Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Development of megaspores, microspores, and male and female gametophytes in Daphne tangutica Maxim an economically valuable shrub

Xinyao Kou
HeXi University
Fang Yan
xt_34@163.com

HeXi University
Ziwen Zheng
Zushima Original Plant Application Technology Research Institute, Hexi University,
Chaoqiang Zhang
Gansu Taikang Pharmaceutical Company Limited
Haiping Wang
Gansu Taikang Pharmaceutical Company Limited
LiTing Ma
HeXi University
Jingwei Bao
HeXi University
Tao Xu
HeXi University
Weijun Zhao
Gansu Province Qilian Mountains Water Conservation Forest Research Institute

Short Report

Keywords: Daphne tangutica Maxim, Embryology, Microspore, Polygonum-type embryo sac, Tapetal remnants, Pollen abortion, Asynchronous development

Posted Date: February 2nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8639277/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Daphne tangutica Maxim is a perennial evergreen shrub with significant ornamental and economic value, but its low fruit set rate restricts its development and utilization. To improve breeding and conservation strategies, there is an urgent need to clarify its gametophyte development process and the causes of abortion. Using wild populations as materials, paraffin sectioning and microscopic observation were employed to systematically conduct cytological observations and descriptions of megasporogenesis and microsporogenesis, male gametophyte development, the type of anther wall development, and embryo sac formation, among others. The microspores of *D. tangutica* are tetrahedral in shape, and mature pollen is mainly of the bicellular type; the anther wall shows basic-type development. The megaspore develops into a Polygonum-type and ultimately forms a Polygonum-type embryo sac, indicating that the female gametophyte functions are basically intact. During the late stage of pollen development, residual material appears in the tapetum (forming continuous or semi-continuous band-like structures along the inner wall of the anther), accompanied by partial anther abortion and the production of non-viable pollen, resulting in a reduced number of fertile pollen grains. Environmental stresses at high altitude, such as low temperatures, strong winds, a short growing season, and restricted pollinator activity, may exacerbate the occurrence of developmental asynchrony and pollen abortion. This study systematically elucidates for the first time the developmental processes of the megaspores and male and female gametophytes of *D. tangutica*, revealing that pollen abortion associated with asynchronous male development and delayed tapetum degradation is one of the main factors leading to low fruit set, whereas the female gametophyte is generally normal. The research findings provide a theoretical basis for embryological studies of *Thymelaeaceae* plants and offer support for determining appropriate pollination timing, improving propagation and breeding schemes, and further investigating the mechanisms of abortion.

1.Introduction

Daphne tangutica Maxim is a perennial, evergreen small shrub belonging to the genus Daphne Linn. of the family Thymelaeaceae. The plant is 60–100 centimeters tall, with clustered, disorderly branches. Leaves are simple, alternate, lanceolate, with revolute margins. Inflorescences emerge with the new leaves; the flowers are bisexual. Its floral morphology is distinctive and brightly colored: the corolla margin is often pale purple, while the inner side is usually white. The drupes are typically red at maturity, ripening between July and August[1]. This species is mainly distributed in Shaanxi, Gansu, Qinghai and the Tibet Autonomous Region of China, occurring at forest edges, mountainous areas, valleys and shrublands at altitudes of about 3,000 meters[2]. As a rare evergreen shrub of the cold, sparsely vegetated belt in northwest China, the yellow-flowered daphne plays a key role in local soil conservation and water resource retention[3]. *D. tangutica* contains a variety of chemical constituents, such as coumarin derivatives[5,6], lignans, flavonoids[7,8] and diterpene and triterpene esters[9–11], and exhibits pharmacological activities including anti-inflammatory, antibacterial, antimalarial, antihypertensive, sedative and hypnotic effects[11].

Gametophyte formation is a fundamental prerequisite for sexual differentiation and has been extensively characterized in numerous plant species. Sexual flower development is often accompanied by selective abortion of reproductive organs. For instance, in Chloroluma gonocarpa (*Sapotaceae*), staminate flowers are unisexual due to underdeveloped stigma/style and ovule abortion following meiosis[12]. In G*inkgo biloba*, pollination suppresses programmed cell death (PCD) in the nucellus, promoting proper megagametophyte development[13]. Detailed investigations of megasporogenesis have been conducted in Liparis elliptica[14], while studies on *Epidendrum denticulatum* and *Epidendrum orchidiflorum* revealed extensive embryo degeneration, likely attributed to late-acting genetic incompatibilities[15]. In Meliaceae, staminate flowers of female plants produce inviable pollen grains due to tapetal necrosis, resulting in coalescence of pollen into an amorphous mass within anthers[16]. Similarly, *megasporogenesis* and *microsporogenesis* abnormalities have been documented in *Pilostyles berteroi*. However, the developmental timing of floral organ degeneration in *D. tangutica* remains poorly characterized and warrants further investigation.

Understanding the regulation of flower development in *D. tangutica* has significant implications for germplasm innovation and horticultural advancement. Therefore, this study aimed to: (1) systematically characterize the morphological development of floral organs, and (2) investigate male and female gametophyte development to identify the critical period(s) of reproductive organ degeneration. Through comprehensive analysis of megaspore, microspore, and gametophyte development, this work contributes to enhanced taxonomic understanding of the genus.

2.Materials and Methods

2.1. Plant Material and Study Site

For each developmental stage, at least 25 ovules were randomly collected from different trees and processed. Ovules were fixed in 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS) (10× PBS: 1.3 mM NaCl, 70 mM Na_2HPO_4, 30 mM NaH_2PO_4; pH 7.4) with vacuum infiltration, followed by overnight incubation in fixative at 4°C. Samples were subsequently dehydrated through a graded ethanol series (30%, 50%, 70%, 85%, and 95% in distilled water, 1 h per step), followed by substitution with a graded xylene series (25%, 50%, 75%, and 100%, 1 h per step). Xylene was then gradually replaced with Paraplast Plus (Fisher Scientific), with solvent changes performed daily. Embedded ovules were stored at 4°C until sectioning. Serial sections of 10 μm thickness were cut using a Leica RM2125RT microtome. Sections were deparaffinized and rehydrated, then stained with 1% safranin for 30 min, followed by counterstaining with 0.5% fast green for 2 min. Observations and imaging were performed using a Shenzhen AOSVI T2-3M180 stereo microscope.

2.2. Sampling and Sample Collection

At each analysis stage, a minimum of 25 ovules were randomly collected from different plants for processing. The ovules were fixed using vacuum infiltration in phosphate-buffered saline (PBS)

containing 4% (w/v) paraformaldehyde (10×PBS formulation: 1.3M NaCl, 70 mM Na_2HPO_4, 30mM NaH_2PO_4; pH 7.4), followed by overnight preservation in the fixative at 4°C. The next day, the samples were dehydrated through an ethanol gradient (30%, 50%, 70%, 85%, and 95% ethanol in sterile distilled water, 1 hour per step), followed by ethanol replacement with a xylene gradient (25%, 50%, 75%, and 100%, 1 hour per step). Finally, xylene was gradually replaced with Paraplast Plus (Fisher Scientific) by changing once daily. The embedded ovules were stored at 4°C until processing, and 10-μm-thick sections were cut using a Leica RM2125RT microtome. After dewaxing and rehydration, the slides were stained with 1% safranin for 30 minutes, followed by counterstaining with 0.5% fast green for 2 minutes. Imaging and observation were performed using a Shenzhen AOSVI T2-3M180 stereomicroscope.

3.Results

3.1. External Morphology

The inflorescences of *D. tangutica* are terminal or axillary capitula with short pedicels. The development of the inflorescence occurs simultaneously with the emergence of new leaves and lateral shoots (Fig. 1A-B). Each inflorescence is composed of 9–11 small flowers (Fig. 1C-E). The corolla is cross-shaped and radially symmetrical, typically with four petals. The outer petals are purplish-red, and some petals are light purple (Fig. 1C). The inner side of the petals is white, and the petal blades are lanceolate (Fig. 1D). Basal bracts of the inflorescence axis number 8–15; the basal bracts are pointed and scale-like, hard and resilient, yellow-green, with fine, elongated hairs on the outer surface.

In *D. tangutica*, individual flowers are bisexual, with eight stamens and one pistil. The eight stamens are arranged in two whorls (upper and lower); because the filaments are extremely short, the anthers of the upper whorl are located near the mouth of the corolla tube while those of the lower whorl are positioned in the middle of the corolla tube, and the distance between the upper and lower whorl anthers is 1.430 ± 0.23 mm (Fig. 2A). Due to the extremely short style, the pistil is located at the base of the corolla, beneath the anthers of the lower whorl of stamens; the hairs on the stigma of the pistil are covered with a mucilage that can adhere to the anthers. During the bract-splitting stage, the outer side of the petals turns light purple and the petals open slightly; in the early flowering stage, the inner side of the petals is white and fully expanded, and at this time four yellow anthers of the upper whorl can be observed at the mouth of the corolla tube (Fig. 2B-C). From the early flowering stage to the full bloom stage, the petals change from green to purple; from the full bloom stage to the late flowering stage, the ovary at the base of the corolla tube is green and ovoid (Fig. 2E), and the petals wither and curl, with the color gradually changing from light purple to yellow-green, and finally to light brown (Fig. 1E).

