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峨眉双蝴蝶的胚胎学研究*

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摘要: 首次报道了峨眉双蝴蝶 *Tripterospermum cordatum* (Marq.) H. Smith 的胚胎学特征, 研究结果用以讨论双蝴蝶属的系统演化关系。主要研究结果如下: 花药四室; 药壁发育为双子叶型; 绒毡层属单型起源, 细胞具单核, 属腺质型绒毡层, 药隔处的绒毡层细胞形成类胎座, 其余部位的绒毡层细胞仍为一层细胞; 在花药成熟时, 花药的药室内壁纤维状加厚且柱状伸长, 表皮细胞减缩退化, 纤维状加厚不明显。成熟花粉为 3 - 细胞型。子房为 2 心皮, 1 室, 侧膜胎座。胚珠为 4 或 8 列的 Hypertropous 胚珠类型。大孢子母细胞减数分裂形成的 4 个大孢子呈直线式排列, 合点端的大孢子具功能。胚囊发育为蓼型。珠孔受精。胚乳发育为核型。胚胎发育为茄型酸浆 Ⅱ 变型。通过比较双蝴蝶属与蔓龙胆属和龙胆属的胚胎学特征表明, 双蝴蝶属在一些重要的胚胎学特征上与蔓龙胆属和龙胆属均存在较大差异, 故将该属作为一独立属处理较为合适。

关键词: 峨眉双蝴蝶; 胚胎学; 系统关系
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Embryology of Tripterospermum cordatum (Gentianaceae)*

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Abstract: The present paper reports embryological characters of *Tripteropsermum cordatum* (Marq.) H. Smith for the first time and discusses the systematic relationships of *Tripterospermum*. Anthers are tetrasporangiate. The development of anther walls conforms to the Dicotyledonous type. The tapetal 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 to form placenoids. The anther wall has only one middle layer; the endothecium persists and its cells become pillar and fibrous, and the epidermis degenerates. Cytokinesis at meiosis of microsporocytes is of the simultaneous type and the microspore tetrads are tetrahedral. Pollen grains are 3 -

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celled. The ovary is bicarpellary and unilocular. The placentation is of reduced or typical parietal placentae with 4 or 8 rows of hypertropous ovules. 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 Solanad type Physalis Ⅱ variation type. Compared with *Gentiana* and *Crawfurdia*, *Tripterospermum* is different from *Gentiana* and *Crawfurdia* in embryological characters. The embryological data indicate that it is better to treat *Tripterospermum* as an independent genus.

Key words: *Tripterospermum cordatum*; Embryology; Systematic relationships

Tripterospermum was established by Blume in 1826 based on the species *Tripterospermum trinerve*. *Tripterospermum* was reexamined by Marquand in 1931 and 1937, who did not accept the genus *Tripterospermum* and merged it into *Gentiana*. Clarke (1875) transferred *Tripterospermum* to *Crawfurdia* Wall., and divided this genus into 2 sections *Dipterospermum* Clarke and *Tripterospermum* (Blume) Clarke. However, the genus is retained as independent one by many authors (Ho & Liu, 1990; Ho, 1988; Wu, 1984; Smith, 1965; Gilg, 1895 ect.). This genus has never been embryologically investigated. The objective of the present paper is to report the results of observations on embryology of genus *Tripterospermum* and to discuss systematic implications of the embryological data.

1 Materials and Methods

Material investigated for the present study was collected from Tianquan, Sichuan Province, China. The voucher (Liu Jian - quan 270) is deposited 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 the conventional method and sectioned at the thickness of 6～12μm. Sections were stained with safranin - fast green, and then observed and photographed under Olympus BH2.

