川东獐牙菜小孢子发生和雄配子体形成

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摘 要 报道了川东獐牙菜(*Swertia davidii* Franch.)小孢子发生和雄配子体形成的过程。主要结果如下:花药四室,药壁发育为基本型;绒毡层异型起源,属于腺质型绒毡层,药室内具有的退化绒毡层核是早期该细胞有丝分裂凸入药室中央并原位退化形成的;中层细胞 3 层;药室内壁退化;花药壁表皮宿存,细胞柱状伸长,纤维状加厚。小孢母细胞减数分裂为同时型,四分体排列方式主要为四面体形和左右对称型,少数为"T"形和十字交叉形;成熟花粉为 2-细胞类型。

关键词 川东獐牙菜;小孢子;雄配子体

The genesis of microspore and the formation of male gametophyte in *Swertia davidii* Franch.

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Abstract The present paper firstly reports the microsporogenesis and the formation of male gametophyte in *Swertia davidii* Franch.. The main results showed that anther is tetrasporangiate. The development of anther walls conforms to the Basic type and comprises of epidermis, endothecium, three middle layers and tapetum at the mature stage. The tapetum cells have dual origin and belong to the glandular type. The degenerating tapetum nuclei in the middle of anther locules are from the tapetum cells, which undergo mitosis, then intrude into the anther locules and degenerate in situ at the early stage. Three middle layers are ephemeral. Endothecium degenerates shortly after differentiating; epidermis persists and develops to become histogram elongated and fibrous-thickening. The cytokinesis of the microspore mother cells in meiosis is of the Simultaneous type. Most of the microspore tetrads are tetrahedral and there are still a few other types, such as isobilateral, dilateral and "T"-shaped. Pollen grains are 2-celled when shed.

Key words *Swertia davidii* Franch.; microspore; male gametophyte

Swertia davidii Franch. is annual herbage that belongs to *S.* section *Ophelia* (D. Don ex G. Don) Benth. et Hook (Gentianaceae)[1]. Hacknames include fish-gallbladder-grass, water-ganoder, and stool-grass etc. *S. davidii* Franch. has been widely used in curing jaundice hepatitis, dysentery, pneumonia, tonsillitis and gynecological inflammation at civilian of HuNan, HuBei, Szechwan and Guizhou in China. There is few research of basal biology in *S. davidii* Franch., excepting its introduction in cultivation[3] and tissue culture[4], and the study on the aspect of generative biology is blank. The embryological data of *S. davidii* Franch. can provide useful information for artificial propagation and exploiture.

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1 Materials and Methods

Materials were collected from Zhangjiajie Maoyan River basin, Hunan province, China(110°16′E, 29°06′N, Alt 150m). The voucher (Huang Hengyu 159) is deposited in the Herbarium of the Biology Department of Jishou University.

Either entire or partial flower buds and flowers at different development stage were collected and fixed in the FAA (50% alcohol:glacial acetic acid:formaldehyde = 89:6:5). Fixed materials were stained in Ehrfichs hematoaylin($C_{16}H_{14}O_6 \cdot 3H_2O$, American) after washed with water. The materials were dehydrated in ten grades of alcohol and transparentized with five grades of dimethylbenzene, and then were infiltrated and embedded in paraffin. After that anthers and flower buds were sectioned at the thickness of 5 ~ 8μm, using a Spcencer rotary microtome model 820. Observation and photographs were taken under Zeiss-Axioskop2 photomicroscope.

2 Observations and Results

The base of flower is 4 in *S. davidii* Franch.. The flower primordium forms perianth and staminate primordium in sequence in early phase of budding. And then the apical meristerm of flower primordium is transformed into staminate, the base of the staminate primordium forms filament while the top forms anther. In *S. davidii* Franch., each anther contains four sporangia that the rear pair of which are smaller (Plate Ⅰ:1). At that stage, the surface of anther is a layer of flat epidermal cells, inside which there are some meristematic cells of similar modility. With the development of anther, four patches of tissue are differentiated from the main mass of cells. The archesporial cells in each patch, which are differentiated below the epidermis, characterized by their large size, dense cytoplasm and conspicuous nuclei (Plate Ⅰ:2). They undergo periclinal division to produce primary parietal layer towards epidermis and primary sporogenous cells towards inside (Plate Ⅰ:3).

