from the micropyle and enters the funicular tissue. At the point where the haustorium leaves the micropyle and enters the funicular tissue, it enlarges and becomes densely protoplasmic. Two large nuclei are present in the globular mass of cytoplasm. The antipodal embryo sac haustorium contains one large endosperm nucleus (Plate XIV, Figure 64). As the embryo enlarges and the suspensor develops, there is a concentration of cellular endosperm between the embryo and the micropylar end of the sac. Only a few endosperm nuclei are found at the periphery of the sac in the chalazal region. The micropylar haustorium continues to

expand and the chalazal haustorium degenerates (Plate XIV, Figure 65: Plate XV, Figures 66 and 67).

The micropylar haustorium continues its development throughout the differentiation of the embryo. Its two nuclei enlarge, but they remain in the mass of cytoplasm between the micropyle and the funiculus. Cytoplasmic projections extend from this area and penetrate the funicular and placental tissues (Plate XV, Figures 68 and 69).

I. Sultani var. Beauty of Klettgau (red-flowered) and

I. Sultani var. ———————— (pink-flowered).

Both these varieties of I. Sultani are identical in ovule development. The photographs and drawings of the ovule development and degeneration are of I. Sultani var. Beauty of Klettgau (red-flowered).

The development of the embryo sac follows the pattern of Polygonum Type (Plate XVI and XVII, Figures 70-79).

Pollen tubes penetrate the mature embryo sac and the usual fusions of egg and sperm nucleus and polar nuclei and sperm occur. However, the zygote begins to degenerate shortly after fertilization is completed, and the endosperm nucleus remains undivided in the sac (Plate XVII, Figure 79). Degeneracy of the whole embryo sac follows.

Comparison of the Embryo Sacs and Haustoria in these Species.

The length of the mature embryo sac was used as a basis for comparing relative sizes of the embryo sacs. Accordingly, the sac of I. capensis (200 micra long) is the largest, those of I. glandulifera

(191.7 micra) and of I. Walleriana (137.7 micra) are intermediate and that of I. Sultani (123 micra) is the smallest.

All four species are alike in having monosporic 8-nucleate embryo sac development.

Two archesporial cells are common in I. glandulifera and I. Walleriana, but they are not found in I. capensis and I. Sultani. In all species the archesporial cell becomes the megaspore mother cell without cutting off a parietal cell. Also, the megaspore mother cell divides meiotically to give first a dyad and later a tetrad of megaspores. The chalazal cell of the tetrad becomes the functional megaspore in I. capensis, I. glandulifera and I. Walleriana. However, in I. Sultani the inner cell of the two chalazal megaspores gives rise to the embryo sac.

The organized embryo sacs of all the species studied contained an egg apparatus at the micropylar end, two polar nuclei near the center of the sac and three antipodal cells at the chalazal.

The synergids of I. capensis and I. glandulifera differ from those of the other species studied in that they develop haustoria. In. I. capensis these haustorial projections extend up the micropylar canal to a length of 80 micra. In. I. glandulifera, haustorial extension is less marked and the synergids disappear soon after fertilization. The synergids of I. Walleriana and I. Sultani do not elongate and they do not persist after fertilization.

The polar nuclei of I. glandulifera are much larger than those in the other species studied. In the mature sac each polar nucleus is

about 16.3 micra in diameter, while in the other species the diameter of each polar nucleus is about 6.4 to 7.8 micra. The diameter of the polar fusion nucleus of I. glandulifera is 25.6 micra, that of I. capensis is 14.2 micra, that of I. Walleriana is 13.5 micra and that of I. Sultani is 14.9 micra.

Although the embryo sac of I. capensis elongates considerably there is no evidence of antipodal haustoria. In I. glandulifera, I. Walleriana and I. Sultani, however, prominent antipodal embryo sac haustoria are present. The haustorium in I. glandulifera is far more extensive, vacuolated and branching than in I. Walleriana and I. Sultani. Also in I. glandulifera, the haustorium extends into the funicular tissue, whereas in I. Walleriana and I. Sultani it projects only to the vascular trace leading from the funiculus to the chalazal end of the embryo sac.

In I. glandulifera there was neither development of the embryo nor of the endosperm after a few initial divisions of the endosperm nucleus. In I. Sultani neither embryo nor endosperm develops. In other words, ovule development ceases at or immediately after the fertilization stage. On the other hand, embryo and endosperm development proceeded in I. capensis and I. Walleriana.

The polar nuclei of I. capensis, I. glandulifera and I. Sultani begin to fuse as the pollen tube enters the sac. Both nucleoli are still present until the sperm nucleus reaches them and then complete fusion occurs. However, the polar nuclei of I. Walleriana do not fuse until the sperm nucleus fuses with them.

Endosperm nuclear division occurs in advance of that of the zygote in I. capensis, I. glandulifera and I. Walleriana. The endosperm of I. capensis and I. Walleriana is cellular, with a wall separating the sac into two chambers after the first division of the endosperm nucleus. In I. capensis and I. Walleriana embryo formation is of the Asterad Type.

Micropylar endosperm haustoria occur in I. capensis, I. glandulifera and I. Walleriana. In I. capensis the haustorium arises from two micropylar endosperm cells. These two cells produce two cytoplasmic extensions which penetrate the micropylar canal and fuse. Divisions of the original two nuclei follow and the result is a multinucleate haustorium. The main haustorium invades the tissues bordering the micropylar canal by means of small projections. However, the haustorium extends only half way along the micropylar canal and begins to degenerate by the time the embryo has begun to differentiate. In I. glandulifera the free nuclear endosperm concentrated around the micropylar end of the embryo sac produces cytoplasmic projections which penetrate the layer of nucellar cells bordering the sac.

The endosperm haustoria in I. capensis and I. Walleriana arise from two micropylar endosperm cells. The haustorium of I. capensis becomes multinucleate by the division of the two endosperm nuclei, while that of I. Walleriana remains binucleate throughout its development. However, the haustorium itself remains binucleate and its two nuclei migrate into the haustorium. The micropylar haustorium of I. Walleriana is much more extensive than that of I. capensis penetrating the funicular and placental tissues. This haustorium persists until the cotyledons of the embryo have nearly filled the sac. In all three cases, the haustoria are intracellular.

VIII. DISCUSSION.

Origin and Development of the Embryo Sac.

In all Impatiens species reported to date, the archesporial cell gives rise directly to the megaspore mother cell (Ottley, 1918; Dahlgren, 1934; and Steffen, 1951).

There is some difference of opinion, however, as to the origin of the embryo sac. Raitt (1916), Caroll (1919), Dahlgren (1934), and Steffen (1951) agree that the embryo sac has a monosporic origin. Ottley (1918) suggested that the embryo sac of I. Sultani "seems to arise directly from the megaspore mother cell as in Lilium", but later in the same paper she mentioned that "the embryo sac is thus derived from two megaspores." The observations of the present study are in line with the findings of the former authors.

A tetrad of megaspores is formed in I. capensis, I. glandulifera, I. Walleriana and I. Sultani. Steffen (1951), Caroll (1919), and Raitt (1916) observed the same situation in I. glandulifera, I. capensis and I. pallida. Dahlgren (1934) mentioned the formation of a dyad but he did not follow its further development.

In all species reported the functional megaspore divides by three synchronous divisions to form a normal 8-nucleate embryo sac.

Fertilization and Endosperm Formation.

Embryo and endosperm development proceeds successfully in I. capensis and I. Walleriana. Endosperm development in these two species

follows the pattern reported for I. glandulifera (Dahlgren, 1934) and I. Sultani (Lebon, 1929). In I. glandulifera and I. Sultani ovule development ceases at or immediately after the fertilization stage.

Haustoria.

Haustoria are of six types: megaspore, synergid, antipodal, embryo sac, suspensor, and endosperm. By far the most common type of haustoria is that formed from the endosperm and it has been reported in such families as the Scropulariaceae (Krishna Iyengar, 1940b), Bignoniaceae and Orobanchaceae (Crété, 1955), Diapensaceae and Empetraceae (Samuelsson, 1913) and the Balsaminaceae (Ottley, 1918; Dahlgren, 1934; and Steffen, 1951).

Synergid Haustoria

Although synergid haustoria are not of common occurrence, they have been reported in the Compositae (Dahlgren, 1924 and Schurhoff, 1926). Steffen (1951) has noted the elongation and persistence of synergids in I. glandulifera. The synergid haustoria in I. capensis and I. glandulifera differ from those reported for the Compositae in that they are longer and the nuclei remain in the basal portion instead of migrating toward the micropylar end of the haustorium.

Embryo Sac Haustoria.

In I. glandulifera, I. Walleriana and I. Sultani, the chalazal end of the embryo sac pushes through the nucellar tissue just

before or during fertilization and appears as an haustorium (Plate VIII, Figure 35b; Plate IX, Figure 41; Plate XIII, Figure 61; and Plate XVII, Figure 79).

Dahlgren (1934) noted the elongation of the sac in I. glandulifera just before endosperm formation and considered it an endospermal haustorium because endosperm nuclei subsequently were observed in it. In the writer's opinion, since this haustorium originates as a projection of the whole embryo sac at the 8-nucleate stage of development, it should be termed an embryo sac haustorium rather than an endosperm haustorium. Ottley (1918) noted an antipodal embryo sac haustorium in I. Sultani which Lebon (1929) considered to be an endosperm haustorium formed from the chalazal cell of the endosperm. However, the present investigator believes it should be termed an embryo sac haustorium since the mature sac itself develops into the chalazal tissues.

Endosperm Haustoria.

The micropylar endosperm haustorium of I. glandulifera is unicellar and uninucleate (Dahlgren, 1934). Although the haustoria of I. capensis and I. Walleriana originate from two cells, fusion of the two cells yields a unicellular binucleate haustorium very early in development. While the haustorium of I. capensis becomes multinucleate later, that of I. Walleriana remains binucleate throughout. Raitt (1916) and Ottley (1918) reported that I. pallida and I. Sultani had multinucleate haustoria. These workers believed the endosperm was

nuclear and thus did not signify whether these haustoria were unicellular or otherwise. Although Lebon (1929) reported cellular endosperm in I. Sultani, he did not note the existence of a micropylar haustorium. Venkateswarlu and Atchutarmamurti (1956a) reported that Hydrocera triflora, the only member of the second genus of the Balsaminaceae, developed a unicellular multinucleate endosperm haustorium.

If Krishna Iyengar's (1940b) phylogenetic system of classification based on the origin and nature of the micropylar haustorium is applied to this family, it can be concluded that the Balsaminaceae is more advanced than primitive because of the reduction in the number of cells and nuclei composing the micropylar haustorium.

Krishna (1939) and other authors suggested that the haustoria in some cases may not have a nutritional function because they frequently degenerate when nutritive materials are most needed. However, most workers have observed that haustorial structures function as nutritive mechanisms and accumlate materials from the cells through which they pass or come in contact (Samuelsson, 1913; Kausik, 1938b; Rau, 1951b; Subramanyam, 1951; Maheshwari, 1954; and Steffen, 1954). In the present study the observations confirm the absorbing action of the haustoria because of the effect which they produce both on the tissues which they actually penetrate and those lying in the general area of penetration. The penetration and absorption of the vascular tissue in I. glandulifera by haustoria would seem to be clear indication of their nutritive role. All the cells of these regions are depleted of their contents. In the cases where cells are invaded by the haustoria

both cell walls and contents disappear.

The method of penetration, the depletion of protoplasm and the nutritional function of the haustoria constitute a separate physiological problem.

From the present observations, it is evident that the embryo sac of Impatiens is Polygonum Type with the formation of synergid, embryo sac and endosperm haustoria. There are anatomical and cytological indications that these haustoria have a nutritional function.

