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条纹龙胆的胚胎学研究*

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摘 要: 首次报道了条纹龙胆(*Gentiana striata* Maxim.)的胚胎学特征, 研究结果用以讨论龙胆属狭蕊组(*Gentiana* Sect. Stenogyne)的系统演化关系。主要研究结果如下: 花药四室; 药壁发育为双子叶型; 绒毡层细胞仅来源于初生壁细胞, 故绒毡层起源属单型起源, 细胞具单核, 原位退化, 属腺质型绒毡层, 药隔处的绒毡层细胞经多次平周分裂形成 2 层至多层的绒毡层细胞, 其余部位的绒毡层细胞仍为 1 层细胞; 中层细胞 1 层; 在花药成熟时, 花药的表皮细胞和药室内壁均部分纤维状加厚且柱状伸长。小孢子母细胞减数分裂为同时型, 四分体的排列主要为四面体形; 成熟花粉为 3-细胞型。子房为 2 心皮, 1 室, 侧膜胎座。胚珠 4 列。薄珠心, 单珠被, 珠心基部产生珠被原基, 进而形成珠被, 条纹龙胆仅有 1 层珠被。珠被沿珠心向上生长并将珠心包围, 于胚珠顶部形成珠孔。胚珠在发育过程中, 整个胚珠的本体倒转, 而且珠柄继续生长并弯曲, 使珠孔与合点端的连线与珠柄垂直, 形成 Hypertropous 胚珠。大孢子母细胞减数分裂形成的 4 个大孢子呈直列式排列, 合点端的大孢子具功能。胚囊发育为蓼型。极核在受精前融合为次生核, 反足细胞 3 个、多宿存。雄蕊先熟。珠孔受精。胚乳发育为核型。胚胎发育为茄型酸浆Ⅱ变型。通过比较龙胆属狭蕊组与龙胆属其它组和双蝴蝶属的胚胎学特征表明, 龙胆属狭蕊组在一些重要的胚胎学特征上与双蝴蝶属较相似, 而与龙胆属其它组存在较大差异, 故建议应将龙胆属狭蕊组从龙胆属中移出。

关键词: 条纹龙胆; 胚胎学; 系统位置
中图分类号: Q 944.4 **文献标识码**: A

Embryology of Gentiana striata (Gentianaceae)

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Abstract: This paper describes embryological characters of *Gentiana striata* Maxim. For

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the first time, The results are used to discuss the systematic position of *Gentiana* Sect. Stenogyne. Anthers are tetrasporangiate. The development of anther walls conforms to the dicotyledonous type. The tapetum cells origin from the primary parietal cells, and thus the tapetum is of single origin. The development of the tapetum with uninucleate cells is of the Glandular type. The tapetal cells on the connective side show radial elongation or periclinal division and intrude into the anther locule. There are one middle layers. Parts of the epiderm is and endothecium persist and the cells become pillar and fibrous. Cytokinesis in the microsporocyte meiosis is simultaneous type and the microspore tetrads are tetrahedral. Pollen grains are 3-celled. The ovary is bicarpellary and unilocular. The placentation is of reduced parietal placentae with 4 rows of ovules. The type of ovule is Hypertropous. The ovule is unitegmic and tenuinucellar. The embryo sac originates from a single-archesporial cell. The one chalazal megaspore in linear tetrad becomes functional. The development of embryo sac is of the Polygonum type. Before fertilization, two polar nuclei fuse into a secondary nucleus. Three antipodal cells persist. Flowers are protandrous. Fertilization is porogamous. The development of the endosperm is of the Nuclear type. The embryogeny corresponds to the Soland type Physalis Ⅱ variation type. The present paper indicates that *Gentiana* Sect. Stenogyne is more similar to *Tripterospermum* than to other sections of *Gentiana* in embryological characters. Sect. Stenogyne might be separated from *Gentiana*.