2.1 The formation and differentiation of anther wall

The primary parietal cell undergo periclinal division (Plate Ⅰ:4) to produce outer layer and inner layer (Plate Ⅰ:5) forming three layers with the epidermis (Plate Ⅰ:6). Either outer layer or inner layer divides again to produce several layers (Plate Ⅰ:7). At first, the form and configuration of the cells of these several layers are similar, and then differentiation will take place. At the time when primary sporogenous cell appears, the mature anther wall consists of six layer cells: epidermis, endothecium, three middle layers and one tapetum (Plate Ⅰ:8,9). It indicates that the outer and inner layer derive from primary parietal cells. The outer layer undergoes division to produce endothecium and middle layer, and the inner layer divides to produce middle layer and tapetum. The middle layer is make up of the cells from the outer and inner layer. According to Davis's compartmentalization[5], the ontogeny of microsporangial wall is the Basic type.

The innermost layer of anther wall is tapetum with dual origin that mainly from the inner layer of primary paries and partly from connective cells. Tapetum starts to differentiate at phase of early sporogenous cell and its cells gradually augment while its protoplasm gradually manifold in the course of development of microspore mother cell. The tapetal cells become the most complete upgrowth at phase of microspore mother cell and their size are similar to microsporocytes inside them and are larger than those of the outer. Here, the tapetal cells present close squareness and have the feature of glandular cell (Plate Ⅰ:10). In the course of development, some tapetal cells undergo periclinal division to produce some cells protruding in the anther chamber, which form so-called "quasi-placenta" or "cross-grid" (Plate Ⅰ:10,11,12). It is observed that most tapetal cells are uninuclear, but it is also observed that minority are binuclear or multinucleate.

The tapetal cells begin to separate each other when meiosis Ⅰ of microporocyte is over. At the microspore tetrad, the tapetal cells disaggregate intensively and degenerate in situ. The degenerated nuclei of tapetal cells in the anther chamber are formed by the same layer cells that enter it at early time but they are not formed by degenerated tapetal cells of circum-

ference that enter it (Plate Ⅱ :13,14). It is not found that amalgamation occurs and periplasmodium appear when tapetal cells disaggregate. The tapetal cells are single till disappearing. At middle stage of uninucleate microspore, the tapetal cells disappear completely (Plate Ⅱ :15). It is obvious that tapetum belongs to the Glandular type.

The middle layers accomplish differentiation at time of secondary sporogenous cell, including three layers, of which cells are of smaller size, bigger nucleus and squareness or flat rectangle (Plate Ⅰ :8, 9). At time of microsporocyte, the middle layer near tapetum begins disintegrating (Plate Ⅰ :10), and at the stage of microspore tetrad the middle layer cells disaggregate more quickly, only have one or two layer cells left(Plate Ⅱ :1,2). At middle stage of uninucleate microspore, the middle layers disappear completely and only leave the degenerated vestiges (Plate Ⅱ : 15). The endothecium come into being at time of secondary sporogenous cell while cells arrange close and present strip shape (Plate Ⅰ :9). The cells of endothecium are most developed when microsporocyte formed. Henceforth, they don 't grow anymore and their shape becomes prolate with anther growing (Plate Ⅱ :13,14). The endothecium only leave the vestiges of kraurosis when shed (Plate Ⅱ :16).

The epidermis only includes single-layer cells that arrange closely. The exterior of epidermal cells often possess horny layer which play a role in protection. With anther growing, the size of epidermal cells are increasing and display U-like thickenings on radial and inner tangential walls (Plate Ⅰ :10). At the microspore tetrad, the epidermal cells reach the highest level of development, which show intensively fibrous thickenings (Plate Ⅱ :14). As the anther matures, the walls between adjacent pairs of chambers break down so that only two larger sacs remain by the time the epidermal cells lose water and retrogress (Plate Ⅱ :16). The epidermal cells in the joint of two anther chambers are not thickened and become the focus of stress where anther wall dehisce and the mature pollens emit when shed.

