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EMBRYOLOGICAL STUDIES IN LORANTHACEAE: GENUS *TRIPODANTHUS* [1]

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I. INTRODUCTION

The *Loranthaceae* are very peculiar in their way of life, floral structure and reproduction. Such particularities have aroused the interest of researchers and a considerable number of studies have been carried out in the field of embryology. Despite this, many genera remain unknown in this respect.

There is no study on the embryology of *Tripodanthus* (EICHL.) TIEGH., being the objective of this work. Species of this genus are aerial parasites, but they sometimes parasitize roots. They occur in the central-west, east and south regions of Brazil, reaching Uruguay, Paraguay, Argentina and Peru; (EICHLER, 1866/1868; ABBIATTI, 1943; RIZZINI, 1968).

II. MATERIALS AND METHODS

1. *Botanical material.* - *Tripodanthus acutifolius* (R. et P.) TIEGH, [= *Phrygilanthus acutifolius* (R. et. P.) ElCHL.] From Porto Alegre, RS, Brazil and *Tripodanthus flagellaris* (CHAM et SCHLECHT.) TIEGH. [= *Phrygilanthus flagellaris* (CHAM. et SCHLECHT.) EICHL.], From Cuesta Blanca, Córdoba, Argentina.

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2. *Methods.* - Buds were collected at various stages of development and fruits of *Tripodanthus acutifolius* and *Tripodanthus flagellaris* flowers.The various organs were fixed in FAA 50 and Farmer's fluid. Subsequently, they were submitted to ethanolic dehydration and to infiltration and inclusion in paraffin (SASS, 1951). The coloring methods used were those of safranin and "fast-green" (SASS, 1951) and safranin, "fastgreen" and hematoxylin from Heidenhain (CONN *et al.,* 1960). Both gave good results, especially the latter.

For the palynological observations, acetolized material was used, according to the technique of ERDTMAN (1966).

III. RESULTS

1. *Microsporangium*, *microsporogenesis and male gametophyte.* - The anther consists of 4 microsporangia, which are individually opened by a longitudinal slit. The wall, according to its origin, is of the monocotyledon type of DAVIS (1966). In this case, the primary parietal layer divides periclinally giving rise to two secondary parietal layers. The external one will constitute the endothecium and the internal one undergoes a new parietal division, forming the middle layer and the tapetum. The wall of the anther is, therefore, constituted of 4 strata, namely: epidermis, endothecium, middle layer and tapetum.

The epidermal cells, with development, increase in size, becoming well vacuolated and their walls thicken. This stratum remains intact, even in the dehiscent anther (Fig. 1 A-G). The endothecium consists initially of small, vacuolated cells that elongate radially as development proceeds. Occasionally, the cells that make up this stratum divide periclinally, which is why in some areas two layers occur. In the mature anther this stratum is constituted by cells of different sizes, and there are still variations as to the distribution of the fibrous thickenings. The cells located in the area near the dehiscence zone are higher and have all their thickened walls, i.e., the radial ones as well as the external and internal tangentials. Those located in a more distal area are somewhat smaller and thickenings occur in the radial walls and the inner tangential (Fig. 1 A-G). The middle layer is ephemeral and is formed by a single stratum of cells that, however, presents double in certain areas (Fig 1A, B, D, E). The tapetum is glandular and its cells are uninucleated. This stratum is practically consumed during the formation of the pollen grains, and the Ubisch orbits, juxtaposed to the tangential, inner wall of the endothetium and to the pollen grains (Figure 1 AG) can be evidenced. The microsporangial ablation region consists of small, vacuolated cells with no thickening (Fig. 1 H).

Sporogenic cells, in each microsporangium, undergo mitotic divisions giving rise to a large number of pollen mother cells (Fig. 1A, B, D, E). These, in section, have a polygonal contour and are distinguished from tapetum cells by their larger nucleus and dense cytoplasm (Fig. 1 I). The meiotic cytokinesis is of the simultaneous type and thus results in tetrahedral tetrads (Fig. 1 J). The newly formed microspores are triangular with the concave sides, occupying the nucleus and the central position. Microspores with four angles were occasionally observed. During maturation, the microspore increases in volume. Internally to the wall of callose, it develops the intine, thin and uniform and the exine, thicker and ornate in the region of the mesocolpium. (Fig. 1 K). The nucleus divides and originates the vegetative and generative cells. It has a large nucleus and a reduced cytoplasm, surrounded by an evident hyaline-like wall (Fig. 9E). Dissemination occurs at this stage.

The pollen grains are small, 3-, zonocolporate, syncolpate, NPC 345 (ERDTMAN) and STRAKA, 1961) the surface pilate in the region of the mesocolpium and psilate in the other parts

of the grain.The amb is triangular, with concave sides and obtuse vertices (Fig. 2 AD). The nexine 2 retains more or less a constant thickness and the nexine 1 is thickened in the region of the mesocolpium. The sexine is formed by stacks of variable heights; the longest are situated exactly in the center of the mesocolpium. Grains are angle-apertured. The aperatures are compound; the colpi have smooth and rectilinear margins in the polar zones, being more or less circular in the equatorial zones. They are not very noticeable and are located in the center of the equatorial zone.

2. *Mechanism of anther dehiscence.* - As already mentioned, the endothecium presents two regions that differ in the size of its cells and location of the thickenings in the cellular walls. Most endothecial cells are large and thickened on all walls; a few cells, opposite the zone of dehiscence, are characterized by thickening only on the radial and inner tangential walls. The first, that is, cells with thickenings in all its walls, takes part in the opening mechanism of the microsporangium, acting as a rigid door. The cells mentioned second, four to five longitudinal rows and having thin external tangential walls, are the ones that fulfill the mechanical opening function, acting as a flap. The functionality of this system has been tested by placing the anther under a binocular scope and irradiating it with a lamp in order to produce desiccation, with which the anther effectively opens. This process is shown schematically in Figure 2 E-G.

3. *Megasporangium, megasporogenesis and female gametophyte.* - The longitudinal and transverse sections of buds at different stages of development show that the ovary is trilocular, with each locule being occupied by a non-integumentated hemi-anatropous ovum. The locules are connected by a compitum. This, initially, is hollow and ample and is in communication with the style channel. With development it forms a mameliform structure, constituted by vacuolated cells with thin walls. This structure ends up obliterating the cavity in the region of the compitum, so that it becomes virtual (Figs 3A-I, 4A, C). The style is solid and consists of small, compactly arranged cells. This tissue accumulates starch (Fig. 5A), which will serve as a food source for the development of the megagametophytes.

In each ovule, a multicellular archesporium is differentiated, subepidermally, whose cells are distinguished from the neighboring ones by its larger nucleus and more dense cytoplasm. These cells lengthen and function directly as megaspore mother cells. Some of them undergo meiosis and give rise to linear tetrads of megaspores, all of which are potentially functional. Up to three tetrads were observed in each ovule (Fig. 3AI, 4A-E).In analogy with other angiosperms, the side in which the multicellular archesporium differs can be considered micropylar, although, due to the lack of integuments, there is no micropyle. Due to the great degeneration of the sporogenous tissue, mother cells of megaspores, dyads and tetrads, it was not possible to observe embryo sacs in bi- and tetranucleate stages. Nevertheless, it was possible to determine that the growth of the embryo sacs takes a direction opposite to the "micropylar" zone. In fact, they go to the style first crossing the chalazal zone of the ovule and then the upper part of the ovary, finally penetrating the style. The existence of hexanucleated embryo sacs suggests that the nuclei of the tetranuclear embryo sac do not divide at the same time. The lower nuclei divide first and organize into the antipodals and the lower polar nucleus, while the upper nuclei remain individual for a certain time, thus resulting in a hexanucleated embryo sac (Fig. 5 BE). During the process of formation of the female gametophyte, a group of cells that have thickened and lignified walls differentiates at the base of the ovary. However, for reasons that will be explained in the discussion, this structure was called the pelvis (Fig. 5F, 8A, H). After reaching the upper third of the style, the upper nuclei of the hexanuclear embryo sac divide and organize into the egg apparatus and the upper polar nucleus (Fig. 6A), thus forming the octonucleated embryo sac. The egg cell has a

spherical contour, with the nucleus located at the top and the vacuole at the basal thereof. The synergids show a nucleus and vacuole located in the same position of the egg cell (Figs. 6A, B; 9Ac). The antipodals are large, one-walled, and vacuolated. When viewed from the side, they appear to be two, one of them binucleate (Fig. 5D), however, when viewed from the front one can see two at the top and one at the chalazal zone (Fig. 5E). The polar nuclei lie at the ends of the central cell. The embryo sac may form a caecum in the basal region, growing towards the base of the ovary, but leaving the antipodals "in situ" (Fig. 5A). At the mature gametophyte stage, the parenchyma cells that extend from the base of the embryo sac to the pelvis accumulate starch; this will be further digested by the cytoplasmic activity around the endospermogenetic nucleus (Fig. 5A). The starch also occurs in the nectary and in the other tissues of the ovary, but in a much smaller quantity. About 9 to 14 female gametophytes may be present in the upper third of the style, with their egg apparatus, located at different heights (Fig. 6D).

