獐牙菜小孢子发生及雄配子体发育

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摘 要：用石蜡切片法对獐牙菜小孢子发生及雄配子体发育过程进行首次观察研究。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层细胞有丝分裂凸入药室中央并原位退化形成的；中层细胞 2 层；药室内壁同表皮同时宿存，细胞柱状伸长，纤维状加厚。小孢子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，少数为左右对称形和十字交叉形；成熟花粉多为 2-细胞类型，偶见 3-细胞型，具三萌发孔。

关键词：獐牙菜；小孢子；雄配子体

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Microsporogenesis and the development of male gametophyte in *Swertia bimaculata*

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Abstract: Microsporogenesis and the development of male gametophyte in *Swertia bimaculata* were studied by the method of paraffin section for the first time in the present paper. The main results can be concluded as follows: Anthers are tetrasporangiate, the development of anther walls conforms to the basic type and comprises of epidermis, endothecium, two middle layers and tapetum at mature stage. The tapetum has dual origin and belongs 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. Two middle layers are ephemeral; endothecium and epidermis persists and develops to become fibrous-thickening. The cytokinesis of the microspore mother cell 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. Pollen grain is mainly 2-celled type when shed, occasionally 3-celled type, and it has three apertures.

Key words: *Swertia bimaculata*; microspore; male gametophyte

Swertia bimaculata, also named Da Ku-Cao, Hei Jie-Ku-Cao, Zou Dan-Cao in Chinese, is an annual herbage that belongs to Ser. *Macultae* T. N. Ho et S. W. Liu of sect. *Ophelia* of *Swertia* (Gentianaceae). It distributes mainly in Hunan, Yunnan, Guizhou, Tibet, Hubei and Sichuan (Delectis Florae Reipublicae Popularis Sinicae, Agendae Academiae Sinicae Edita, 1988). Most of species in *Swertia* are used as traditional medicine in China, Japan, India, and Nepal with a long history, for they are bitter and cold in property, and have the effect of heat-clearing and detoxifying, cholagogic and strengthening, killing insect. The whole plant can

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be used as medicine in curing digestive system diseases such as hepatitis, cholecystitis, gastropathy and so on. In recent years, it's found that there are some species of this genus showing high bioactivity in anti-virus, decreasing blood glucose and relieving gastric ulcer, curing hepatitis and alopecia, thus, more and more people pay attention to these species in Gentianaceae for the great medical value they have(Chen *et al.*, 1998). *S. bimaculata* is widely used in curing jaundice hepatitis, pneumonia, tonsillitis, gynecologic inflammation in civilian of Hunan. However, the wild resources decrease greatly owing to coyoting and habitat destruction. There is few research of basal biology in *S. bimaculata*, excepting its seed germination (Long & Huang, 2008), and the study on the aspect of generative biology is blank. In order to protect the wild resources and achieve the sustainable use of resources, we give the report on the microsporogenesis and the development of male gametophyte of *S. bimaculata* and accumulate embryological data of *Swertia* in order to offer evidence for systematic taxonomy and protectiong biology. Furthermore, it will provide basic data that is beneficial for introduction and artificial cultivation of this genus.

1 Materials and Methods

Material for the present study was collected from Wanba@shan Forestry Centre of Longshan County of Xiangxi Tujia and Miao Autonomous, Hunan Province, China(109°38′ E, 29°46′ N, Alt: 1396.2±11 m). The voucher(Long hua177)is deposited in the Herbarium of Biological resources and Environmental Science Department of Jishou University and identified by Zhang Dai-gui of Jishou University.

Flower buds and flowers at different development stages were collected and fixed in the modified FAA (50% alcohol : glacial acetic acid : formaldehyde=89 : 6 : 5). These materials were washed with water and then stained in Ehrfich's hematoxyfin together. After that, they were dehydrated in ten grades of alcohol, transparentized with five grades of xylene and embedded in paraffin. These materials were serially sectioned by microtome(Microm) at the thickness of 5—8μm. Sections were mounted on slides in Neutral balsam, observed and photographed with microscope(Leica DM2000).

2 Observation and Results

S. bimaculata is a pentamerous flower (Plate I: 1). In the early phase of budding, perianth and staminate primordium were formed from flower primordium in sequence. The basal part and terminal part of staminate primordium are differentiated to form filaments and anthers separately. Each anther has four microsporangium, and at the early stage of anthers, the surface of anther is composed of a layer of flat epidermal cells, inside which there are a mass of meristematic cells with similar morpha. Thereafter, the anther become quadripartite , for the tissue of four patches differentiate faster. Archesporial cells differentiate below the epidermis in each patch, having larger size and denser cytoplasm(Plate I: 2). These cells divide periclinally to produce primary parietal cells outward and primary sporogenous cells inward(Plate I: 3).