2.2 Microsporogenesis

The primary sporogenous cells undergo mitosis resulting in secondary sporogenous cells (Plate Ⅱ : 17). In comparing with surrounding wall cells, the secondary sporogenous cells have larger size, greater nuclear-cytoplasmic ratio, dense cytoplasm, and deeper color; moreover, they arrange closely and don 't have obvious vacuoles and present polygon (Plate Ⅱ : 18). The polygonal secondary sporogneous cells turn into circular microspore mother cells with development (Plate Ⅱ :19).

Microspore mother cells are of short duration, which undergo meiosis as soon as they are formed. The cytokinesis of the microspore mother cells is the Simultaneous type resulting in tetrads that are surrounded by common callose and each is seperated by callose respectively. In this way, the microspore cells, tetrads or microspores are in a state of isolated comparatively. The function of callose surrounding tetrads seems to be " molecule-boult " which allows many nutriments entering but prevents macromolecular substance. It controls the intercourse of substance among cells and keeps the independency of microspores through interallelic recombination and non-allelic independent assortment in meiosis. Most of the microspore tetrads are tetrahedral (Plate Ⅱ :20) and there are still a few other types, such as isobilateral (Plate Ⅱ :21), dilateral (Plate Ⅱ :23) and " T "-shaped (Plate Ⅱ :22).

2.3 Male gametophyte

At late period of microspore tetrads, the common callose of microspore tetrads begins to attenuate (Plate Ⅱ :24). At last, because of the callosal dissolution, four microspores separate respectively and are released to anther chamber that permeate secretion of tapetum. The microspores have synthesized their own cytoderm when they were in common callose. The microspores that have just been released are still the shape at the time of tetrads, of which the interface is plane and free-face is spherical surface (Plate Ⅲ : 25). This period lasts longer time. Since then, the microspores gradually growing into round-shaping, with a centering nucleus, dense protoplasm, thin cytoderm, and an unclear germinal aperture. With the development of microspore, the wall of which is becoming visible and germinal aperture is also become

obvious (Plate Ⅲ:26,27).

As most angiosperms, the microspore nucleus of *S. davidii* Franch. will experience a series of movements while it is developing. The microspore just formed has a centeral nucleus and dense protoplasm. (Plate Ⅲ:28). With developing, many small vacuoles appear in the protoplasm (Plate Ⅲ:29,30), which gather to become a large vacuole that locates in the center of cell, and protoplasm then distribute in circumambience of cytoderm, and nucleus moves randomly from center to one side of wall (Plate Ⅲ:31, 32). Before long, microspore enters mitosis prophase near cytoderm (Plate Ⅲ:33). The dividing direction of mitosis mainly has two sorts: one is centripetal division (Plate Ⅲ:34), the other is along mural division (Plate Ⅲ:35). Furthermore, the division of microspores in the same chamber is not simultameous. The microspore divides into two nuclei with unequal size (Plate Ⅲ:36). Then the two nuclei undergo cytokinesis to produce a small generative cell and a large vegetative cell (Plate Ⅲ:37), the bigger one contains large vacuole and most protoplasm of former microspore, and the small one only have a little protoplasm. This may be named as the early phase of two-celled pollen.

Through a period time of growth, the combination of pollen tone up, protoplasm continue to increase, the large vacuole disappears, while nucleus fills out and moves from fringe to center with protoplasm increasing. Here, it can be seen that vegetative cell and generative cell are seperated by visible cytoderm (Plate Ⅲ:38). Anon, the one side of generative cell disengages pollen wall and extrudes internally. Finally, generative cell is divorced from pollen wall (Plate Ⅲ:39). In this course, the wall of generative cell gradually dissolves, and the nucleus ceaselessly augments (Plate Ⅲ:40). At last, generative cell is in the protoplasm of vegetative cell. Here, generative cell shows sphericity, and its wall disappears completely (Plate Ⅳ:41). Soon afterward, generative nucleus lay aboard vegetative nucleus. Thus, the mature two-celled pollen has completed (Plate Ⅳ:42). The generative cell divides to produce two sperms in the pollen tube. However, it has been observed that several generative nucleus of mature pollens divides to form three-celled pollen before shedding.

