武汉植物学研究 1998,16(4):289～293
Journal of Wuhan Botanical Research

EMBRYOLOGY OF THE GENUS *THLADIANTHA* (CUCURBITACEAE)

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Abstract The main processes of life cycle of the genus *Thladiantha* were first investigated. Several important characters of embryology were observed and described as follows: secretory tapetum, simultaneous cytokinesis, microspore tetras, tetrahedral and isobilateral, two-celled pollen grains, bitegminous, crassinucellate, polygonad type embryo sac, micropylar fertilization, embryogenesis onagrad type.

Key words *Thladiantha*, Embryology, Cucurbitaceae

The genus *Thladiantha* Bunge belongs to the subtribe *Thladianthinae* of tribe *Joliffferae* of family Cucurbitaceae[1]. There are 22 species in this genus, all of them are distributed in China, only a few species are dispersal to the other countries of East and South-east Asia[2]. The systematic data dealt with *Thladiantha* were mainly obtained from the fields of palynology[3], Cytology[4], anatomy[5,6], and systematics[1,7~9], the information on embryology of *Thladiantha* had not been previously reported[10~14]. This paper reported several embryological aspects of *Thladiantha* and attempted to provide useful information for the further research of the Cucurbitaceae.

1 Materials and Methods

Table 1 The vouche specimens for the embryological study in the genus *Thladiantha* Bunge

Species	Locality	No.	Collector
T. capitata	Mt. Emei, Sichuan	95, 96	J. Q. Li
T. cordifolia	Luoping, Yunnan	250	J. Q. Li
T. hookeri	Dechang, Sichuan	160	J. Q. Li
T. pustulata var. *pustulata*	Songming, Yunnan	17	J. Q. Li

Samples were identified and collected in the fields from South-west China (Table 1), and fixed in formalinacetic acid-alcohol (FAA). The dehydration was acomplished by using an alcohol series of 50%, 75%, 85%, 95% and 100%. The dehydrated tissues were then embedded with paraffin. The embedded tissues were cut 8～15 μm in thickness and

收稿日:1997-06-26,修回日:1997-07-29。第一作者:男,44 岁,研究员(博士),从事植物系统学方面研究。

stained with safranin and fast green.

2 Observation and Results

2.1 Microsporangium and microsporogenesis

Filaments of the paired stamens were connivent at the base (Pl. I, fig. 1). Prior to the connective developed, a transverse section was observed, the anther comprised a homogeneous mass of cells surrounded by the epidermis cells. As connective cells developed, the wall cells began to be differentiated. Original parietal cells were divided through a series of periclinal and anticlinal orientations and at last they formed the anther wall. At the same time, a series of divisions of the primary sporogenous cells took place to form a number of microspore mother cells (Pl. I, fig. 2).

The anther wall comprised four layers: (1)The epidermis, typically one cell thick. Its cells were usually uniform and did not have intercellular space. (2)The endothecium, one cell thick, the cells were similar to those of the epidermis in the young stage. When pollen grains shed, the tapetum disappeared gradually and the endothecium cells increased their size rapidly (Pl. I, fig. 8). (3)The middle layer normally two layers of cells. The cells were longer and flatter than those of the anther wall. At last the inner layer of cells became a narrow band pressed by the other layer (Pl. I, fig. 8). (4)The tapetum, one layer of cells or two in some regions. The tapetum cells had dense cytoplasm and prominent nuclei. The cells were binucleate in most cases, but a few were trinucleate. They can be classified as the secretory tapetum (Pl. I, fig. 8). Simultaneous cytokinensis in the microspore mother cells followed meiosis. The tetras were tetrahedral, isobilateral (Pl. I, fig. 3～7). The pollen grains were two-celled when they were shed (Pl. I, fig. 9).