Key words: *Gentiana striata*; Embryology; Systematic position

1　Introduction

Gentiana Sect. Stenogyne was established by Franchet in 1884[1] and was revised by Kusnezov in 1894[2]. It is a most disputable and poorly known section among the 15 sections in genus *Gentiana*. About this section, several authors had different suggestions on its systematic relationships[3~7]. This paper systematically reports embryology of *Gentiana striata* for the first time. The purpose of present investigation is to discuss the systematic position of the section based on embryological characters.

2　Materials and methods

Material investigated was collected from Pingan County, Qinghai Province, China. The voucher (Lu Xue-feng 094) is preserved in the Herbarium of Northwest Plateau Institute of Biology, Chinese Academy of Sciences, China (HNWP).

Anthers, ovules and seeds at different stages of development were fixed in acetic and absolute ethanol in a proportion of 1∶3. After being stained in Ehrfich's hematoxyfin, the material was embeded in paraffin by conventional method and sectioned at the

thickness of 6～12 μm. Sections were stained with safranin-fast green and observed and photographed under Olympus BH2.

3 Observations

3.1 The development of stamens

3.1.1 Microsporangia and microsporogensis Flowers of *Gentiana striata* are 5 stamens. Anthers are tetrasporangiate. At the early stage of development, 4 rows of archesporial cells differentiate under epidermis in each lobes of transections of anthers (Plate Ⅰ, 1). Archesporial cells are recognizable by their dense cytoplasm and conspicuous nuclei. These cells divide periclinally forming an outer primary parietal cells and inner primary sporogenous cells (Plate Ⅰ, 2). The primary parietal cells divide periclinally and anticlinally forming two layers of the secondary parietal cells. The inner secondary parietal cells give rise to the tapetum. Thus, the tapetum is of a single origin. The outer secondary parietal cells form a subepidermal endothecium and a middle layer by periclinal and anticlinal division. Anther wall is composed of four layers: epidermis, endothecium, middle layer and tapetum (Plate Ⅰ, 3). Endothecium and middle layer originate from the primary parietal cells. The development of the microsporangial wall thus conforms to the dicotylendonous type[8].

Cells of the tapetum on the connective side show radial elongation or periclinal division and intrude into the anther locule. Tapetal cells are uninucleate throught out their development. At about the time of pollen tetrads, the walls of the tapetal cells become indistinct and the tapetal cells degenerate at their original site (Plate Ⅰ, 4). The tapetal cells degenerate completely at the stage of 1-nucleate pollen grains and nucleus near the wall. Thus, the tapetum of *Gentiana striata* is glandular.

The middle layer is crushed during meiosis of microsporocytes.

As the anther matures, Parts of the epidermis and endothecium of the anther wall persist and the cells become pillar and fibrous (Plate Ⅰ, 5、7).

Simultaneously with changes taking place in the wall of microsporangia, the primary sporogenous cells undergo mitosis forming the secondary sporogenous cells, from which microsporocytes are derived. Meiosis in each microsporocyte results in a microspore terad. The cytokinesis is of the simultaneous type. Microspore tetrads are tetrahedral (Plate Ⅰ, 4).

3.1.2 Male gametogenesis Microspores are separated from the tetrad as a uninucleate pollen grain (Plate Ⅰ, 5). Each microspore has a dense cytoplasm with a prominent and centrally placed nucleus. As central vacuole develops, the nucleus takes a peripheral position. The first division of the microspore nucleus results in the formation of two unequal cells, a large vegetative one and a smaller generative one. The generative cell gives rise to two sperms by mitosis. Pollen grains are 3-celled at time of anther

dehiscence (Plate Ⅰ, 6).

3.2 The development of pistil

3.2.1 Macrosporangium and macrosporogensis The ovary is superior, bicarpellary, syncarpous and unilocular with parietal placentae. There are 4 rows of ovules in the transection of ovary. (Plate Ⅲ, 10). The integument initiates by periclinal and oblique division at the base of nucellus. The ovule of *Gentiana striata* is unitegmic. The integument reaches to the top of nucellus and forms a micropyle by continued division. When the whole ovule body reverses, and the raphe elongates and curves during the course of development, the hypertropous form occurs (Plate Ⅲ, 11). Thus, the type of the ovule is hypertropous[9].