2.1 The formation and differentiation of the anther wall

Two layers(outer layer and inner layer) were differentiated in the periclinal division of primary parietal cells(Plate I: 4), forming three layers together with epidermis(Plate I: 5). Whereafter, through the division of the two layers, anther wall has fully differentiated into five layers(Plate I: 6, 7, 8) when secondary sporogenous cells appear (Plate I: 9): epidermis, endothecium, two middle layers and one tapetum. It indicates that both of the outer and inner layers derive from primary parietal cells, and through further division they differentiate into endothecium and middle layer, and middle layer and tapetum respectively. The middle layer is make up of the cells from the outer and inner layers. According to Davis (1966)'s compartmentalization, the development of anther wall conforms to the basic type.

Tapetum is the innermost layer in anther wall, and it is mainly formed from the inner primary parietal cells and partly from the connective cells. Tapetum cells are formed at phase of early sporogenous cells, during the process of microspore mother cell development, its cells

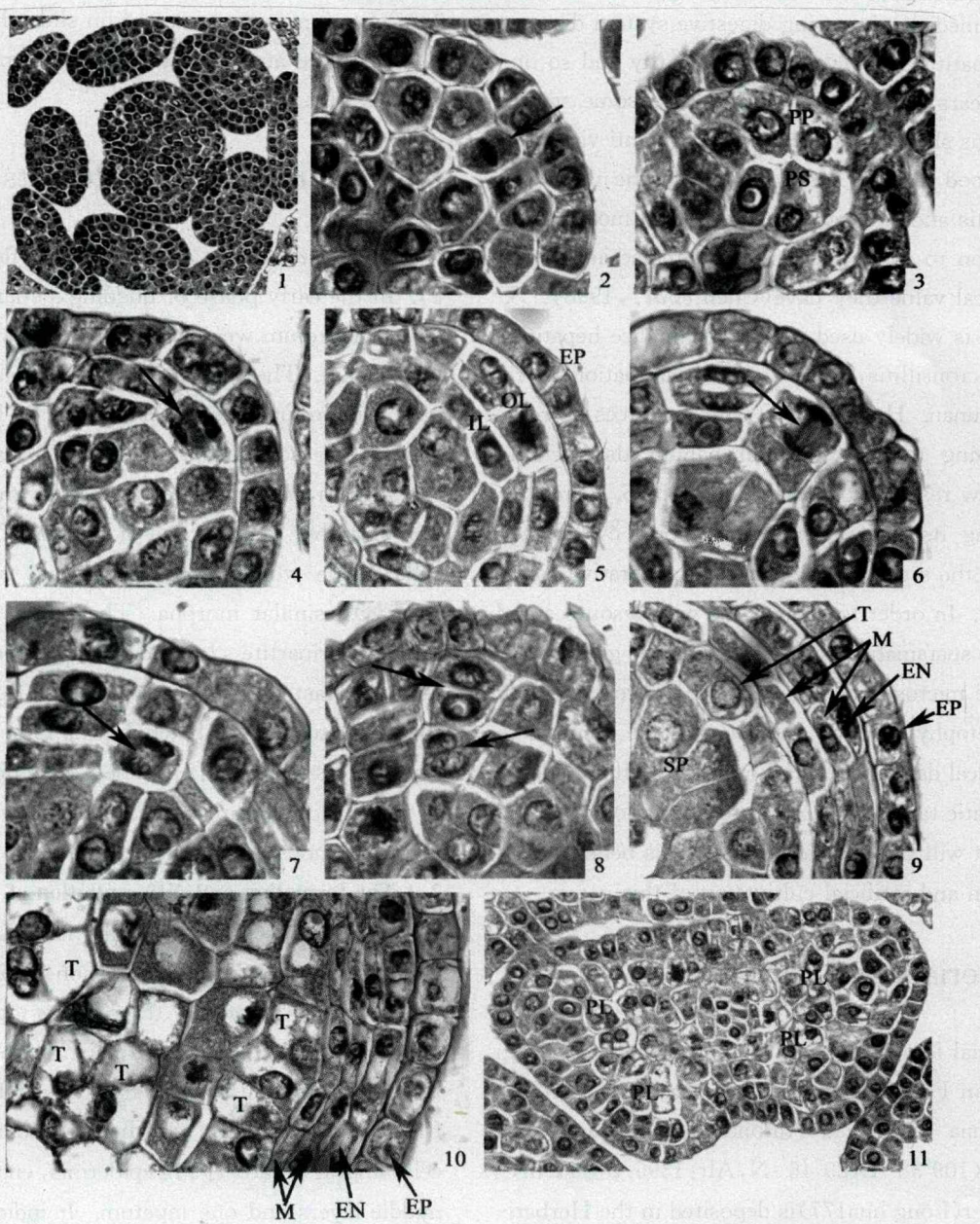

Plate I **1.** Transection of a young anther, showing five anthers×200; **2.** Male archesporium(↑)×1000; **3.** Microspore archesporial cells divide periclinally to form primary sporogenous cells(PS) and primary parietal cells(PP)×1000; **4.** Primary parietal cell dividing(↑)×1000; **5.** Outer and the inner layers from primary parietal cell dividing×1000; **6.** Outer layer of the primary wall dividing(↑)×1000; **7.** Inner layer of the primary wall dividing(↑)×1000; **8.** Outer and inner layer of the primary wall dividing(↑)all×1000; **9.** Five layers anther wall during the period of secondary sporoginous cell×1000; **10.** Tapetum cells during microspore mother cells(T)×1000; **11.** "Trabeculae" and "Placentoid" from the tapetum×400. **ANT.** Antipodal cells; **EP.** Epidermis; **EN.** Endothelium; **M.** Middle layer; **PL.** "Placentoid" originating from the tapetum cells; **PP.** Primary parietal cell; **PS.** Primary sporogenous cell; **SS.** Secondary sporogenous cell; **T.** Tapetum.