2.2 Megasporangium and megasporogenesis

In the Cucurbitaceae, gynoecium was mostly three carpels united to form a compound, inferior ovary with intruded, usually much enlarged parietal placentas (Pl. Ⅱ, fig. 1). In very few cases, carpels were joined in the center, the ovary was plurilocular[15].

The archesporial cell underwent a periclinal division to form a primary parietal cell at the top and a sporogenous cell at the base. The former was further divided to form a group of nucellar tissue cells which made the sporogenous cell backward to the 4th or 5th layer below nucellar epidermis. The latter, just as both the out and inner integuments were differentiated, elongated directly to form a megaspore mother cell (Pl. Ⅱ, fig. 2). So examined tissues showed a typical crassinucellate ovule. Sometimes the megaspore mother cells were aberrant, and two or three megaspore mother cells may be found in one nucellus (Pl. Ⅱ, fig. 3). At this stage, the outer integument enveloped the nucellus completely but the inner one covered only 2/3 of the nucellus, the meiosis of the megaspore mother cell took place and the linear tetrad then was formed (Pl. Ⅱ, fig. 4). The chalazal, one of the four megaspores, was developed into the functional megaspore as usual and the rest degenerated gradually and disappeared.

2.3 Embryo sac

The duration of the functional megaspore or the single nucleate embryo sac was very short. The single nucleate embryo sac having a very big nucleus soon proceeded three successive divisions to form an eight-nucleate embryo sac of the Polygonad type. At the binucleate stage, two nuclei were situated in the middle, then moved to the each pole in the embryo sac, respectively (Pl. Ⅱ, fig. 5). At the four-nucleate stage, the four nuclei grouping into two pairs were arranged at the each end of the sac (Pl. Ⅱ, fig. 6). Finally, the eight-nucleate embryo sac expanded rapidly (Pl. Ⅱ, fig. 7). The eight nuclei in two groups, four nuclei each, were located at the each pole of the sac at first, then moved to their positions immediately (Pl. Ⅰ, fig. 7, 8). In plant species, the three antipodals were normally positioned chalazal, the egg nuclecus together with two synergid nuclei approached to the micropyle. Two of the rest polar nuclei moved to the middle of the embryo sac. In *Thladiantha*, the polar nuclei moved more close to the egg nucleus. Most of the antipodal cells, single nucleate, disappeared before fertilization, on the contrary, the synergid cell just developed fully into the biggest size at the same time.

An irregular situation sometimes occurred in the embryo sac formation. For example, in the species *Thladiantha cordifolia*, the megaspore mother cell was not undergone meiosis to form directly (four)six-nucleate embryo sac, and the nucellus tissue were almost completely disappeared, only a ring of the epidermis was remained (Pl. Ⅲ, fig. 6).

2.4 Fertilization

As embryo sac was mature, a long nucellar beak of the nucellus tissue was formed at the tip. The exostome and endostome were originated from the outer and inner integuments respectively. After the petals fell off, fertilization took place. A pollen tube entered embryo sac through the micropylar canal and reached to the nucellus tissue at last (Pl. Ⅲ, fig. 3).

2.5 Endosperm

Endosperm formation was of the nuclear type and endosperm was well developed before the zygotic division. The three nuclei fusion occurred to form a primary endosperm nucleus (Pl. Ⅲ, fig. 1, 2) which soon underwent a nucleus division into two nuclei. The nuclei then proceed to a free-nucleus stage by a series of successive nuclear division. Usually the free nuclei remained a very short time and soon enveloped by a common thin protoplasmic membrane. They moved peripherally along the membrane and each of the nuclei was surrounded by a mass of cytoplast and became individual cell by the separating. Thus, this process resulted in the endosperm cells filled with almost all the space formed by the nucellus tissues degeneration when the zygote began to division. A few samples showed that a group of free endosperm nuclei surrounded the zygote or suspensor cells and they were easily confused each others (Pl. Ⅳ, fig. 9).