At the stage of microsporocyte, a single hypodermal archesporial cell differentiates in the young nucellus and functions directly as the megasporocyte (Plate Ⅱ, 1) which is characterized by large nucleus and dense cytoplasm. Thus, the ovule of *G. striata* is tenuinucellate. The megasporocyte undergoes meiosis forming a linear tetrad of megaspores (Plate Ⅱ, 2～4). The three micropylar megaspores eventually degenerated, while the chalazal one become functional (Plate Ⅱ, 5).

3.2.2 Femal-gametophyte A 7-celled and 8-nucleate femal gametophyte of the Polygonum type is formed by three mitotic divisions of the functional megaspore. The 1-nucleate embryo sac (Plate Ⅱ, 5) is formed by enlargement of the megaspore and increase in vacuole and in size of the nucleus. The 2-nucleate embryo sac (Plate Ⅱ, 6) is formed by one mitotic division of the 1-nucleate embryo sac. The 2-nucleate embryo sac become 4-nucleate embryo sac (Plate Ⅱ, 7) by another mitotic division. The 4-nucleate embryo sac undergoes mitotic division to form the 8-nucleate embryo sac in which three lie in the micropylar end, two near of the center, and three in the chalazal end. The three micropylar nuclei become the egg and the two synergids. The two median nuclei become the polar nuclei. The chalazal nuclei become the three antipodals. The polar nuclei fuse at the center and the resulting secondary nucleus then moves close to the egg apparatus (Plate Ⅱ, 8～10).

In the mature 8- nucleate embryo sac, the egg cell is recognized by nucleus at the chalazal end and a large vacuole at the micropylar end. The two synergids have obvious filiform apparatus (Plate Ⅱ, 8) and are recognized by their nuclei at the micropylar end and a large vacuole at the chalazal end. The three antipodal cells are stained densely and their nuclei each may divided into two (Plate Ⅱ, 10). The antipodal cells persist until the stage of 4-celled proembryo.

3.3 Fertilization

The fertilization is porogamous (Plate Ⅱ, 11). The pollen tube discharges two sperms. One of sperms fuses with the egg nucleus forming the zygote and the other with the secondary nucleus forming the primary endosperm nucleus. We observed that the

sperm nucleus entered into the secondary nucleus and fusion between them would be happening (Plate Ⅲ, 1). Although fusion between the egg nucleus and sperm nucleus was not found, we speculated that two nuclei had fused because the zygote nucleus is larger than the egg nucleus (Plate Ⅲ, 2). The primary endosperm nucleus is larger than the zygote nucleus. The first division of the primary endosperm nucleus precedes that of the zygote.

3.4 Endosperm

The development of the endosperm of *G. striata* is of Nuclear type. The primary endosperm nucleus gives rise to 2 free endosperm nuclei. A large number of free nuclei form by a series of successive divisions of the two free endosperm nuclei (Plate Ⅲ, 7). At the stage of multi-celled proembyo, wall formation of endosperm initiates from the micropylar end to the chalazal end. After the formation of endosperm cell wall, endosperm cells move to the center of the embryo sac and surrounded the proembryo. A few of endosperm cells is absorbed by the embryo during the development of the latter.

3.5 Embryo and seed coat

The zygote has a large nucleus, conspicuous nucleolus, dense cytoplasm and small vacuole. The zygote divides transversely forming a terminal cell (Ca) and a basal cell (Cb) (Plate Ⅰ.). The Ca and Cb (Plate Ⅲ, 3) undergo transverse division forming a 4-celled linear proembryo-these cells are designated L, L′, M, Ci (Plate Ⅲ, 4～5). The L and L′ divide transversely forming a 6-celled linear proembryo (Plate Ⅲ, 6)- they are designated L_1, L_2, L_1', L_2', M, Ci. The L_1 and L_2 divide vertically and transversely forming the primordia of the cotyledons (Pco), stem apex (Pvt), and hypocotyl (Phy). By vertical and transverse divisions, the L_1' and L_2' give rise to primordia of central cylinder of stem (Icc) and central cylinder of root (Iec). The M produces the rot cap (Co) and the suspensor (S) by vertical and transverse divisions. The cell Ci does not divide.