2 Observations

2.1 Microsporogensis and male gametogenesis

Flowers of *Tripterospermum cordatum* were bisexual and protandrous. Anthers were tetrasporangiate. At early stage of development, 4 rows of archesporial cells differentiated under epidermis of anthers. Archesporial cells were recognizable by their dense cytoplasm and conspicuous nuclei. These cells divided periclinally forming an outer primary parietal cells and inner primary sporogenous cells (Plate Ⅰ: 1). The primary parietal cells divided periclinally and anticlinally forming two layers of the secondary parietal cells. The inner secondary parietal cells gave rise to the tapetum. Thus, the tapetum was of single origin. The outer secondary parietal cells formed a subepidermal endothecium and a middle layer by periclinal and anticlinal division. Anther wall was composed of four layers: epidermis,

endothecium , middle layer and tapetum (Plate Ⅰ: 2) . Endothecium and middle layer originated from the primary parietal cells. The development of the microsporangial wall thus conforms to the dicotylendonous type (Davis , 1966) .

Cells of the tapetum on the connective side showed radial elongation or periclinal division and intruded into the anther locule. At this region where divisions occur , the tapetum becomes 2 to 3 layers and appears as trabeculae and placenoids (Steffen *et al* , 1958) . Tapetal cells were uninucleate throughout their development. At about the time of pollen tetrads , walls of the tapetal cells became indistinct and the tapetal cells degenerated at their original site (Plate Ⅰ: 3～4) . The tapetal cells degenerated completely at the stage of 1 - nucleate pollen grains (Plate Ⅰ: 7) . Thus , the tapetum of *Tripterospermum cordatum* is glandular.

The middle layer was crushed during meiosis of microsporocytes. As the anther matures , the endothecium of the anther wall persisted and the cells became pillar and fibrous. The epidermis degenerated. (Plate Ⅰ: 8) .

Simultaneously with changes in the wall of microsporangia , the primary sporogenous cells underwent mitosis , forming secondary sporogenous cells , from which microsporocytes were derived. Meiosis in each microsporocyte resulted in a microspore terad. The cytokinesis is of the simultaneous type. Microspore tetrads were tetrahedral (Plate Ⅰ: 4～5) . Microspores were separated from the tetrad as a uninucleate pollen grain (Plate Ⅰ: 7) . Each microspore had a dense cytoplasm with a prominent and centrally placed nucleus. As central vacuole developed , the nucleus took a peripheral position. The first division of the microspore nucleus resulted in the formation of two unequal cells , a large vegetative one and a smaller generative one. The generative cell gave rise to two sperms by mitosis. Pollen grains were 3 - celled at time of anther dehiscence (Plate Ⅰ: 6) .

2. 2 **Megasporogenesis and Femal - gametogenesis**

2. 2. 1 Macrosporangium and macrosporogensis　The ovary was superior , bicarpellary , syncarpous and unilocular with parietal placentae. There were 4 rows of ovules in the transection of ovary. (Plate Ⅲ: 13) . The integument initiated by periclinal and oblique division at the base of nucellus. The ovule of *Tripterospermum cordatum* is unitegmic. The integument reached the top of nucellus and formed 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. Thus , the type of ovule is hypertropous (Plate Ⅲ: 14) .

At the stage of microsporocyte , a single hypodermal archesporial cell differentiated in the young nucellus and functioned directly as the megasporocyte (Plate Ⅱ: 1) which was characterized by large nucleus and dense cytoplasm. Thus , the ovule of *T. cordatum* was tenuinucellate. The megasporocyte underwent meiosis forming a linear tetrad of megaspores (Plate Ⅱ: 2～3) . The three micropylar megaspores eventually degenerated , while the chalazal one became functional (Plate Ⅱ: 4) .

2. 2. 2 Embryo sac and femal - gametophyte　A 7 - celled and 8 - nucleate femal gametophyte of the Polygonum type formed by three mitotic divisions of the functional megaspore (Plate Ⅱ: 5～7) . The three micropylar nuclei became the egg and the two synergids. The two median nuclei became the polar

nuclei. The chalazal nuclei became the three antipodals. The polar nuclei fused at the center and the resulted secondary nucleus then moved close to the egg apparatus (Plate Ⅱ: 8～9 and Plate Ⅲ: 1～2).