IX. SUMMARY

1. Embryo sac development in I. capensis, I. glandulifera, I. Walleriana and I. Sultani is of the Polygonum Type.

2. The archesporial cell gives rise to the megaspore mother cell directly without cutting off a tapetal cell.

3. The chalazal cell of the tetrad formed by meiosis becomes the functional megaspore in I. capensis, I. glandulifera and I. Walleriana, while the third megaspore from the micropylar end of the tetrad gives rise to the embryo sac in I. Sultani.

4. The nucleus of the functional megaspore undergoes three mitotic divisions to form the 8-nucleate embryo sac.

5. The mature embryo sac consists of an egg apparatus (two synergids and an egg) at the micropylar end of the sac, two polar nuclei near the center of the sac, and three ephemeral antipodal cells at the chalazal end of the sac.

6. The synergids of I. capensis and I. glandulifera elongate forming haustoria which frequently persist up to the division of the zygote.

7. The antipodal end of the sac in I. glandulifera, I. Walleriana and I. Sultani forms an embryo sac haustorium before fertilization and after endosperm formation an endosperm nucleus migrates into the haustorium.

8. Double fertilization occurs in all species. In I. glandulifera and I. Sultani the zygote degenerates without dividing. The

endosperm nucleus in I. glandulifera divides a number of times before the degeneration of the sac. The endosperm nucleus in I. Sultani degenerates without dividing.

9. The embryo of I. capensis and I. Walleriana is of the Asterad Type.

10. The endosperm is cellular. The endosperm nucleus divides and the sac forms a micropylar chamber and a chalazal chamber. Cellular divisions occur in the micropylar chamber and free nuclear divisions in the chalazal chamber.

11. The micropylar endosperm haustoria in I. capensis and I. Walleriana arise from two micropylar cells of the endosperm and in both cases they are intracellular.

12. The two haustoria in I. capensis fuse and invade the tissues in the vicinity of the micropylar canal. The two endosperm nuclei in the mother cells of the haustorium remain at the base of the haustorium and divide giving a multinucleate haustorium.

13. The two haustoria of I. Walleriana fuse and two endosperm nuclei migrate into it. The haustorium becomes very extensive penetrating the cells of the funiculus and the placenta.

14. The first stage in the formation of the intracellular micropylar endosperm haustoria was noted in I. glandulifera.

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I. CAPENSIS

PLATE I.

Figure 1. The archesporial cell is the enlarged cell just below the nucellar epidermis. Note the inner integument arising as a small protuberance from the nucellar tissue at the base of the archesporial cell. (1500X).

Figure 2. The megaspore mother cell at interphase before the onset of meiosis. The inner integument has increased in size. (1400X).

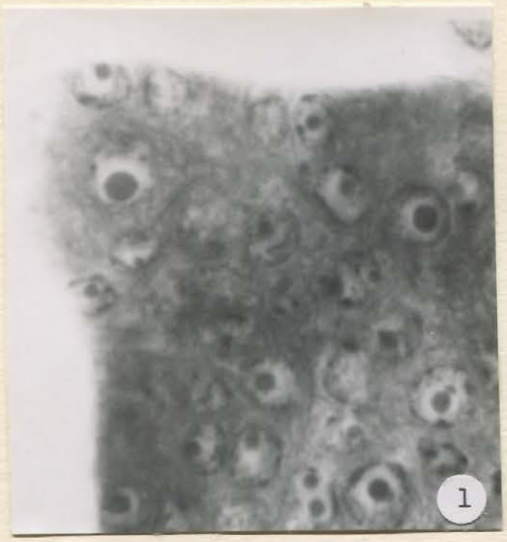
Figure 3. The megaspore mother cell at pachytene of prophase 1. the outer integument can be seen arising from the nucellar tissue below the inner integument. (650X).

Figure 4. Late prophase 1. The inner integument surrounds the the megaspore mother cell. The outer integument has increased in length. (650X).

Figure 5. The megaspore mother cell at anaphase 1. (1500X).

Figure 6. The top nucleus or cell of the dyad formed by meiosis has divided and the micropylar cell is already degenerating. (1400X).

Plate 1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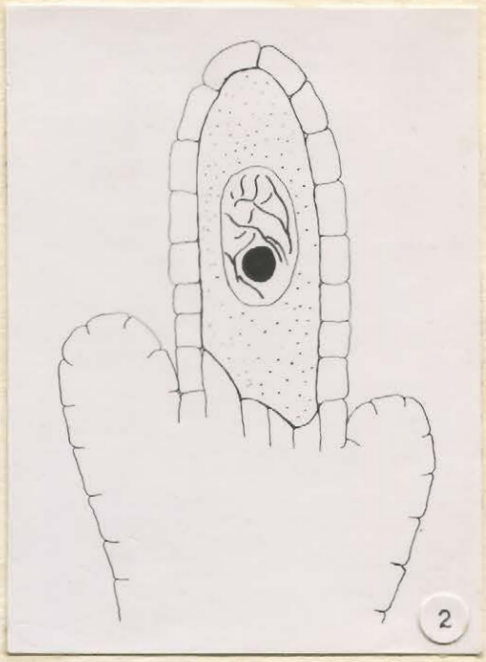
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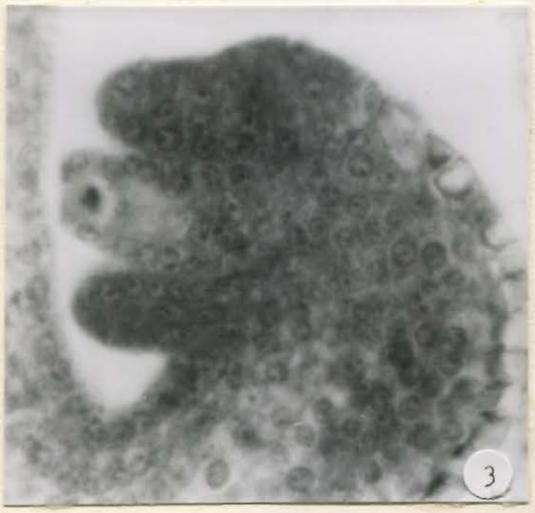
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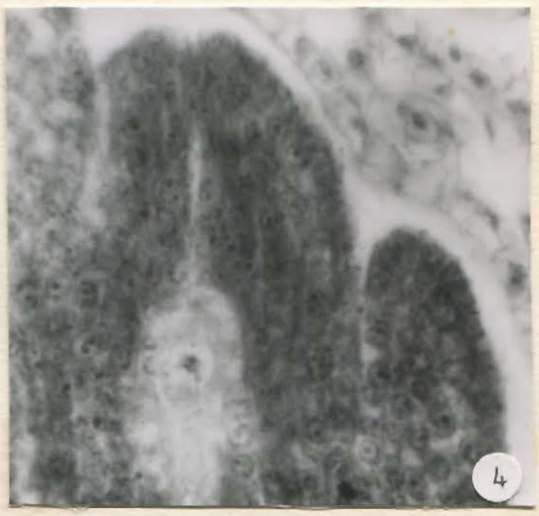
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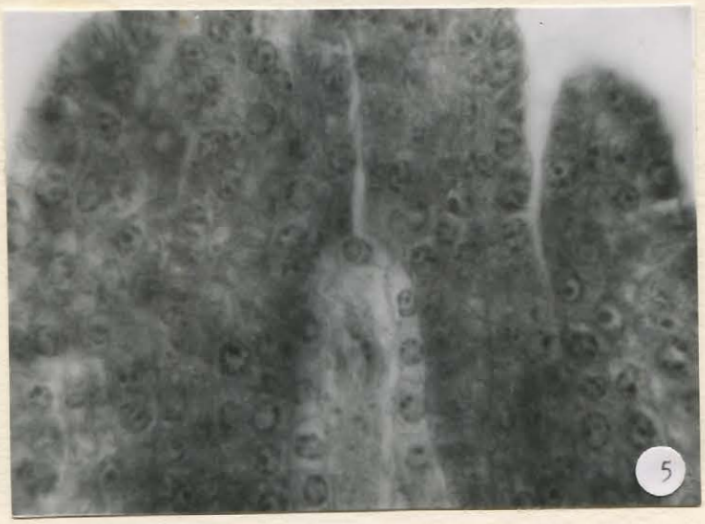
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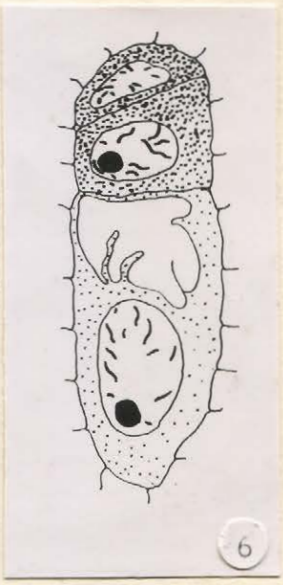
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I. CAPENSIS

PLATE II.

Figure 7. A T-shaped tetrad of megaspores. The three micropylar megaspores are degenerating. The chalazal functional megaspore is in late mitotic prophase. The spindle of the last meiotic division can still be observed. (1400X).

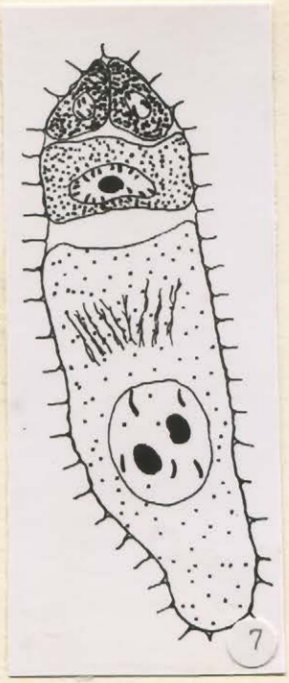
Figure 8. A T-shaped tetrad of megaspores. (1400X).

Figure 9. A linear tetrad of megaspores. The three micropylar megaspores are degenerating. The functional megaspore is in anaphase of the first mitotic division. (1000X).

Figure 10. The 2-nucleate embryo sac consists of a primary micropylar nucleus and a primary chalazal nucleus separated by a vacuole. Three degenerating megaspores are still present. (1500X).

Figure 11. Anaphase of the 2-nucleate embryo sac with the three degenerating megaspores still observable. (1000X).