Thus, in this species, the cell Cb of 2-celled proembryo does not contribute to the formation of the entire dicotyledonary embryo. The cell L of 4-celled proembryo contributes to the development of cotyledons, stem apex and hypocotyls. Cells of the third generation of the proembryo after three turns of divisions are composed of 6 cells. The embryogeny corresponds to the Physalis II variation of Solanad type[10].

In mature seeds, the embryo is cordate (Plate Ⅲ, 8). During the development of embryo sac, integument cells at both micropylar and chalazal ends divide forming outgrowth which gives rise to the wing (Plate Ⅲ, 11). The epiderm is of the integument becomes the seed coat (Plate Ⅲ, 9). The inner layers of the integument are absorbed.

4 Discussion

Gentiana Sect. Stenogyne was included in genus *Gentiana* since the section was established. The section was firstly reexamined by Smith[7] who suggested that it should

be related to *Tripterospermum*. Love & Love[6] considered the section as subgenus of the genus *Tripterospermum*. Yuan *et al*.[4] suggested that the section should be segregated from the genus *Gentiana* according to ITS phylogeny.

Table 1　Comparison of embryological characters of Sect. Stenogyne with other sections of Gentiana **and** Tripterospermum

Characters	Sect. Stenogyne	Other section of *Gentiana*	Tripterospermum
Anther			
Number of sporangia	4	4	4
Epidermis	Persistent	Reduced	Reduced
Endothecium	Not all reduced	Persistent	Persistent
Middle layers	1	2	1 or 2
Tapetum: Origin	Single	Dual	Single
Tapetum: Type	Glandular	Glandular	Well developed
Tapetum: Placentoid	Developed	Well developed	Well developed
Number of nuclei in the tapetal cell	1	1	1
Meiosis of microsporocyte	Simultaneous	Simultaneous	Simultaneous
Shape of microspore	Tetrahedral	Tetrahedral	Tetrahedral
Number of cells in a mature pollen	3	3	3
Ovule			
No. of ovule rows	4	10～30	4 or 8
Type	Hypertropous	Anatropous	Hypertropous or anatropous
No. of integument	1	1	1
No. of archesporia	1	1	1
Nucellus	Tenuinucellate	Tenuinucellate	Tenuinucellate
Type of megasporogenesis	Polygonum	Polygonum	Polygonum
Filiform apparatus of synergids	Present	Present	Absent or present
Antipodal cells	Persistent	Persistent	Persistent
No. of antipodal cells	3	3(4, 8)	3
Character of antipodal cells	Not enlarged	Not enlarged	Not enlarged
Seeds			
Type of endosperm formation	Nuclear	Nuclear	Nuclear
Type of embryogeny	Soland subtype physalis Ⅱ	Soland subtype physalis Ⅱ	Soland subtype physalis Ⅱ
Appendages of seed	Winged	Wingless (rarely winged)	Wingless or winged
Exotesta	Sclerechymatous	1 absent	1, sclerechymatous
Endotesta	Absent		Present or absent

Compared Sect. Stenogyne with other sections of *Gentiana*[11] and *Tripterospermum*[12], lots of similar embryological characters exist among Sect. Stenogyne, other sections of *Gentiana* and *Tripterospermum* (Table 1). such as tetrasporangiate anthers; dicotyledonous type of microsporangial development; simultaneous cytokinesis in the microsporocytes; the tetrahedral microspore tetrads; 3-celled pollen; superior, bicarpellary and unilocular ovary; unitegmic and tenuinucellar ovule; polygonum type of megagametophyte; porogamous fertilization; nuclear endosperm; the Physalis Ⅱ variation of Solanad type of embryogeny. Besides above