In the mature 8 - nucleate embryo sac, the egg cell was recognized by nucleus at the chalazal end and a large vacuole at the micropylar end. The two synergids (Plate Ⅱ: 8) were recognized by their nuclei at the micropylar end and a large vacuole at the chalazal end. The three antipodal cells were stained densely and their nuclei each may divided into two (Plate Ⅲ: 2). The antipodal cells persisted until the stage of 4 - celled proembryo.

2．3 **Fertilization**

The fertilization was porogamous. The pollen tube entered the megagametopyte by the micropyle. Although we did not catch the course of fusion between the sperm and egg or secondary nucleus, we inferred occurring fusion between male and femal nucleus. Because the nucleoli of zygote and primary endosperm nucleus are larger than that of the egg and secondary nucleus (Plate Ⅲ: 3). The first division of the primary endosperm nucleus preceded that of the zygote.

2．4 **Endosperm**

The development of endosperm of *T. cordatum* is of Nuclear type. The primary endosperm nucleus gave rise to 2 free endosperm nuclei. A large number of free nuclei formed by a series of successive divisions of the two free endosperm nuclei (Plate Ⅲ: 5). At the stage of multi - celled proembyo, wall formation of endosperm cells initiated from the micropylar end to the chalazal end. After the formation of endosperm cell wall, endosperm cells moved to the center of the embryo sac and surrounded the proembryo (Plate Ⅲ: 10). A few of endosperm cells were absorbed by the embryo during the development of the latter.

2．5 **Embryo and seed coat**

The zygote had a large nucleus, conspicuous nucleolus, dense cytoplasm and small vacuole (Plate Ⅲ: 3). The zygote divided transversely forming a terminal cell (Ca) and a basal cell (Cb). The Ca and Cb (Plate Ⅲ: 4～5) underwent transverse division forming a 4 - celled linear proembryo - these cells are designated L, L', M, Ci (Plate Ⅲ: 6). The L and L' divided transversely forming a 6 - celled linear proembryo (Plate Ⅲ: 7～9) —they are designated L_1, L_2, L_1', L_2', M, Ci. The L_1 and L_2 divided vertically and transversely forming primordia of cotyledons (Pco), stem apex (Pvt) and hypocotyl (Phy). By vertical and transverse divisions, the cells L_1' and L_2' gave rise to primordia of central cylinder of stem (Icc), central cylinder of root (Iec) and rot cap (Co). The cells M and Ci produced the suspensor (S) by vertical and transverse divisions.

Thus, in this species, the cell Cb of 2 - celled proembryo did not contribute to the formation of the entire dicotyledonary embryo. The cell L of 4 - celled proembryo contributed to the development of cotyledons, stem apex and hypocotyls. The proembryo after three turns of divisions were composed of 6 cells. The embryogeny corresponds to the Physalis II variation of Solanad type (Johansen, 1950).

In mature seeds, the embryo was globular (Plate Ⅲ: 11). The epidermis of the integument became the exotesta (Plate Ⅲ: 12). Most inner layers of the integument were absorbed. Two and five

layers of inner integument became the endotesta (Plate Ⅲ: 12).