Before fertilization, the lower polar nucleus migrates toward the upper polar nucleus located in the style where they merge (Fig. 9D). The secondary nucleus is thus located near the egg cell (Figs. 6B; 9A, B).

4. Endospermogenesis. - It was not possible to observe the union of the egg cell with one of the male gametes, since all the preparations had the zygote formed. However, it was observed the union of the other male gamete with the secondary nucleus, which occurred later. This aspect is shown in Figure 6C. Once the fusion has occurred, the endospermogenetic nucleus migrates toward the ovary (Fig. 6E, F), directing towards the pelvis. In this route, the amiliferous tissue existing in the central region of the ovary is consumed. Upon reaching the pelvis, the nucleus divides resulting "ab initio" a cellular endosperm (Fig. 6 H). New divisions occur resulting in an irregular structure. The basal cells are thin and have a dense cytoplasm; the upper cells are elongated, vacuolated and show a serial arrangement. (Fig. 7A, B). The cross sections of the fruit reveal the existence of several endosperm groups; these, later, merge, resulting in a compound structure. In the globular embryo stage, the endosperm is organized and differentiated into three regions: 1, epidermis, consisting of small cells, with dense cytoplasm; 2, central region, with large cells, rich in starch and 3, region adjacent to the embryo, consisting of small, vacuolated and partially digested cells (Fig. 7 F). The mature endosperm enveloped the entire embryo (Fig. 8 I). It was also verified the occurrence of cellular filaments, uniseriate, at different heights of the ovary and style. These cells were interpreted as probably resulting from nucleus and endospermogenetic divisions, before reaching the pelvis (Fig. 6C).

5. Embryogenesis. - The initial stage of embryo development was not observed. Figure 7C shows an embryo with 16 cells arranged in two strands of 8. The basal cells of the suspensor are larger and vacuolated; the embryonic cells themselves are smaller and have dense cytoplasm. During its growth the embryo passes through the endosperm (Fig. 7A, B), growing towards the pelvis. The globular embryo (Fig. 7D, E) is endowed with a multicellular, secondary suspensor, as wide as the embryo itself. Although two pro-embryos have been observed in the young fruit, only one develops. The mature embryo consists of the hypocotyl-radicular axis and two cotyledons, free for their entire length in *Tripodanthus acutifolius* (Fig. 8 I) and partially fused in *Tripodanthus flagellaris* (Fig. 9 F). In the radicular region, the secondary suspensor remains (Fig. 8 I).

6. Fruit. - The wall of the fruit is initially parenchymatous. After fertilization it is differentiated into four distinct zones, namely: 1, external parenchyma zone; 2, viscin zone; 3, internal parenchyma zone, and 4, vascular area (Fig. 8A). The external parenchymatic zone is of succulent in nature and is constituted by small, thin-walled cells. The cells of the viscin zone are

initially small and with dense cytoplasm. With the development of the fruit, such cells elongate radially and their cytoplasm becomes vacuolated. This phenomenon is more pronounced in the upper third of the fruit. The viscin extends throughout the fruit, externally to the internal parenchyma. In the upper part of the fruit, it is vascular bundles that supply the androecium and perianth. The internal parenchyma is composed of thin, vacuolated walls. These cells are larger in the middle and lower thirds of the fruit. The vascular zone is constituted by the vascular bundles that supply the perianth and androecium and by the parenchymatic cells associated with them. The sclereids are confined to the basal area of the fruit. The pelvis can be evidenced in the different stages of development of the fruit (Fig. 8 A, H, I).

IV. DISCUSSION AND CONCLUSIONS

The wall and the microsporangium of the *Loranthaceae* is found to be constituted by 4 to 6 layers. In *Tripodanthus acutifolius* it is formed by 4 layers, as occurs with other species of the family *Loranthaceae* (MAHESHWARI and SINGH, 1952; DIXIT, 1958, 1961; PRAKASH, 1961; RAJ, 1970; VENTURELLI, 1981). The presence of uninucleated cells on the tapetum, as described in *Tripodanthus acutifolius,* is reported only for *Dendrophthoe falcata* (L. *)* F. ETTINGSH. (SING H, 1952) and *Barathranthus axanthus* (KORTH.) MIQ. (PRAKASH, 1963). In the other *Loranthaceae* species such cells have 2, 3 or 4 nuclei. The absence of fibrous thickening in the endothecial cells is only found for *Macrosolen cochinchinensis* VAN TIEGH. (MAHESHWARI and SINGH, 1952), *Lepeostegeres gemmiflorus* (BL.) BL. (Dixit, 1958), *Elythranthe* (AGRAWAL, 1953 apud JOHRl and BHATNAGAR, 1972) and *Amylotheca dictyophleba* VAN TIEGH. (RAJ 1970). In other species of the family, endothecial cells have thickened radial and inner tangential walls, as is. also, common among Angiosperms. In this aspect, *Tripodanthus* differs from the other species studied, since the endothecium presents two distinct regions as the occurrence of thickenings in its walls. As described, the arrangement of these thickenings conditions [affects] the mode in which anther dehiscence occurs, a fact not yet mentioned for the family. It is therefore interesting that this subject be investigated in other species in order to establish the possible phylogenetic implications.

The pollen grains are triradiate, as is common among the *Loranthaceae.* Occasionally, tetradradiate grains were found, as also reported by JOHRI *et al.* (1957), Nayayana (1958 a, b), PRAKASH (1963) and RAJ (1970) for the species analyzed by them. Regarding sculpture, the observations made in *Tripodanthus* differ from those of BARTH (1972), since the author cites the occurrence of bacula, instead of stacks, in the sexine.

The trilocular ovary as described for *Tripodanthus acutifolius* is also mentioned for *Macrosolen cochinchinensis* (MAHESHWARI and SINGH, 1952), *Nuytsia floribunda* (LABILL) R. BR. (NARAYANA, 1958a) and *Lepeostegeres gemmiflorus* (DIXIT, 1958b). In each ovule, several archesporial cells differ subepidermally, as occurs in the species mentioned above. The stylar channel, in the species studied, is solid and the transmission tissue is rich in starch. This situation was only mentioned previously by SMART (1952) for *Tupeia* CHAM. et SCHLECHT. and by VENTURELLl (1981) for *Struthanthus vulgaris* MART. In the other species studied the style is hollow. Several embryo sacs are formed in *Tripodanthus*, as is usual among the *Loranthaceae.* It was also observed the occurrence of embryo sacs in the hexanucleate stage, a frequent situation in the family in question. MAHESHWARI (1950) mentions that in the egg cell, the nucleus and greater part of the cytoplasm occupy the basal portion of the cell, placing the vacuole in the upper region. In this respect, *Tripodanthus* differs

not only from the other species of the family analyzed so far, but also from most known Angiosperms, since, as described, the nucleus and vacuole occupy in the egg cell a position similar to that occupied by the nucleus and vacuole of the synergids. Also in *Struthanthus vulgaris* described previously by VENTURELLI (1981) this situation can be observed, although in this species the vacuoles are small. It is possible that this particularity is related to the fact that in *Loranthaceae* the first division of the zygote is longitudinal and not transverse as in most Angiosperms. Dixit (1958b) and RAJ (1970) describe only two antipodals, one of which is binucleate, in the species analyzed by them. Dixit (1958a, 1961) also mentions that in the species of *Tolypanthus* and *Amyema*, analyzed by them, 3 or 2 antipodals are formed; in this case, the upper one is binucleate. A situation similar to that described by DIXlT was observed in *Tripodanthus acutifolius.* It is, however, always 3 cells. The apparent occurrence of 2 cells, of which the upper one is binucleate, depends on the position of the section, as observed in this work.