The pollen grains of *S. davidii* Franch. begin to bourgeon before shedding. The most pollen grains are uniporous sprouting (Plate Ⅳ:43) but it can also been observed as biporose or triporate sprouting (Plate Ⅳ:44,45). However, only one pollen tube can grow up, the others stop growing at midway, which accords with the pollen grains of artificial cultivation.

3 Discussions

It has been reported that there are Glandular and Amoeboid type in tapetum of Gentianaceae plants[7]. The reports of almost all Amoeboid tapetum have mentioned various of changes in early time of development of tapetum[8,9], such as undergoing various divisions to produce some cells protruding in the anther chamber while form "quasi-placenta" or "cross-grid". However, in reports of Glandular tapetum have also mentioned development and presence of similar structure [10~15]. I think they are of the same type, which results from different understanding of the degeneration of tapetum in early time. Through this study, I consider these sorts of tapetum should not be Amoeboid but Glandular. The tapetum of all these species (including *S. davidii* Franch.) elongate lengthways and pile into anther chamber while tapetum are regarded as two or three layers and sometimes also form "quasi-placenta" or "cross-grid" in early tine of development. With development of pollen, these entering anther chamber tapetal cells disaggregate in situ and consist in the circumambience of pollen, resulting in being easily regarded that the peripheral tapetal cells "flow" into chamber. I haven't seen any reports and also haven't observed the amalgamation of tapetal cells occurs. Till disappearing, the tapetal cells are always single and don't form periplasmodia. Such course has also been debated in the study of *Gastrodia elata* Bl.

As to the function of tapetum, someone argued that it was the developed nourishment for microspore mother cells' development, and someone considered

it was developed nourishment of microspores. According to my observation, it was the time that meiosis was over and microspores have just shaped when tapetal cells began disaggregating. Therefore, I think tapetum is likely to be the nourishment for the development of microspore in *S. davidii* Franch.. The initiatory time of tapetum disaggregation has consanguineous relations with the disappearance of middle layers, which would be different in various plants. Not only do tapetal cells disappear completely, but also most endothecium is absorbed. Furthermore, the epidermis seems to replace the protective action of endothecium as well as developed nourishment of microspore. A series of behaviors of anther wall have ensured development of microspore mother cells and microspores.

The pollens are 2-celled in *S. davidii* Franch.. In comparing with 3-celled pollen, the livingness of 2-celled pollen is obvious stronger. Through comparing with 3-celled pollen of Podophylloideae (Berberidaceae)[19,20], the followings may be the reasons for why the livingness of the 2-celled pollens are stronger than that of 3-celled: ①3-celled pollen consume more stored nourishments in the course of producing two sperms during the last mitosis; ②Generally, the ektexine of 3-celled pollen is thinner and pigment is fewer, so its resistivity is lower; ③3-celled pollen is in an active state of metabolizability while 2-celled pollen is in a state of dormancy. Consequently, their perdurable ability is different.