Figure 12. The organized 8-nucleate embryo sac. The egg apparatus at the micropylar end of the sac consists of two elongated synergids and an egg which extends a short distance below them. The two polar nuclei are moving towards each other from opposite ends of the sac. Three degenerating antipodals are still recognizable at the chalazal end of the sac. The nucellar cells surrounding the sac have been absorbed. (1000X).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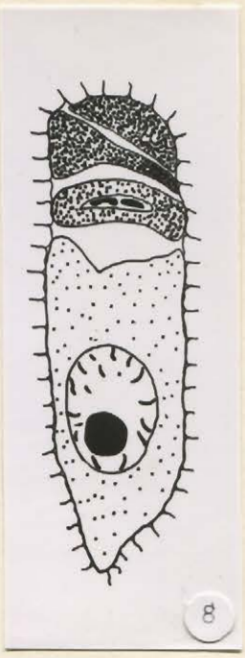
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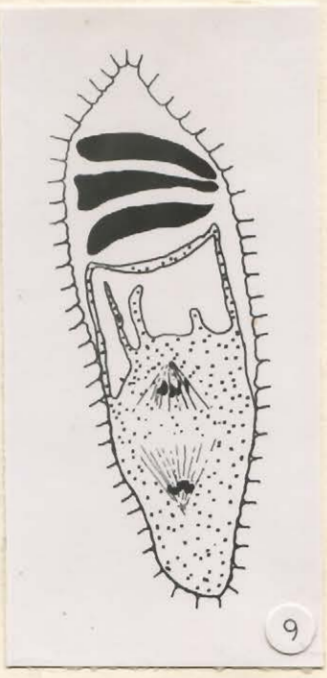
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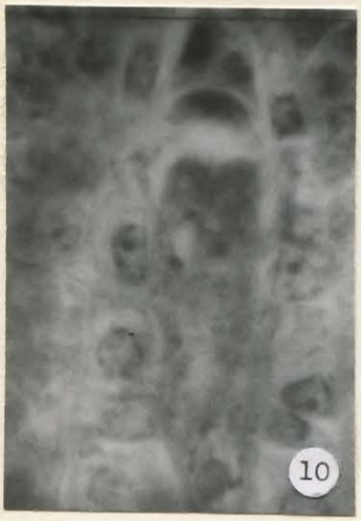
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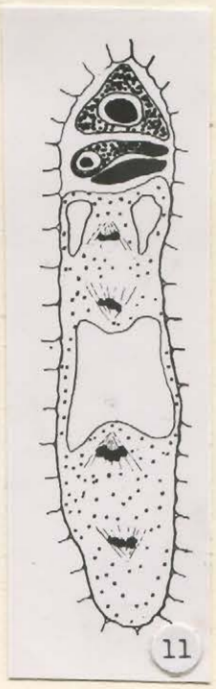
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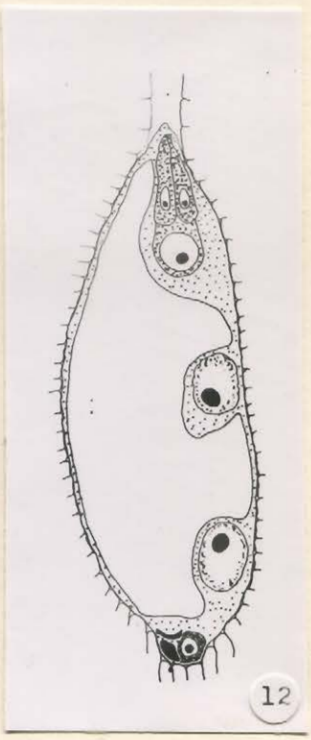
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I. CAPENSIS

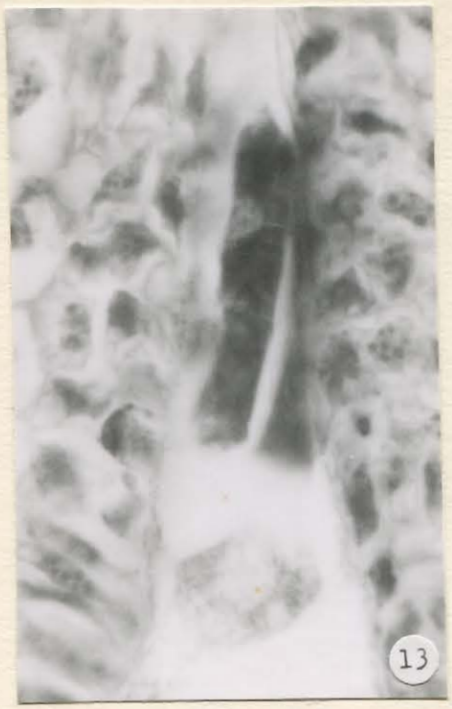
PLATE III.

Figure 13. One synergid and the outline of the egg are visible. The other synergid lies to the left of the visible synergid. A large vacuole is situated at the tip of the synergid and the nucleus lies above it. The tip is somewhat club-shaped. This synergid is 31.9 micra. long. (1500X).

Figure 14. Elongating synergids. One synergid overlaps the other. A vacuole is present between the nucleus and the micropylar end of the synergids. The ends of the synergids which are entering the micropylar canal are tapered. (1000X).

Figure 15. The synergids extend five-eighths of the way along the micropylar canal. The nucellar tissue surrounding the micropylar canal has disappeared and the integumentary cells next to the canal show signs of depletion. (1000X).

Figure 16. In this section the egg and one synergid can be noted. The micropylar end of the sac has broadened considerably. The sac is now 200 micra long. (1500X).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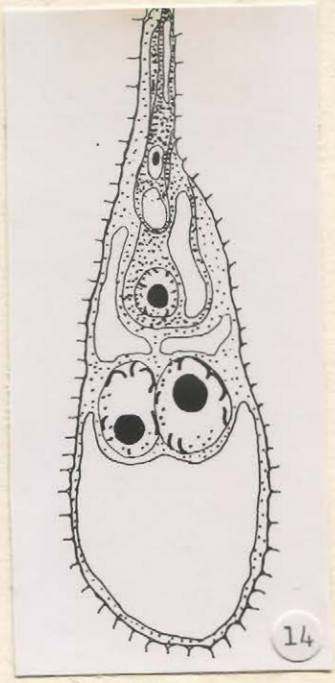
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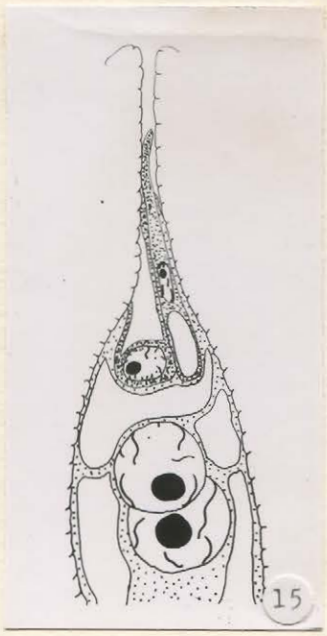
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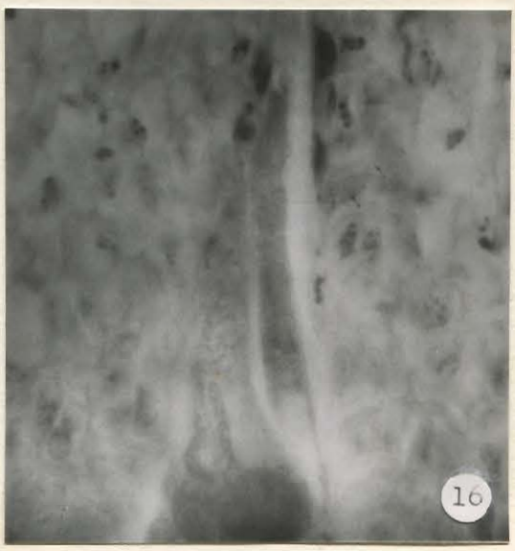
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I. CAPENSIS

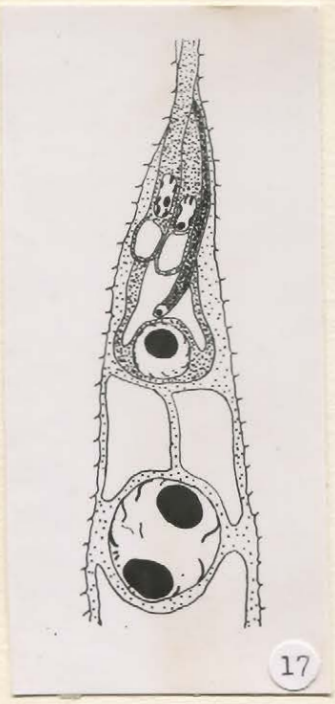
PLATE IV.

Figure 17. The pollen tube has entered the sac to one side of the synergids and its tip lies near the egg. The two polar nuclei are beginning to fuse. (1000X).

Figure 18. The remnants of one synergid can be observed behind the pollen tube. The tube nucleus and the sperm nuclei cannot be seen clearly. (1500X).

Figure 19. One synergid is still present. A sperm nucleus lies near the egg. The polar nuclei and the sperm nucleus have fused. (1000X).

Figure 20. More than one pollen tube is entering the embryo sac, one near the egg and one further up the micropylar canal. (1000X).



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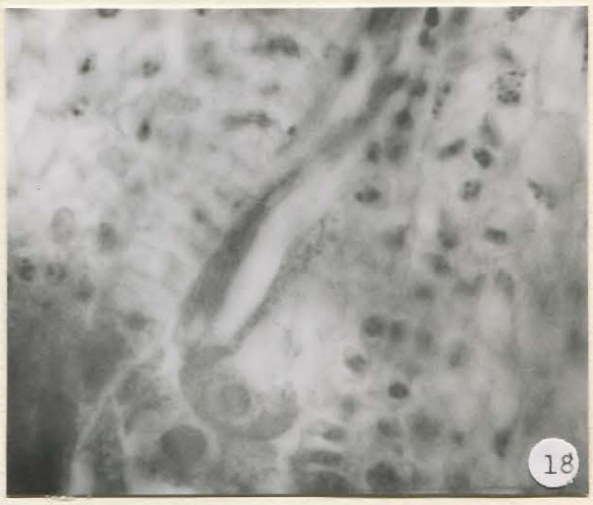

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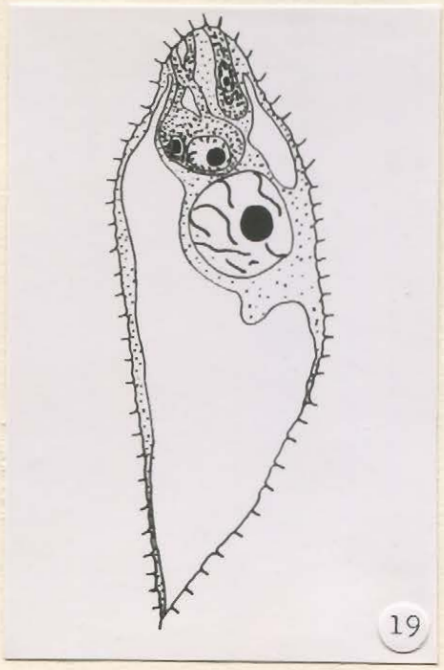

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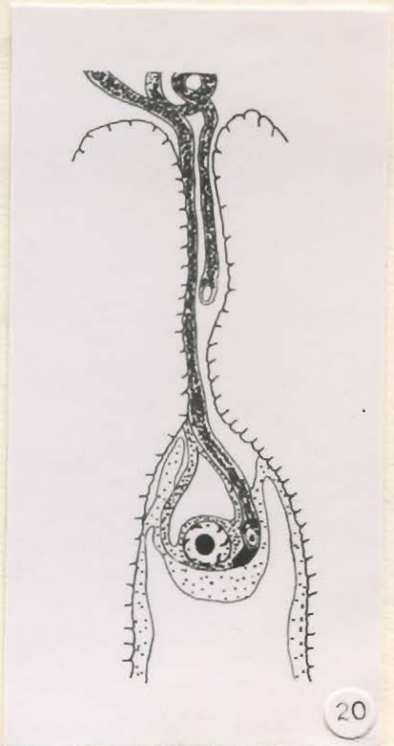

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I. CAPENSIS

PLATE V.

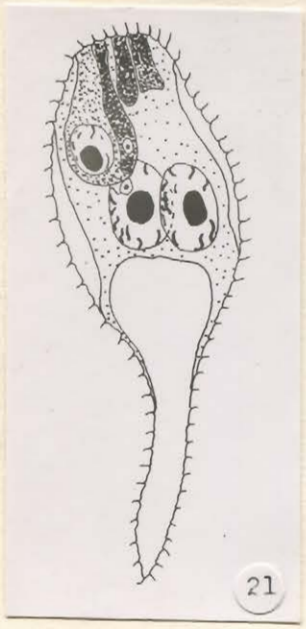
Figure 21. Double fertilization. (1000X).

Figure 22. Two sperm nuclei near the egg. (1000X).

Figure 23. The endosperm nucleus at metaphase. The zygote is still undivided. The remnants of the pollen tube can be seen at the micropylar end of the sac, while a degenerating synergid is situated at the side of the zygote. (1000X).

Figure 24. The sac is divided into two chambers, a small micropylar chamber and a large chalazal chamber. Cellular divisions take place in the micropylar chamber and nuclear divisions take place in the chalazal chamber. The zygote cannot be seen in the section. At the micropylar end of the sac the degenerating pollen tube and synergid are still distinguishable. (1000X).