similar embryological characters, there are other similar characters between Sect. Stenogyne and other sections of *Gentiana* such as uninucleate tapetal cells; obvious filiform apparatus in synergids; 3 antipodal cells. But Sect. Stenogyne is different from other sections of *Gentiana* in some important embryological characters as follow: (1) In the development of anther wall, Sect. Stenogyne has one middle layer, parts of epidermis and endothecium are fibrous; Other sections of *Gentiana* have two middle layers, fibrous endothecium and degenerated epidermis. (2) In the differentiation of tapetum, Sect. Stenogyne has single origin of tapetum which shows radial elongation or periclinal division and intrude into the anther locule; other sections of *Gentiana* have dual origin of tapetum, tapetal cells form trabeculae[13] and placenoids.[14] (3) Sect. Stenogyne has 4 rows of hypertropous ovules in the transection of ovary and reduced parietal placentae; other sections of *Gentiana* have 20～30 rows of anatropous ovules and superficial placentae[15].

Besides similar embryological characters among three taxa, Sect. Stenogyne has more similar characters to *Tripterospermum* than to other sections of *Gentiana*. These characters are one middle layer, single origin of tapetum, obvious filiform apparatus in synergids (in *Tripterospermum* Sect. Platyspermum,[12] synergids don't have obvious filiform apparatus), 4 rows of hypertropous ovules in the transection of ovary (8 rows of anatropous ovules in T. Sect. Platyspermum), 3 antipodal cells. But Sect. Stenogyne is different from *Tripterospermum* in some embryological characters as follow: (1) In the development of anther wall, parts of epidermis and endothecium of Sect. Stenogyne are fibrous; endothecium of *Tripterospermum* is fibrous and epidermis degenerates. (2) In the differentiation of tapetum, Sect. Stenogyne has single origin of tapetum which shows radial elongation or periclinal division and intrude into the anther locule; in *Tripterospermum* tapetal cells form placenoids[14].

From above comparison of embryological characters among Sect. Stenogyne, other sections of *Gentiana* and *Tripterospermum*, the results indicate that Sect. Stenogyne is more similar to *Tripterospermum* than to other sections of *Gentiana* in embryological characters. It appears probable that Sect. Stenogyne might be more closely related to *Tripterospermum* than to other sections of *Gentiana* in respect of embryological characters. It might be reasonable to separate Sect. Stenogyne from the genus *Gentiana*. However, We don't want to make a taxonomic attempt upon this section until we obtain new data on the section.