2.6 Embryogeny

The embryogeny of the genus *Thladiantha* was the Onagrad type. Two-celled proembryo was formed by the first transverse division of the zygote (Pl. Ⅳ, fig. 1). The apical cell toward chalaza was bigger than the basal cell toward the micropyle(Pl. Ⅳ, fig. 2). A four-celled (T-typed) proembryo was formed by a transverse division of the basal cell and a vertical division of the apical cell (Pl. Ⅳ, fig. 3～7). Then the two cells toward micropyle underwent further divisions to give rise to a long suspensor(Pl. Ⅳ, fig. 4,5). At the same time, the two cells toward chalaza divided at right angles to the previous division plane to form a quadrant (Pl. Ⅳ, fig. 6). The further division of the quadrant cells was transversely oriented and the division produced an octant. Following divisions proceed irregularly, and at last a globate young embryo was formed(Pl. Ⅳ, fig. 8). Occasionally the polyembryony was observed, but embryogenic type was not clear(Pl. Ⅲ, fig. 5).

2.7 Seed coat formation

At the later stage of young embryo development, the differentiation of seed coat was observed(Pl. Ⅲ, fig. 4). The two-layered inner integument cells pressing each other became a very thin layer, and at last developed into a membranous inner seed coat. The outer integument(eight-nine layers of cells) cells were fully developed and the cells of the 7th layer were much elongated. The lignification further resulted in the outer layer harder. When the seed became mature, it was enfolded by a yellowish and carnified aril which was formed from the tissue of the podosperm.

3 Conclusions

The results presented in the paper are generally agreed to the embryological data of the Cucurbitaceae reviewed by G. L. Davis[13]. A new character that the microspore tetras included both tetrahedral and isobilateral had been observed in Cucurbitaceae. Two closely related genera *Thladiantha* and *Momordica*[16] were also found to be very similar in embryological characters. This result is also agreed with the conception of the subtribe *Thladianthinae* proposed by C. Jeffrey[1].

Acknowledgments This work is a part of my doctoral dissertation. I am very grateful to my advisers: Professor Wu Zhengyi, Kunming Institute of Botany, the Chinese Academy of Sciences, and Professor Lu Anming, Institute of Botany, the Chinese Academy of Sciences for guidance and encouragement. I am greatly indebted to above mentioned institutes for providing of facilities, and especially to Professor Pan Kaiyu and Miss Wen Jie for their providing experimental facilities to me.

References

1 Jeffrey C. The Cucurbitaceae of eastern Asia. London: Royal Botanic Gardens, Kew, 1980.

2 Li J Q. On the systematics of *Thladiantha*(Cucurbitaceae). *Acta Bot Yunn*, 1997, **19**(2): 103～127

3 Zhang Z Y, Lu A M. Pollen morphology of the subtribe *Thladianthinae* (Cucurbitaceae) and its taxonomic significance. *Cathaya*, 1989, **1**: 23～36

4 Li J Q, Wu Z Y, Lu A M. Cytological observation on the plants of *Thladianthinae* (Cucurbitaceae). *Acta Bot Yunn*, 1993, **15**(1): 101～104

5 Li J Q, Wu Z Y, Lu A M. Comparative anatomy of leaf blade-midribes of the subtribe *Thladianthinae* (Cucurbitaceae). *Acta Bot Yunn*, 1992, **14**(4): 418～422

6 Li J Q. Comparative anatomy of nutritive organs of the subtribe *Thladianthinae* (Cucurbitaceae). *J Wuhan Bot Res*, 1995, **13**(2): 107～112

7 Lu A M, Zhang Z Y. A revision of genus *Thladiantha* Bunge (Cucurbitaceae). *Bull Bot Res*, 1981, **1**(1～2): 61～96

8 Li J Q, Wu Z Y, Lu A M. New taxa of the genus *Thladiantha* (Cucurbitaceae). *Acta Bot Yunn*, 1992, **4**(2): 133～134

9 Li J Q. A revision of the genus *Siraitia* Merr. and two new genera of Cucurbitaceae. *Acta Phytotax Sin*, 1993, **31**(1): 45～55