MAHESHWARl (1950) defines the hypostace as a group of nucellar cells, with particular characteristics, located directly below the chalazal zone of the eggs. This structure is reported for several members of the *Loranthaceae* family (SlNGH, 1952; MARESHWARI and SlNGH, 1952; DIXIT, 1958a, 1958b, 1961; JOHRI *et al.,* 1957; NARAYANA, 1956, 1958a, 1958b; PRAKASH, 1960, 1961, 1963, JOHRI and PRAKASH, 1965, RAJ, 1970, VENTURELLI, 1981). In the species under study, a group of lignified walls with an aspect and function similar to that performed by the hypostase, but of a carpel nature, is distinguished in the basal region of the ovary, which is why it is not homologous to hypostase. In view of this, such a structure was termed pelvis, alluding to its vessel shape. As the occurrence of this structure appears to be a characteristic trait of the *Loranthaceae*, it would be interesting for it to be analyzed more carefully, since it is very likely that any and all citations of hypostase occurrence should in fact refer to the pelvis

The presence of more than one pro-embryo, as observed in *Tripodanthus acutifolius*, seems to be the rule in the family; only one reaches maturity, as is usual. The cotyledons are free throughout their length in certain species of *Loranthaceae* (MAHESHWARI and SINGH, 1952; DIXIT 1958b; PRAKASH, 1960, 1961; RAJ, 1970) or partially fused in others (SINGH, 1952; AGRAWAL, 1954; NARAYANA, 1956, 1958a, JOHRI *et al.,* 1957, DlXlT, 1958a, 1961, PRAKASH, 1963, JOHRI and PRAKASH, 1965). Among the species studied, *Tripodanthus acutifolius* has free cotyledons, while *Tripodanthus flagellaris* has them partially fused. Multicellular suspension and composite endosperm, as seems to be rule among *Loranthaceae,* were also observed in *Tripodanthus acutifolius.*

Regarding the fruit, the four regions described for other *Loranthaceae* were observed in *Tripodanthus acutifolius*. The viscin zone is more pronounced in the upper third of the fruit, where it directly surrounds the vascular tissues supplying the perianth and androecium. A similar situation was described for *Macrosolen cochinchinensis* by MAHESHWARI and SINGH (1952).

V. REFERENCES

ABBIATTl, D. 1943. Synopsis of the Argentine Lorantáceas. *Agent Magazine. Agron.* 10: 1-25.

AGRAWAL, J. S. 1954. Female gametophyte, endosperm, and embryo of *Scurrula* Linn. Indian Science Congress, 41st. Proceedings 3: 137..

BARTH, M. O. 1972. Systematic catalog of tree plants Southern Brazil - Xl. *Loranthaceae* and *Olacaceae*. *Mem. Inst. Oswaldo* Cruz 70 (1): 49-57.

CONN, H. J., MA DARROW and V. M. EMMEL. 1960. *Staining procedures.* I-XII, 1-289. The Williams & Willkins, Baltimore.

DAVIS, GL 1966. *Systematl embryology of the angiosperms.* I-VIII, 1-528. JOHN WILEY & SONS, New York.

DlXIT, SN 1958a. Morphological and embryological studies in the Loranthaceae family. IV. *Amyema* Van-Tiegh. Phytomorphology 8: 346-364.

---- 1958b. Morphological and embryological studies in the Loranthaceae family. V. *Lepeostegeres gem miflorus* BI. *Phytomorphology* 8 (3-4); 365-376.

---- 1961. Morphological and embryological studies in the Loranthaceae family. VIII. *Tolypanthus* Bl. *Phytomorphology* 11 (4); 335-345.

EICHLER, GA. 1866/68. Loranthaceae. ln *Flora brasiliensis* (Martius, KFP ed.) Frid. Fleischer in Comm; Lipsiae vol. 5, p. 1-135.

ERDMAN, G. 1966. *Pollen morphology and taxonomy.* I-XII, 1-553. Hafner Publishing, New York.

ERDMAN, G. and H. STRAKA. 1961. Cormophyte spore classification. *Förh*. *Geol. Fören*. *Stockholm* 33 (1): 65-78.

JOHRI, BM, JS AGRAWAL and S. GARG. 1957. Morphological and embryo gica I studies in the famil and *Loranthaceae* - I. *Helicanthes elastica*(Desr.) Dans. *Phytomorphology* 7 (3-4): 336-354.

JOHRI, BM and S. PRAKASH. 1965. Morphological and embryogical studies in the family *Loranthaceae* - XI. *Tapinostema acaciae* (Zucc.) Van Tiegh. *Phytomorphology* 15 (2): 150-158.

JOHRI, BM & BHATNAGAR. S.P. 1972. Botanical monograph no. 8. *Loranthaceae* 1-155. Council of Scientific & Industrial Research, New Delhi.

MAHESHWARI, P. 1950. *An introduction to the embryology of angiosperms*. 7a. ed. IX, 1-453. Tata McGraw-Hill, New Delhi.

MAHESHWARl, P. & SlNGH, B. 1952. Embryology of *Macrosolen cochinchinensis. Bot. Gaz.* 14: 20-32.

NARAYANA, R. 1956. Morphological and embryological studies in the Loranthaceae-Loranthoideae. III. *Dendrophthoe neelgherrensis* (W. & *A.*) Van Tiegh. *Half - Yrly Jour. Mysore Univ.* 16: 185-204.

---- 1958a. Morphological and embryological studies in the family Loranthaceae II - *Lysiana exocarpi* (Behr.) Van Tiegh. *Phytomorphology 8:* 146-148.

---- 1958b. Morphological and embryological studies in the Loranthaceae family. III - *Nuytsia floribunda* (Labill.) R. Br. *Phytomorphology* 8: 306-323.

PRAKASH, S. 1960. Morphological and embryo lo gica I studies in the famil and *Loranthaceae* - VI. *Peraxilla tetrapetala* (L. f.) Van Tiegh.*Phytomorphology* 10 : 224-234.

---- 1961. Morphological and embryological studies in the family Loranthaceae. VII - *Atkinsonia ligustrina* (Cunningh.) FV Muell. *Phytomorphology* 11: 325-335.

---- 1963. Morphological and embryological studies in the family Loranthaceae - X. *Barathranthus axanthus* (Korth.) Miq. Phytomorphology 13: 97-103.

RAJ, B. 1970. Morphological and embryological studies in the family Loranthaceae - XIll. *Amylotheca dictyophleba* Van Tiegh. *Oesterr. Bot.* Z. 118: 417-430.

RIZZINI, CT 1968. Lorantáceas. *Flora illustrated Catarinense* 1: 1-44.

SASS, JE 1951. *Botanical microtechnique.* 2 a. I. I-XI, 1-228: The Iowa State College, Iowa.

SING H, B. 1952. A contribution to the floral morphology and embryology of *Dendrophthoe falcata* (L. f.) Ettingsh. J. Linn. *Soc. Bot.* 5.3: 449-474.
SMART, C. 1952. Th e Iife-history of *Tupeia* Cham & Schlechtd. *Trans. Roy. Soc. New Zealand Bot.* 79: 459-466.
VENTURELLl, M. 1981. Embryology of *Struthanthus vulgaris* (Loranthaceae-Loranthoideae). *Kurtziana* 14: 73-100.