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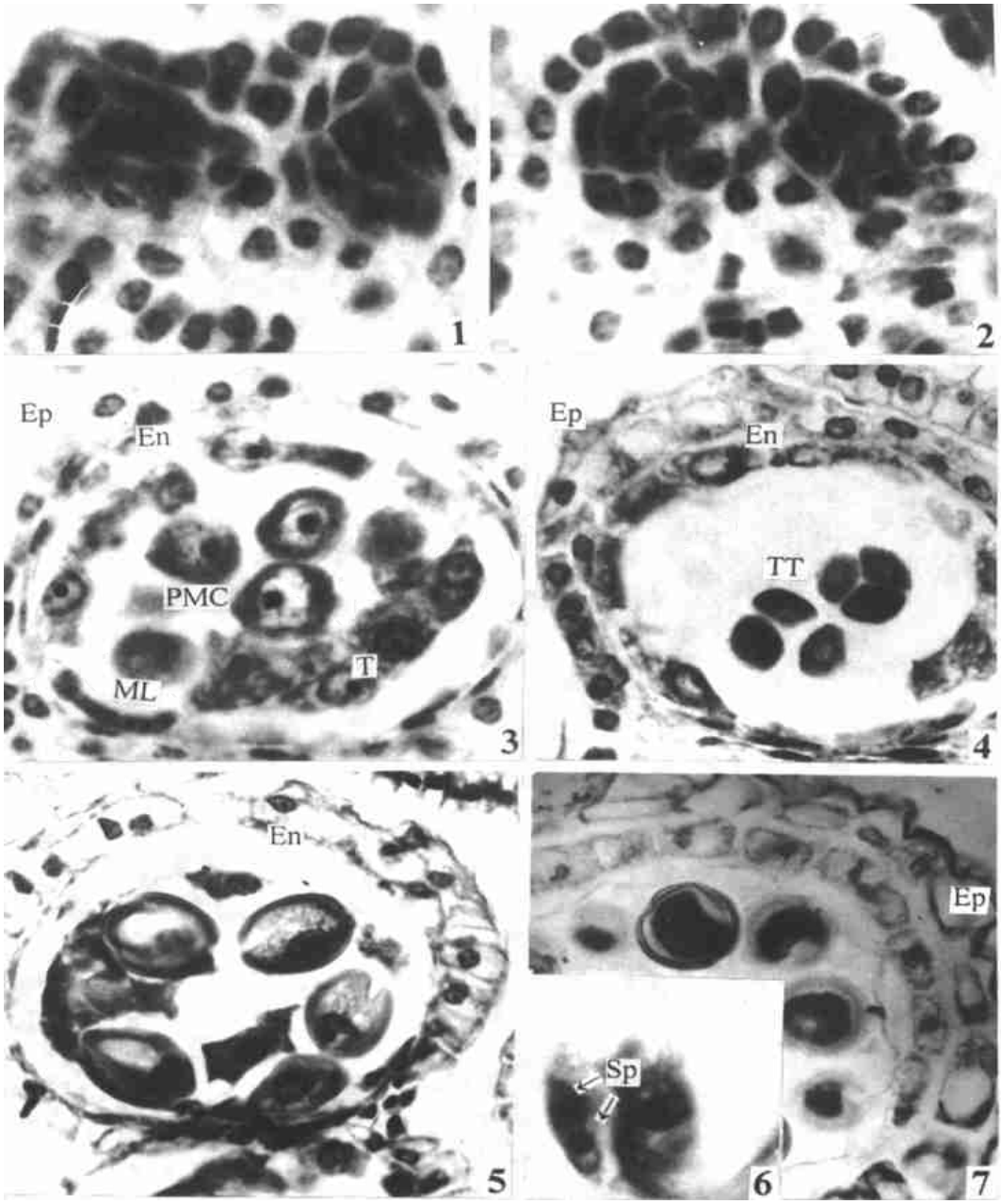
Explanation of Plates:

Plate Ⅰ. Anther and microsporogenesis of *Gentiana striata*. 1. The archesporial cells differentiated under epidermis. 2. The primary parietal cells undergoing periclinal divisions (PMC, microsporocyte). 3. Four layers of anther wall cells: epidermis (Ep), endothecium (En), middle layer (ML), and tapetum (T). 4. Tetrahedral tetrad (TT) and anther wall. 5. 1-nucleate pollen grain and anther wall. 6. 3-celled pollen grains. 7. Anther wall before releasing pollen, showing fibrous thickened epidermis and partially fibrous thickened endothecium. (1, 2 ×812; 3～5, 7 ×478; 6 ×1 280)

Plate Ⅱ. Ovaries and early development of the embryo sac of *Gentiana striata*. 1. A unitegmic ovule and a megasporocyte. 2. Metaphase I of meiosis in macrosporocyte. 3. Anaphase II of meiosis in macrosporocyte. 4. Linear megaspore tetrad. 5. The functional chalazal megaspore (FM), with the other three degenerating. A. 1-nucleate embryo sac and showing the three degenerated megaspores (DM). 6. Two nucleate embryo sac. 7. The 4-nucleate embryo sac. 8～10. Consecutive transections of an 8-nucleate embryo sac showing an egg (E), two synergids (Sy), secondary nucleus (SN) and antipodal cells (Ant). 11. Showing pollen tube (PT). (1～7 ×867; 8～10 ×788; 11 ×296)

Plate Ⅲ. Mature embryo sac, fertilization and ovule tape of *Gentiana striata*. 1. The sperm (Sp) in the secondary nucleus. 2. The zygote (Z) and the primary endosperm nucleus (Pn). 3. The terminal cell (Ca) and basal cell (Cb), showing two celled proembryo. 4-5. Consecutive sections of a linear 4-celled proembryo. 6. A linear 6-celled proembryo. 7. A multicellular proembryo and free nucleate endosperm. 8. Embryo (Emb) at the cardio-shaped stage when seeds released from capsule. 9. A layer of testa and wing of seed. 10. four rows of ovule. 11. Showing hypertropous ovule. (1～2 ×788; 3～7 ×290; 8× 233; 9 ×46; 10 ×97; 11 ×197)