3.2 Development of anther wall

Through observation it was found that *D. tangutica* is monoecious; a single flower has eight stamens, each stamen bearing four microsporangia, and the microsporangia are butterfly-shaped (Fig. 3L), The two microsporangia are connected by a septum. In the flower bud differentiation stage, flower buds

form; at this time a group of cells with a high capacity for division is produced, and the apices of the stamen primordia differentiate to form anther primordia. The outer side of the anther primordium is a single layer of epidermal cells, while the inner side is mainly meristematic tissue. At the four corners on the inner side of the epidermis, sporogenous cells undergo periclinal divisions to form two layers of cells: the outer cells become primary wall cells, peripheral marginal cells, and the inner cells form larger primary sporogenous cells (Fig. 3A-C). After the primary wall cells continue to divide, inner and outer two-tier secondary wall cells are formed (Fig. 3C-D). At this stage the anther wall is composed of one layer of endothecium lining the locule, two middle layers (two cell layers) and one layer of tapetum, respectively (Fig. 3E). According to Davis's criteria for anther wall development in angiosperms, an anther wall with a two-layered middle layer corresponds to the basic type[17].

From the early flowering stage to the full bloom stage, a series of changes occurred in the anther wall; The developmental stages of the anther wall corresponded to the developmental stages of the microspores. During this period, the primary sporogenous cells underwent multiple mitotic divisions to develop into microspore mother cells. The early tapetum cells were uninucleate and arranged in an orderly manner, with dense cytoplasm in the tapetal cells (Fig. 3F-H). By mid-April when the anther wall differentiation was complete, the tapetum cells became enlarged and appeared binucleate or multinucleate. The innermost layer of tapetum cells exhibited irregular arrangement, while the middle layer cells appeared flattened (Fig. 3H). At the beginning of April, during the early stage of anther development, the cells are arranged in an orderly manner. After entering the bicellular pollen stage, the epidermal cells exhibit an irregular, wavy arrangement (Fig. 3H-I). In the full bloom stage, its cuticular protective units (epidermal cells) contract and degenerate, then undergo programmed cell death, disappearing by the time the pollen matures (Fig. 3L). The middle-layer cells initially have a double-layered structure; by the uninucleate microspore marginal stage the outer (external) layer lyses and disappears (Fig. 3I), callose deposition appears in the intercellular spaces, and the inner layer cells enlarge and compress the middle-layer cells (Fig. 3H-J). From the full bloom stage to the end of the late flowering stage., the middle-layer cells disappear (Fig. 3G-K). During the early flowering stage of D. tangutica, the endothecium cells elongate longitudinally (Fig. 3G-I). At the full flowering stage, localized band-like thickenings occur in the cell walls. At this mature stage, only the fibrous layer remains in the anther wall, and the pollen sacs rupture to release pollen grains (Fig. 3J-K). From the early flowering stage to the full bloom stage, during the tetrad to free microspore stage the tapetal cells at the cell walls are clearly visible; as the inner wall of the anther locule thickens and the middle layer cells disintegrate, the well-developed tapetal cells also begin to disintegrate (Fig. 3G). The tapetum is contracted at the tetrad stage; at this time the tapetum cell cytoplasm becomes dense and the number of nuclei increases (Fig. 3H). During the reproductive cell division stage at the early flowering stage of D. tangutica (Fig. 3F-H), the tapetum cell cytoplasm extends into the inner wall of the locule (Fig. 3I). From full bloom to the end of flowering stage, the anthers develop and mature, and their structure undergoes a series of changes to facilitate pollen release. At this stage, the inner wall cells of the locule exhibit pronounced fibrous thickening, forming a fibrous layer that provides the mechanical force for anther dehiscence; the middle layer cells have completely degraded and the tapetum has largely completed degradation, but in

portions of the tapetum that have not detached from the anther wall it has not fully disintegrated and still shows filamentous remnants. Epidermal cells were markedly thickened, and the outer epidermis exhibited irregular protrusions (Fig. 3J). The four microsporangia undergo structural reorganization following degradation of the tapetum: the septal tissue between two adjacent pollen sacs on each side gradually differentiates, causing them to fuse into two larger pollen sacs (Fig. 3K). Subsequently, the pollen sac splits open at the middle, releasing mature pollen. As the anther ages further, its opening continues to enlarge to ensure the pollen is released smoothly (Fig. 3L).

3.3. Microspore Formation and Development

Microspore mother cells originate from the spore-forming cells of the sporogenous tissue; At the bract-splitting stage, the spore-forming cells within the sporogenous tissue differentiate to produce primary sporogenous cells (Fig. 4A-B); These primary sporogenous cells subsequently divided and differentiated to form microspore mother cells (Fig. 4C). The microspore mother cells were characterized by relatively large nuclei, densely distributed organelles, and normal cellular structure.

When the microspore mother cells enter meiosis, the nuclei of the adjacent tapetum layer become polyploid. The microspore mother cells complete the separation of homologous chromosomes during meiosis I, forming binucleate cells (Fig. 4B, D), and then rapidly enter meiosis II, during which sister chromatids separate (Fig. 4E-F). During meiosis II, the chromosomes move toward the poles along the spindle fibers (Fig. 4G).As the nucleus divides, cytokinesis occurs simultaneously in the cytoplasm, partitioning it into four haploid cells and thus forming a tetrad of microspores (Fig. 4G-H). From the early flowering stage to the full bloom stage, microspores are released from the tetrads and develop into male gametophytes.

Microspore mother cells formed from sporogenous cells through multiple mitotic divisions; the early tapetal cells are uninucleate with dense cytoplasm. The longitudinal view of the tapetum from the microspore tetrad to the free microspore stage is clearly observed (Fig. 5A) and corroborated by (Fig. 3F-H): The developed tapetum cells begin to disintegrate. After the tetrads release microspores, the tapetum becomes multinucleate with dense cytoplasm; tapetum cells gradually vacuolate and form large vacuoles, and the inner tangential walls of the tapetum begin to dissolve. At the mature anther stage, the anther wall is reduced to only the fibrously thickened inner wall of the locule and residual epidermal cells; the tapetum and middle layer cells are completely disintegrated, and the epidermal cells show no obvious thickening (Fig. 5F-G). Epidermal cells are arranged neatly at the early stage; during the bicellular pollen stage the epidermal cells are arranged in a wavy, irregular pattern, then gradually shrink and degenerate, disappearing when the pollen matures (Fig. 3A-L). Observation of Fig. 5C indicates that some microspores are at different stages during development.

3.4. Development of Male Gametophytes

From the full bloom stage to the late flowering stage, after microspore formation the callose wall dissolves and microspores separate from the tetrads to produce free uninucleate microspores. At this

time the microspore cells just separated from the tetrads increase in volume, causing uneven thinning of the cell wall; the position of the nucleus within the microspore cell changes and the nucleus enlarges (Fig. 3I). Subsequently, due to peripheral depletion and increased internal pressure causing cytoplasm accumulation and formation of a large central vacuole, the nucleus in the microspore moved with the cytoplasm from the center to one side of the cell (Fig. 6A), and the nucleus was squeezed toward the pollen side (Fig. 3K). The nucleus closer to the vacuole is the vegetative nucleus, while the one near the pollen wall is the generative nucleus (Fig. 3I). The larger vegetative cell with thick cell wall and dense cytoplasm undergoes cytokinesis, while the smaller generative cell is adjacent to the vegetative cell (Fig. 6A). The vesiculated tapetum begins to dissolve, with tapetal cell cytoplasm gradually being released into the anther locule (Fig. 6B). At this stage, some pollen grains mature into abnormal pollen with internal blank spaces (Fig. 3J) or exhibit obvious deformities leading to pollen abortion (Fig. 3K).

The callose wall of the microspore tetrad begins to dissolve, and the tetrad microspores gradually separate, forming free uninucleate microspores (Fig. 7A). Early uninucleate microspores are enlarged and have dense cytoplasm (Fig. 7B); the nucleus is centrally located and relatively large. Subsequently, the cytoplasm vacuolates and continuously acquires nutrients from the tapetal cells, gradually forming a large central vacuole; the nucleus is pushed to one side of the pollen grain, forming the uninucleate peripheral stage, and microspore divisions are asynchronous within the same anther (Fig. 7B-C). After the cytoplasmic filling of the uninucleate pollen grain is complete, a mitotic division follows, forming two nuclei (Fig. 7E-F); the nucleus near the central vacuole is the vegetative nucleus, and the one adjacent to the pollen wall is the generative nucleus. At this stage the nuclei of the two cells are distinct, the vegetative cell is larger in volume, and the generative cell detaches from the wall and is free within the vegetative cell. At this time the cytoplasm was sparse (Fig. 6A), the region containing the reproductive cells was located at the end away from the germ pore, and the pollen was nearly spherical. Mature pollen showed three germ pores, thick cell walls, and dense cytoplasm; some pollen had sparse cytoplasm, lacked inclusions, and had lost viability (Fig. 6B).