VI. SUMMARY

The present paper deals with studies on the embryology and pollen grains of *Tripodanthus acutifolius* (R. et P.) TlEGH. and *Tripodanthus flagellaris* (CHAM. et SCHLECHT.) TIEGH. The anther wall originates according to the Monocotyledoneous type and comprises 4 layers: epidermis, endothecium, one middle layer and a glandular tapetum which remains uninucleate. The type of anther debiscence and the unusual kind of wall thickenings showed by the endothecial cellS are described for the first time for the family. Pollen grains are sincolporate. The exine is pilate on the mesocolpe. The ovary is 3-locular, each locuie having one hemianatropous ovule. A multicellular archesporium differentiates in each ovule primordium. The sporogenous cells function directly as rnegaspore mother cells. The megaspores are arranged in linear rows. The 6-nucleate condition precedes the 8-nucleate stage. The 3 antipodal cells are already conspicuous at the 6-nucleate stage. Nine to fourteen embryo sacs elongate upward, reaching up to two-thirds of the length of the style. ln the egg cell the nucleus Iies in the upper part and the vacuole in the lower, and the same occurs in the synergids. The endosperrn is cellular. The adjacent endosperms of several embryo sacs fuse to forrm a composite endosperm. Same proembryos develop concomitantly but only one reaches maturity. The mature embryo is green and comprises the hypocotyl-root axis and two cotyledons; these are free in *Tripodanthus acutifolius* but in *Tripodanthus flagellaris* they are fused in the upper half. A structure called "pelvis" differentiates at the base of the ovary, and has a function similar to that of the hypostase. The fruit wall comprises 4 zones; the outer one is fleshy. ln the upper region of the fruit the viscin zone surrounds the vascular bundles of the perianth and the androecium.

VI. RESUMO

O presente trabalho descreve a embriologia de *Tripodanthus* (EICHL.) TlEGH. A parede da antem, quanto ao seu desenvolvimento, pertence ao tipo Monocotiledôneo e é formada pela epiderme, endotécio, camada média e tapete glandular, uninucleado. As células do endotécio diferem quanto a ocorrência de espessamentos em suas paredes, o que condiciona um tipo de deiscencill (linda não descrita para a família. Os grãos de pólen são sincolporados, com exina pilada na região do mesocolpo. O ovário é trilocular, com um óvulo heminátropo por lóculo. Em cada óvulo se diferencia um arquespório multicelular. As células esporogênicas funcionam diretamente como célulasmãe de megásporos. As tétrades são lineares, sendo todos os megásporos potencialmente funcionais. O estádio hexanucleado precede o octonucleado, durante a formação do gametófilo feminino. Formam-se de 9 a 14 sacos embrionários, os quais atingem o terço superior do estilete; este é sólido e rico em amido. A oosfera apresenta o núcleo e vacúolo localizados na mesma posição daqueles das sinérgides. O endosperma é celular e resulta da fusão dos endospermas adjacentes, de vários sacos embrionários. Observou-se a formação de alguns pro-embriões, porém, apenas um atinge a maturidade. O embrião maduro é reto, clorofilado e compreende o eixo hipocótiloradicular e dois cotilédones livres em *Tripodanthus acutifolius* e parcialmente soldados em *Tripodanthus flagellaris.* Na base do ovário diferencia-se

a pelvis; esta desempenha função semelhante à da hipóstase. A parede do fruto acha-se constituída de 4 regiões, sendo a externa de natureza parenquimática, suculenta. Na região superior do fruto, a viscina envolve os feixes vasculares que suprem o perianto e androceu.

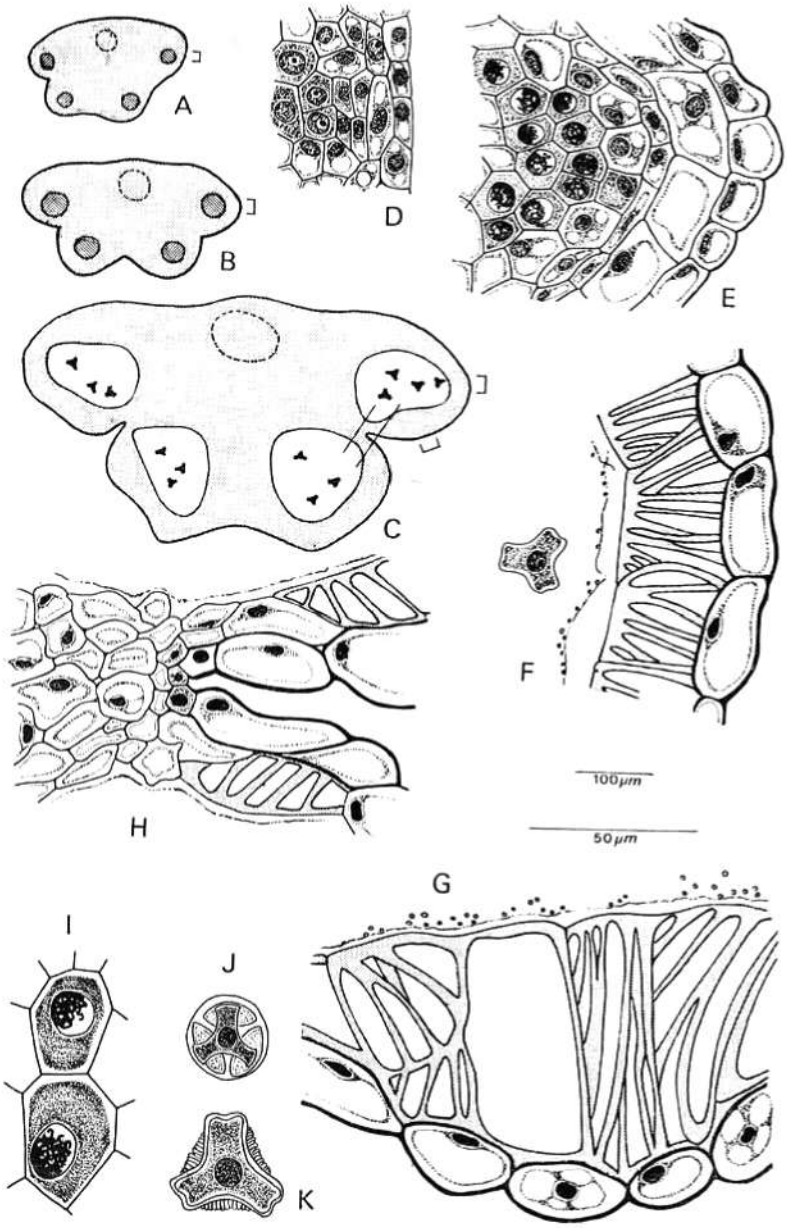

FIG. 1. - *Tripodanthus acutifolius*. A, B, C: schematic representation of transverse sections of the anther at different stages of development; D: detail of an area of the anther represented in figure A, showing the origin of the tapetum and the middle layer; E: detail of an area of the anther represented in Figure B. The epidermis, endothecium, middle layer, tapetum and pollen mother cells are visible in synapse; F and G: detail of the wall of the dehiscing anther, whose areas are indicated in figure C, in the right thecum, in the upper pollen sac, respectively. Figure F shows the thickening present in the radial and inner tangential walls and in Figure G the presence of these thickenings on all walls. Also visible are Ubisch's orbits; H: detail of the anther dehiscence region, the area of which is indicated on the right-hand side of figure C; I: pollen mother cells; J: tetrahedral tetrad; K: microspore.The 100 µm scale corresponds to the figures A - C and that of 50 µm to figures D - K.