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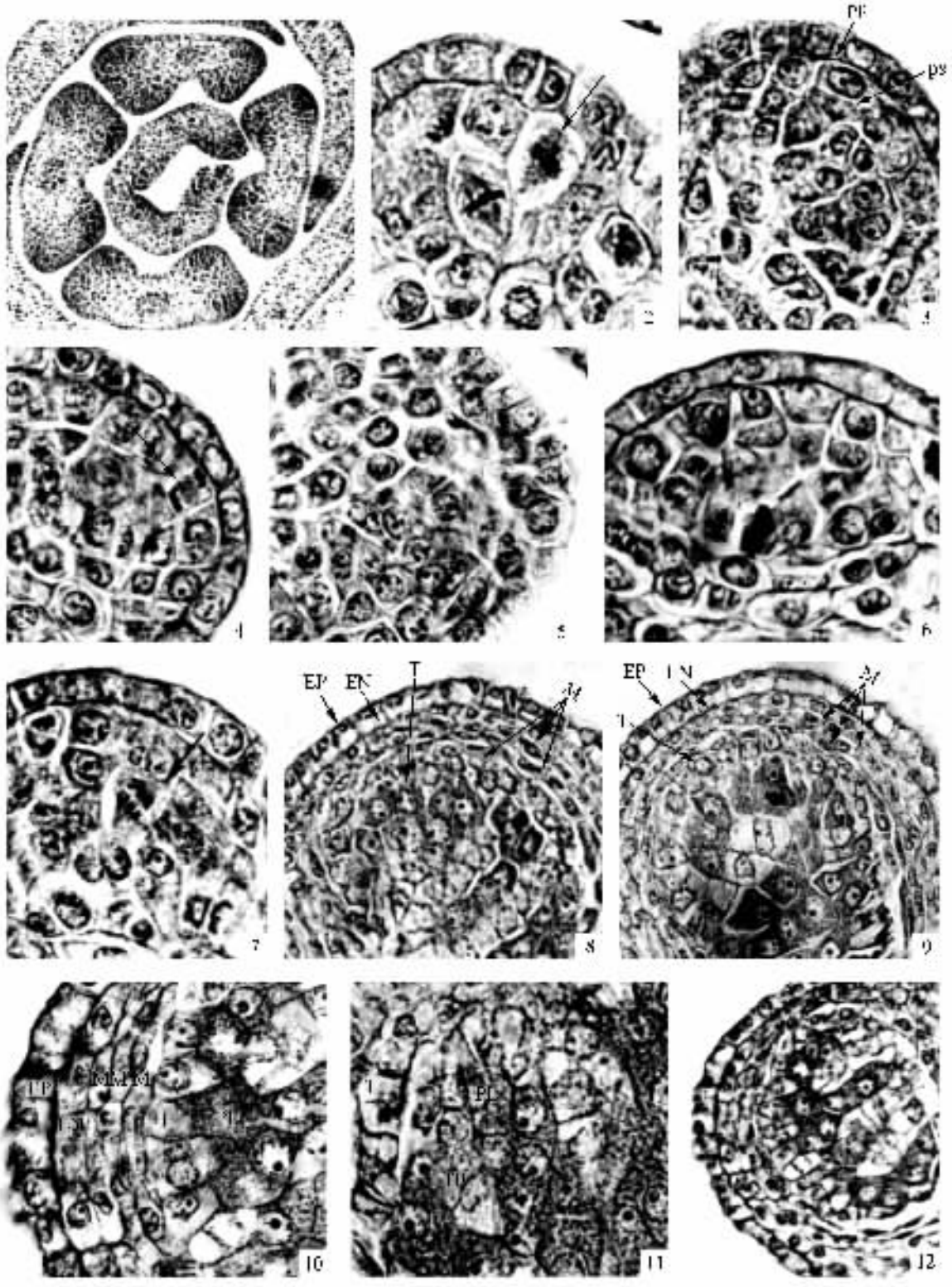

Plate Ⅰ　1. The transection of a young anther, showing tetrasporangiate ×130; 2. Male archesporium(↑) ×1 250; 3. The microspore archesporial cells divide periclinally to form primary sporogenous cells(PS) and primary parietal cells(PP) ×1 250; 4. The primary parietal cell dividing(↑) ×1 250; 5. The out and the inner layers from primary parietal cell dividing ×1 250; 6. The anther wall including epidermis, out layer and inner layer ×1 250; 7. The inner layer of the primary wall dividing(↑) ×1 250; 8,9. Six layers anther wall in the time of secondary sporoginous cell ×500; 10. Six layers anther wall in the time of microspore mother cells ×1 250; 11. "Trabeculae" and "Placentoid" from the tapetum ×1 250; 12. The tapetum cells protruding the anther chamber in the time of microspore mother cells ×500