3.5. Megaspore Formation

D. tangutica is a plant with bisexual flowers; it has one carpel, a unilocular ovary, a single ovule, and the ovule is attached to the base of the ovary chamber. The ovule primordia at the base of the locule develop into nucellar tissue, and sporogenous cells appear beneath the nucellar epidermis. Compared with surrounding cells, the sporogenous cells have dense cytoplasm, a conspicuous nucleus, and a large cell size (Fig. 8A). Peripheral cells and sporogenous cells arise from the periclinal divisions of archesporial cells, and the megaspore mother cell is further formed from the sporogenous cells (Fig. 8B). Inner and outer integuments gradually form around the nucellar tissue (Fig. 8C). Proliferation of peripheral cells causes the megaspore mother cell to separate from the nucellar epidermis, resulting in the formation of a thick-nucellus ovule (Fig. 8C-D). After meiosis, a tetrad of megaspores appears (Fig. 8E); the megaspores increase in cell volume through differentiation, at which time the nuclei are easily observed (Fig. 8F). The non-degenerated megaspore moves to the micropylar end to form a functional megaspore (Fig. 8G), which then develops into a mononucleate embryo sac (Fig. 8H).

3.6. Female Gametophyte Development

From the early flowering stage to the late flowering stage, the uninucleate embryo sac undergoes three mitotic divisions to become a 2-nucleate embryo sac (Fig. 9A), a 4-nucleate embryo sac (Fig. 9C), and an 8-nucleate embryo sac (Fig. 9D). In the two-nucleate embryo sac, the sac is slightly centrally located (Fig. 9B); in the four-nucleate embryo sac, the embryo sac cavity is enlarged (Fig. 9C). During the mitotic transition from a 4-nucleate embryo sac to an 8-nucleate embryo sac, cellularization was completed rapidly; 7-celled, 8-nucleate mature embryo sacs were observed on serial sections of the same material (Fig. 9D-F), *D. tangutica* forms a mature Polygonum-type embryo sac structure, i.e., the female gametophyte is functionally complete.

3.7 Timeline of reproductive organ development in *D. tangutica*

Based on the flowering period and the developmental stages of the reproductive organs of *D. tangutica*, summarize its developmental timing (Fig. 1). Paraffin sections of male and female reproductive organs at different stages do not represent the development of the pistil and stamen from the same flower; they should be examined and analyzed separately.

From mid-March to early April, during this period *D. tangutica* develops from the floral bud differentiation stage (Fig. 1A1) to the bract-opening stage (Fig. 1B1); the flower buds noticeably enlarge and the outer bracts split. The inner small flowers show signs of emerging; as the weather warms and conditions become suitable for development, the leaves deepen in color to a dark green. This stage takes about 20 days. The anther primordium (Fig. 1A2) develops to form a complete anther wall (Fig. 1B2); the pistil develops from sporogenous cells (Fig. 1A3) into a megaspore mother cell (Fig. 1B3). In mid-April, the external morphology of the flower reaches the early flowering stage (Fig. 1C1), with some of the small flowers in a cluster beginning to open; this stage lasts approximately 4–6 days. The primary sporogenous cell undergoes mitosis to develop into a two-celled pollen grain (Fig. 1C2); meanwhile, the megaspore mother cell begins meiosis (Fig. 1C3). In mid to late April, the external morphology reaches the full bloom stage stage, all the individual flowers are fully open and the petals completely unfolded. This period lasts about 8–10 days. The pollen in the pollen sacs is essentially mature, and four smaller Medicinal septa form two larger Medicinal septa. And the two large pollen sacs showed signs of splitting and releasing pollen; the megaspore mother cells entered the mitotic stage. By late April, the flowers have developed to the late flowering stage. After the anther has finished shedding pollen, it begins to wither and degenerate; meanwhile, the megaspore mother cell has already formed.

4. Discussion

The results showed that the development of *D. tangutica* involves tetrahedral microspores and bicellular pollen; the development of the anther wall is of the basic type, similar to plants such as *Taraxacum officinale* F.H.Wigg. and *Datura stramonium* L.[18–19]. Megasporocyte development is similar to that of

plants such as *Polygonum cuspidatum* and *Oryza sativa* L.[20–21]. We found that within the same flower, in the same locule of a single anther or among different microsporangia of the same anther, the developmental stages of male gametophytes were often at different stages. In studies on medicinal materials such as *Pogostemon cablin* (Blanco) Benth[22], it was found that the microspore mother cells in the anther were in the same meiotic stage, showing perfect synchrony. In studies of *Lilium* spp[23], *Asteraceae*[24], *Pennisetum ciliare* (L.) Link[25], and *Triticum aestivum* L.[26], asynchrony in anther development was found, and the fertilization mode of *D. tangutica* was determined to be facultative outcrossing[27]. Under such harsh and highly variable ecological conditions, the above strategies dispersing reproductive risk across time and increasing the chances of successful fertilization, may constitute a “protective” reproductive measure, that is, increasing the probability of obtaining effective pollination within a single flowering period in unfavorable environments, thereby enhancing the stability of the population’s continued reproduction and renewal[28]. This interesting phenomenon of asynchronous development may be a reproductive strategy mechanism to appropriately prolong the pollination period and promote outcrossing, providing assurance for the successful reproduction of the species, and thus having certain positive significance for its evolution[29–31]. The asynchrony in the development of these anthers may be an adaptive strategy to prolong the pollination period[32–34], but it may also reduce fruit set due to a decline in pollen quality associated with developmental disorders. Structural abnormalities[35–37], resource competition[38–40], hormonal imbalance[41–44], and other mechanisms can directly or indirectly lead to asynchronous microspore development or even abortion. High-altitude shrubs may adapt to environmental stress by reducing the allocation of resources to reproduction, and this resource trade-off exacerbates partial microspore abortion[45]. *D. tangutica* is mainly distributed in western China, growing at an altitude of about 3000 m, and faces environmental stresses such as low temperatures, strong winds, a short growing season, and restricted pollinator activity[46]. Its asynchrony in late pollen development may also be related to the plateau environment[47–48]. Overall, asynchronous anther development may serve as a beneficial reproductive strategy by extending the pollination window and increasing opportunities for outcrossing, but it may also reduce reproductive success due to developmental abnormalities or environmental stress; therefore, interpreting this phenomenon requires considering evidence for both its adaptive and pathological aspects. During plant growth and development, reproduction is a very important part; abortion at any stage will lead to plant sterility/abortion[49,50]. Abortion is generally divided into female abortion[51], male abortion[52], and seed abortion[39,52].

The male gametophyte is very important in sexual reproduction[53], and its normal development ensures successful plant reproduction. Medicinal plant species such as *Pogostemon cablin* (Blanco) Benth.[54] and *Ginkgo biloba* L.[55] have been studied, but no systematic embryological study of *D. tangutica* has been conducted. Male sterility is mainly due to abnormal development of the tapetum[16,36], leading to anther abortion and empty pollen sacs. Tapetum degeneration usually begins during the tetrad stage or the late uninucleate pollen stage and is completely degraded when the pollen matures [57]. In *D. tangutica*,

after pollen maturation, the anther wall still shows residual continuous or semi-continuous band-like tapetum distributed along the inner wall. This phenomenon is inconsistent with the general pattern in angiosperms whereby the tapetum typically completes programmed degradation before pollen maturation. Tapetal degradation may be delayed or impeded, thereby affecting pollen development and maturation and ultimately leading to abortion[58–60]. The tapetum, as the innermost cell layer of the anther wall, plays a key role during pollen development, including providing nutrients, participating in pollen wall formation, and achieving timely extension and delay through programmed cell death (PCD)[59,61]. This interfered with the normal development of the plant. This is consistent with pollen abortion in medicinal plants such as *Salvia miltiorrhiza* Bunge[62] and *Allium sativum* L.[63] caused by delayed degradation or persistence of the tapetum. We linked the developmental status of *D. tangutica* with time and found that its developmental stages clearly correspond to the flower's external morphology. For example, the development of the anthers and ovules corresponds to the external morphology of the flower. These distinct stage characteristics will help us infer the developmental stage of *D. tangutica* based on external features. According to our observations, pollen maturation and embryo sac maturation are essentially synchronous, and pollen does not mature at the same time, which increases the window for pollination and the likelihood of successful pollination. By examining the stages of pollen and embryo sac maturation, better breeding strategies can be proposed to improve breeding efficiency and quality.

This study represents the first systematic investigation of the development of microspores and megaspores and the differentiation characteristics of male and female gametophytes in *D. tangutica*. The conclusions obtained fill a gap in embryological research on *Thymelaeaceae* plants and provide an important theoretical basis for subsequent studies on the reproductive developmental mechanisms of this family.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors have no conflicts of interest to declare.

Funding

This study was supported by the Gansu Provincial Department of Education Graduate Student "Innovation Star" Program (Project No.: 2026CXZX-997) and the President's Fund Project of Hexi

University (Project No.: CXTD2025009).