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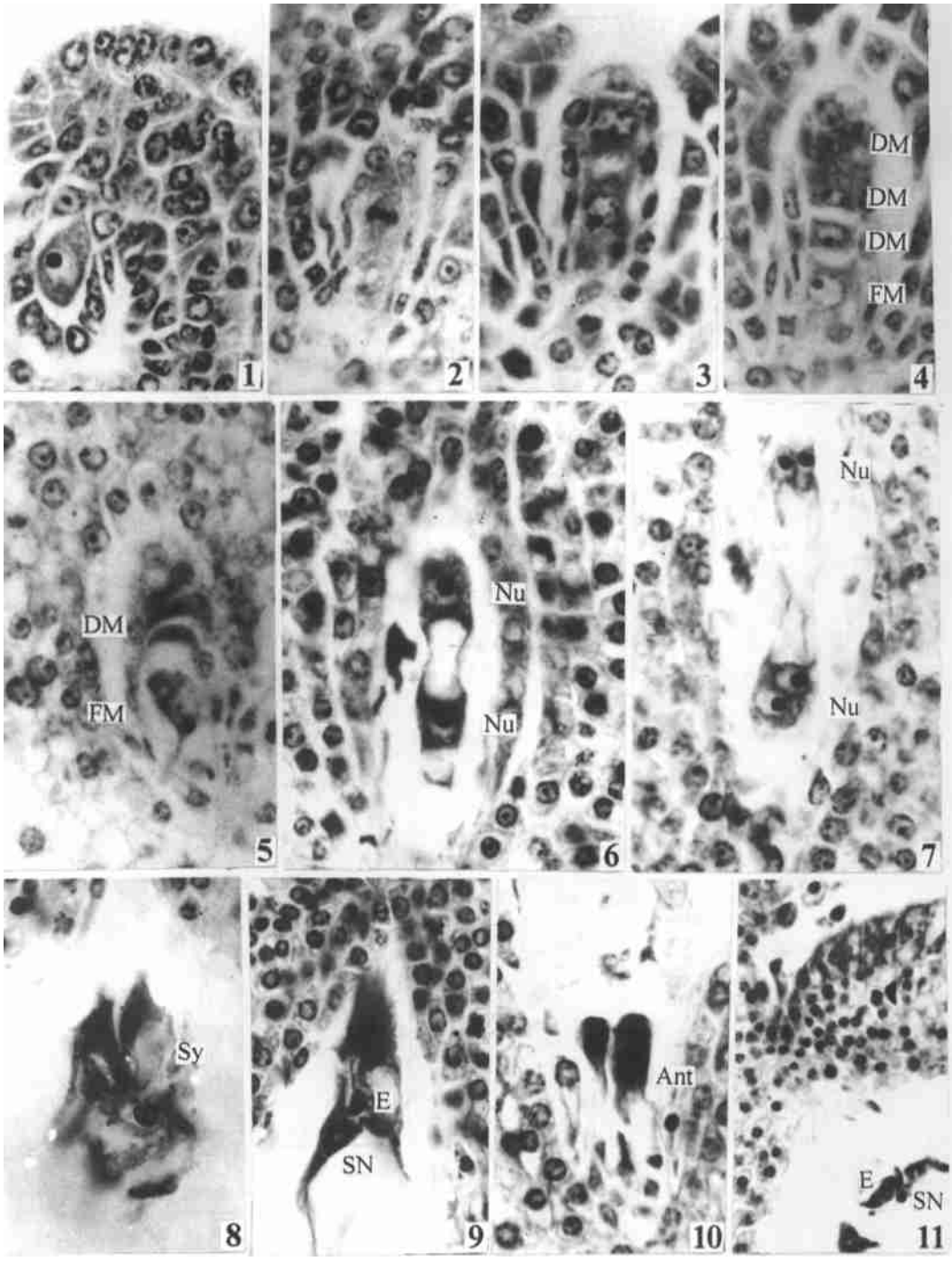