and cell nuclei augment, protoplasm manifold gradually and undergo endogenetic mitosis and amitosis. The tapetal cells become the most complete development, featured the glandular cell, at phase of microsporocytes. Meanwhile, the size of tapetal cells are similar to microsporocytes but are much larger than those of other layers and present close squareness(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: 11; Plate II: 12, 13). It is observed that the majorities of tapetal cells are uninucleate and the minorities of tapetal cells are binucleate or multinucleate. Tapetal cells start to separate from each

other at the end phase of microsporocytes (Plate II: 14), degenerate obviously and disaggregate intensively at their original site in the time of tetrads. The degenerated nuclei of tapetal cells in the anther chamber are formed by the degenerated layer cells which enter it at early time (Plate II: 15) but not formed by circumference tapetal cells which enter it after degeneration. It is not found that amalgamation occurs and periplasmodium appear when tapetal cells disaggregate from large numbers of sections. The tapetal cells are single till disappearing. At intermediate phase of uninucleate microspore, the tapetal cells disappear completely (Plate II: 16). Therefore, the tapetum is similar to the Glandular type.

The middle layers accomplishes differentiation during secondary sporogenous cells, it included two layers of cells with smaller size, bigger nucleus and shaped in flat rectangle (Plate I: 9). When the meiophase I of microsporocytes is over, the middle layers cells degenerate quickly and left one layer only (Plate II: 14), then, it gets degenerated completely at the early stage of the formation of 2-celled pollen grain.

The endothecium is formed at the stage of secondary sporogenous cells while cells arrange close and present strip shape (Plate I: 9). The endothecial cells are most developed when microsporocytes are formed (Plate I: 10). After that, the endothecium does not develop any more and is pulled as flat and long with the expansion of anther (Plate II: 14, 15). At the early stage of the formation of 2-celled pollen grain, the endothecial cells nearby the connective tissue become vacuolization to present rectangle shape while there is only sere vestige left outside till shedding (Plate II: 17).

The epidermis is formed the earliest and only includes single-layer cells that arrange closely. What's more, the exterior of epidermal cells often possess horny layer which plays a role in protection. Before long, the epidermal cells augment, the radial wall and tangential wall elongate to present in the shape of quadrangle or rectangle (Plate I: 10). At time of tetrads, the epidermal cells reach the highest level of development and become fibrous-thickening at the inside tangential wall (Plate II: 15). At shedding, the connective tissue between the two anthers chambers at the same side disappears, resulting in the connectivity of two anther chambers (Plate II: 18). It is observed that the epidermal cells in the joint of two anther chambers are not thickened and become the focus of force which is the place where anther wall dehisce and the mature pollens emit.

2. 2 Microsporogenesis

The primary sporogenous cells derived from archesporial cells undergo mitosis resulting in secondary sporogenous cells. Comparing with those surrounding wall cells, the secondary sporogenous cells have larger size, higher nuclear-cytoplasmic ratio, dense cytoplasm, and deeper color. Moreover, they arrange closely, present polygon but don't have obvious vacuoles (Plate II: 9). The polygonal secondary sporogenous cells turn into circular microspore mother cells with development (Plate II: 19, 20).

Microsporogenesis of *S. bimaculata* is derived from the meiotic divisions of microsporocytes like other angiosperms. In the process of meiosis, the structure of chromosome has a series of variations as other plants and the meiosis is not synchronized. The process is described in detail as follows: Prophase I (Plate II: 20): According to numerous observations, prophase I can be observed in different period of materials, indicating this period lasts longer time.

Metaphase I (Plate II: 21): The dumpy chromosomes are arranged on the equatorial plane in the center of microsporocytes with prominent spindle fibers after the prophase I is over.

Anaphase I (Plate II: 22): Chromosomes are separated from each other and move to the two poles. Spindle fibers are observed connecting with the two groups of chromosomes.

Telophase I, prophase II (Plate II: 23): The nuclear membrane appears again and new nucleus is formed gradually after the two groups of chromosomes arrive to the two poles. But no cell wall is formed and a binucleate cell appears without the period of dyad. When the meiophase I is over, cells can not divide for a while and chromosomes do not disappear completely. But in a few minutes, those cells keep on dividing and enter