Figure 25. The first division of the zygote is transverse. (1000X).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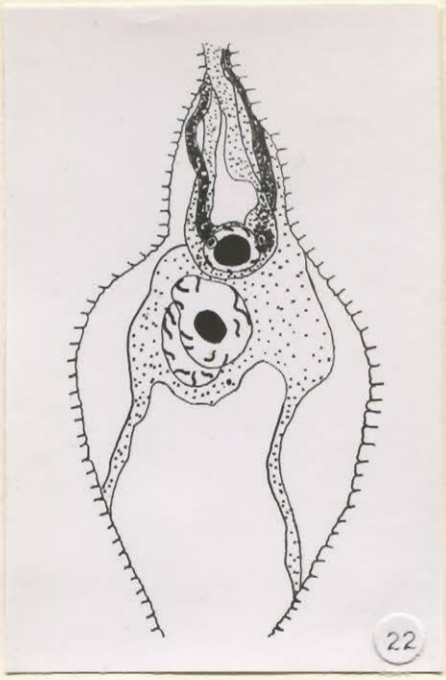
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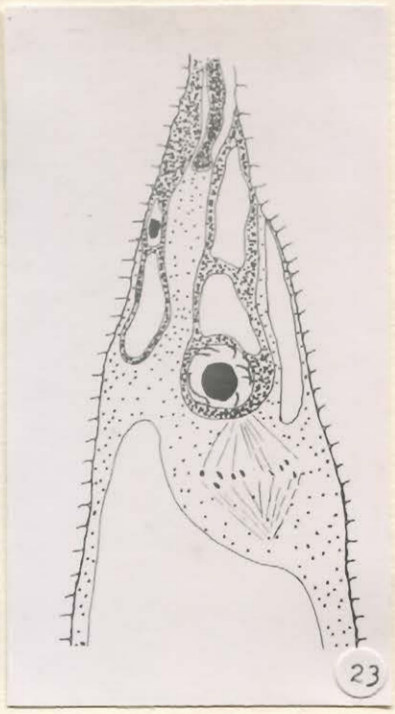
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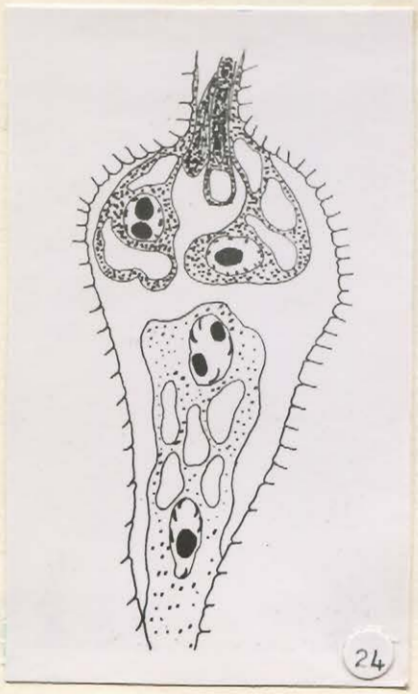
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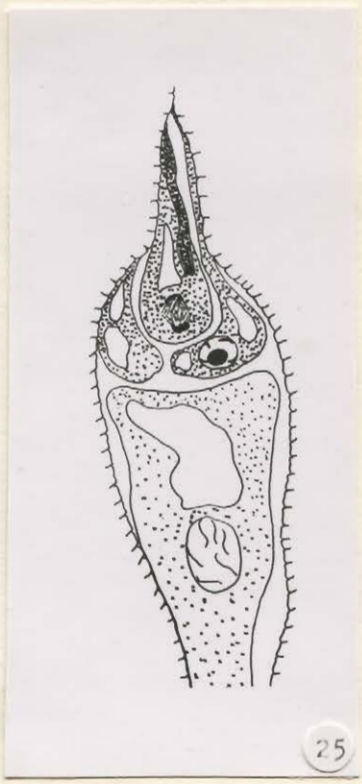
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I. CAPENSIS

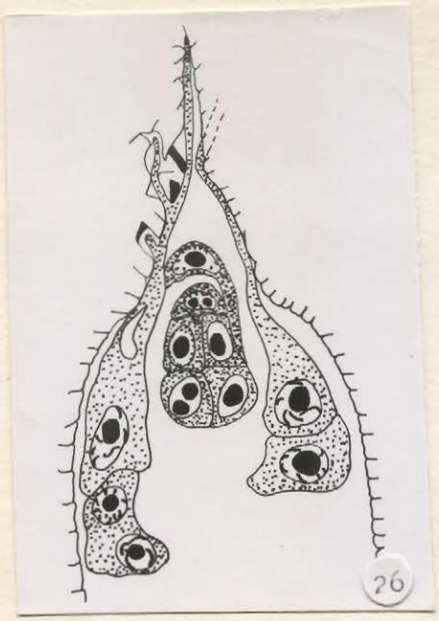
PLATE VI.

Figure 26. The two micropylar endosperm haustoria extending into the micropylar canal and branching in the surrounding tissues. (1000X).

Figure 27. The embryo is a rounded mass of cells. The two micropylar haustoria have fused and the resulting haustorium is multinucleate. (650X).

Figure 28. The micropylar haustorium is degenerating. The surrounding integumentary tissue is depleted of its contents. (600X).

Figure 29. The cotyledons have differentiated and only a peripheral layer of endosperm is present. (130X).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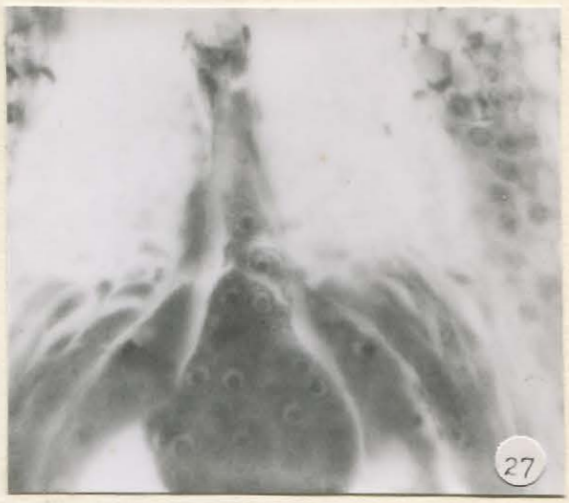
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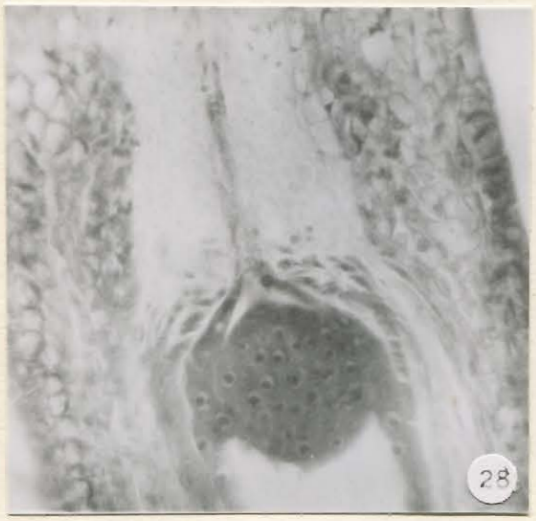
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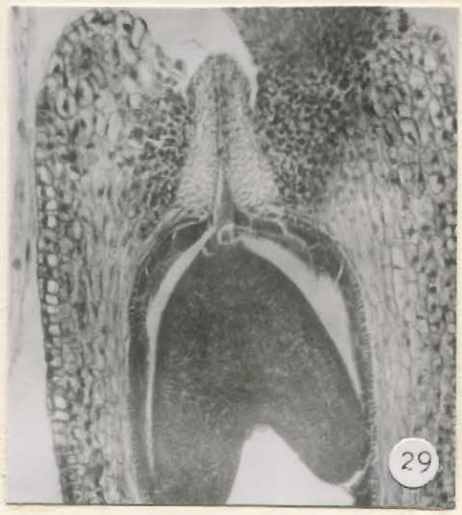
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I. GLANDULIFERA

PLATE VII.

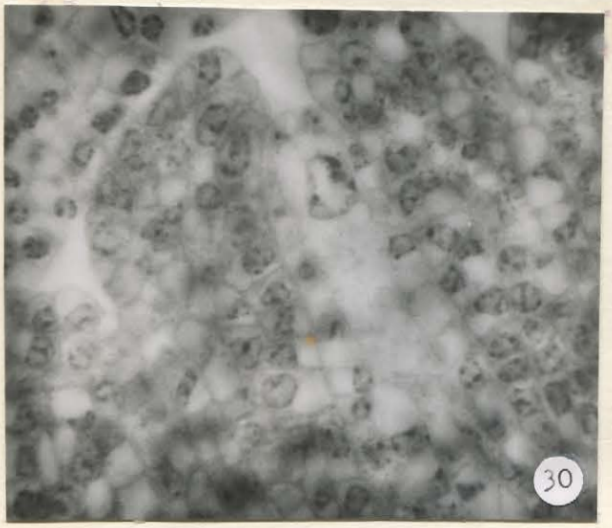
Figure 30. The megaspore mother cell is surrounded by a layer of nucellar cells at the sides and at the micropylar end. The inner integument almost surrounds it and the outer integument is also well advanced. (1500X).

Figure 31. A double archesporium. The micropylar archesporial cell has divided once almost at right angles to the axis of the embryo sac. The chalazal archesporial cell has divided to form a linear tetrad and the chalazal functional megaspore has proceeded to the 2-nucleate embryo sac stage (only one nucleus is visible). (1400X).

Figure 32. An orientated embryo sac that has no vacuoles. (1400X).

Figure 33. A vacuolated embryo sac. One antipodal cell is still present. (1000X).

Figure 34. A mature embryo sac in which the synergids have lengthened, the egg has protruded into the embryo sac and the polar nuclei lie very close together. A small protuberance can be seen at the chalazal end of the sac in which the antipodals are degenerating. (1000X).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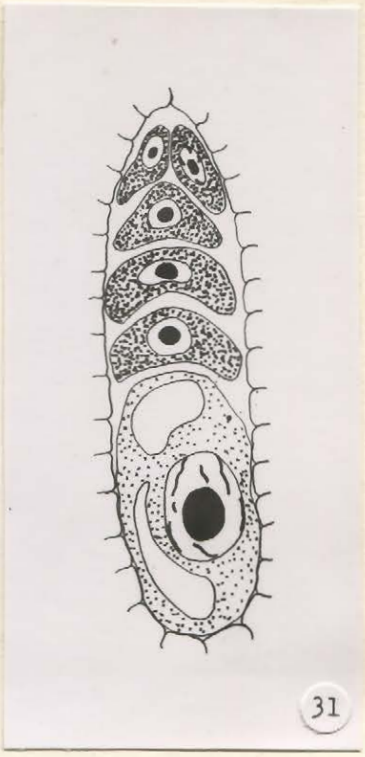
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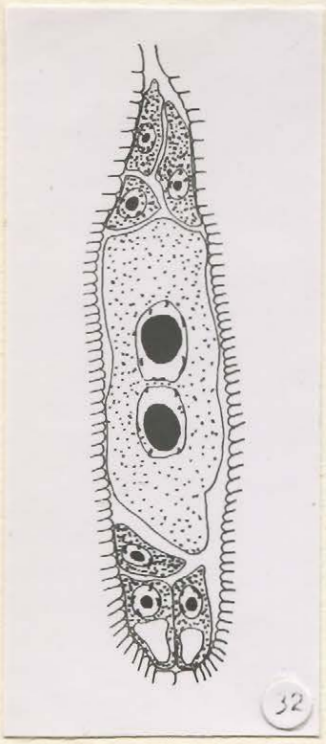
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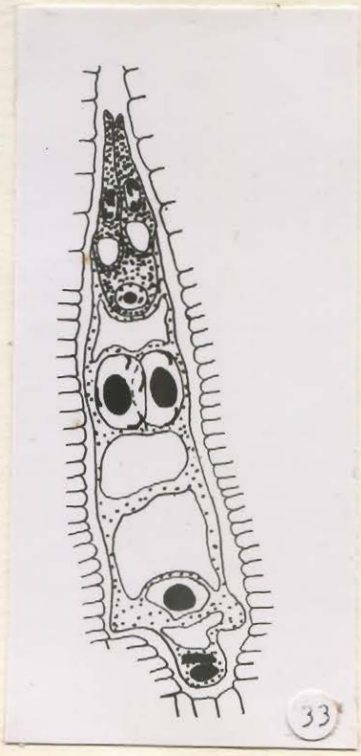
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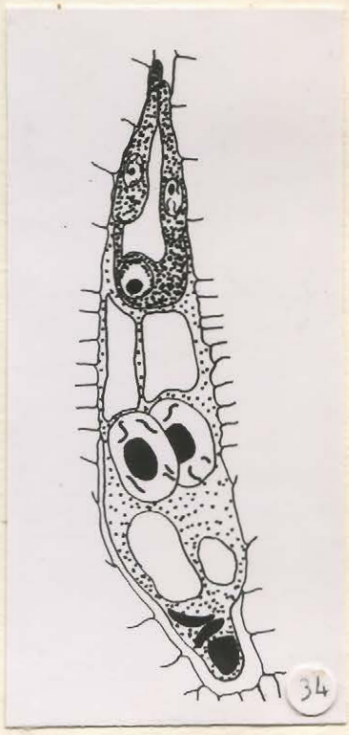
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I. GLANDULIFERA