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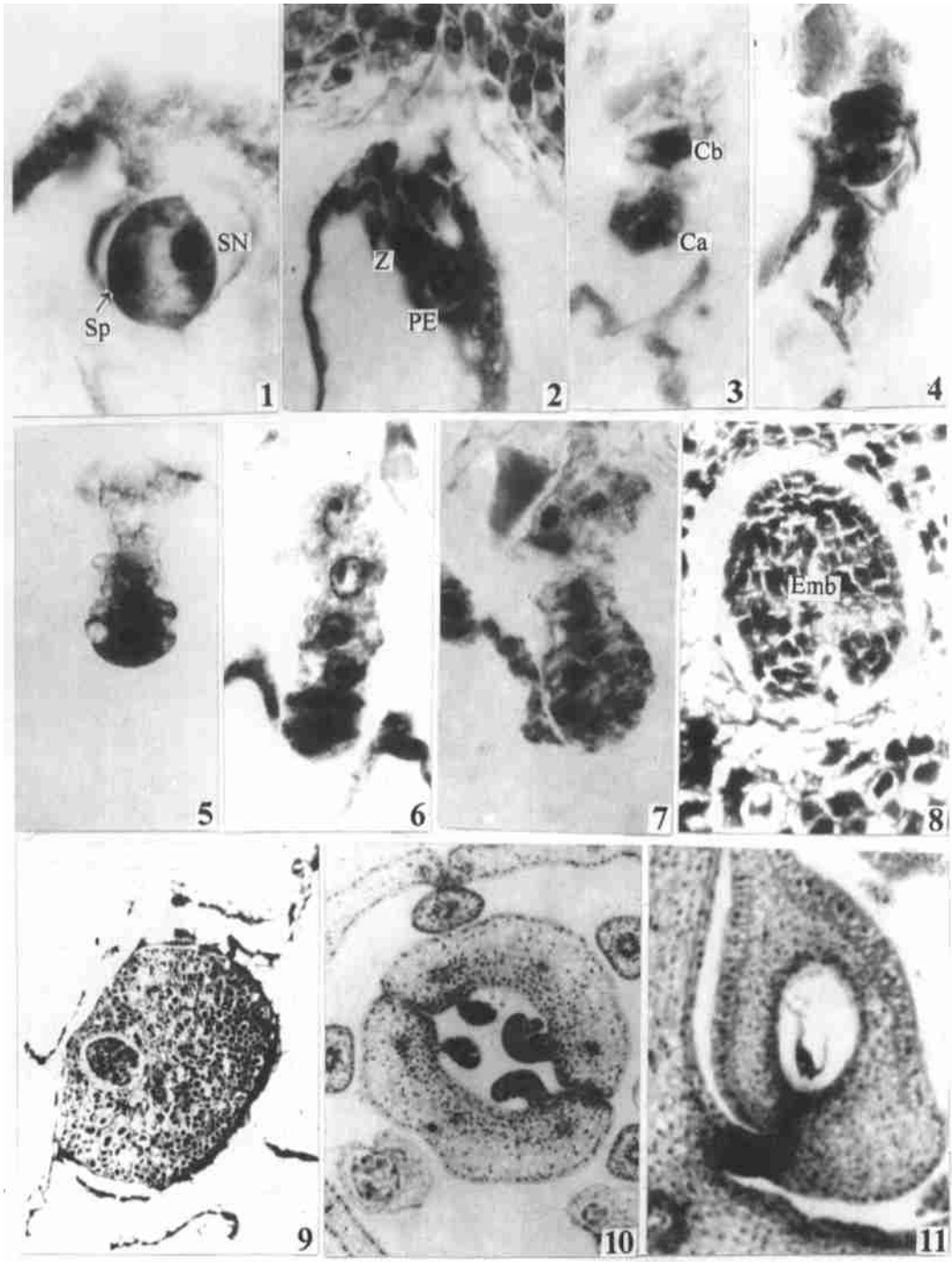

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