3 Discussion

Tripterospermum not only was placed in the genus *Gentiana* by Marquand (1937, 1931), but also was placed in the genus *Crawfurdia* (Clarke, 1875). Ho *et al*. (1999) * summarizes embryological characters of *Gentiana*: tetrasporangiate anthers; dicotyledonous type of microsporangium development; dual tapetal origin; binucleate and multinucleate tapetal cells; trabecula and placenoids formed by division of tapetal cells; glandular tapetum, 2 middle layers; persistent epidermis in the mature anther; simultaneous cytokinesis at meiosis of the microsporocytes; tetrahedral microspore tetrads; 2- or 3-celled pollen; superior, bicarpellary and unilocular ovary with superficial placentae; anatropous, unitegmic and tenuinucellar, ovules 20～30 in number; Polygonum type of megagametophytes; porogamous fertilization; nuclear endosperm; embryogeny of the Physalis variation of Solanad type; globular embryo in mature seeds. Although there are numerous similar embryological characters between *Tripterospermum* and *Gentiana*, *Tripterospermum* is different from *Gentiana* in embryology: (1) In the anther wall, *Tripterospermum* has one or two middle layers; while *Gentiana* has always two middle layers; (2) In differentiation of tapetum, *Tripterospermum* has single origin of tapetum, while *Gentiana* has dual origin of tapetum, and (3) *Tripterospermum* has 4 or 8 rows of hypertropous ovules in the transection of ovary and reduced or typical parietal placentae; whereas *Gentiana* has 20～30 rows of anatropous ovules and superficial placentae (Gopal *et al*., 1962).

Tripterospermum has more similar embryological characters to *Crawfurdia* ** than to *Gentiana*. But *Tripterospermum* is different from *Crawfurdia* in embryology: (1) In the anther wall, *Tripterospermum* has fibrous endothecium, while *Crawfurdia* has fibrous epidermis; (2) In the differentiation of tapetum, tapetal cells of *Tripterospermum* form placenoids (Steffen *et al*., 1958), while the tapetal cells of *Crawfurdia* only show radial elongation; and (3) *Tripterospermum* has hypertropous ovules; while *Crawfurdia* has anatropous ovule.

From above comparision of embryological characters among *Tripterospermum*, *Gentiana* and *Crawfurdia*, the results indicate that *Tripterospermum* is neither similar to *Gentiana* nor to *Crawfurdia* embryologically. We support the treatment of *Tripterospermum* as a distinct genus. The evolutionary trends of embryological characters have been elucidated by many authors (Hohri *et al*., 1984, 1992; Tobe, 1989). There exist both advanced and primitive characters in a single genus, *Tripterospermum*, *Gentiana* or *Crawfurdia*. Thus, the systematic relationships among *Tripterospermum*, *Gentiana* and *Crawfurdia*, can not be discussed only with embryological characters.

References

Davis G L, 1966. Systematic Embryology of the Angiosperms [M]. New York: John Wiley & Sons Inc.

* Ho T N, Liu J Q, 1999. Embryology of *Gentiana lawrencei* var. *farreri*. Acta Botanica Boreali - Occidentali Sinica (in Press)

** Chen Shi - long, 1997. Ph. D. Thesis: Studies on Systematics of *Crawfurdia* Wall. and Its Related Taxa in the Gentianaceae.

Eames A J, 1961. Morphology of the Angiosperms [M]. New York: McGrAw - Hill Book Company Inc.

Gilg E, 1895. Gentianaceae [M]. In: Engler A, Prantl K (eds.) Die Naturlichen Pflanzenfamilien, **4** (2). Leipzig: Engelmann, 50～62

Gopal K G, Puri V, 1962. Morphology of the flower of some Gentianaceae with special reference to placentation [J]. *Bot Gaz*, **124**: 42～57

Ho T N, 1988. Gentianaceae [M]. In: Flora Reipublicae Popularis Sinica Vol. 62. Beijing: Science Press, 1～418

Ho T N, Liu S W, 1990. The infrageneric classification of *Gentiana* (Gentianaceae) [J]. *Bull Br Mus Bot*, **20**: 169～192

Ho Ting - nong, Liu Shang - wu, Lu Xue - feng, 1996. A phylogenetic analysis of *Gentiana* (Gentianaceae) [J]. *Acta Phytotaxonomia Sinica* (**植物分类学报**), **34** (5): 505～530

Johansen D A, 1950. Plant Embryology [M]. Waltham, Mass U S A

Johri B M, 1984. Embryology of Angiosperm [M]. Berlin: Springer - Verlag.

Johri B M, Ambegaokar D K, Srivastava P S, 1992. Comparative Embryology of Angiosperms [M]. Berlin: Springer - Verlag.