10 Chakravarty H L. Morphology of the staminate flowers in the Cucurbitaceae with special reference to the evolution of the stamens. *Lloydia*, 1958, **21**: 49～87

11 Singh D. Development of embryo in the Cucurbitaceae. *J Ind Bot* Soc, 1961, **40**: 620～623

12 Chopra R N. Female gametophyte and endosperm of some members of the Cucurbitaceae. *Phytomorphology*, 1965, **15**(3): 217～223

13 Davis G L. Systematic embryology of the angiosperms. New York: John Wiley & Sons, 1966.

14 Li D Z. Systematics and evolution of *Hemsleya* (Cucurbitaceae). Kunming: Yunnan Science and Technology Press, 1993. 18～20

15 Cronquist A. An integrated system of classification of flowering plants. New York: Columbia Univ Press, 1981. 422～425

16 Kirkwood J E. The comparative embryology of the Cucurbitaceae. *Bull N Y Bot Gard*, 1904, **3**: 314～402

赤爬属的胚胎学研究

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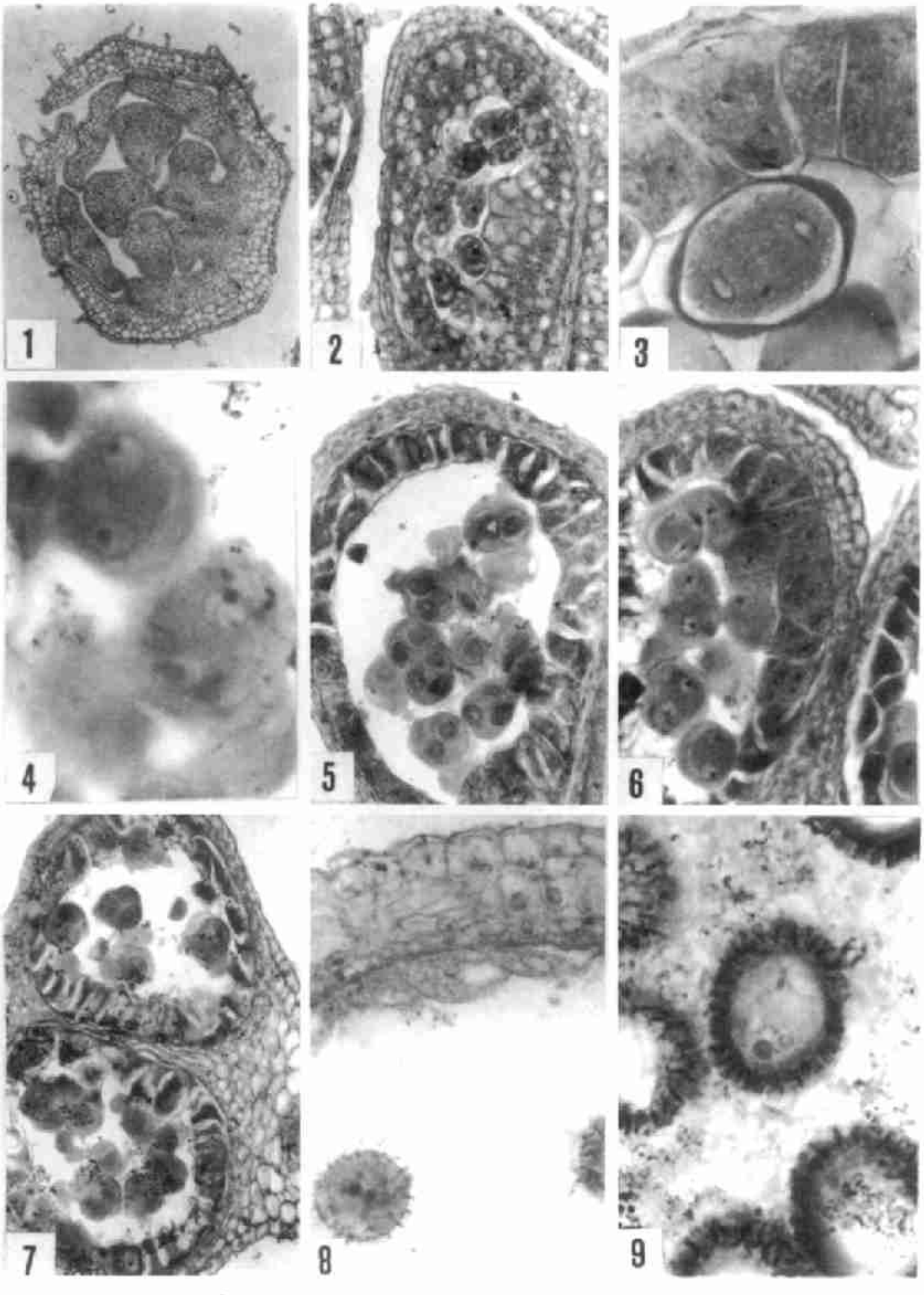
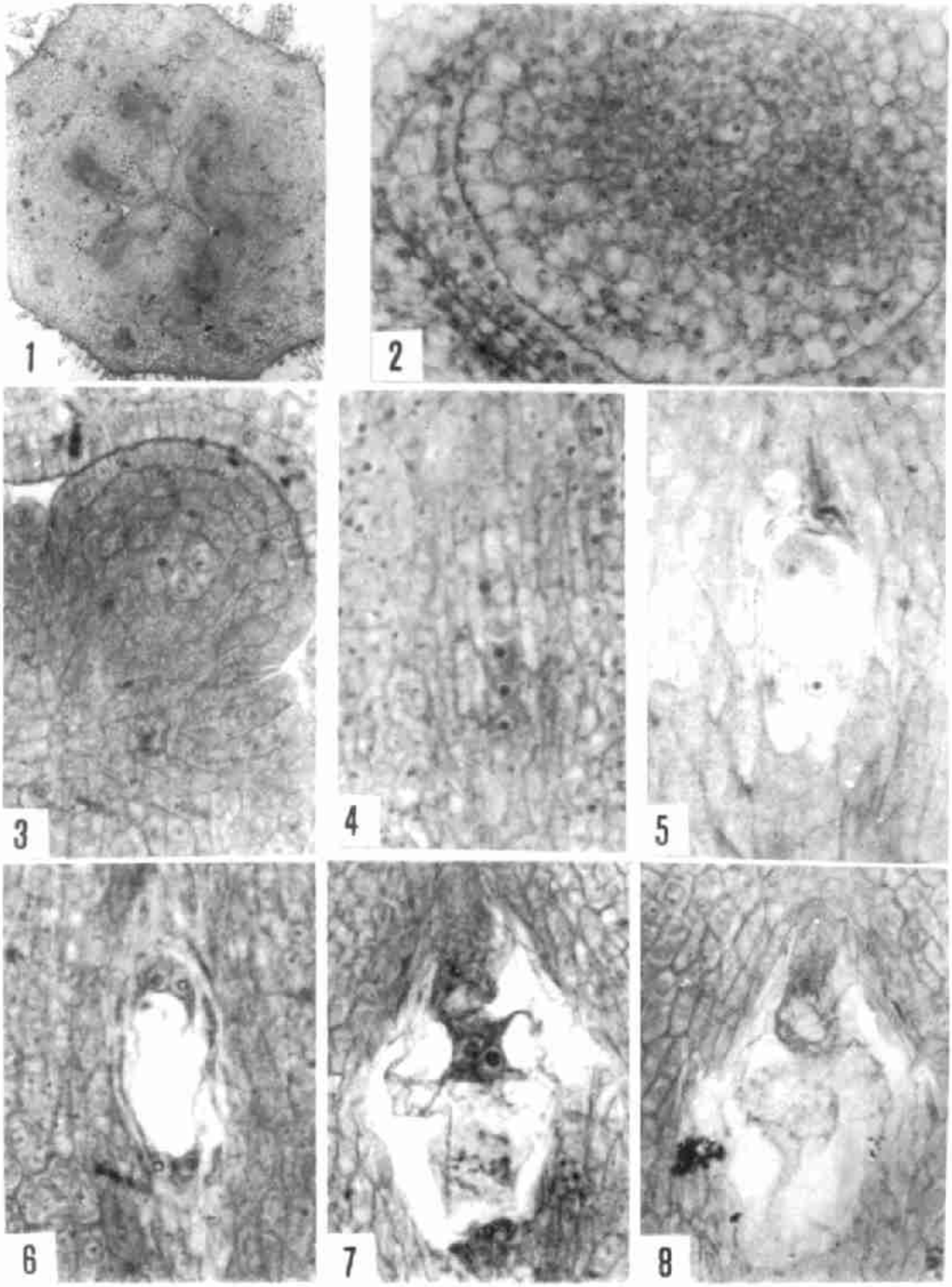
摘 要