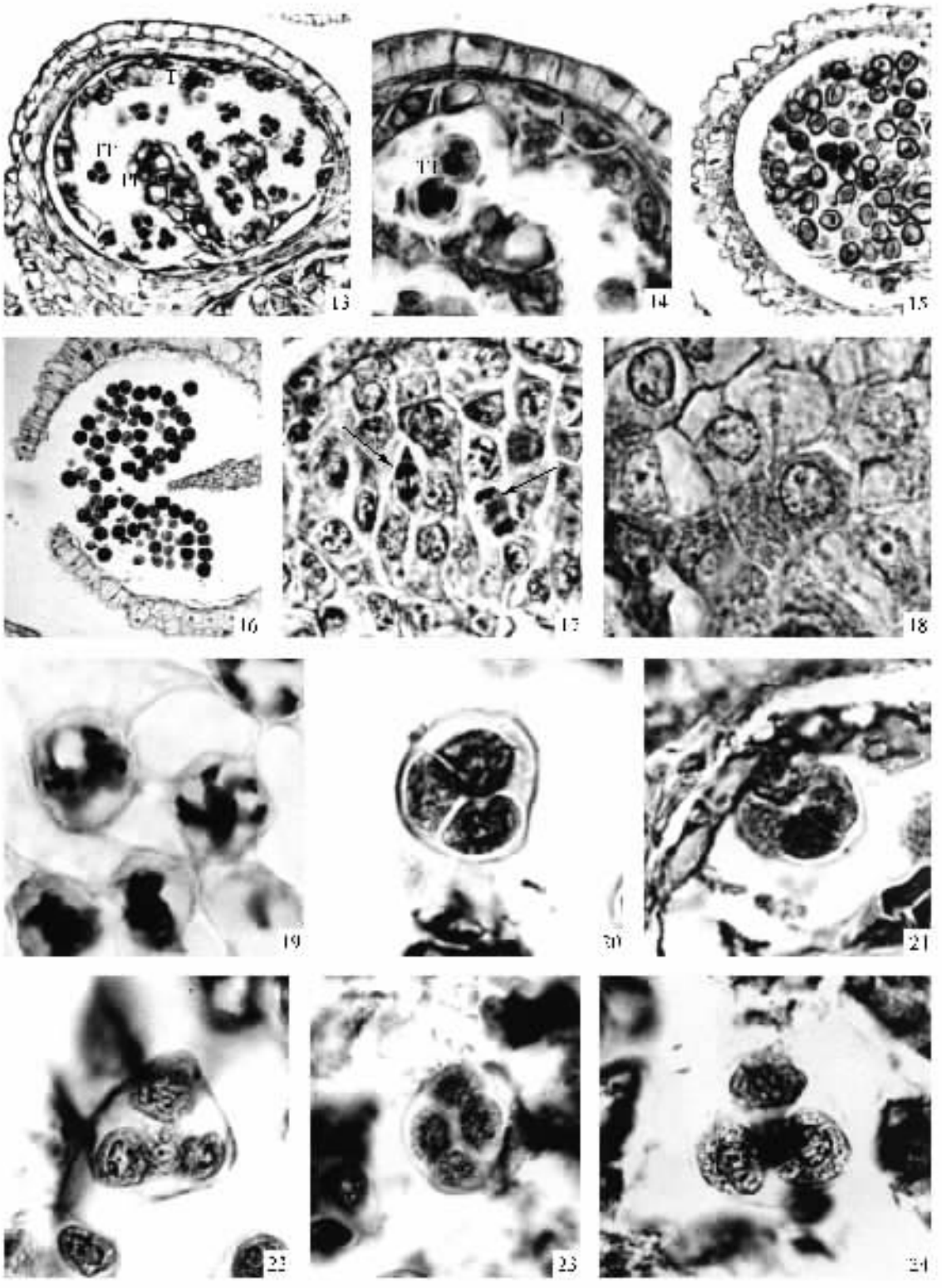

Plate Ⅱ 13. The anther wall in the stage of microspore tetrads ×250 ; 14. Degenerating of the tapetum at original sites, noting the remaining nuclei of the tapetum in the middle of the anther chamber ×500 ; 15. The anther wall in the stage of uninucleate microspore ×250 ; 16. The anther wall of the time of the pollen spread ×125 ; 17. The mitosis of the primary sporobenous cell(↑) ×1 250 ; 18. Secondary sporoginous cells ×1 250 ; 19. Microspore mother cells ×1 250 ; 20. Tetrahedral microspore tetrads ×1 250 ; 21. Isobilateral microspore tetrads ×1 250 ; 22. "T" shape microspore tetrads ×1 250 ; 23. Dilateral microspore tetrads ×1 250 ; 24. The terminal microspore tetrad ×1 250