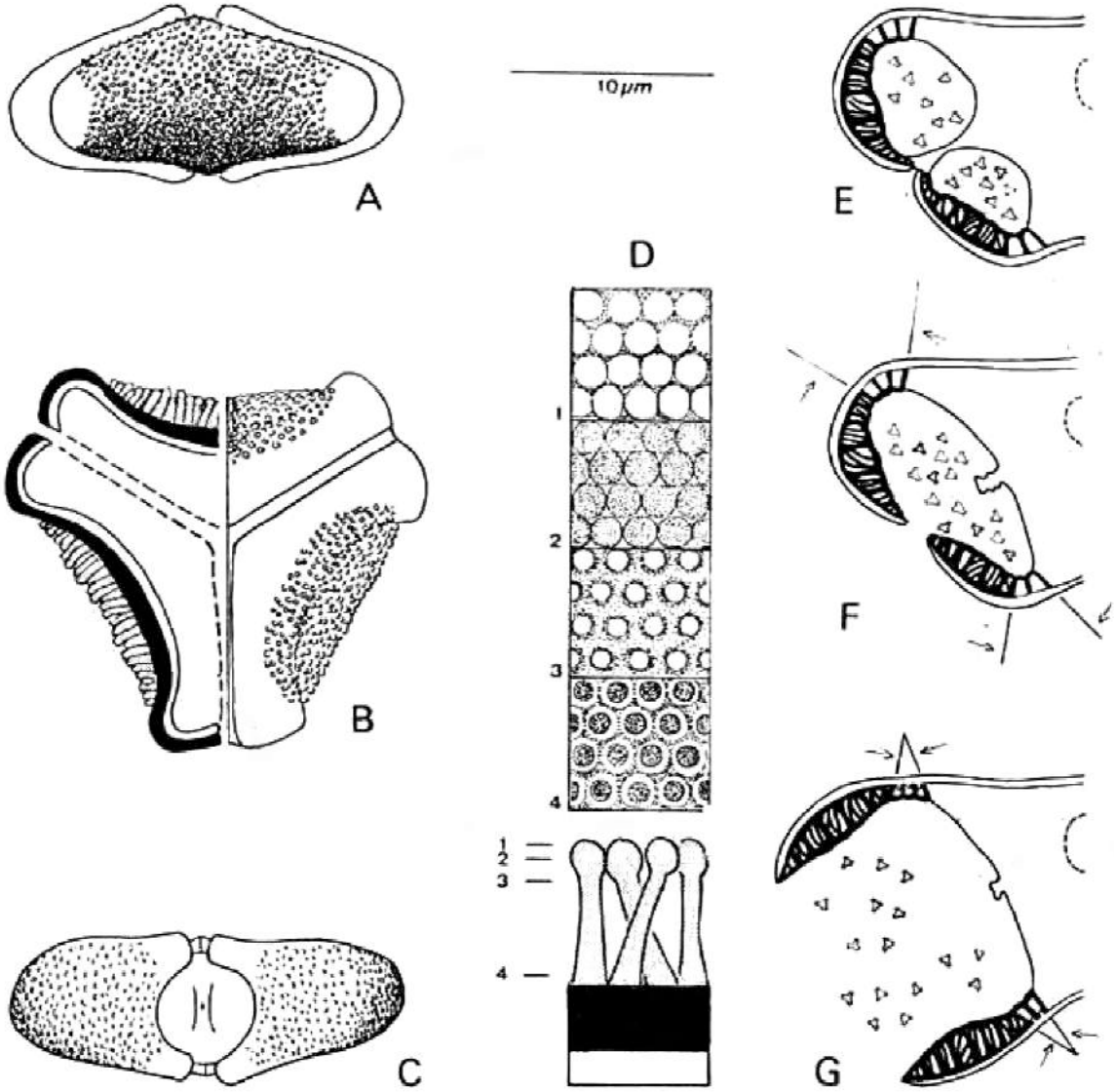

FIG. 2. - *Tripodanthus acutifolius.* A and C: equatorial view of the pollen grain; B: Polar view of the pollen grain, showing the optical section, the pilate surface and the three openings joining at the poles; D: detail of anther wall and LO; E-G: representation and schematic of the anther opening. The 10µm scale corresponds to the figures A-C.

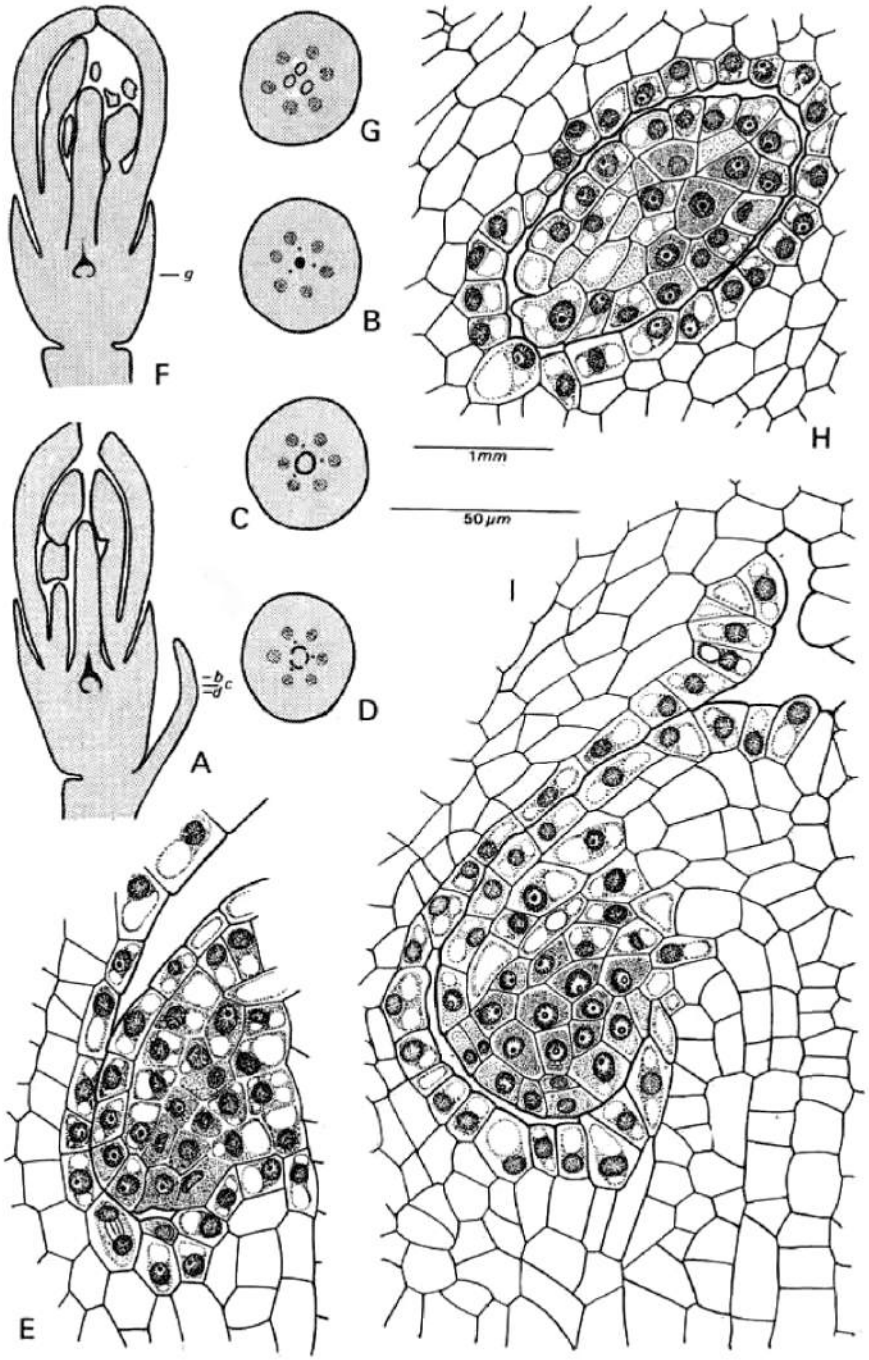

FIG. 3. - *Tripodanthus acutifolius*. A and F: schematic representation of medium longitudinal sections of young flower buds. The sections reveal the ovules, the mamelon, and the region of the compitum; B-D: schematic representation of cross - sections through the ovary, whose heights are marked in figure A; E: detail of the longitudinal section of one of the eggs represented in figure A; G: schematic representation of the transverse section of the ovary, whose height is marked in figure F. H: detail of the cross-section of one of the eggs represented in figure G; I: detail of the longitudinal section of one of the eggs represented in figure F. The scale of 1 mm corresponds to figures A, B, C, D, F, G and that of 50 µm to figures E, H, I.