首次描述了葫芦科赤爬属植物生活史的主要过程以及胚胎学的重要特征，它们是：腺质绒毡层，胞质分裂同时型，小孢子四分体四面体型和等面体型，花粉粒散发时具两细胞，双珠被，厚珠心，蓼型胚囊，珠孔端受精，胚胎发育柳叶菜型。

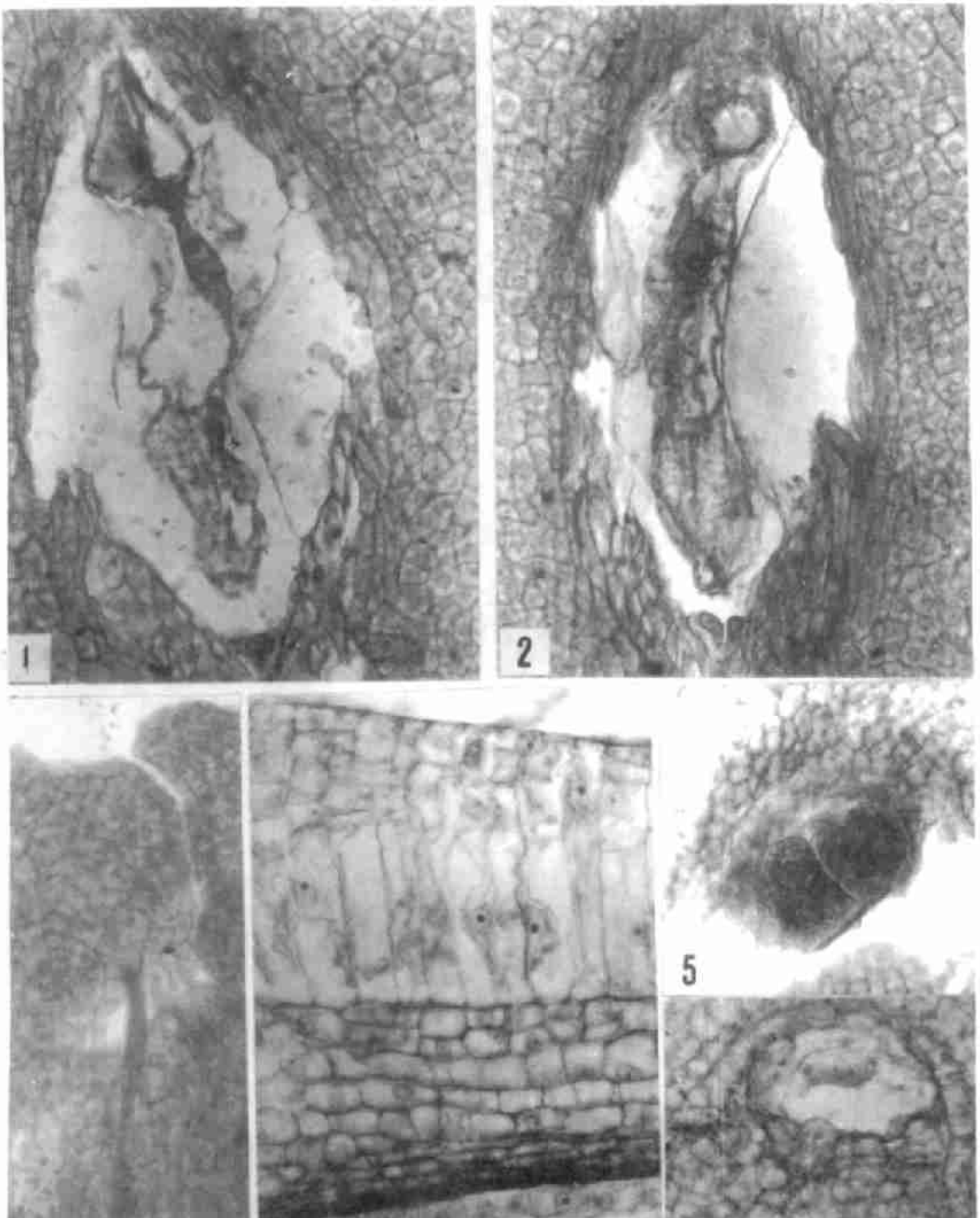
关键词 赤爬属，胚胎学，葫芦科



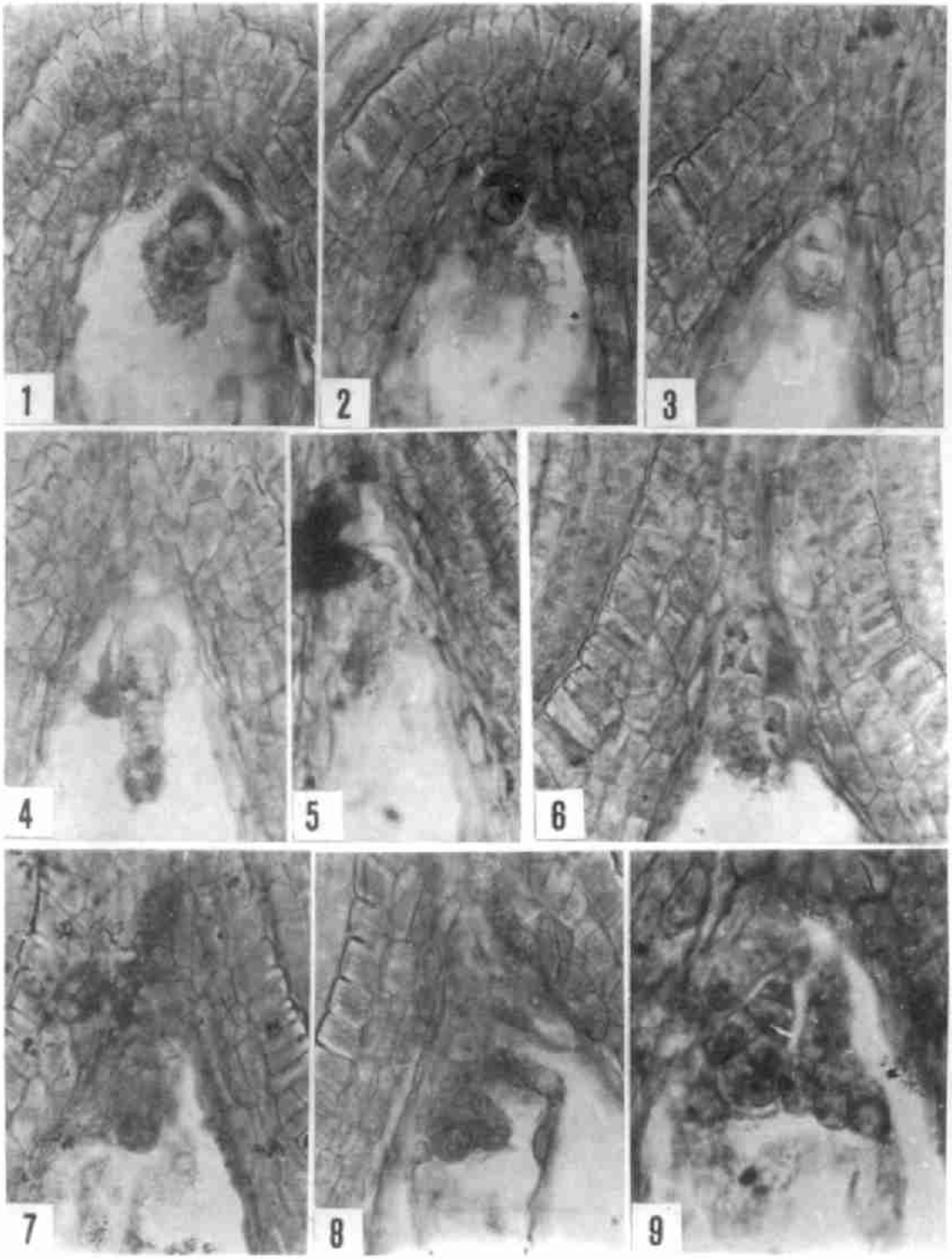
1~9: Microsporangium and microsporogensis of *Thladiantha*. 1. A transverse section of young male flower (× ca. 200); 2. A longitudinal section of an anther, showing microspore mother cells and the cellular layers of anther wall (× 800); 3. Meiosis of microspore mother cell (× 2 500); 4.6. Simultaneous type cytokinesis (4. × 2 500, 6. × 1 000); 5, 7. Microspore tetras, showing tetrahedral and isolateral; secretory tapetum (5. × 800, 7. × 504); 8. Constitution of the anther wall in the later stage of microsporogensis, showing