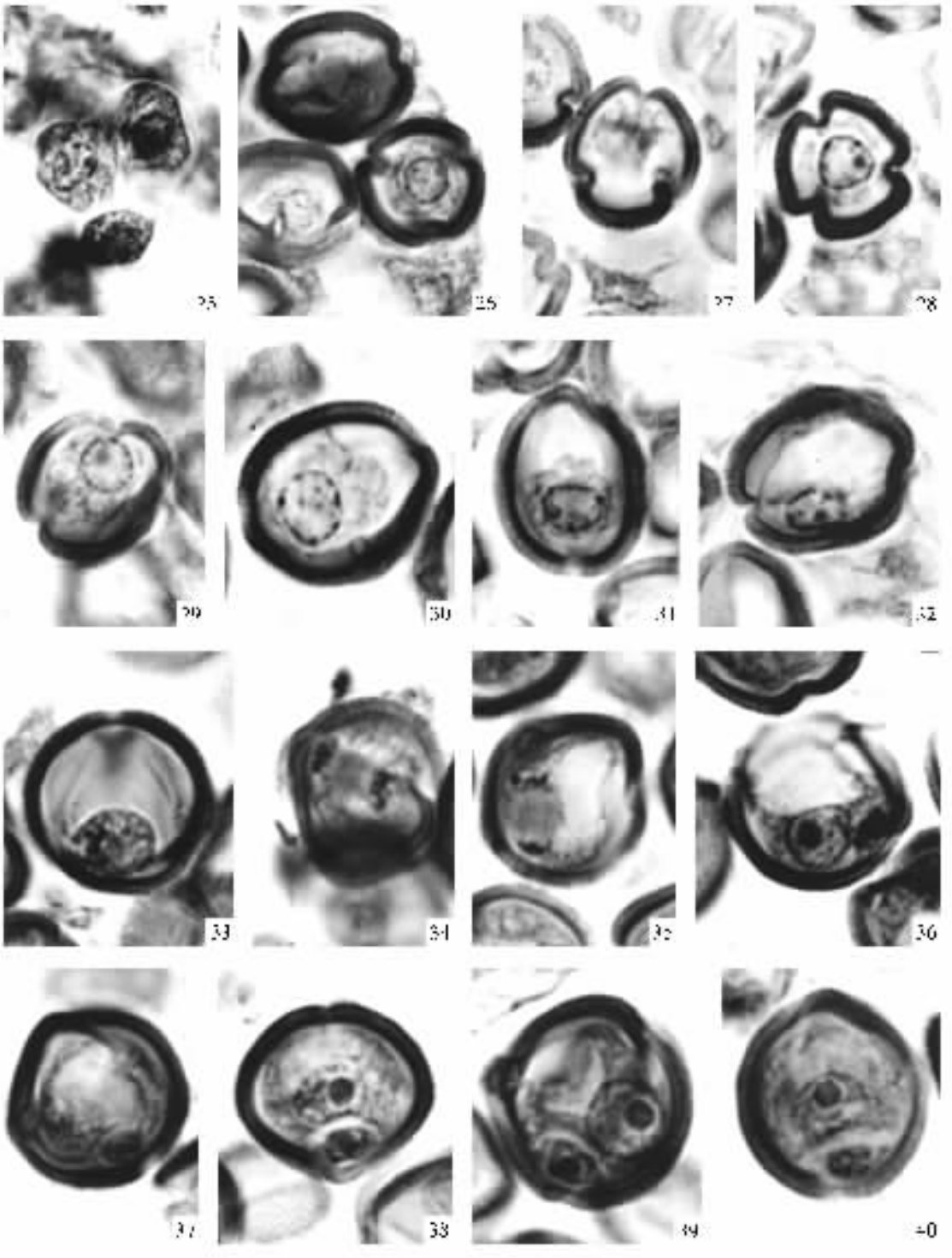

Plate Ⅲ　25. The microspore had just formed from microspore tetrads ×1 250 ;26. The early microspore ×1 250 ;27. The microspore that had just formed had soon appeared three apertures ×1 250 ;28. The early microspore had more developed ×1 250 ;29. Some small vacuoles appear, entering the middle of microspore ×1 250 ;30. Vacuoles had gradually increased in microspore ×1 250 ;31. Vacuolate period of uninucleate microspore ×1 250 ;32. The terminal microspore ×1 250 ;33. The uninucleate microspore at the prophase of mitosis ×1 250 ;34. The uninucleate microspore was radially dividing ×1 250 ;35. The uninucleate microspore was breadthwise dividing ×1 250 ;36. A big nucleus and a small nucleus appeared after dividing ×1 250 ;37. Cell