Love A, Love D, 1976. The Natural Genera of Gentianaceae [M]. In: Kachroo P. (ed.), Recent Advanced in Botany, Prof. P N Mehra Commemorative Volume 205～222. Dehra Dun, Delhi.

Marquand C V B, 1931. New Asiatic *Gentianas*: II [J]. *Bull Misc Inf Roy Gard Kew*, 1931: 68～88

Marquand C V B, 1937. The *Gentianas* of China [J]. *Bull Misc Inf Roy Gard Kew*, 1937: 134～180

Pringle J S, 1978. Sectional and subgeneric names in *Gentiana* (Gentianaceae) [J]. *Sida*, **7**: 232～247

Smith H, 1965. Notes on Gentianaceae [J]. *Not Bot Gard Edinb*, **26** (2): 253

Steffen K, Landmann W, 1958. Entwicklungsgeschichtliche und cytologische Untersuchungen am Balkentapetum von *Gentiana cruciata* und *Impatiens glandulifera* [J]. *Planta*, **50**: 423～460

Tobe H, 1989. The embryology of angiosperms: its broad application to the systematic and evolutionary study [J]. *Bot Mag* (Tokyo), **102**: 351～367

Wu Qing - ru, 1984. Taxa et Combinationes Nove *Tripterospermum* et *Crawfurdia* [J]. *E Flora Sinica*, **4** (3): 129～139

Yuan Y M, Kupfer P, 1995. Molecular phylogenetics of the subtribe Gentianinea (Gentianaceae) inferred from the sequences of internal transcribed spacers (ITS) of nuclear ribosomal DNA [J]. *Pl Syst Evol*, **196**: 207～226