Author Contribution

F.Y and X.Y.K. designed the research. X.Y.K, Z.W.Z, C.Q.Z. H.P.W, L.T.M, J.W.B, T.X.and W.J.Z performed the experiments. X.Y.K and Z.W.Z. analyzed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Data Availability

The data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Ji YZ, Lei Y, Li XL, Zhao WZ (2018) Establishment of the tissue culture and regeneration system of Daphne tangutica. Northwest Univ 33:94–99 https://doi: 10. 3969/ as sn. 100U I = 746 1, 20106, U6, 16
2. Zhou ZM, Lv B, Yan F, Zhang CY, Wang HP, Wang QL, Wang JD, Xu T, Zhang CM, Chen Y (2022) Preliminary study on the active substances of different medicinal parts of Daphne tangutica Maxim of different planting years. Chin Agric Sci Bull 38:53–59. https://doi:10.11924/j.issn.1000-6850.casb2021-0570
3. Di XH, Zheng H, Yang YZ, Meng N, Yang QF, Zhu JY, Yan DN, Li ZZ, Li RN (2024) A new method to quantify the impacts of human activity on soil conservation service. Environ Manage 368:1–11. https://doi.org/10.1016/j.jenvman.2024.122257
4. Li YT, Jiao J, Zhang Y, Huang CB, Wang HD, Wang B, Su X, Song H, Zhao MS, Jiang DX, Wang JQ, Jiang Q (2021) Clinical efficacy of cortex daphnes (Zushima) patch in patients with symptomatic knee osteoarthritis: a multicenter non-Inferiority randomized controlled clinical trial. Front Pharmacol 12:1–11. https://doi:10.3389/fphar.2021.646310
5. Guo YJ, Zhang HQ, Zhe L, Du Y, Li D, Fang H, You J, Yu LJ, Li R (2023) Up-regulated CD38 by daphnetin alleviates lipopolysaccharide-induced lung injury via inhibiting MAPK/NF-κB/NLRP3 pathway. Cell Commun Signal 21:1–11. https://doi.org/10.1186/s12964-023-01041-3
6. Syed AM, Kundu S, Ram C, Kulhari U, Kumar A, Mugale MN, Mohapatra P, Murty US, Sahu BD (2023) Up-regulation of Nrf2/HO-1 and inhibition of TGF-β1/Smad2/3 signaling axis by daphnetin alleviates transverse aortic constriction-induced cardiac remodeling in mice. Free Radical Bio Med 186:17–30. https://doi.org/10.1016/j.freeradbiomed.2022.04.019
7. Xu WC, Shen JG, Jiang JQ (2021) Phytochemical and biological studies of the plants from the genus Daphne. Chem Biodivers 8:1215–1233. https://doi:10.1002/cbdv.201000117

8. Gupta DP, Park SH, Lee YS, Lee YS, Lee S, Lim S, Byun j, Cho IK, Song GJ (2023) Daphne genkwa flower extract promotes the neuroprotective effects of microglia. Phytomedicine 108:1–9. https://doi.org/10.1016/j.phymed.2022.154486
9. Bai Z, Liu JY, Mi Y, Zhou D, Chen G, Liang D, Li N, Hou Y (2021) Acutissimalignan b from traditional herbal medicine Daphne kiusiana var. atrocaulis (rehd.) F. maekawa inhibits neuroinflammation via NF-κB signaling pathway. Phytomedicine 84:1–13. https://doi.org/10.1016/j.phymed.2021.153508
10. Liu J, Yin X, Kou CX, Thimmappa R, Hua X, Xue ZY (2024) Classification, biosynthesis, and biological functions of triterpene esters in plants. Plant Commun 5:1–27. https://doi:10.1016/j.xplc.2024.100845
11. Zhang W, Shen YH, Lou ZY, Liu RH, Zhang C, Fu P, Shan L, Zhang WD (2007) Two new flavanes and bioactive compounds from Daphne tangutica Maxim. Nat Prod Res 21:1021–1026. https://doi:10.1080/14786410701371603
12. Judkevich MD, Alayón Luaces P, Gonzalez AM, Judkevich MD, Luaces PA, Gonzalez AM (2024) How pollen and ovule development underlay dioecy in Chloroluma gonocarpa (Sapotaceae). J Plant Res 137:1061–1072. https://doi.org/10.1007/s10265-024-01579-4
13. Muto A, Talarico E, D'Apice G, Di Marzo M, Moschin S, Nigris S, Babolin N, Greco E, Araniti F, Chiappetta A, Colombo L, Baldan B, Bruno L (2024) Development of pollinated and unpollinated ovules in Ginkgo biloba: unravelling the role of pollen in ovule tissue maturation. J Exp Bot 75:3351–3367. https://doi.org/10.1093/jxb/erae102
14. Ryabchenko AS, Kolomeitseva GL, Babosha AV, Koval VA (2024) Megasporogenesis, megagametogenesis and embryogenesis of Liparis elliptica (Orchidaceae), with special note to the development of unique unitegmal ovule. Protoplasma 261:411–424. https://doi.org/10.1007/s00709-023-01906-x
15. Alves MF, Pinheiro F, da Silva Graciano D, De Toni KLG, Baumgratz JFA (2025) Permeability of postzygotic barriers: embryology of a partially fertile Epidendrum (Orchidaceae) hybrid. Plant Biol 27:40–51. https://doi.org/10.1111/plb.13748
16. Gama RL, Leite El Ottra JH, Pirani JR, Demarco D (2022) Gynodioecy in Trichilia (Meliaceae) and a peculiar case of male sterility due to tapetal necrotic cell death. Braz J Bot 45:449–462. https://doi.org/10.1007/s40415-021-00746-4
17. Carrizo, García C (2002) Anther Wall Formation in Solanaceae Species. ANN BOT-LONDON 90:701–706. https://doi.org/10.1093/aob/mcf248
18. Yuan Gz, Wu Z, Liu Xy, Li T, Teng NJ (2021) Characterization and functional analysis of LoUDT1, a bHLH transcription factor related to anther development in the lily oriental hybrid Siberia (Lilium spp.). Plant physiology and biochemistry. PPB 166:1087–1095. https://doi.org/10.1016/j.plaphy.2021.07.018
19. Shirkhani Z, Chehregani RA, Gholami M (2019) Sporogenesis and gametophytes development in Datura stramonium L. (Solanaceae). Braz J Bot 42:107–117. https://doi.org/10.1007/s40415-019-00514-5

20. Shi L, Li C, Lv G, Li X, Feng W, Bi Y, Wang W, Wang Y, Zhu L, Tang W, Fu Y (2024) The adaptor protein ECAP, the corepressor LEUNIG, and the transcription factor BEH3 interact and regulate microsporocyte generation in Arabidopsis. Plant Cell 36:2531–2549. https://doi.org/10.1093/plcell/koae086
21. Nguyen VNT, Usman B, Kim EJ, Shim SH, Jeon JS, Jung KH (2024) An ATP-binding cassette transporter, OsABCB24, is involved in female gametophyte development and early seed growth in rice. Physiol Plant 176:1–10. https://doi.org/10.1111/ppl.14354
22. Wang XB, Ge XJ, Zhong WX, Wu DD, Yan ZY, Zhan RY, Chen LK, Li H (2025) Construction of a single-cell transcriptome atlas for Pogostemon cablin embryoids reveals PcNAC048 as a dual regulator coordinating lateral root morphogenesis and patchouli alcohol biosynthesis. Commun Biol 16:1–42. https://doi.org/10.1038/s42003-025-09434-5
23. Yuan GZ, Wu Z, Liu XY, Li T, Teng NJ (2021) Characterization and functional analysis of LoUDT1, a bHLH transcription factor related to anther development in the lily oriental hybrid Siberia (*Lilium* spp). Plant Physiol biochemistry: PPB 166:1087–1095. https://doi.org/10.1016/j.plaphy.2021.07.018
24. Panigrahi S, Bhatia R, PM G, Budhlakoti V, Hossain F, Shasany AK, Kumar G (2025) Standardization of microspore developmental stages and plant growth regulators for induction of haploid through anther culture in Chrysanthemum genotypes. Vitro Cell Dev-Pl 61:981–9995. https://doi.org/10.1007/s11627-025-10578-6
25. Sharma R, Geeta R, Bhat V (2014) Asynchronous male/female gametophyte development in facultative apomictic plants of Cenchrus ciliaris (Poaceae). S. AFR. J BOT 91:19–31. https://doi.org/10.1007/s11627-025-10578-6
26. Bennett MD, Rao MK, Smith JB, Bayliss MW (1973) Cell development in the anther, the ovule, and the young seed of Triticum aestivum L. var. Chin Spring Philos Trans R Soc Lond B Biol Sci 266:399–381. https://doi.org/10.1098/rstb.1973.0036
27. He R, Yan F, Zhu RY, Zhang CY, Wang HP, Wang QL, Wang JD, XU T, Zhang CM, Chen Y (2024) Studies on flowering phenology and breeding system of Daphne tangutica Maxim. Acta Agrestia Sin 32:188–197. https://doi:10.11733/j.issn.1007-0435.2024.01.020
28. Satake A, Kelly D (2021) Delayed fertilization facilitates flowering time diversity in Fagaceae. Philos Trans R Soc Lond B Biol Sci 367:1–9. https://doi.org/10.1098/rstb.2021.0115
29. Minnaar C, Anderson B (2021) A combination of pollen mosaics on pollinators and floral handedness facilitates the increase of outcross pollen movement. Curr Biol 31:3180–3184. https://doi.org/10.1016/j.cub.2021.04.074
30. Ahrens CW, Watson-Lazowski A, Huang G, Tissue DT, Rymer PD (2022) The roles of divergent and parallel molecular evolution contributing to thermal adaptive strategies in trees. Plant Cell Environ 45:3476–3491. https://doi.org/10.1111/pce.14449
31. Wei N, Kaczorowski RL, Arceo-Gómez G, O'Neill EM, Hayes RA, Ashman TL (2021) Pollinators contribute to the maintenance of flowering plant diversity. Nature 597:688–692.