PLATE VIII.

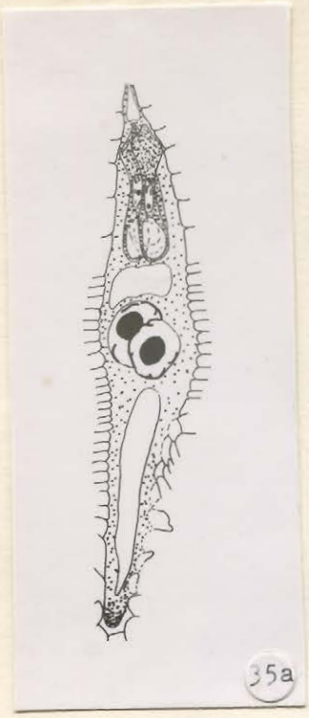
Figure 35a. One synergid lies on top of the egg and the other synergid. The synergids have vacuolated tips and hooked indentations. The membrances between the polar nuclei are almost indistinguishable. The chalazal embryo sac haustorium has begun its development. (1000X).

Figure 35b. The extending tip of the antipodal embryo sac haustorium. (1400X).

Figure 36. The egg, one elongating synergid and one polar nucleus are present. The antipodal haustorium is penetrating the chalazal nucellar tissues. (1500X).

Figure 37. The elongating synergids with their vacuolated tips and hypertrophied nuclei. They are 80.9 micra long. The polar fusion nucleus is present. (1000X).

Figure 38. The pollen tube entering the embryo sac. One synergid is still present as are the two fusing polar nuclei. (1000X).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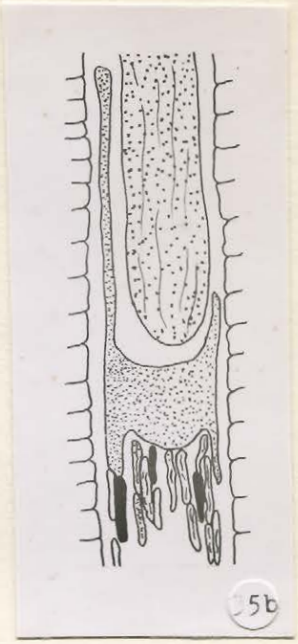
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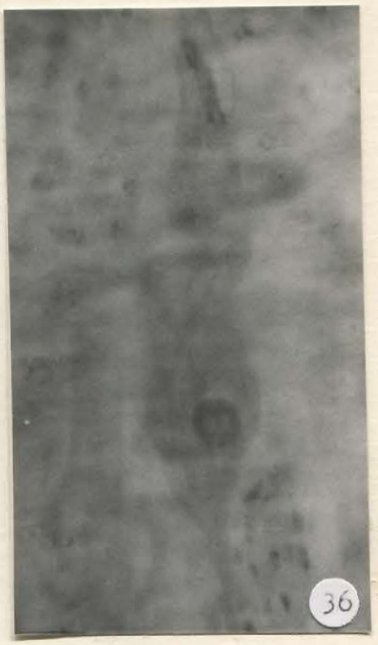
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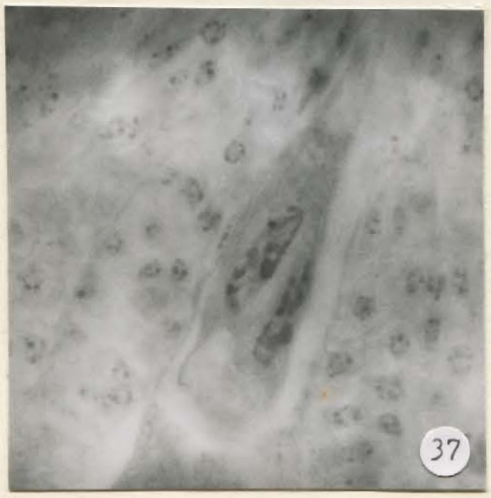
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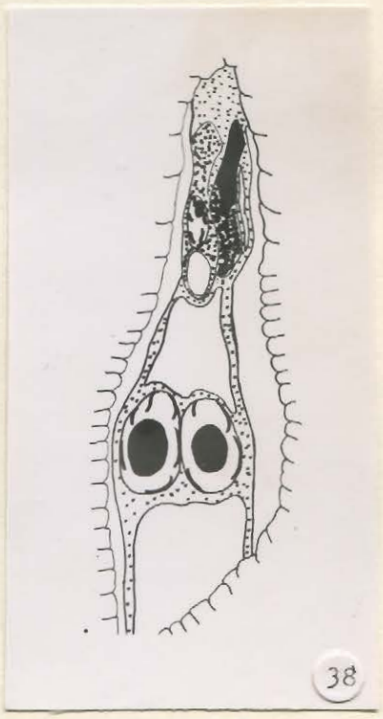
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I. GLANDULIFERA

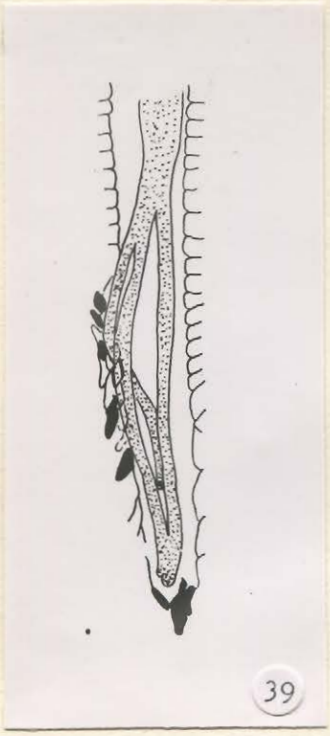
PLATE IX.

Figure 39. The branching antipodal embryo sac haustorium. Note that it penetrates the nucellar cells. (1400X).

Figure 40. A pollen tube and a degenerating synergid are at the micropylar end of the sac. Below these lie the egg and the large polar fusion nucleus. Note the widening of the sac in the vicinity of the fused polar nuclei. (1000X).

Figure 41. The antipodal embryo sac haustorium during fertilization. (650X).

Figure 42. Many pollen tubes surround the egg which is degenerating. (600X).



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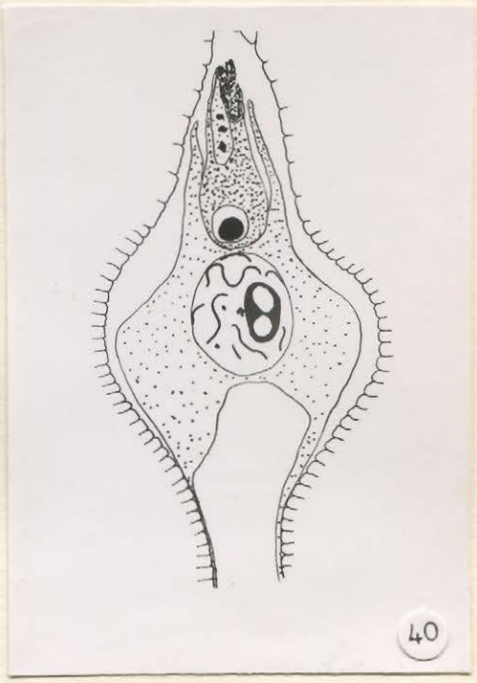

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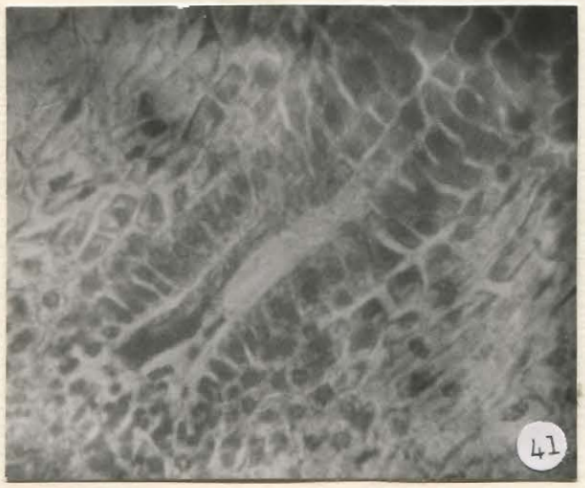

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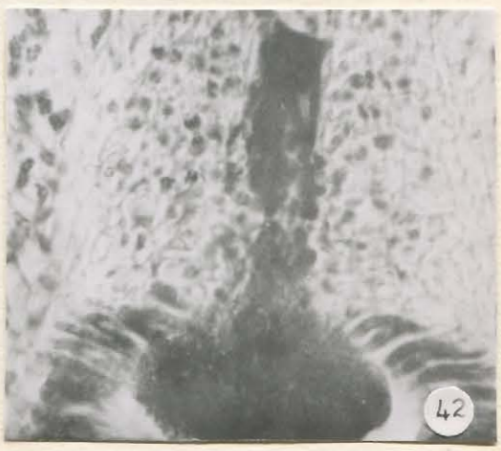

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I. GLANDULIFERA

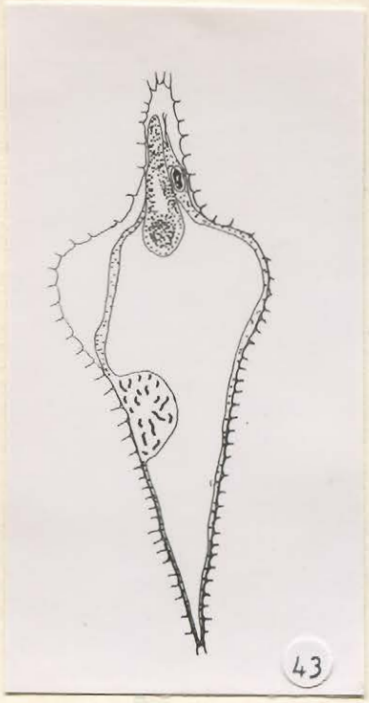
PLATE X.

Figure 43. The degenerating egg. One degenerating synergid is present and the endosperm nucleus lies undivided at the periphery of the sac. (1000X).