remnant endothecium and degenerating tapetum cells (× 1 260); 9. Two-celled pollen grains (× 2 000) (All are *Thladiantha pustulata* var. *pustulata*, except fig. 3 is *T. capitata*)



1～8. Megasporangium, megasporogenesis and embryo sac formation in *Thladiantha hookeri*. 1. A transverse section of ovary, showing the parietal placentation (×ca. 100); 2. Megaspore mother cell, showing the crassinucellate ovule (×1 500); 3. One or more megaspore mother cells (×1 500); 4. Megaspore linear tetras (×1 500); 5. Two-nucleate embryo sac (×2 500); 6. Four-nucleate embryo sac (×2 000); 7, 8. Eight-nucleate embryo sac, successive slides (×1 500)



1～6. Eight-nucleate embryo sac, showing the two polar nuclei contacting each other (×1 500); 2. The primary endosperm nucleus (×1 200); 3. A pollen tube is entering the embryo sac through a micropylar canal (×1 500); 4. Seed coat formation, showing the cells of the inner and outer inter integument (×1 500); 5. Polyembryony (×750); 6. Showing four nuclei in an aberrant embryo sac (×1 500)



1～9. Embryogeny of *Thladiantha cordifolia* (×1 500). 1. A zygote surrounded by free endosperm nuclei; 2. Two-celled proembryo, showing one basal cell and one apical cell; 3, 7. Four-celled (T-typed) proembryo; 4, 5. Successive slides: 4. showing a long suspensor, 5. showing the biggest suspensor cell toward the micropyle; 6. Proembryo at the quadrant stage; 8. Globular proembryo; 9. A quadrant proembryo confuses with free endosperm nuclei