https://doi.org/10.1038/s41586-021-03890-9

32. Liu MY, Zhou YH, Sun JX, Mao F, Yao Q, Li BL, Wang YY, Guo YB, Dong X, Liao SH, Wang P, Hang SB (2023) From the floret to the canopy: High temperature tolerance during flowering. Plant Commun 4:1–29. https://doi.org/10.1016/j.xplc.2023.100629
33. Satake A, Kelly D (2021) Delayed fertilization facilitates flowering time diversity in Fagaceae. Philos T R Soc B 376:1–9. https://doi.org/10.1098/rstb.2021.0115
34. Liu P, Quan X, Song z, Li WH, Wang Y, Gu HY, Xie DX, Yng WC, Dresselhaus T, Zhong S, Qu LJ (2025) A two-step self-pollination mechanism maximizes fertility in Brassicaceae. Cell 188:2845–2855. https://doi.org/10.1016/j.cell.2025.03.021
35. Hu QQ, Yan N, Cui KH, Li GH, Wang WC, Huang JL, Peng SB (2024) Increased panicle nitrogen application improves rice yield by alleviating high-temperature damage during panicle initiation to anther development. Physiol Plant 176:11–17. https://doi.org/10.1111/ppl.14230
36. Tidy AC, Ferjentsikova I, Vizcay-Barrena G, Liu B, Yin WZ, Higgins JD, Xu J, Zhang DB, Geelen D, Wilson ZA (2022) Sporophytic control of pollen meiotic progression is mediated by tapetum expression of ABORTED MICROSPORES. J Exp Bot 73:5543–5558. https://doi.org/10.1093/jxb/erac225
37. Melendez-Alvarez JR, Zhang R, Tian XJ (2023) Growth Feedback Confers Cooperativity in Resource-Competing Synthetic Gene Circuits. Chaos Soliton Fract 173:1–9. https://doi:10.1016/j.chaos.2023.113713
38. Huang TH, Suen DF (2021) Iron insufficiency in floral buds impairs pollen development by disrupting tapetum function. Plant J 108:244–267. https://doi:10.1111/tpj.15438
39. Saini DK, Lima JME, Sunkar R, Doherty C, Jagadish KSV (2025) Plant's developmental decision to either abort a flower or set seed. Trends Plant Sci 30:100–113. https://doi:10.1016/j.tplants.2025.08.015
40. Siemons C, Jonkers S, Vlieg RC, Corral-Martínez P, van Noort J, Boutilier K (2025) Establishment and maintenance of embryogenic cell fate during microspore embryogenesis. Plant J 121:1–20. https://doi.org/10.1111/tpj.17243
41. Zhang L, Jing PP, Geng B, Shi JJ, Zhang JL, Liang D, Yang YJ, Qu YF, Huang JL (2025) Effect of glutathione reductase on photosystem II characterization and reactive oxygen species metabolism in cotton cytoplasmic male sterile line Jin A. Plant J 121:1–19. https://doi.org/10.1111/tpj.17217
42. Liu N, Qian YC, Lu QQ, Han XF, Yang L, Su JH, Zhang Y, Ni M, Shao YM, Mou WS, Zhang SQ, Xu JA (2025) DELLA-ANAC106 reciprocal negative feedback circuit modulates gibberellin homeostasis to regulate male fertility in Arabidopsis. New Phytol 248:305–320. https://doi.org/10.1111/nph.70427
43. Walker CH, Ware A, Šimura J, Ljung K, Wilson Z, Bennett T (2023) Cytokinin signaling regulates two-stage inflorescence arrest in Arabidopsis. Plant Physiol 191:479–459. https://doi.org/10.1093/plphys/kiac514
44. Shi C, Zhao ZF, Zhong YC, Qiao Y, Zhang L, Yang F, Li SY, Li LL, Huang LJ, Tang XC, Luo P, Sun MX (2025) Reprogramming of microspore fate via BBM-BAR1 for highly efficient in vivo haploid

induction. Cell 188:6109–6120. https://doi.org/10.1016/j.cell.2025.08.014

45. Ma S, Wang LJ, Ye LM, Jiang J (2023) Vegetation restoration thresholds under different vegetation types and altitude gradients in the Sichuan-Yunnan ecological shelter. China J Environ Manage 340:1–12. https://doi.org/10.1016/j.jenvman.2023.117910
46. Carrizo García C, Nepi M, Pacini E (2017) It is a matter of timing: asynchrony during pollen development and its consequences on pollen performance in angiosperms—a review. Protoplasma 254:57–73. https://doi.org/10.1007/s00709-016-0950-6
47. Bayr D, Plaza MP, Gilles S, Kolek F, Leier-Wirtz V, Traidl-Hoffmann C, Damialis A (2023) Pollen long-distance transport associated with symptoms in pollen allergics on the German Alps: An old story with a new ending? Sci. Total Environ 881:1–14. https://doi.org/10.1016/j.scitotenv.2023.163310
48. Cun S, Zhang C, Chen JQ, Qian LS, Sun H, Song B (2024) Effects of UV-B radiation on pollen germination and tube growth: A global meta-analysis. Sci Total Environ 915:1–13. https://doi.org/10.1016/j.scitotenv.2024.170097
49. Xie F, Vahldick H, Lin Z, Nowack MK (2022) Killing me softly - Programmed cell death in plant reproduction from sporogenesis to fertilization. Curr Opin Plant Biol 69:1–18. https://doi.org/10.1016/j.pbi.2022.102271
50. Saini DK, Lima JME, Sunkar R, Doherty C, Jagadish KSV (2025) Plant's developmental decision to either abort a flower or set seed. Trends. Plant Sci S1360–1385(25):00255–00259 Advance online publication. https://doi.org/10.1016/j.tplants.2025.08.015
51. Millan-Blanquez M, Simmonds J, Bird N, Manes Y, Uauy C, Boden SA (2025) Stigma longevity is not a major limiting factor in hybrid wheat seed production. J Exp Bot 76:4003–4013. https://doi.org/10.1093/jxb/eraf154
52. Du HS, Zhang XF, Li R, Chen B, Zhang XF, Wang YH, Wang JF, Wang T, Wen CL, Liu N, Geng SS (2025) The natural Msc-4 allele confers genic male sterility via conserved and divergent gene regulatory mechanisms in pepper. Plant Physiol 199:kiaf474. https://doi.org/10.1093/plphys/kiaf474
53. Huang JY, Dong J, Qu LJ (2021) From birth to function: Male gametophyte development in flowering plants. CURR. OPIN. PLANT BIOL 63:1–9. https://doi.org/10.1016/j.pbi.2021.102118
54. Wang XB, Ge XJ, Zhong WX, Wu DD, Yang ZY, Zhan RT, Chen LK, Li H (2025) Construction of a single-cell transcriptome atlas for Pogostemon cablin embryoids reveals PcNAC048 as a dual regulator coordinating lateral root morphogenesis and patchouli alcohol biosynthesis. Commun Biol 12:1–42. https://doi.org/10.1038/s42003-025-09434-5
55. Ma LY, Hu ZJ, Shen WW, Zhang YY, Wang GC, Chang B, Lu JK, Cui YN, Xu HM, Feng Y, Jin B, Zhang X, Wang L, Lin J (2024) Three-dimensional reconstruction and multiomics analysis reveal a unique pattern of embryogenesis in Ginkgo biloba. Plant physiol 196:95–111. https://doi.org/10.1093/plphys/kiae219
56. Pacini E, Dolferus R (2016) The trials and tribulations of the plant male gametophyte—Understanding reproductive stage stress tolerance. In: Abiotic and Biotic Stress in Plants—Recent Advances and Future Perspectives. InTech. http://dx.doi.org/10.5772/61671