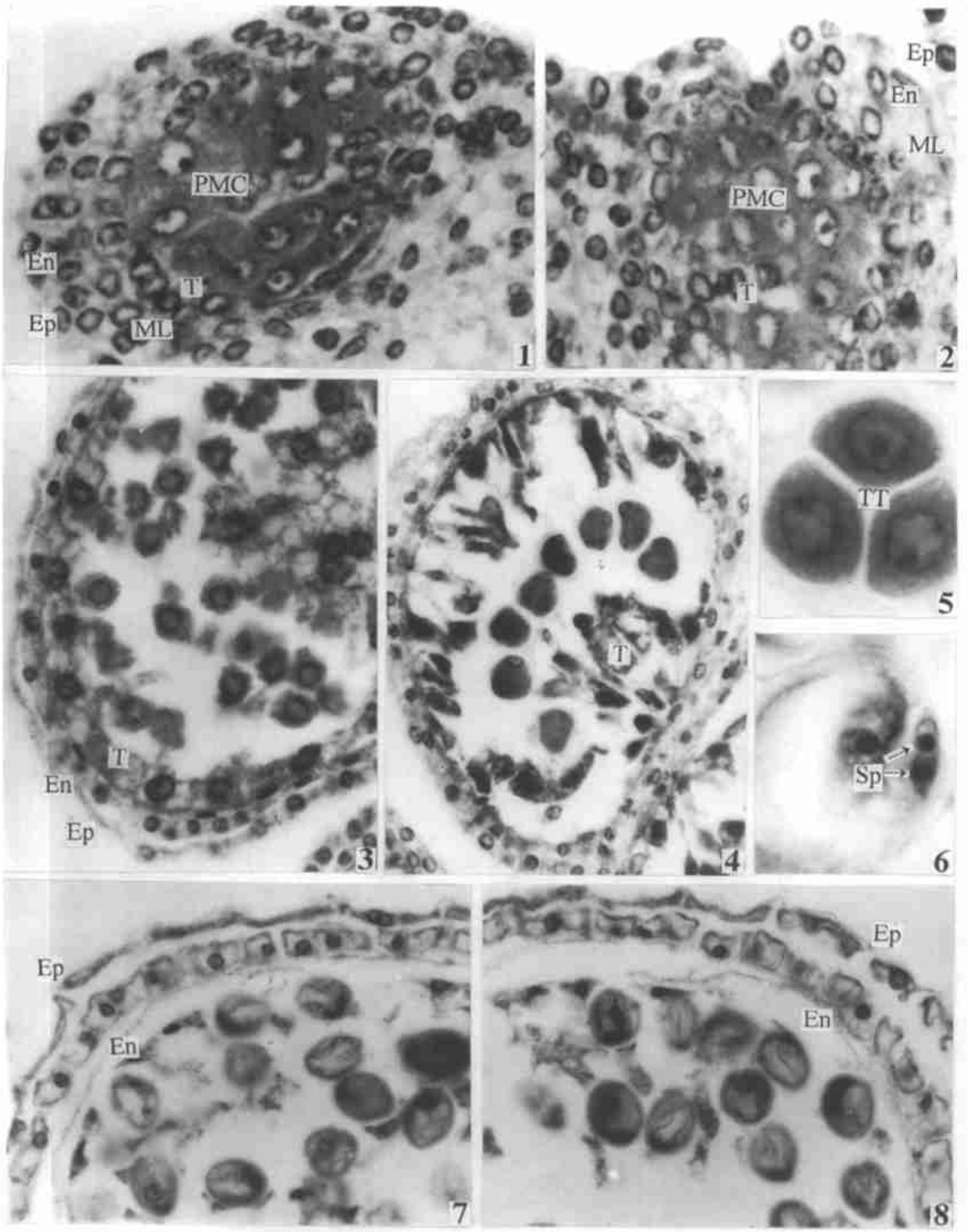
Explanation of Plates

Ant, Antipodal cell. Ca, Terminal cell. Cb, Basal cell. DM, Degenerating megaspore. E, Egg. Emb, Embryo. En, Endothecium. Ep, Epidermis. Fe, Free endosperm nucleus. FM, Functional megaspore. ML, Middle layer. Nu, Nucleus. PMC, Microsporocyte. Pn, Polar nucleus. Sp, Sperm. Sy, Synergid. T, Tapetum. Ts, Testa. TT, Tetrahedral tetrad. Z, Zygote.

Plate Ⅰ 1. Anther wall at early stage of microspocyte. 2. Four layers of anther wall cells: epidermis, endothecium, middle layer, and tapetum. 3. Anther wall at late stage of microsporocyte. 4. Anaphase II of meiosis in microsporocytes. 5. Tetrahedral tetrad. 6. 3 - celled pollen grains. 7. Anther wall of one - nucleate pollen grain and nucleus near the wall. 8. Anther wall before releasing pollen, showing fibrous thickened endothecium and degenerated epidermis. (1～2 ×637; 3～4, 7～8 ×412; 5～6 ×1165)

Plate Ⅱ 1. A unitegmic ovule and a megasporocyte. 2. Anaphase I of meiosis in megasporocyte. 3. Linear megaspore tetrad. 4. The functional chalazal megaspore, with other three degenerating. A 1 - nucleate embryo sac and showing other three degenerated megaspores. 5. Two - nucleate embryo sac. 6～7. The same section of 4 - nucleate embryo sac. 8～9. Consecutive transections of an 8 - nucleate embryo sac showing an egg, two synergids, two polar nuclei. (1～3 ×426; 4～7 ×732; 8～9 ×622)

Plate Ⅲ 1. 1～2. Consecutive transections of an 8 - nucleate embryo sac showing two polar nuclei and antipodal cells. 3. Zygote. 4～5. Consecutive sections of the terminal cell and basal cell, showing two - celled proembryo. 6. A linear 4 - celled proembryo. 7～9. Consecutive sections of a linear 6 - celled proembryo. 10. Walls formed in free endosperm nuclei. 11. Globular embryo at mature stage of berry. 12. Testa at globular embryo stage. 13. Four rows of ovule. 14. Showing hypotropous ovule. (1～2 ×622; 3～9 ×396; 10～11 ×396; 12～13 ×88; 14 ×39)