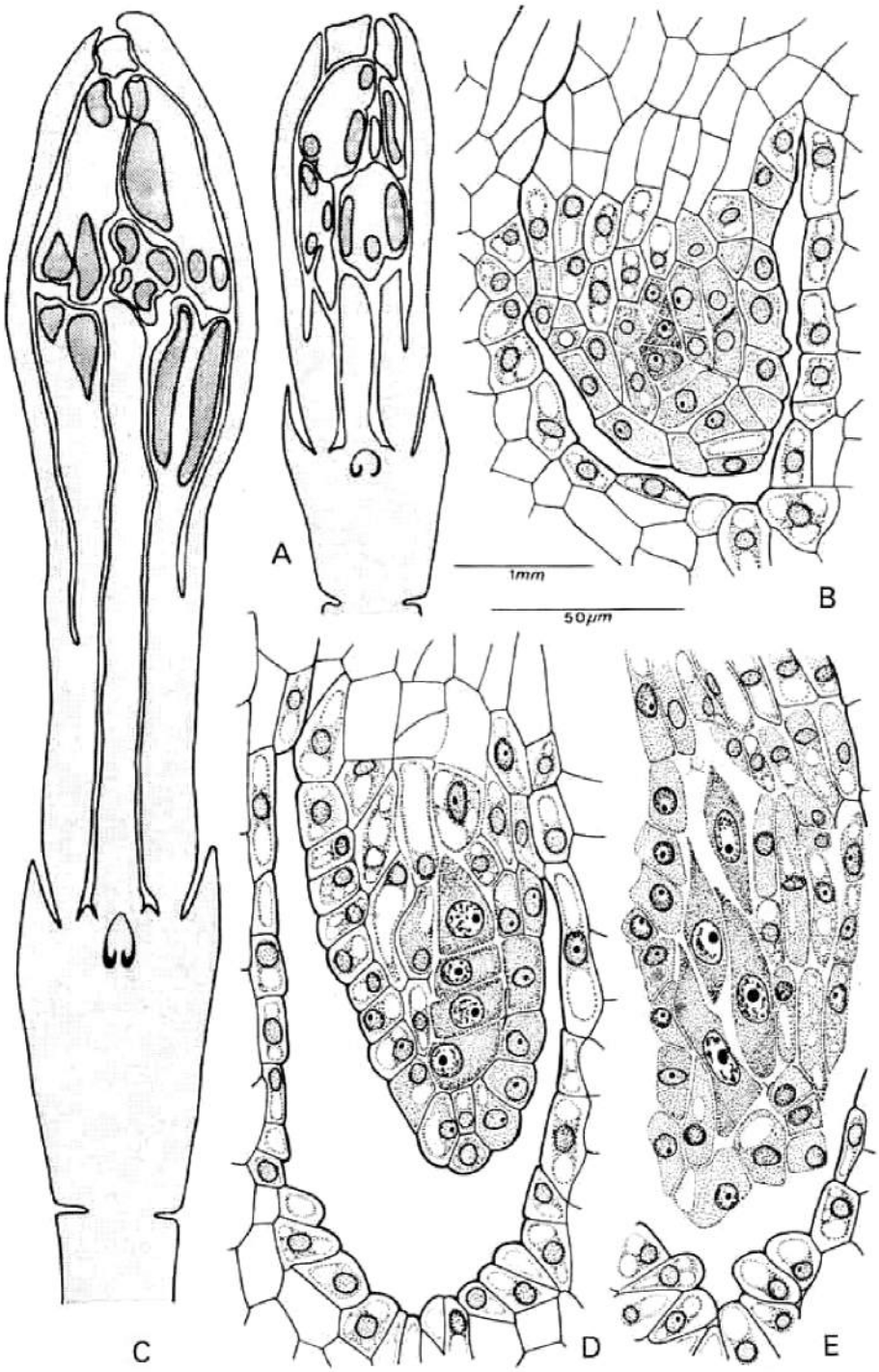

FIG. 4. - *Tripodanthus acutifolius*. A and C: schematic representation of longitudinal sections of the floral bud at progressive stages of ovule development; B and D: details of the ovules shown on the left side of Figures A and C, respectively. It is observed a dyad in the central region of the ovule represented in figure B and a tetrad in figure D; E: detail of an ovule at a later stage than that of figure D, showing 4 and fusiform megaspores.The 1 mm scale corresponds to Figures A and C and that of 50 μm to Figures B, D, E.

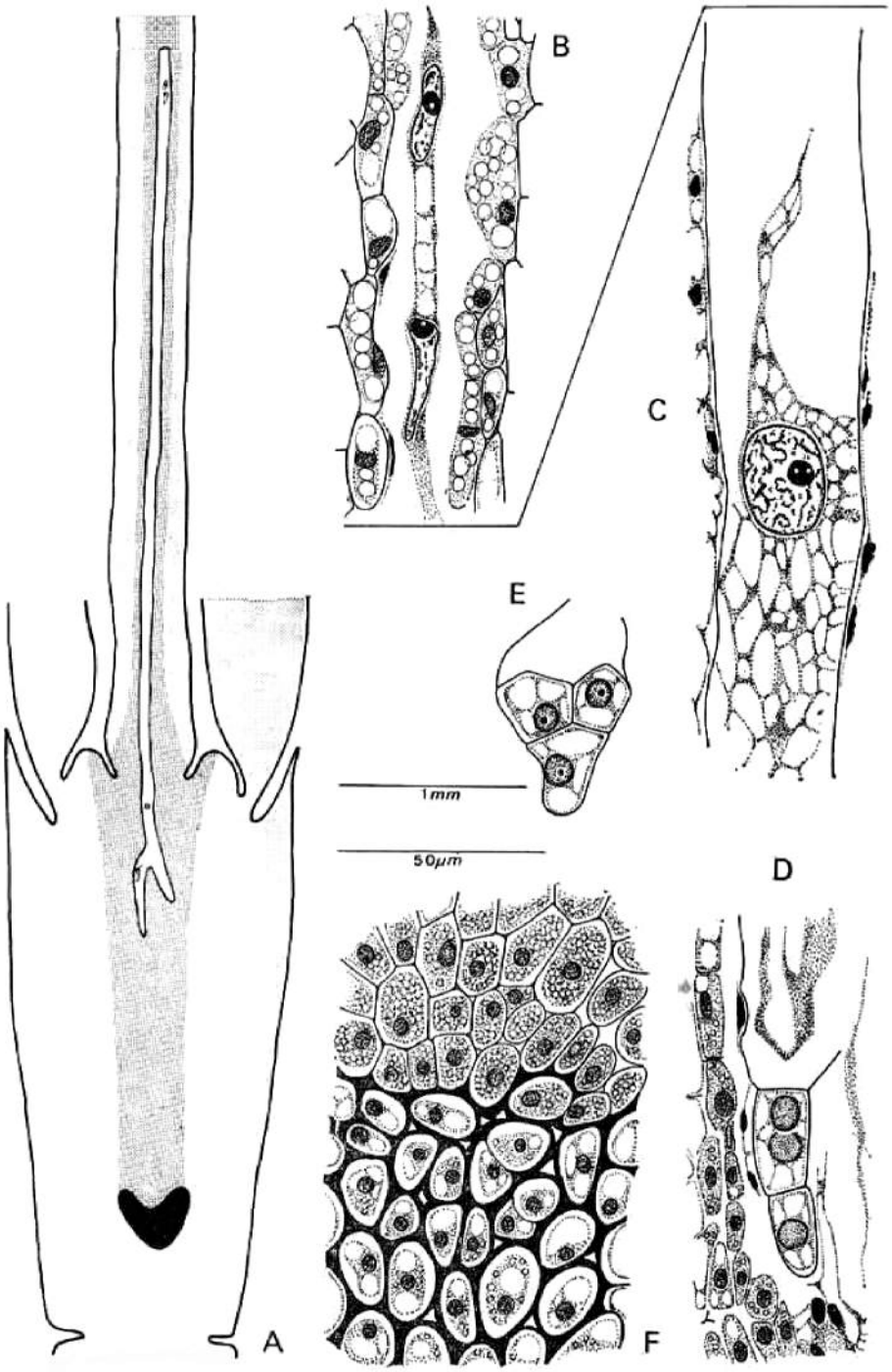

FIG. 5. - *Tripodanthus acutifolius*. A: Schematic representation of the median longitudinal section of the flower, revealing the location of a hexanuclear sac. The pelvis and the amiliferous column are still visible; B: detail of the upper end of the hexanuclear embryo sac shown in Figure A; C and D: details of the hexanuclear sac shown in Figure A. In Figure C is represented the lower polar nucleus and in D the antipodals, side views; E: frontal view of the antipodals. F: Longitudinal section through the pelvis, showing the thickening of the cell walls. The scale 1 mm corresponds to figure A and that of 50 µm to Figures B-F.