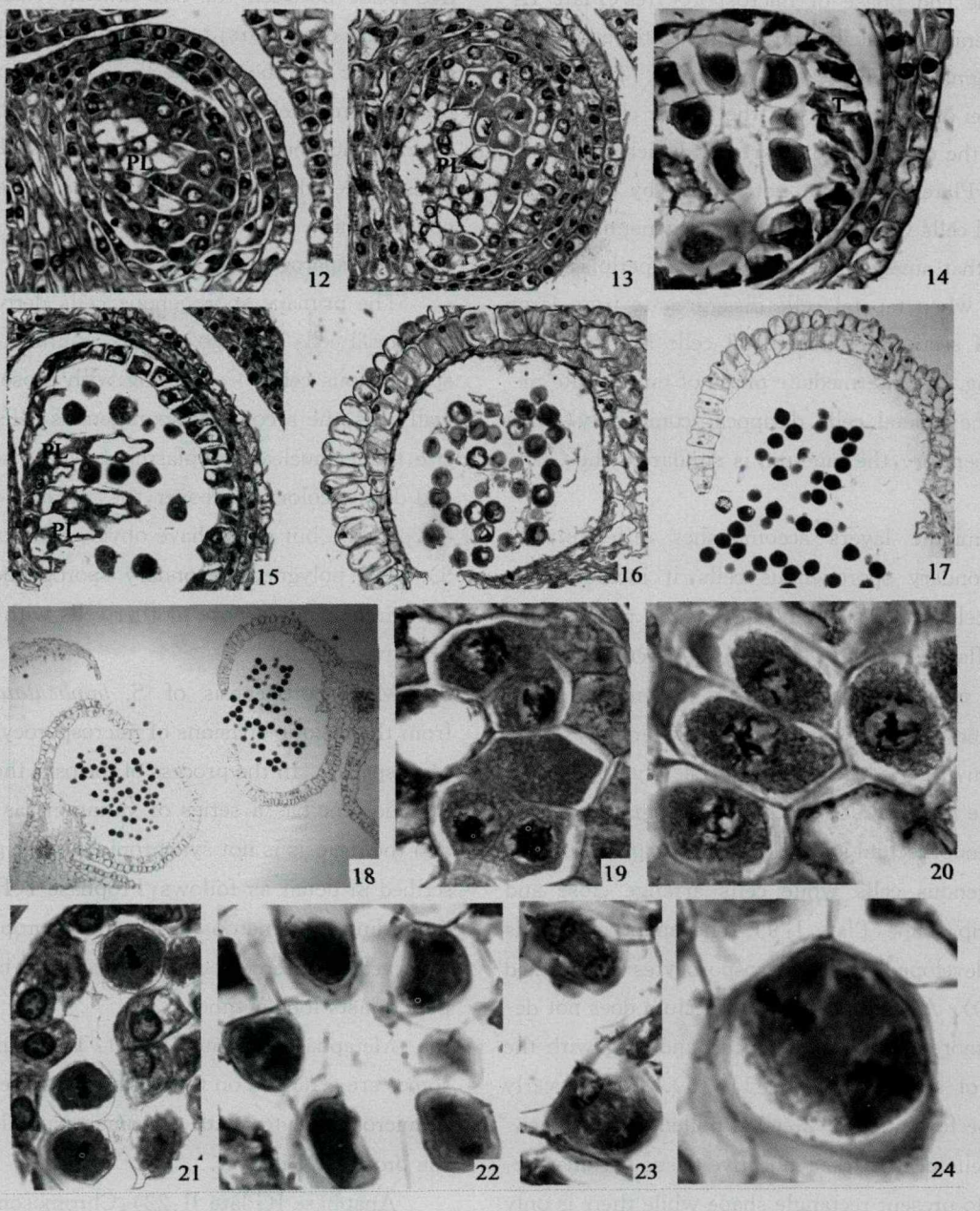

Plate Ⅱ **12,13.** Tapetum cells protruding the anther chamber in the time of microspore mother cells×400. **14.** Ttapetum cells in the stage of microspore miosis×1000; **15.** Degenerating of the tapetum at original sites, noting the remaining nuclei of the tapetum in the middle of the anther chamber ×400; **16.** Anther wall in the stage of uninucleate microspore×400; **17.** Anther wall during the period of pollinating×200; **18.** Anther wall in the pollinating period×100; **19,20.** Microspore mother cells×1000; **21.** Microsporocyte at metaphase Ⅰ of meiosis×1000; **22.** Microsporocyte at anaphase Ⅰ of meiosis×1000; **23.** Microsporocyte at telophase Ⅰ of meiosis×1000; **24.** Microsporocyte at metaphase Ⅱ of meiosis×1000. **PL.** "Placentoid" originating from the tapetum cells; **T.** Tapetum.

into prophase II. In the short period, the nuclear membrane is obvious and some thick chromosomes twist to a mass.

Metaphase II (Plate II: 24): Nuclear membrane disappears; Stumpy chromosomes are arranged in the center of cells; spindle fibers are obvious in the center of cells but not obvious in the pole of cells.

Anaphase II (Plate III: 25): Each group of chromosomes in the two daughter nuclei separates from each other and moves to the two poles. Spindle fibers are observed connecting with each two groups of chromosomes, presenting four umbrellas in shape.

Telophase II (Plate III: 26): Chromosomes disappear gradually after they get to the two poles. Fur-

thermore, nucleolus and nuclear membrane appear; four new nuclei circular are formed; these nuclei are separated from each other by the phragmoplast which appears among them and produce the cell walls, thus four new cells are formed.