wall had appeared between two nuclei ×1 250 ;38. The early 2-celled pollen, showing obvious cell wall ×1 250 ;39. The generative cell pop out entad ×1 250 ;40. The wall of generative cell dissolving ×1 250

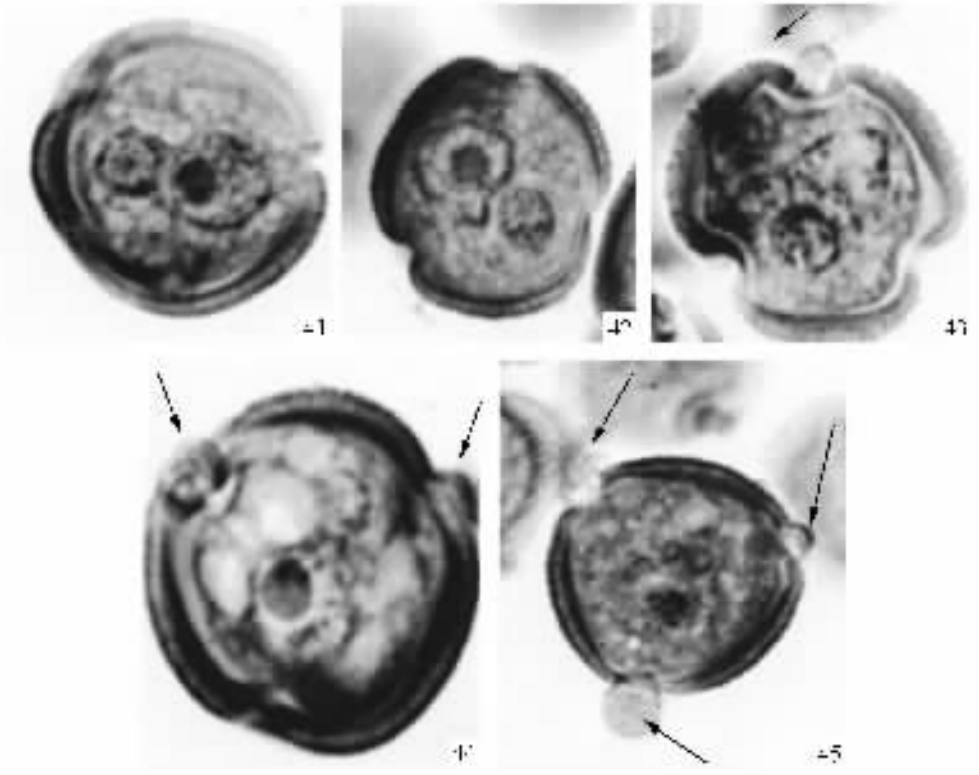

Plate Ⅳ 41. The generative cell aggrandizing and moving central ×1 250 ;42. The mature 2-celled pollen ×1 250 ;43. One aperture bourgeoning (↑) ×1 250 ;44. Two apertures bourgeoning (↑) ×1 250 ;45. Three apertures bourgeoning (↑) ×1 250
EP. Epidermis EN. Endothelium M. Middle layer PL. "Placentoid" originating from the tapetum cells PP. Primary parietal cell PS. Primary sporogenous cell T. Tapetum TR. "Trabeculae" from the tapetum TT. Tetrahedral microspore tetrad

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