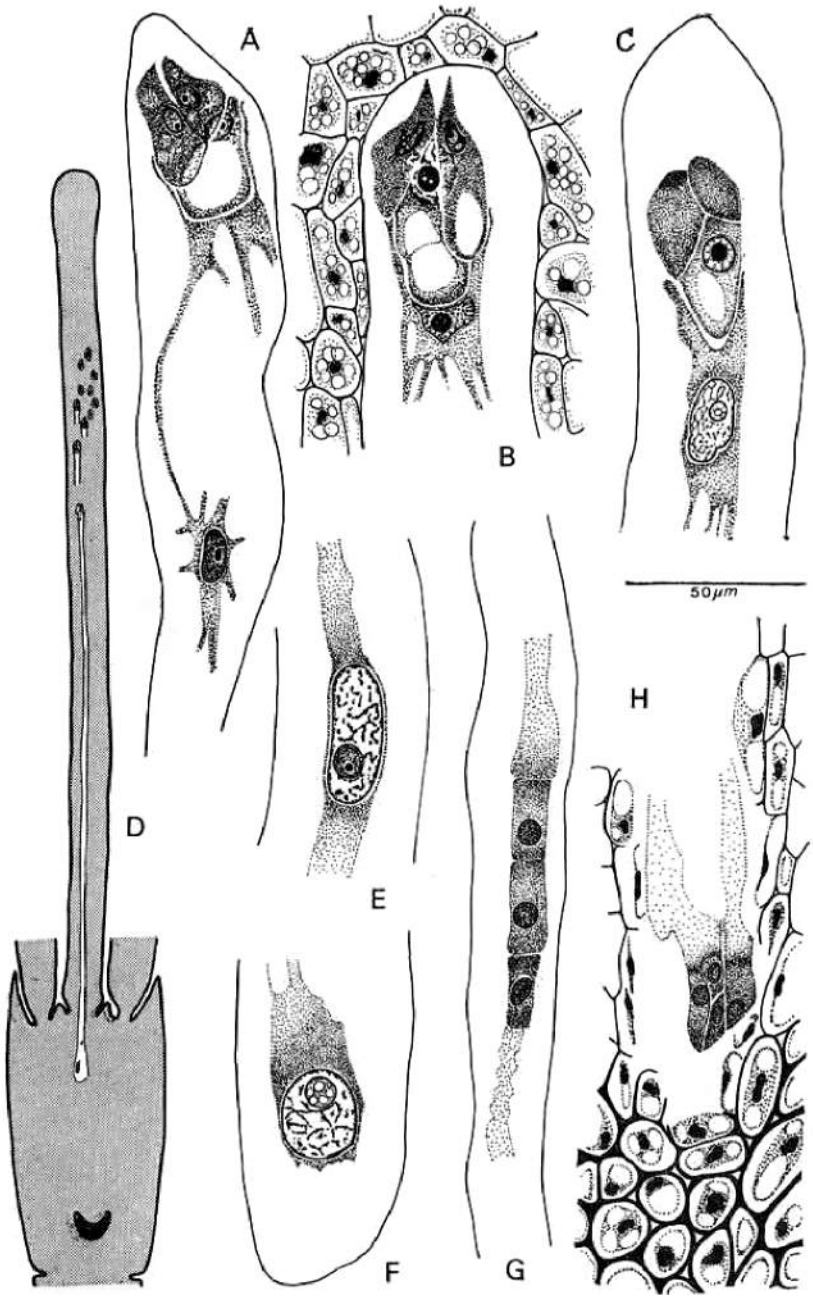

FIG. 6. - *Tripodanthus acutifolius*. A: longitudinal section through the upper extremity of the female gametophyte showing the egg apparatus and the upper polar nucleus. The inverted position of the vacuole and nucleus of the egg cell is shown: B: longitudinal section through the upper end of the female gametophyte showing the egg apparatus and the secondary nucleus: C: longitudinal section through the upper end of the female gametophyte showing the zygote, synergids and and the fusion of the secondary nucleus with one of the male gametes: D: median longitudinal section of the flower showing the height reached by the embryo sacs; E and F: endospermogenetic nuclei located, respectively, in the style and upper portion of the ovary: G: cell filament, located in the style and probably resulting from the divisions of the endosperm nucleus, in that region; H: endosperm with 4 cells, in the region near the pelvis. The scale of 50 µm corresponds to figures A, B, C, E, F, G, H.

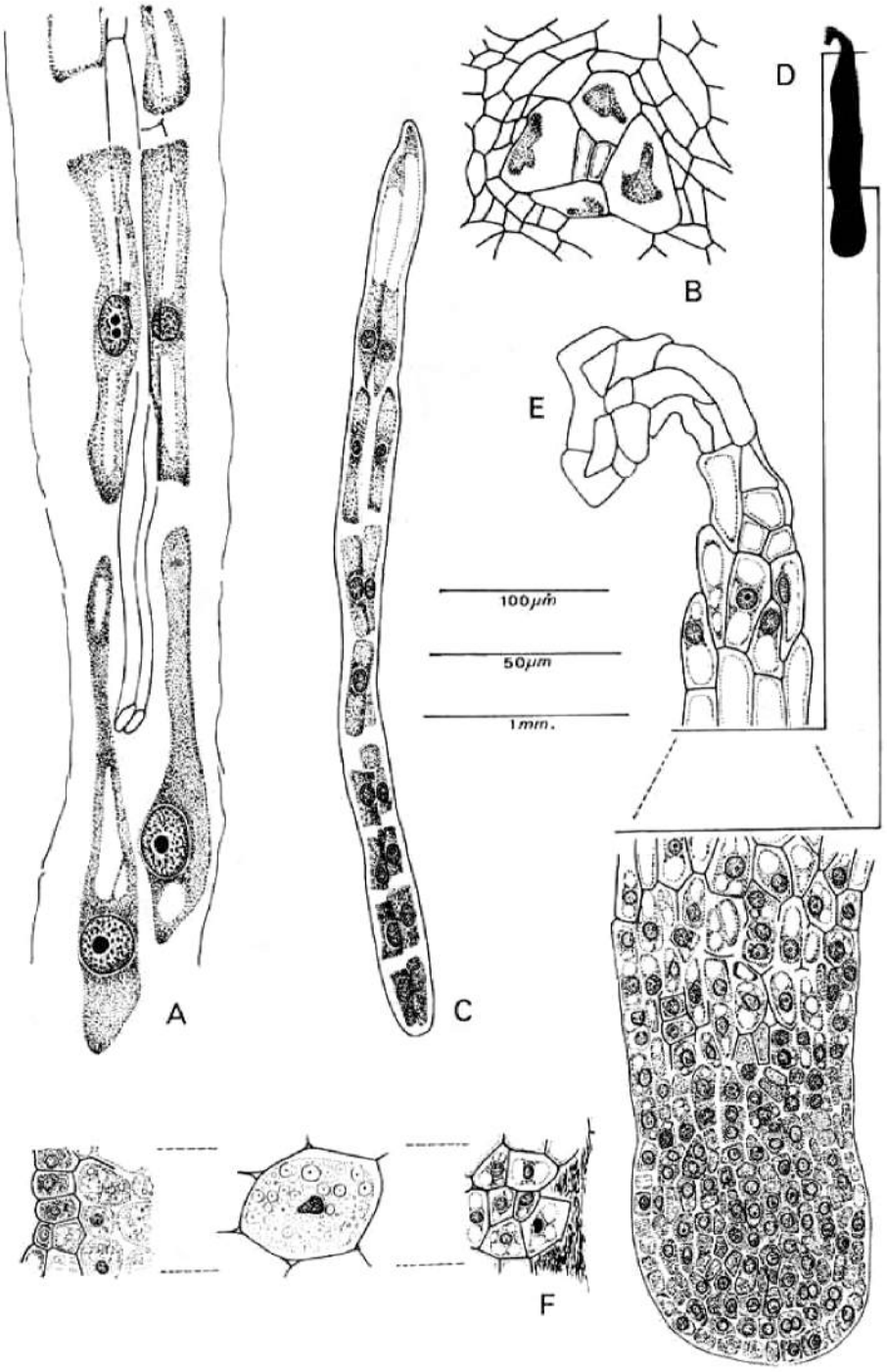

FIG. 7. - *Tripodanthus acutifolius.* A: longitudinal section through the young fruit, showing part of the embryo and endosperm; B: cross section through the young fruit, showing the embryo surrounded by 4 cells of the endosperm; C: embryo with 16 cells: D: globular embryo; E: detail of the median longitudinal section of the embryo represented in figure D, showing the embryo itself and part of the secondary, multicellular suspensor; F: longitudinal section through the mature endosperm showing the epidermis, the middle region, with starch, and the internal part partially digested. The 50 µm scale corresponds to figures A, B, C; 10 µm to Figures E, F and 1 mm to figure D.