The cytokinesis of the microspore mother cell in meiosis is of the simultaneous type according to plates of the meiosis process. Those tetrads are surrounded together by the callose. What's more, there is also callose among the microspores in the same tetrad. In that case, microsporocytes, tetrads or microspores are existed lonely. The functions of the callose surrounding the tetrads are similar to a "molecular sieve", which allow a great deal of nourishment pass while prevent some macromolecular substances pass, controlling the communications of substances between cells. Most of microspore tetrads are tetrahedral (Plate III: 27) and small part of microspore tetrads are dilateral(Plate III: 28)or isobilateral(Plate III:29).

2.3 Male gametophyte

At the late phase of microspore tetrads, cells separate from each other and the expansion of tetrads made the common callose thinner. Finally, the callosal dissolved; these four microspores separated respectively and were released into anther chamber that permeated with secretion of tapetum. The microspores have synthesized their own cytoderm when they are in common callose. The shape of newly released microspores remains, and the interface and the free-face of it are plane and spherical respectively (Plate III: 30). This period lasts for longer time. Since then, the microspores gradually grow 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 becoming obvious(Plate III:31).

Like most angiosperms, the microspore nucleus of *S. bimaculata* will experience a series of movements. The newly released microspore has a central nucleus and dense protoplasm. After a period of development, many small vacuoles appear in the protoplasm (Plate III:32), they gather to become a large vacuole that locates in the center of the cell, and protoplasm then distributes in circumambience of cytoderm, and nucleus moves randomly from center to one side of the wall (Plate III: 33). Before long, microspore enters mitosis prophase near cytoderm. There are mainly two directions of mitosis: 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 simultaneous. The microspore divides into two nuclei with unequal size. Then the two nuclei undergo cytokinesis to produce a small generative cell which only has a little protoplasm and a large vegetative cell which contains large vacuole and most protoplasm of former microspore. This may be named as the early phase of two-celled pollen.

After a period of development, the combination of pollen tone up, protoplasm continuously increased, 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: 36). Anon, one side of generative cell disengages from pollen wall and extrudes internally. Finally, generative cell is divorced. In this course, the wall of generative cell dissolves gradually, and its nucleus augments ceaselessly. At last, generative cell is free in the protoplasm of vegetative cell. Meanwhile, generative cell shows sphericity and its wall disappears completely (Plate III: 37). Before long, generative nucleus lay aboard vegetative nucleus. Thus, the development of two-celled pollen has completed (Plate III: 38). The generative cell divides to produce two sperms in the pollen tube. However, it's also observed that several generative nuclei of mature pollens divides to form three-celled pollen before shedding(Plate III:39).

For *S. bimaculata*, pollen grains germinated before shedding. Most of pollen grains are uniporous aperture, but it can also be observed biporose or triporate aperture(Plate III:40). However, only one pollen tube can grow up, others stop growing in the midway.

2.4 Male abortion

A lot of male abortions are observed from many sections. Abortion is undertaken in some of those microspores when staminate primordium is just formed