Figure 44. The chalazal haustorium has reached the vascular trace, has turned, and has penetrated the funicular tissue. (1000X).

Figure 45. The egg and the sperm nucleus lie at the micropylar end of the sac. The endosperm nuclei are concentrated here also. Cytoplasmic projections from the endosperm can be seen entering the nucellar tissue at this end of the sac. (1000X).

Figure 46. A seed with no embryo or endosperm. (150X).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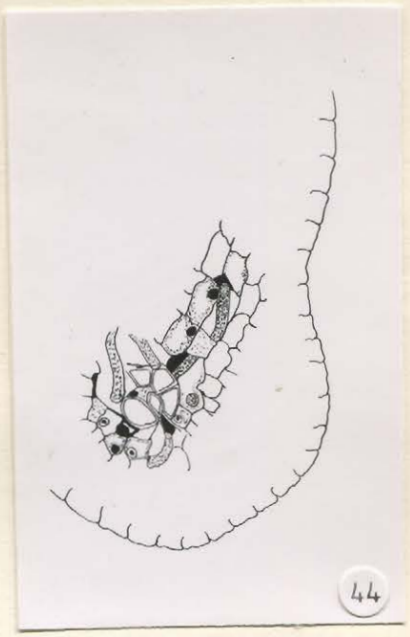
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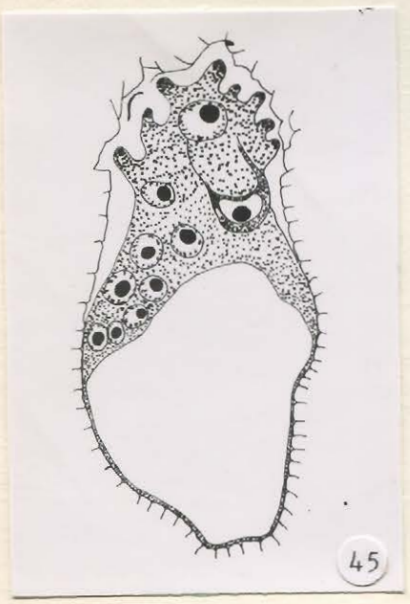
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I. VALERIANA

PLATE XI.

Figure 47. The top ovule has two archesporial cells. In the lower ovule, the differentiated cell beneath the nucellar epidermis is the archesporial cell. (1500X).

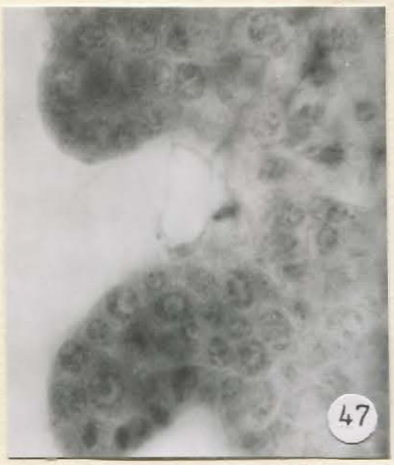
Figure 48. The inner integument has arisen as a tiny protuberance on either side of the ovule below the archesporial cell. (1500X).

Figure 49. The megaspore mother cell in prophase 1. The inner integument has grown just beyond the base of the megaspore. (1500X).

Figure 50. The megaspore mother cell at a later stage. (650X).

Figure 51. The megaspore mother cell dividing. (1500X).

Figure 52. The resulting dyad. (1500X).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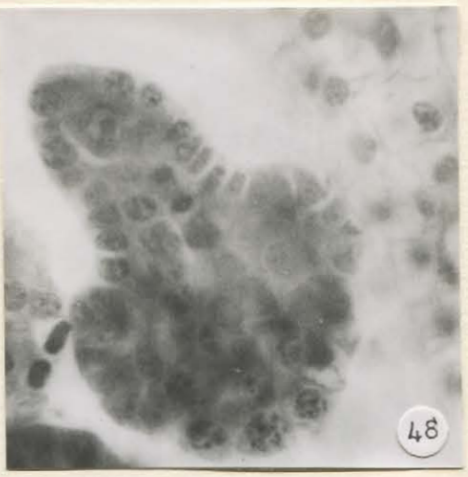
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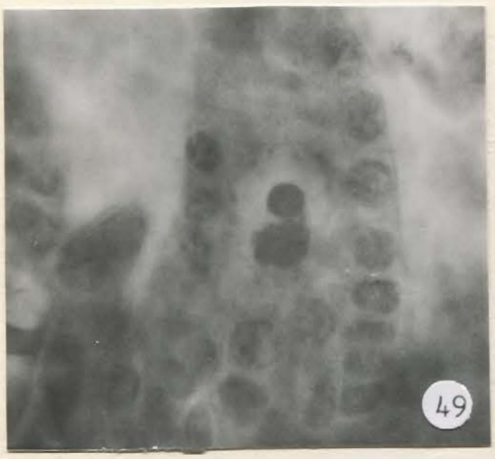
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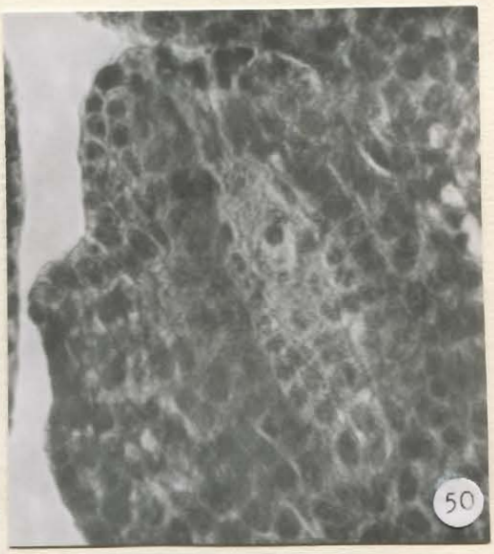
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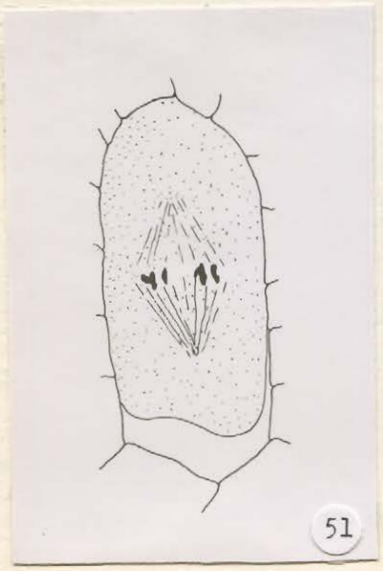
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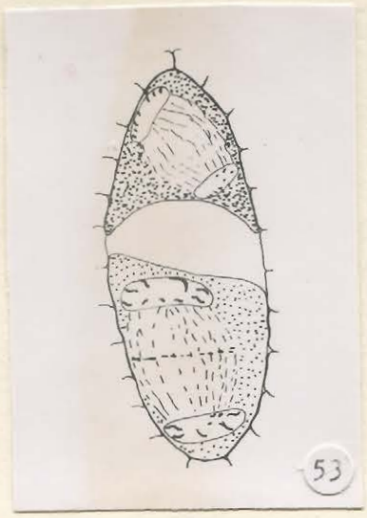
PLATE XII.

Figure 53. The formation of a tetrad of megaspores. Walls are not observable between the two nuclei of the micropylar cell which is degenerating. A wall can be seen across the equator of the spindle in the chalazal cell. (1000X).

Figure 54. The functional megaspore is the chalazal megaspore of the tetrad. The other megaspores appear as a black degenerating cap at the micropylar end of the sac. (650X).

Figure 55. The two nuclei of the 2-nucleate embryo sac are separated from each other by a vacuole. (1500X).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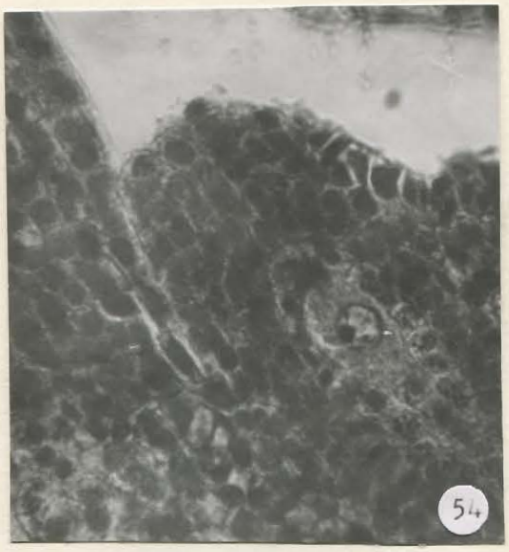
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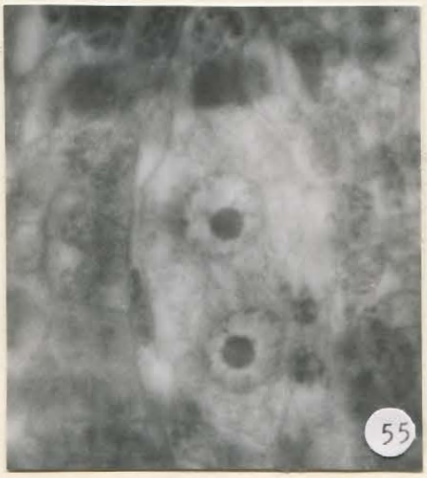
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I. VALLERIANA

PLATE XIII.

Figure 56. The 4-nucleate embryo sac. (1500X).

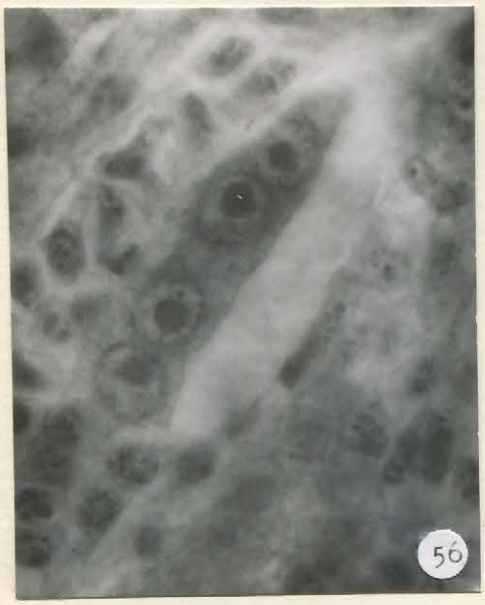
Figure 57. The 8-nucleate embryo sac. (1000X).

Figure 58. The organized embryo sac. The two synergids have vacuoles at their micropylar ends. The egg lies below the synergids (next section). One polar nucleus lies over the other in the middle of the sac. One antipodal cell is longer than the other two and the walls between them are hard to distinguish. (1500X).

Figure 59. The mature embryo sac. Note the elongation towards the antipodal end of the sac. (1000X).

Figure 60. Double fertilization. One sperm nucleus lies to the right of the egg and the other sperm nucleus lies below the two fusing polar nuclei. (1000X).