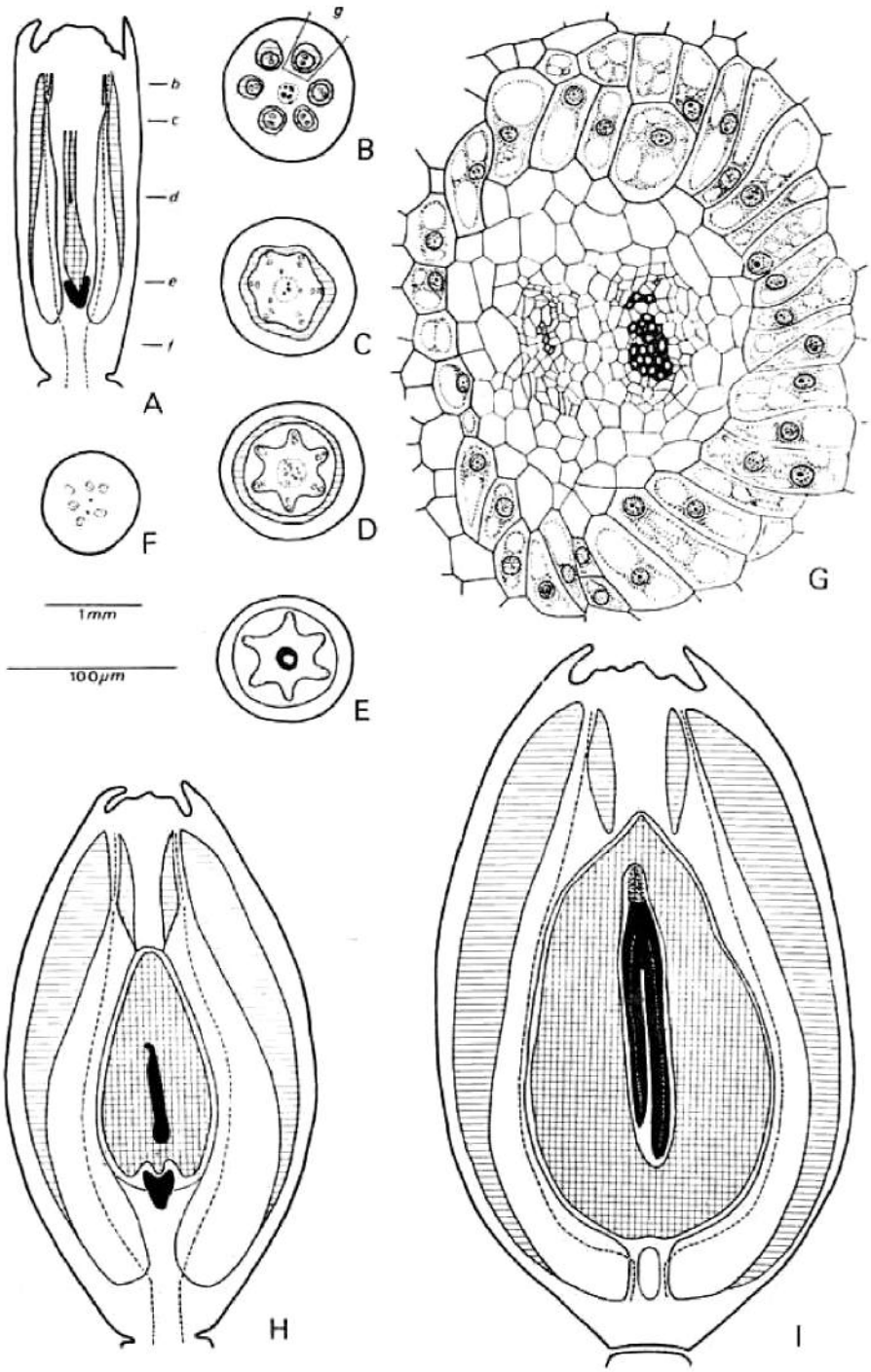

FIG. 8. - *Tripodanthus acutifolius.* A, H, I: Schematic representation of longitudianl sections through the fruit at different stages of embryo development. The location and gradual development of the viscin zone is observed; B-F; cross sections through the young fruit, whose heights are indicated in figure A; G: cross-section through one of the vascular bundles shown in figure B, showing the arrangement of the viscin cells, around the bundle. *Symbolism;* the striped zone corresponds to the viscin, the checkered to the endosperm and the black zone to the embryo and pelvis. The scale of 100 μm corresponds to the figure G and 1 mm to Figures A, B, C, D, E, F, H, I.

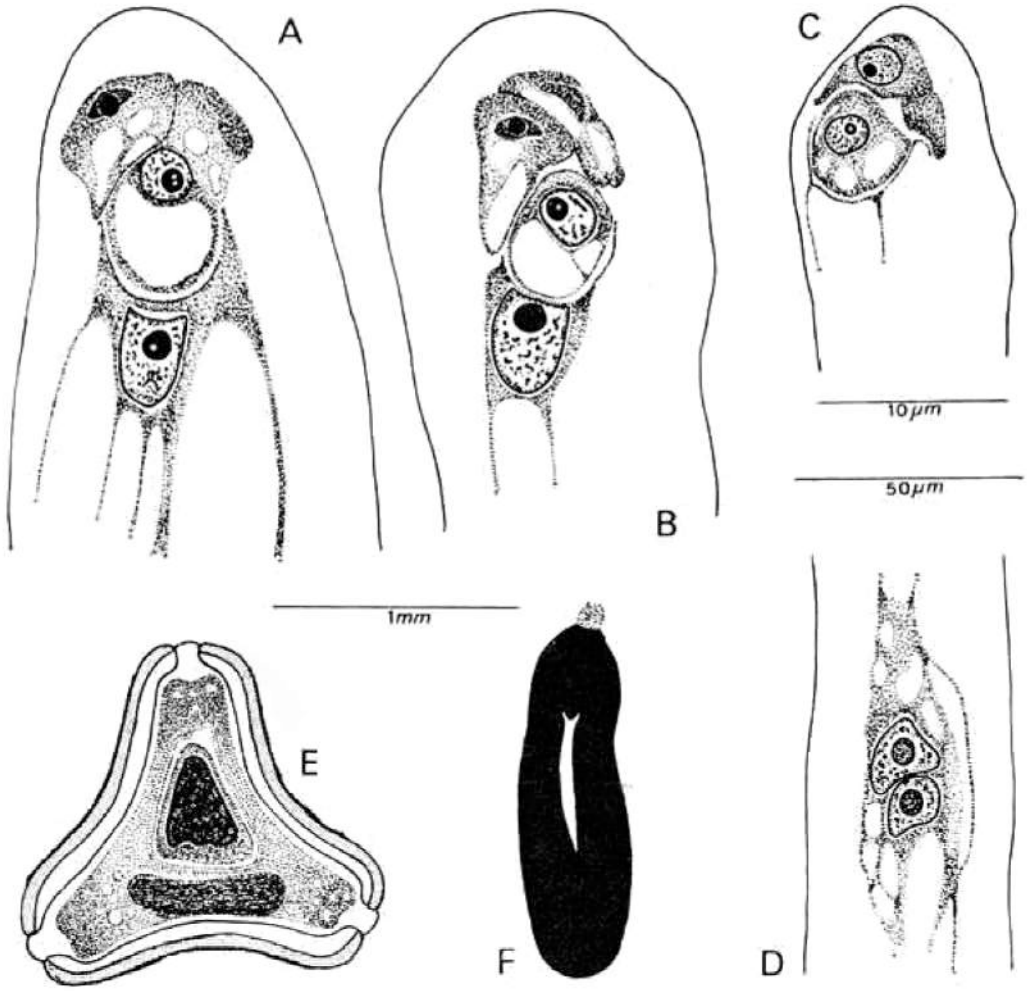

FIG. 9. - *Tripodanthus flagellaris.* A and B: longitudinal section through female gametophytes showing the egg aparatus and the secondary nucleus. The nucleus of the egg cell is located in the upper part of the same and the vacuole in its basal portion; C: longitudinal section through the gametophyte showing the egg apparatus. The egg was is at an initial stage of vacuolation; D: longitudinal section of the polar nuclei, before fusing; E: male gametophyte; F: embryo. Partial fusion of cotyledons is observed. The scale of 10 μm corresponds to figure E, and that of 50 μm to figures A-D, and 1 mm to figure F.