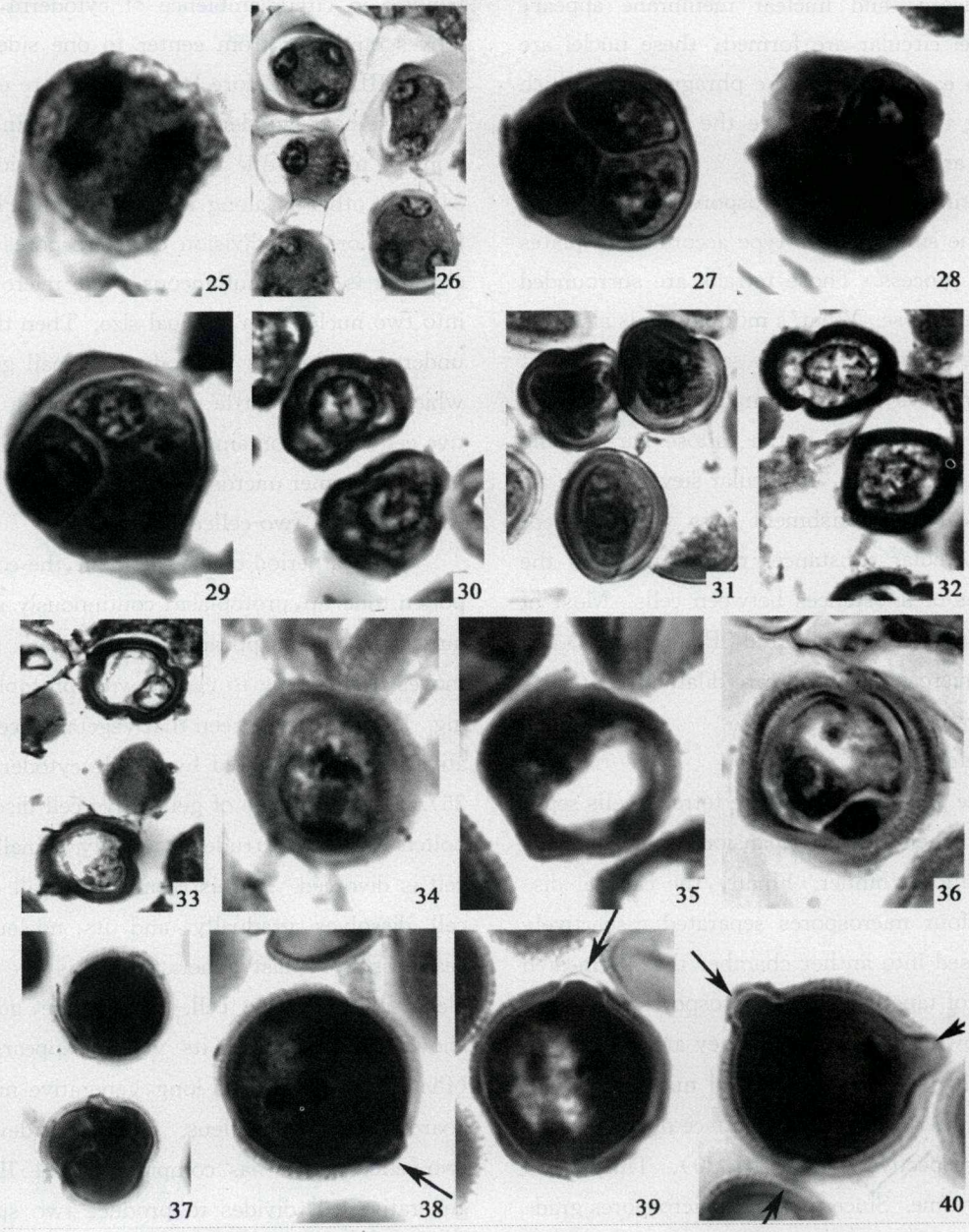

Plate Ⅲ **25.** Microsporocyte at anaphase Ⅱ of meiosis×1000; **26.** Microsporocyte at telophase Ⅱ of meiosis×1000; **27.** Tetrahedral microspore tetrads×1000; **28.** Isobilateral microspore tetrads×1000; **29.** Dilateral microspore tetrads×1000; **30.** Microspore had just formed from microspore tetrads×1000; **31.** Newly released microspore with three apertures×1000; **32.** Some small vacuoles appear, entering the middle of microspore×1000; **33.** Vacuolate period of uninucleate microspore×1000; **34.** Uninucleate microspore was radially dividing×1000; **35.** Uninucleate microspore was breadthwise dividing ×1000; **36.** The early 2-celled pollen, showing obvious cell wall×1000; **37.** Generative cell aggrandizing and moving central×1000; **38.** Mature 2-celled pollen and one aperture bourgeoning(↑)×1000; **39.** Mature 3-celled pollen and one aperture bourgeoning(↑)×1000; **40.** Three apertures bourgeoning(↑)×1000.

(Plate IV:41), while some take place at the phase of microsporocytes(Plate IV: 42, 43). Abortion is also observed in field observations, interestingly, flowers developed on the top of axial shoot and every ramification are normal(Plate IV:44,45), while that under axial shoot and ramification are always abortive(Plate IV: 46-51), this has never been seen in other genera of Gentianaceae.

3 Discussion

In *S. bimaculata*, anthers are tetrasporangiate. The cytokinesis of the microspore mother cell in meiosis is of the simultaneous type. Most of the microspore