Figure 61. The branching antipodal embryo sac haustorium. (1000X).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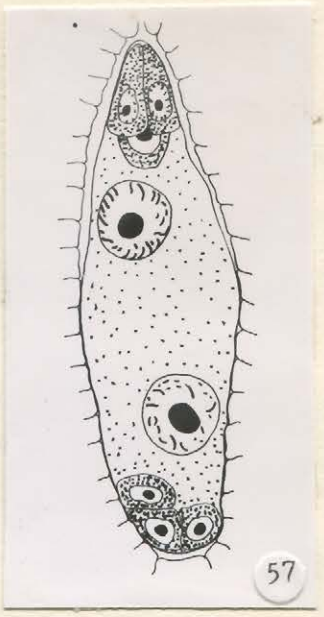
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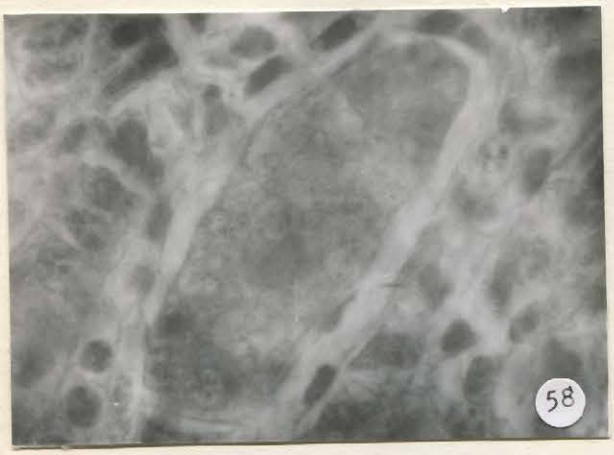
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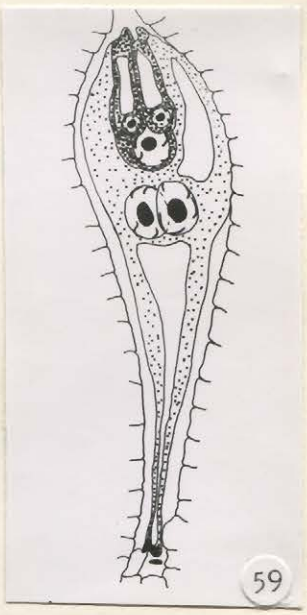
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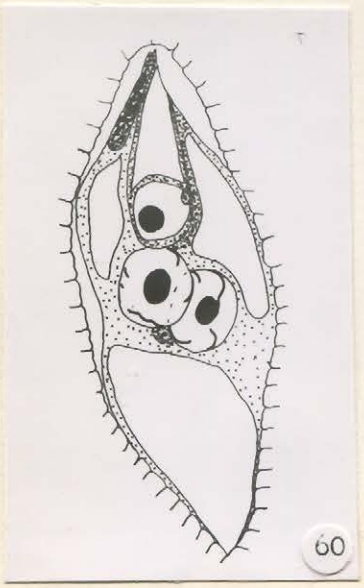
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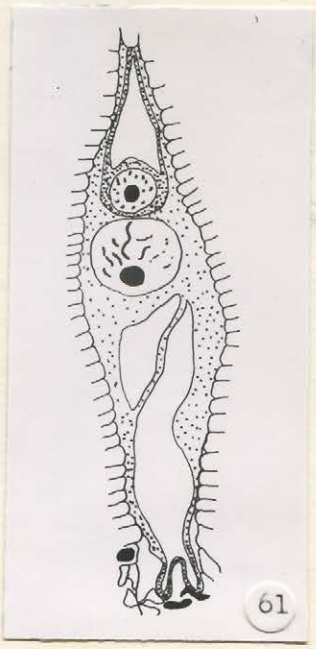

61

I. WALLERIANA

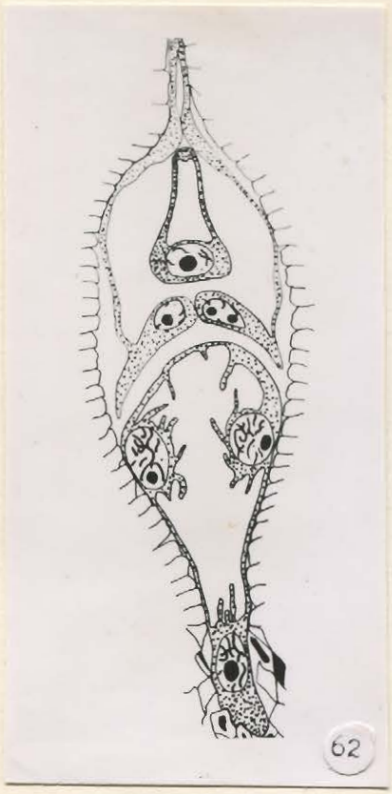
PLATE XIV.

Figure 62. The zygote is still unicellular. Note the two endospermal cells which are sending projections into the micropylar canal. (1400X).

Figure 63. The 2-celled embryo. (1500X).

Figure 64. The 12-celled embryo. The micropylar haustorium has expanded in the region between the micropyle and the funiculus and two large nuclei are present in this globular mass. The chalazal embryo sac haustorium contains one endospermal nucleus. (1000X).

Figure 65. Note the 1-celled suspensor. (1000X).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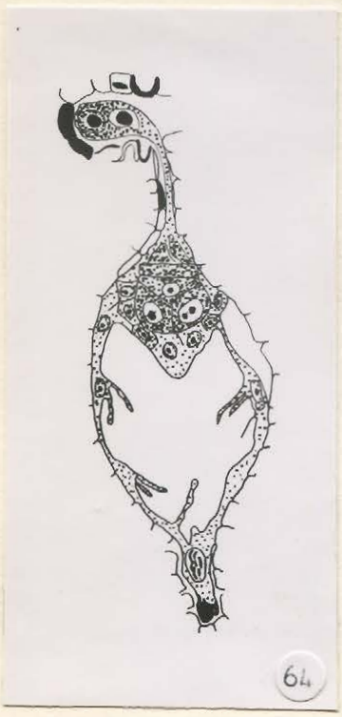
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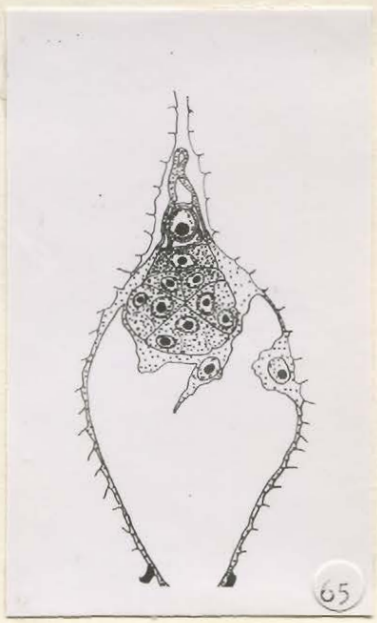

65

I. WALLERIANA

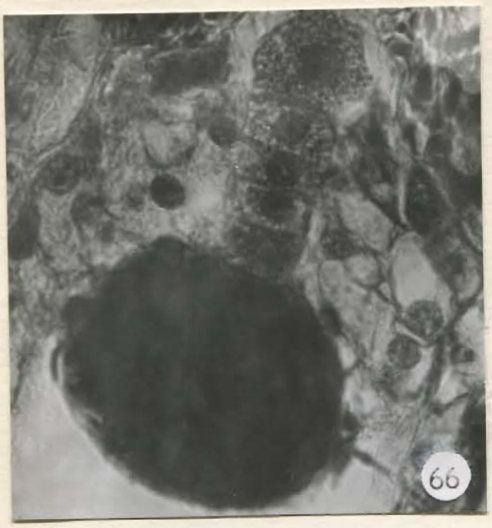
PLATE XV.

Figure 66. The embryo at a later stage. The suspensor is fully developed. Cellular endosperm almost surrounds the developing embryo and nuclear endosperm forms a peripheral layer lining the inside of the sac. (650X).

Figure 67. The embryo at a later stage. Note the micropylar endosperm haustorium with its expanded portion. (600X).

Figure 68. Cotyledons are differentiating. The micropylar endosperm haustorium extends into the funicular tissue. (1000X).

Figure 69. The binucleate micropylar endosperm haustorium with its branches penetrating the funicular cells. (650X).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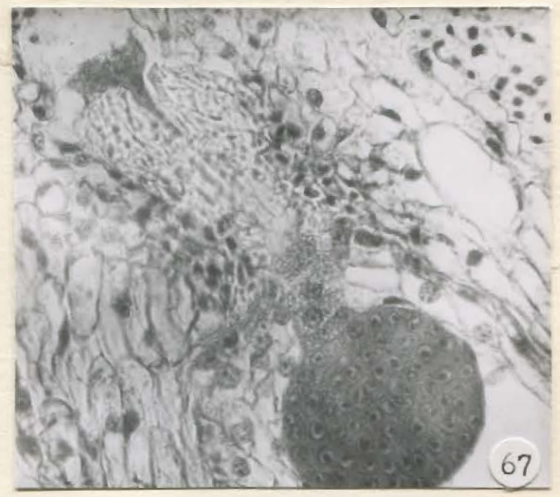
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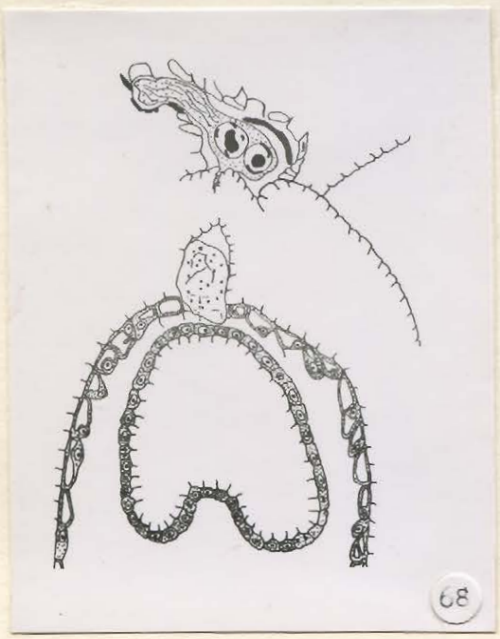
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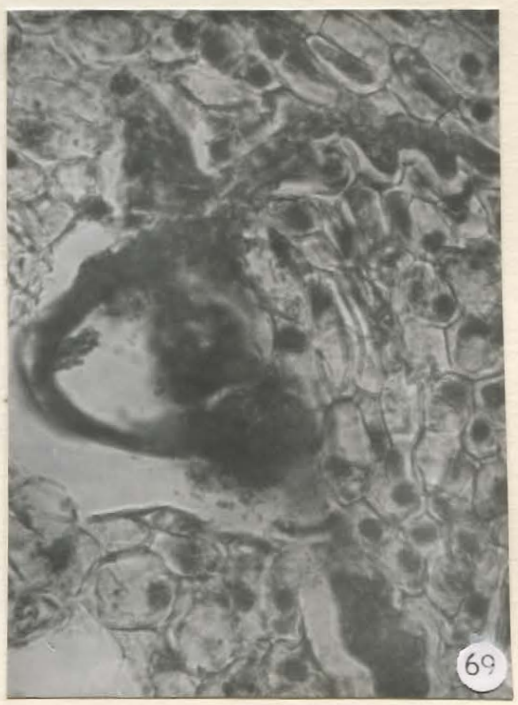
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I. SULTANI

PLATE XVI.

Figure 70. The differentiated archesporial cell lies below the nucellar epidermis. The inner integument can be seen as a small protuberance arising from the nucellar tissue at the base and to the left of the archesporial cell. (1500X).

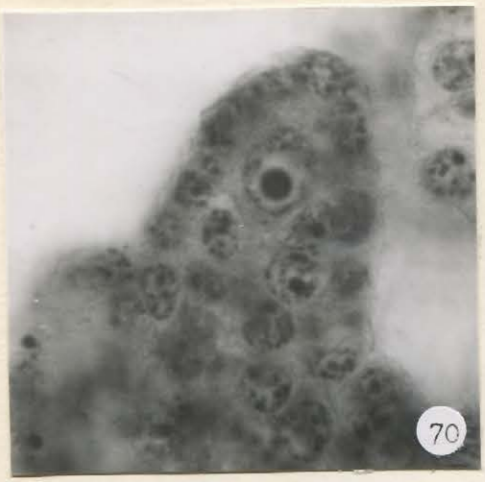
Figure 71. The archesporial cell gives rise directly to the megaspore mother cell which is seen here in late prophase of meiosis 1. The inner integument has grown considerably and the ovule is completely anatropous. (1000X).