57. Zhou CX, An K, Zhang X, Tong BQ, Liu D, Kong DR, Bian FH (2023) Sporogenesis, gametophyte development and embryogenesis in Glehnia littoralis. Bmc Plant Bio 23:1–13. https://doi.org/10.1186/s12870-023-04105-1
58. Zeng JG, Duan MM, Wang YQ, Li GG, You YJ, Shi J, Liu CG, Zhang JY, Xu J, Zhang SQ, Zhao J (2024) Sporophytic control of tapetal development and pollen fertility by a mitogen-activated protein kinase cascade in rice. J Integr Plant Biol 66:1500–1516. https://doi.org/10.1111/jipb.13673
59. Che RH, Hu B, Wang W, Xiao YH, Liu DP, Yin WC, Tong HN, Chu CC (2022) POLLEN STERILITY, a novel suppressor of cell division, is required for timely tapetal programmed cell death in rice. Sci China Life Sci 65:1235–1247. https://doi.org/10.1007/s11427-021-2011-2
60. Wei S, Ma L (2023) Comprehensive Insight into Tapetum-Mediated Pollen Development in Arabidopsis thaliana. Cells 12:1–14. https://doi.org/10.3390/cells12020247
61. Hua M, Yin W, Fernández Gómez J, Tidy A, Xing G, Zong J, Shi S, Wilson ZA Barley TAPETAL DEVELOPMENT and FUNCTION1 (HvTDF1) gene reveals conserved and unique roles in controlling anther tapetum development in dicot and monocot plants. New Phytol. 240: 173–190., Fan B, Chen Q, Zhou SY, Zhang YT, Wang YW, Shang YT, Zhang N, Liu XY, Wang ZY (2023) (2025). Exploring candidate genes related to pollen abortion in garlic (Allium sativum) based on cytological studies and transcriptome sequencing. J. Plant LANT Res. 138: 637–651. https://doi.org/10.1007/s10265-025-01631-x
62. Liao JQ, Zhang ZZ, Shang YK, Jiang YY, Su ZX, Deng XX, Pu X, Yang RW, Zhang L (2023) Anatomy and Comparative Transcriptome Reveal the Mechanism of Male Sterility in Salvia miltiorrhiza. Int. J Mol. Sci. 24: 1–14. https://doi.org/10.3390/ijms241210259 Authorship contribution statement F.Y and X.Y.K. designed the research. X.Y.K, Z.W.Z, C.Q.Z. H.P.W, L.T.M, J.W.B, T.X.and W.J.Z performed the experiments. X.Y.K and Z.W.Z. analyzed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript

Figures

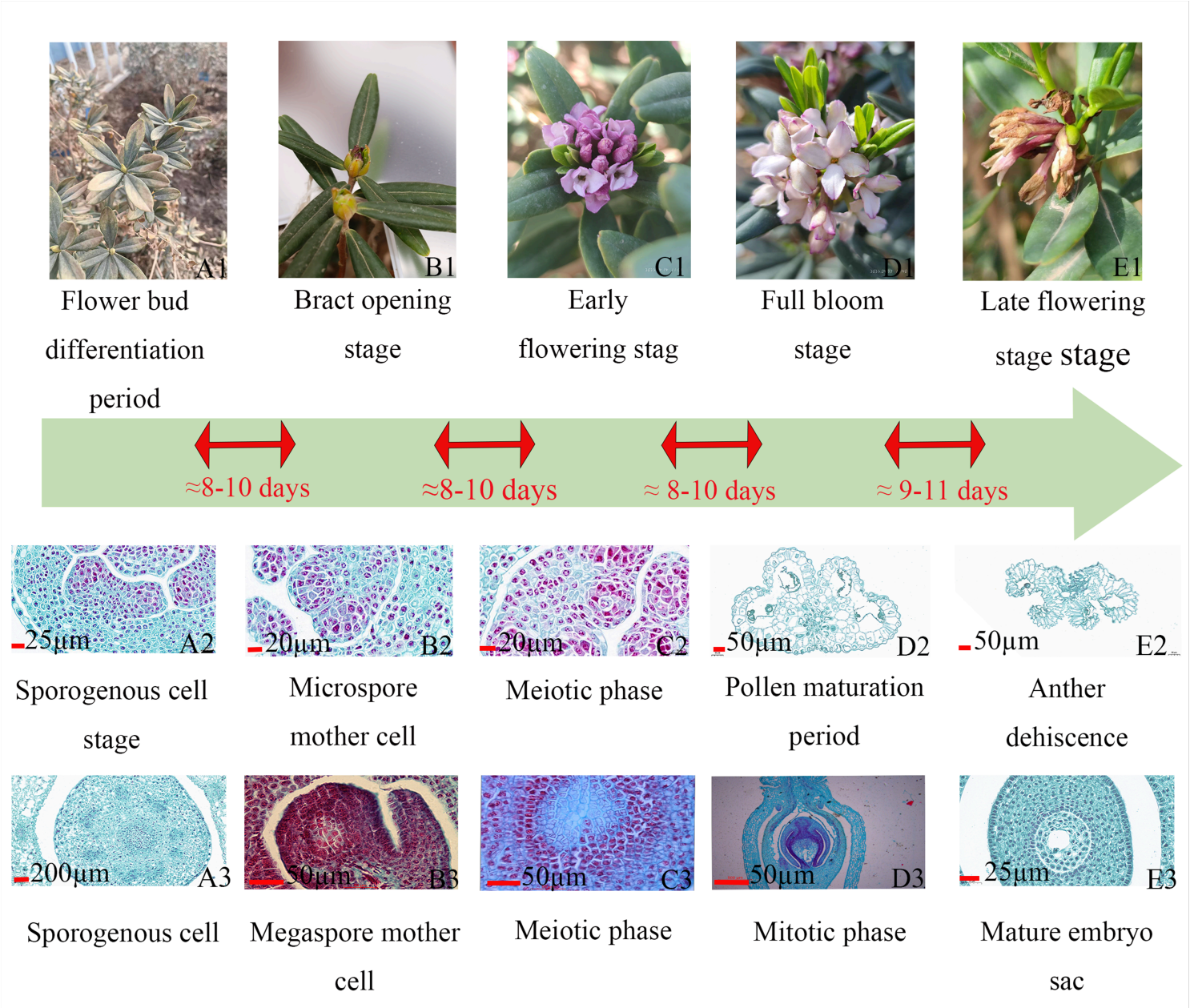


Figure 1

shows the developmental timeline of *D. tangutica*.: (A 1) Flower bud differentiation period; (A 2) Sporogenous cell stage; (A 3) Sporogenous cell; (B 1) Bract opening stage; (B 2) Microspore mother cell; (B 3) Megaspore mother cell; (C 1) Initial flowering period; (C 2) meiotic phase; (C 3) Meiotic phase; (D 1)Full bloom; (D 2) Full bloom; (D 3) Mitotic phase; (E 1) Mature embryo sac; (E 2) Anther dehiscence; (E 3) Mature embryo sac.

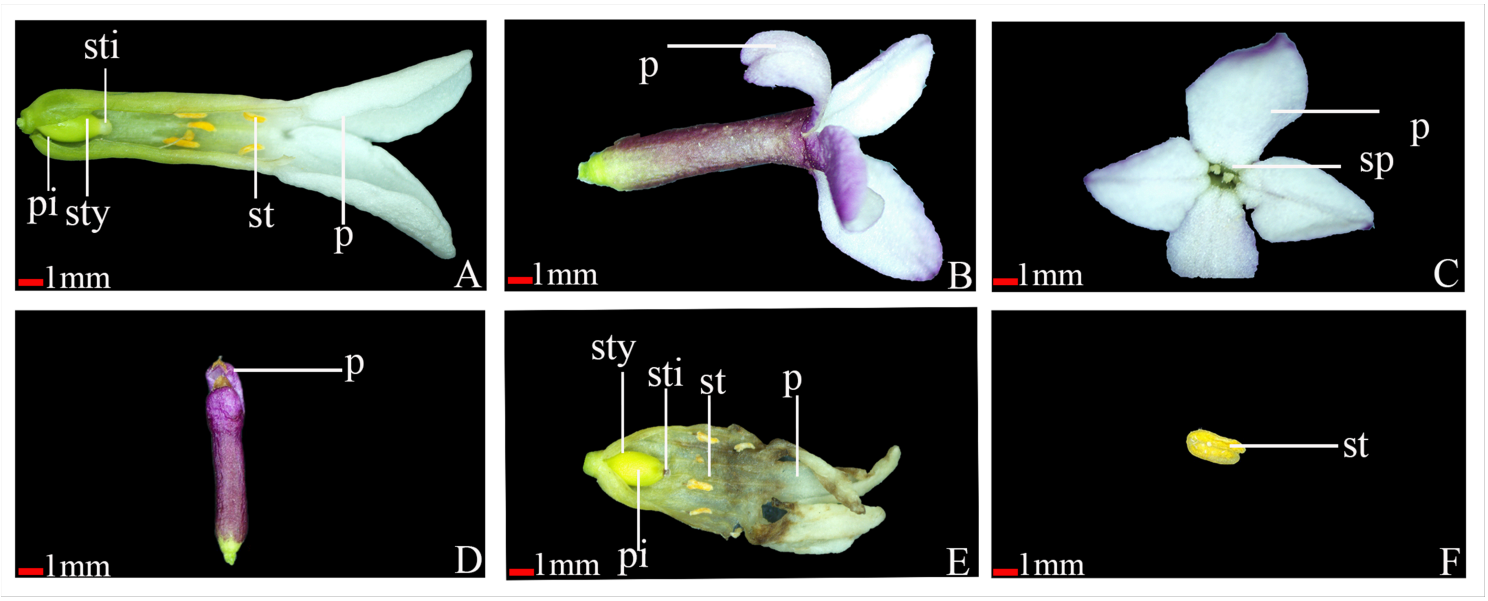


Figure 2

Inflorescence development and floral morphology in *D. tangutica*. as: (A) Flower bud differentiation and bract dehiscence stages; (B) Inflorescence pigmentation stage; (C,D) Early flowering stage, showing lateral and top views; (E,F) Full-bloom stage, showing lateral and top views.