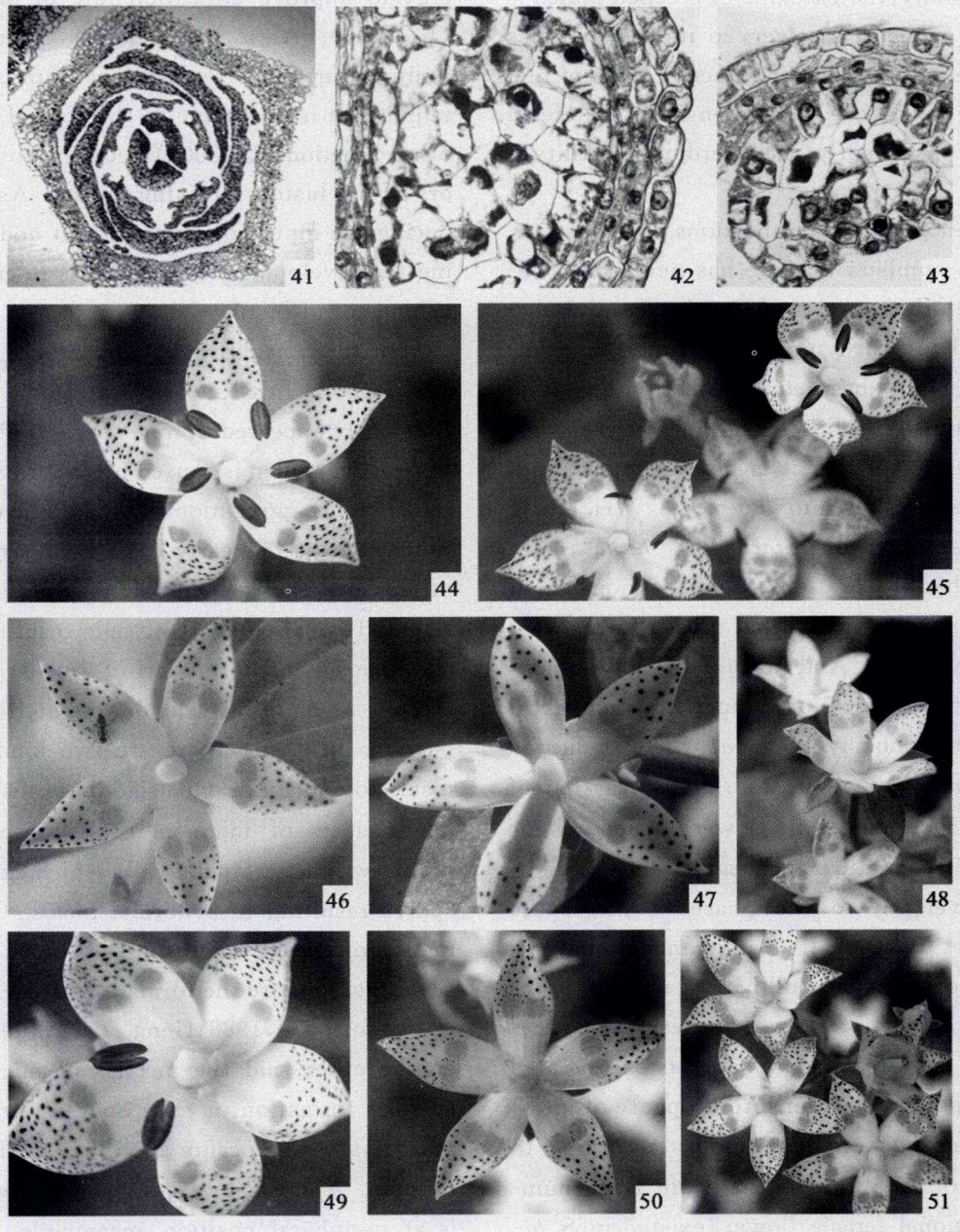

Plate Ⅳ　**41.** Aborted stamen×100; **42,43.** Aborted microsporocyte×1000; **44,45.** Natural flowers; **46-51.** Flowers with aborted stamen.

tetrads are tetrahedral and there are still a few other types, such as isobilateral, dilateral. Pollen grains are mainly 2-celled type when shedding, occasionally 3-celled type. In investigations of more than 2000 angiosperms by Brewbaker, it was observed that pollen grains of 70% of those plants were 2-celled pollen while all of species that develop primarily (Brewbaker, 1967) were 2-celled pollen. Thus, 2-celled pollen was considered as primary characters and 3-celled pollen is derived from 2-celled pollen (Yuan *et al.*, 1991). In *S. bimaculata*, pollen was always 2-celled which indicates that there are some primary characters existed. In comparing with 3-celled pollen, the livingness of 2-celled pollen is obvious stronger. Through comparing with 3-celled pollen, the followings may be the reasons for why the livingness of the 2-celled pollens are stronger than that of 3-celled: ① 3-celled pollen consume more stored nourishments in the course of producing two sperms during the last mi-

tosis;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.

The phenomenon that abortions are undertaken in a large numbers of male has never been observed in the other species of Gentianaceae. But it was reported in Stachyuraceae by some authors. Tang *et al.* (1983) observed that there are at least six species of bisexual flowers in Stachyuraceae had no pollen. It is said that it is bisexual flower but actually differentiated into dioecious. Wei *et al.* (2001) investigated on *Stachyurus himalaicus*, through further observations and discussions about the phenomenon, he proved that those flowers were functional unisexual. And he called them "pseudo-hermaphrodite flower" or "functional unisexual flower". Synchronously, Wei *et al.* (2002) indicated that development of ovule was later than that of staminate in functional male flower. When pollens in the anther are mature and begin to shed, the ovule has developed to the phase of megaspore mother cells. As a result, ovule can not undergo further development and pollinate, it will disappear with the abscission of flower. Whereas, in functional female flower, the staminate has degenerated when microsporocytes enter into prophase of meiosis and can not go on developing. According to field observations, although there are large numbers of "functional female flower" existed in *S. bimaculata* but the evidence that could prove those flowers are differentiated into dioecism is not enough. The phenomenon can be explained by redundancy theory of ecology, it is said that each organ of plant is composed of many members that perform the same function, that means composition of organs are redundant, such as some vegetative organs, stem, leaf and root, as well as those generative organs, stamina, pistil and flowers. Cutting down redundance appropriately can help plant distribute and use those limited nourishment and energy to improve utilization rate of limited energy (Li, 2006). As to living beings, for resources are limited, they increase functional inputs on one certain aspect is inevitable at the cost of reducing the other functions (Li, 2006) according to strategy theory of life history of living beings. As a result, reducing the inputs on nourishment and energy during the development of stamina is to ensure the development of pistil.