Figure 72. The megaspore tetrad in which the micropylar and chalazal megaspores are degenerating. The lower of the two megaspores in the center of the tetrad is the most active. (1400X).

Figure 73. The functional megaspore mother cell. Two degenerating megaspores are situated at the micropylar end of the sac and one at the chalazal end of the sac. (1400X).

Figure 74. This photograph represents the 2-nucleate sac. A vacuole separates the micropylar nucleus from the chalazal nucleus. (1000X).

Figure 75. The 4-nucleate embryo sac. A pair of nuclei is situated at the micropylar end of the sac and separated from the pair of nuclei at the chalazal end of the sac by a vacuole. (1000X).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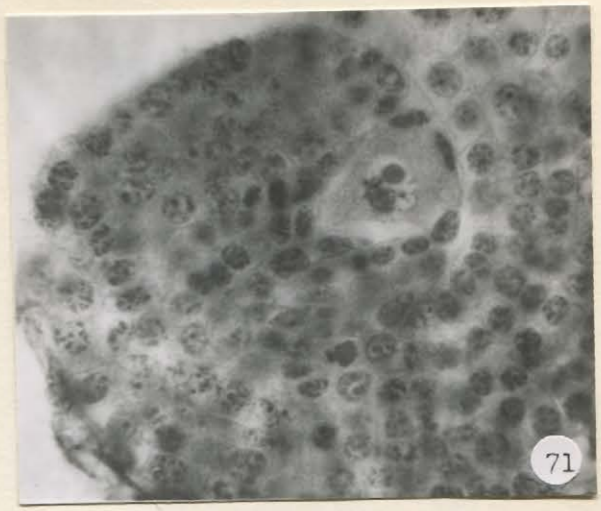
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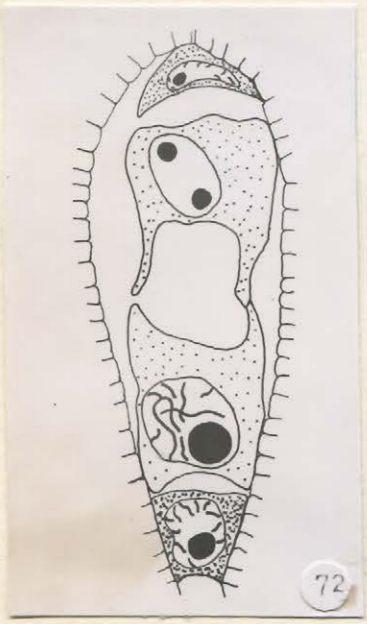
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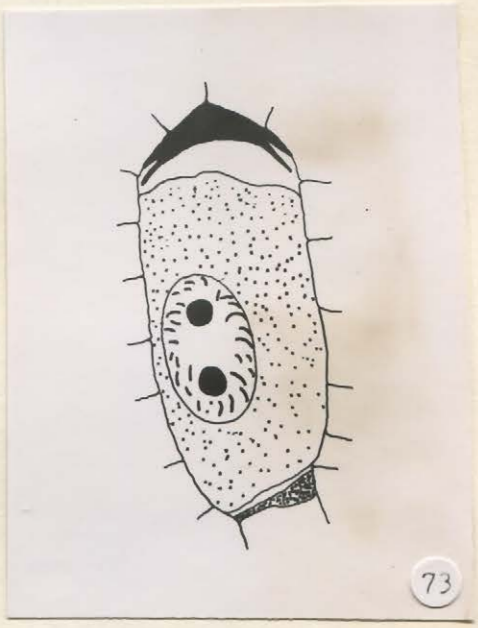
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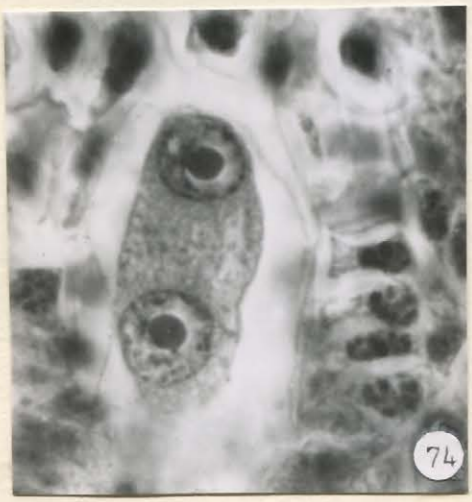
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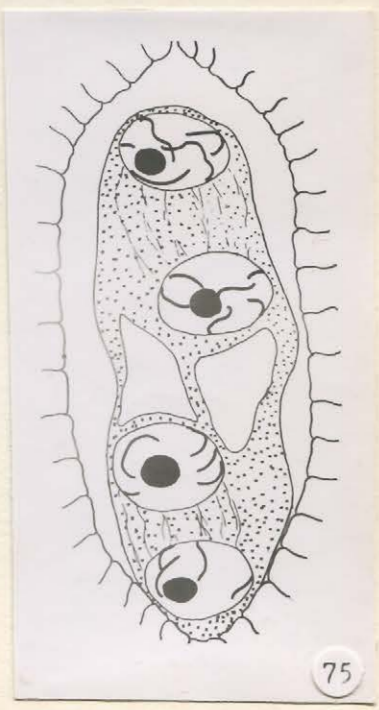

75

I. SULTANI

PLATE XVII.

Figure 76. The 8-nucleate embryo sac. A quartet of nuclei at the micropylar end of the sac is separated from a similar quartet at the antipodal end of the sac by a large vacuole. (1000X).

Figure 77. The mature embryo sac. The egg apparatus is situated at the micropylar end of the sac. The egg lies below the two synergids. The synergids are elongated cells, each with the nucleus near the tip and the vacuole below the nucleus. The two polar nuclei lie side by side near the egg. The three antipodal cells are degenerating at the antipodal end of the sac. (1000X).

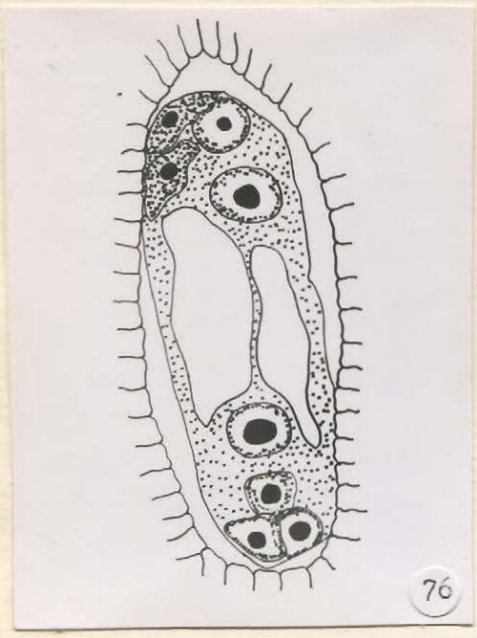
Figure 78. Fertilization. The synergids disappear at the time of fertilization. A sperm nucleus can be seen lying near the egg and also one lies near the fusing polar nuclei. The antipodal end of the sac extends past the degenerating matter of the antipodal cells and can be seen entering the chalazal tissue. (1000X).

Figure 79. The antipodal embryo sac haustorium. This haustorium is curved and its base is 2-lobed. The cells bordering the haustorium are absorbed. (1500X).

PLATE XVII. (contd.).

Figure 80. Post-fertilization. The endosperm nucleus and the embryo sac haustorium are present, but the zygote is degenerating. (1000X).

Figure 81. The endosperm nucleus is degenerating. (1000X).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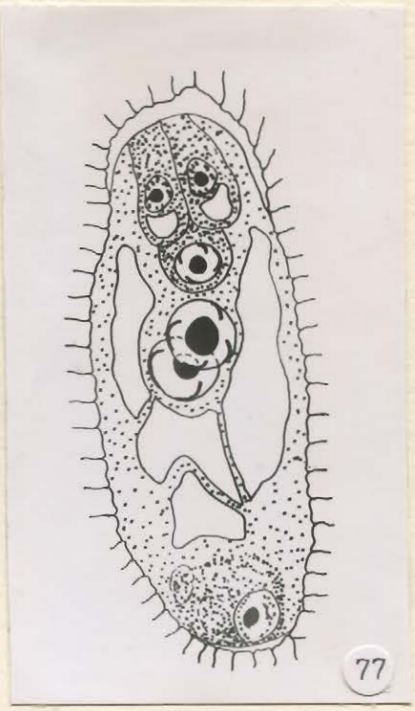
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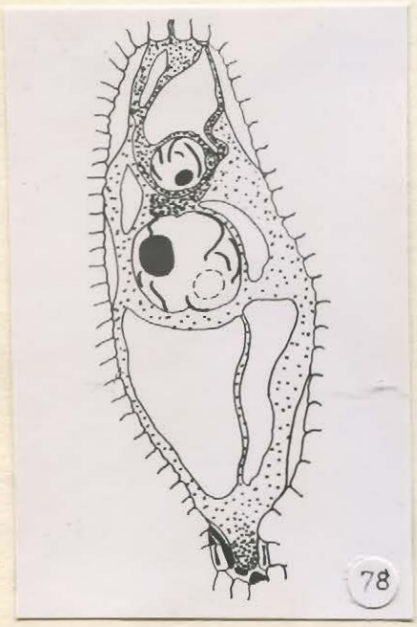
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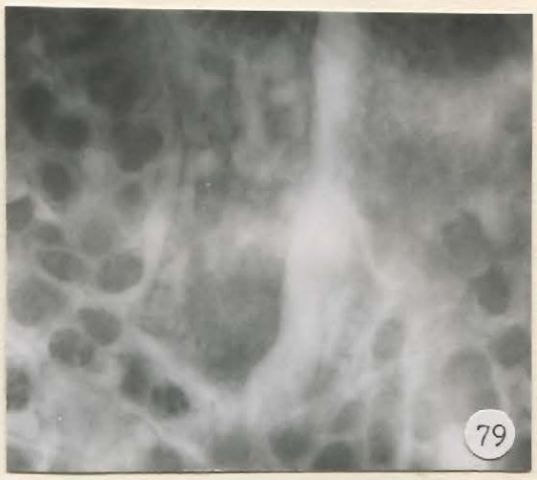
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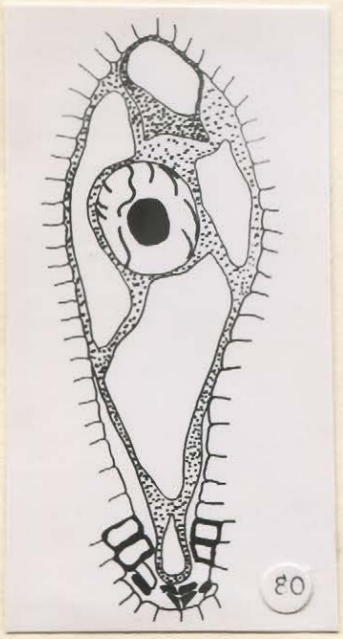
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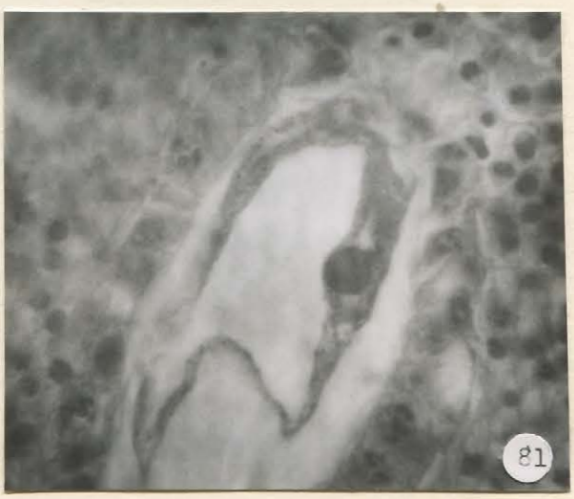
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