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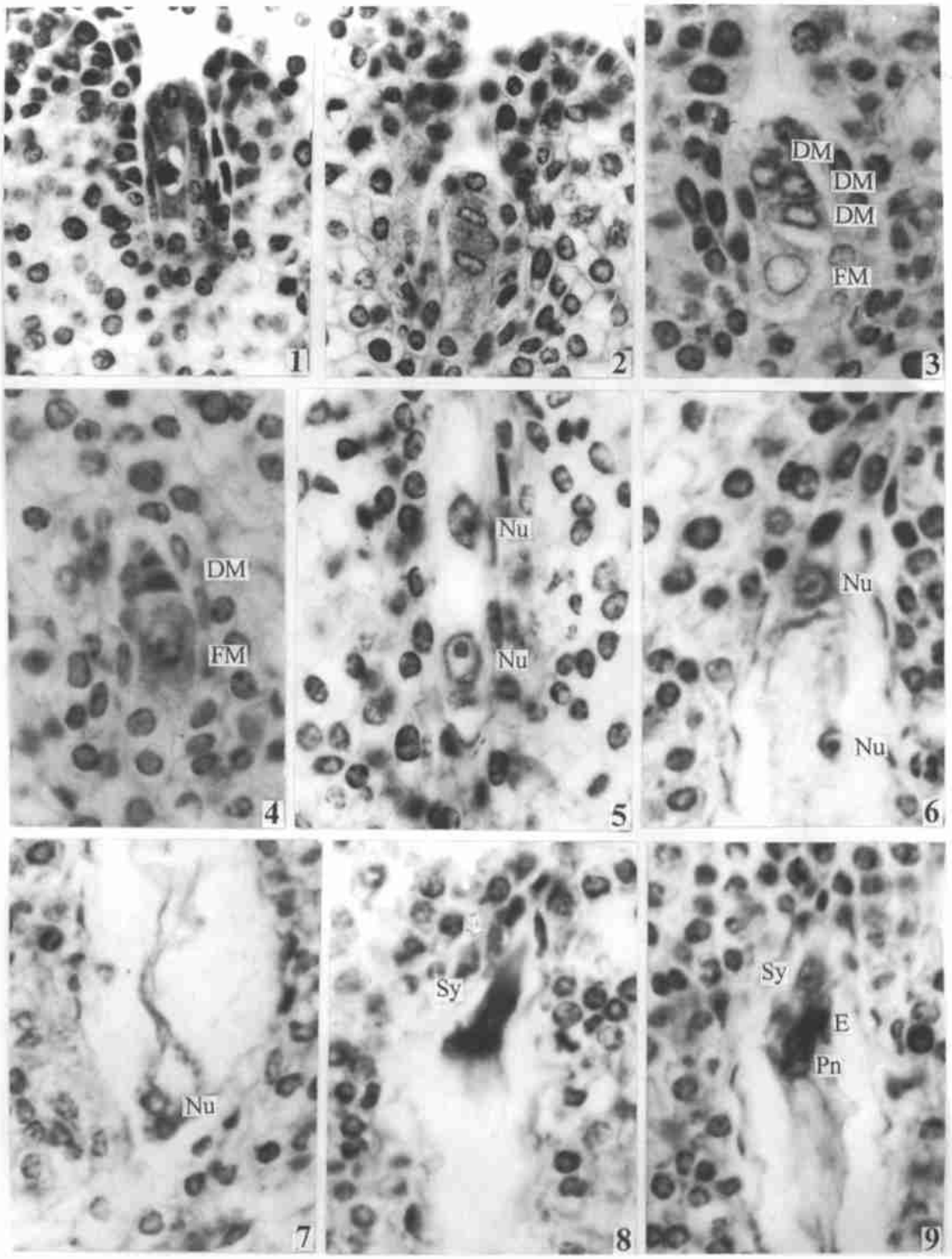