In 1984, Reghuvanshi and Singh (Johri *et al.*, 1992) reported that in *Capsicum*, male abortion was frequently appeared in nine species. The formation of abortive pollen is caused by abnormal function of tapetum, disaggregation of middle layer, degeneration at a relatively early stage or abnormal augment of tapetum, enlargement of cell nucleus, vacuolization and radial elongate of cells resulting in abnormal meiosis and formation of 2-or 4- nucleate microspores. At last, nuclei degenerate resulting in abortion. In China, there are two kinds of mechanisms to explain it. One said it is caused by abnormal meiosis of microsporocytes, because of the highly vaculated tapetal cells which extrude microspores resulting in abnormal meiosis and the formation of natural microspores tetrads. Thus abortion occurs before the formation of microspores tetrads (Wu *et al.*, 1988; Geng *et al.*, 1994). However, the other said the meiosis of microsporocytes and the formation of microspores tetrads were all normal, abortion occurs at the phase of male gametophyte. Abnormal variation begins when nucleus is at peripheral position, meanwhile, tapetal cells take radial enlargement abnormally and extrude pollen grains which make microspores are disaggregated before forming 2-celled pollen resulting in abortion (Wang *et al.*, 2004). According to observations from sections, we agree with the former, via. it is because of abnormal function of tapetum, disaggregation of middle layers, degeneration at a relatively early stage or abnormal augment of tapetum, enlargement of cell nucleus, highly vacuolization and radial elongation of cells.

It is observed that there are always some

dichophysis that are similar to microspores archesporial cells on morpha in microsporangium from investigations on microsporogenesis of Gentianaceae, Scrophulariaceae, Labiatae, Bignoniaceae and Araliaceae, and the origin, evolvement, type of those dichophysis are in debate. Guerin(1926) considered that those dichophysis were derived from archesporial cells according to the observations that dichophysis in microsporangium of Gentianaceae separated the anther into many small lumina or accumulated to a mass. Ralph(1949) also found large numbers of dichophysis in some species of *Swertia* L., and considered that those dichophysis were derived from parenchyma cells of connective and similared to primary parietal cells on function and origin. Steffen *et al.* (1958) reported the dichophysis in *Gentiana* L. again and supported the former. Steffen *et al.* (1958) separated it into two kinds according to morpha: (1) Placentoide which accumulates in angle of anther wall is similar to placenta; (2) Trabeculae which separates the anther chamber into many small lumina is in the center of sporangium and cells elongate longitudinally. Eames(1961) believed that the dichophysis was not derived from archesporial cells, and it needed further observations and confirmations. Bhojwani(1979) and Echilin(1971) indicated that, either inferred from origin or function, the structure was of the characteristic of tapetum. It was considered as sporogenous tissue in the past possibly because tapetal cells and sporogenous cells were similar on morpha at the early stage observed under optical microscope. Rao *et al.* (1983) reported that the structure was derived from sporogenous tissue in Cansora. Zhu *et al.* (1989) and Li *et al.* (1994) reported that in *Gentiana* L. separately and considered it was derived from primary parietal cells and parenchyma cells of connective. We are trying to explain the phenomenon as follows according to early reports and our observations on movements of microspores and tapetum. Firstly, the dichophysis in microsporangium is of characteristic of tapetum without doubting and may be of dual origin which is derived from primary parietal cells and connective tissues. Secondly, both of the tapetal cells that from primary parietal cells and connective tissues undergo abnormal divisions to form Placentoide and Trabeculae. There is only placentoide in *Swertia* L. (Ralph, 1949), while in *Gentiana* L. (Guerin, 1958; Steffen *et al.*, 1958; Zhu *et al.*, 1989; Li *et al.*, 1994) and *Gentianopsis* Ma. (Liu *et al.*, 1997), both types are existed. As to the type of the tapetum discussed above, the previous authors (Guerin, 1926; Ralph, 1949; Steffen *et al.*, 1958) had not discussed. Zhu *et al.* (1989) considered that was of glandular type, while Rao *et al.* (1983) and Li *et al.* (1994) thought that was of amoeboid type. Our observations indicates that the type we discussed is different from typical amoeboid type, for that cell wall of tapetum is destroyed earlier resulting in protoplasmic mass intruding into the anther chamber to form protoplasm surrounding the microspores. It is also different from typical glandular type causing that the cells of tapetum differentiate only to the inner wall of anther but do not intrude into the anther chamber to form placentoide or trabeculae. However, according to the process of degeneration of tapetal cells in *S. bimaculata*, all of those cells degenerate at their original site and protoplasm do not move. Thus the tapetum is more close to glandular type. The structure, function and degeneration of the tapetum need further investigations. Especially, the observations under electron microscope can helpful not only to the understanding of the origin, differentiation and type of the tapetum, but also to the acknowledgement of development and function of tapetum in reproductive biology of angiosperm.

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用，且对其不定芽质量也有一定的促进作用；但是长期使用6-BA会产生玻璃化现象。低浓度的NAA适宜于白花天目地黄不定芽的生根。

本试验成功建立白花天目地黄愈伤组织再生体系，为其在庭院观赏花卉绿化应用提供了可能性；同时在保存地黄属优良种质资源和改良地黄药材品质等方面具有重大意义。

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