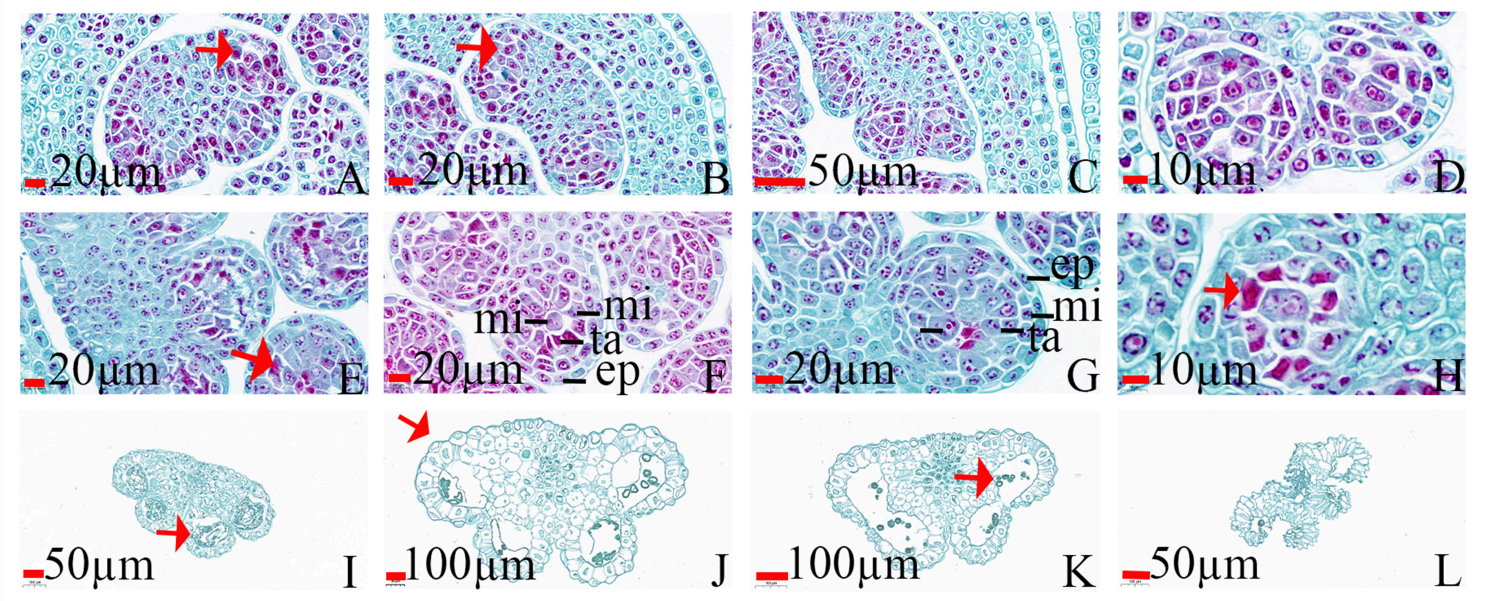


Figure 3

Illustrates the development of the anther wall in *D. tangutica*. they should be listed as: (A) Cross-section of the anther primordium at the archesporial cell stage; (B) Peripheral cells undergoing periclinal division; (C) Transverse section of the young anther showing the differentiation of four microsporangium; (D) Developing anther wall alongside early microspore mother cells; (E) Arrows indicate binuclear or multinuclear tapetum cells; (F) Anther wall with isolated microspores; (G) Disintegration of tapetal cells; (H) Fibrous thickening of the endothecium wall; (I) Transverse section of the anther displaying fibrous

thickening of the endothecium and mature pollen grains; (J-L) Stages of pollen grain maturation. Abbreviations: asc, Archesporial cells; ppc, Primary peripheral cells; ssc, Secondary sporogenous cells; aw, anther wall; en, Endothecium; ep, Epidermis; ml, Middle layer; mi, Microspore; ta, Tapetum; fe, Fibrous endothecium; pg, Pollen grains.

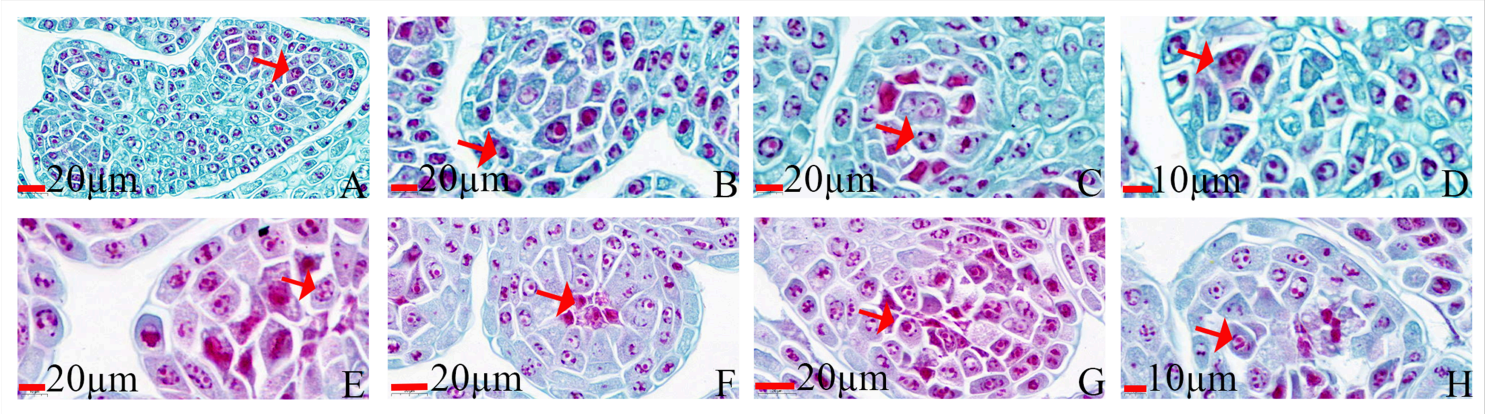


Figure 4

Illustrates the microspore development process. they should be listed as: (A) Microspore mother cell; (B) Late Meiosis I; (C) Dyad stage after Meiosis I; (D) Prophase of Meiosis II; (E) Anaphase II; (F) Late stage of Meiosis II; (G) Illustrates the formation of the partition wall (indicated by arrows); (H) Represents the tetrahedral tetrad.

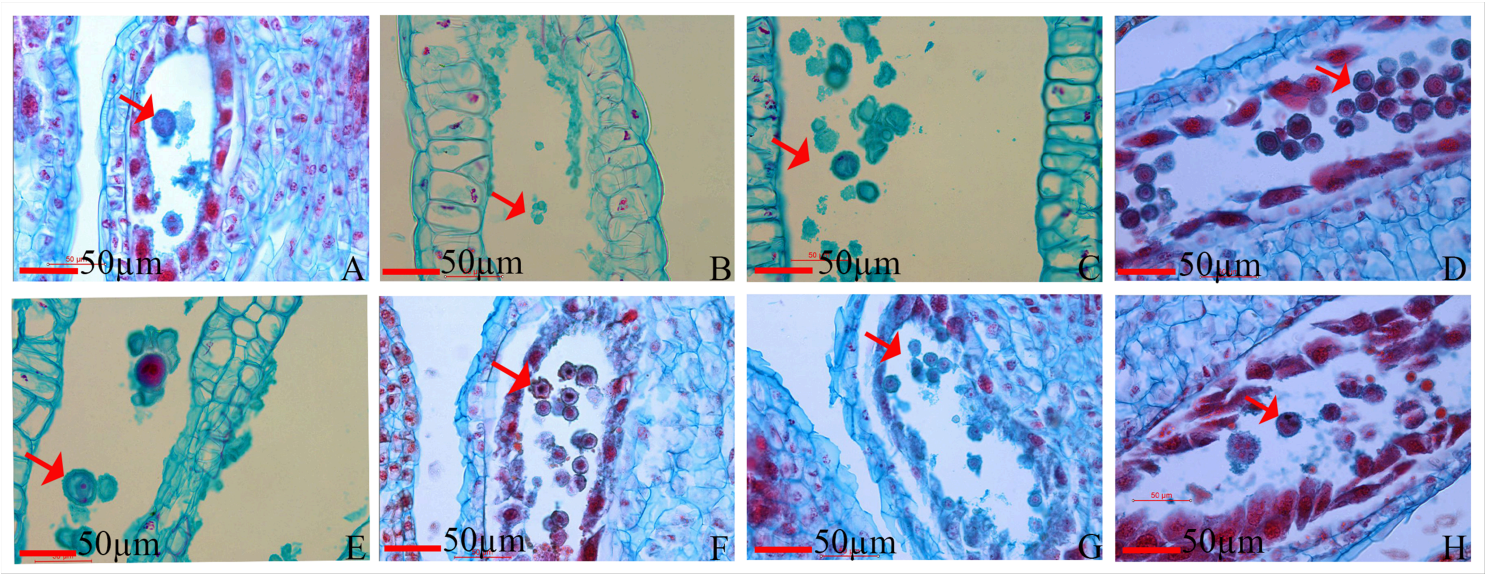


Figure 5

Illustrates the microscopic observation of the microspore development process through a longitudinal section. they should be listed as: (A) Represents microspore mother cells; (B) Indicates late meiosis I; (C) Marks the end of meiosis I; (D) Denotes prophase of meiosis II; (E) Shows anaphase II of meiosis; (F) Depicts late meiosis II; (G) Illustrates the formation of the cell plates; (H) Highlights the tetrad of microspores (as indicated by the arrows).