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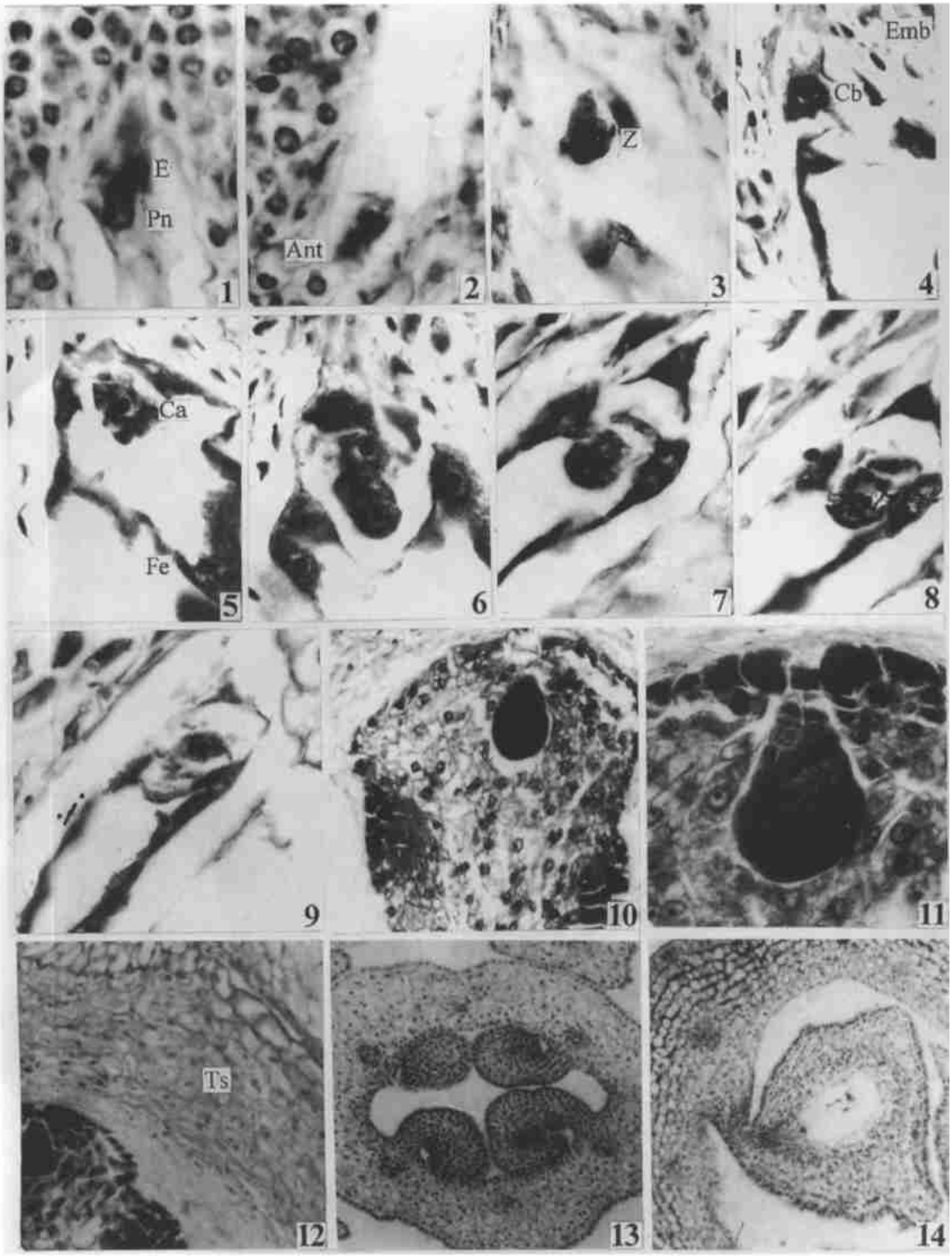

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