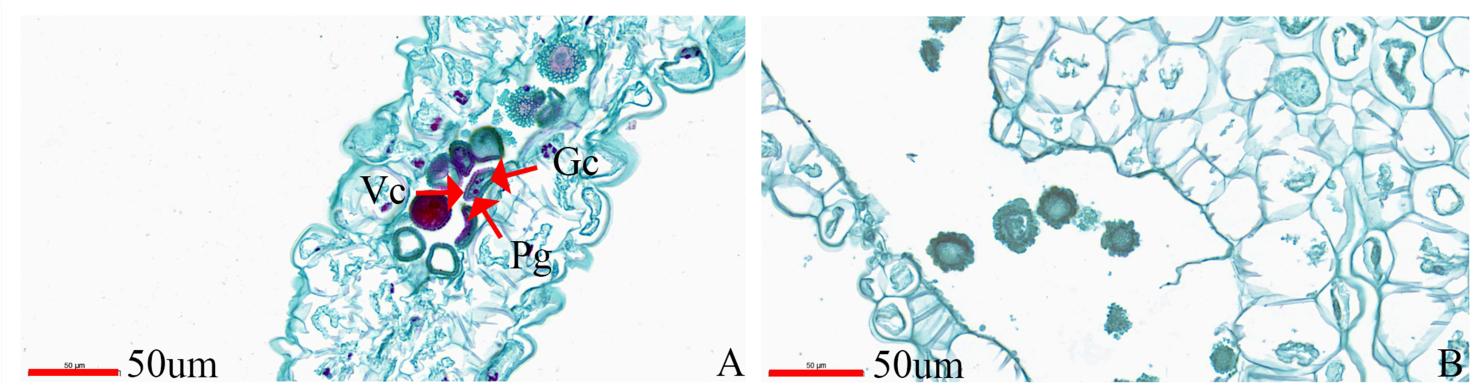


Figure 6

Male gametophyte development. they should be listed as: (A) Indicates the early uninucleate microspore (marked by an arrow); (B) Denotes generative nucleus division (also marked by an arrow); VN, denotes the vegetative nucleus; gc, Refers to the generative nucleus; and gp, Indicates the germination pore.

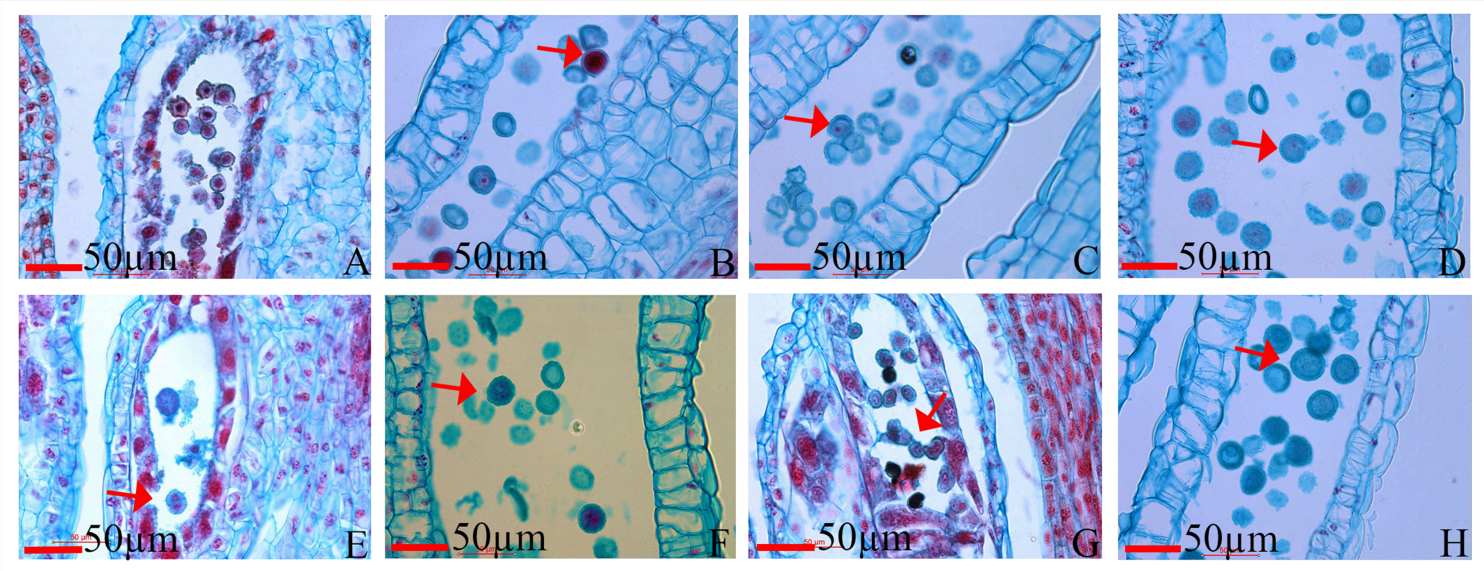


Figure 7

Presents a microscopic observation of the developmental process of the male gametophyte, illustrated through longitudinal sections. they should be listed as: (A) Shows microspores released from the tetrad, as indicated by the arrows. (B) Depicts early mononuclear microspores, also marked by arrows. (C) Mononuclear microspores in the marginal phase are highlighted by arrows. (D-F) Illustrate the process of generative nucleus, with arrows indicating the relevant structures. (G) Features two-cell pollen, (H) Presents a mature pollen grain, with arrows pointing to significant features. Additionally, vC Denotes the nutrient cell, gC Represents the generative nucleus, and gp Indicates the germination pore.

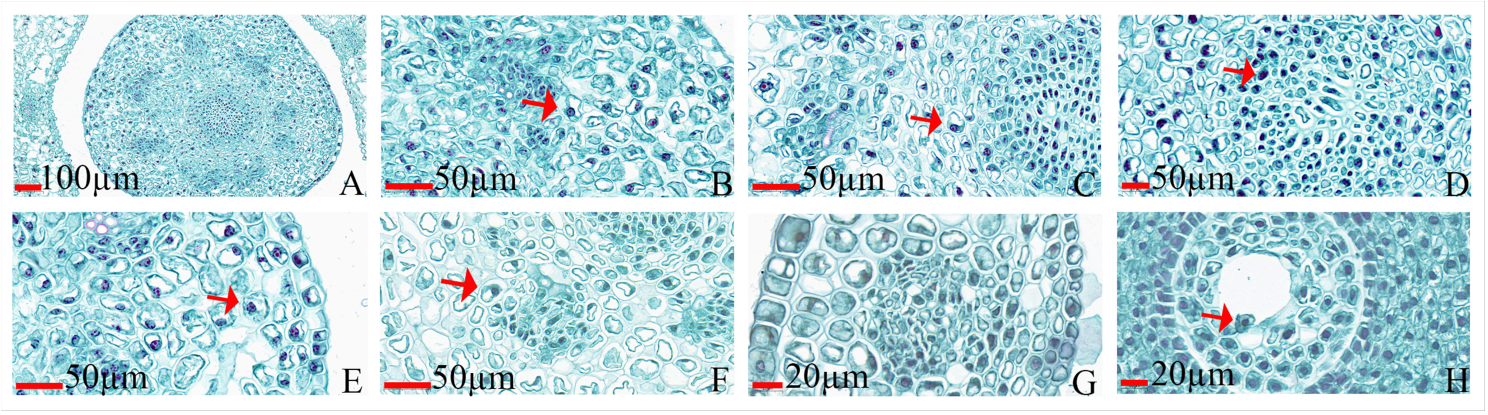


Figure 8

Illustrates the development of the embryo sac and megaspore.they should be listed as: (A) Archesporial cells (indicated by arrows); (B) Megaspore mother cell (indicated by an arrow); (C) Nucellar tissue (indicated by an arrow); (D) Crassinucellate ovule (indicated by an arrow); (E) Megaspore tetrad (indicated by an arrow); (F) Degeneration of three megaspores (indicated by an arrow); (G) Functional megaspore (indicated by an arrow); (H) Mononuclear embryo sac (indicated by an arrow).

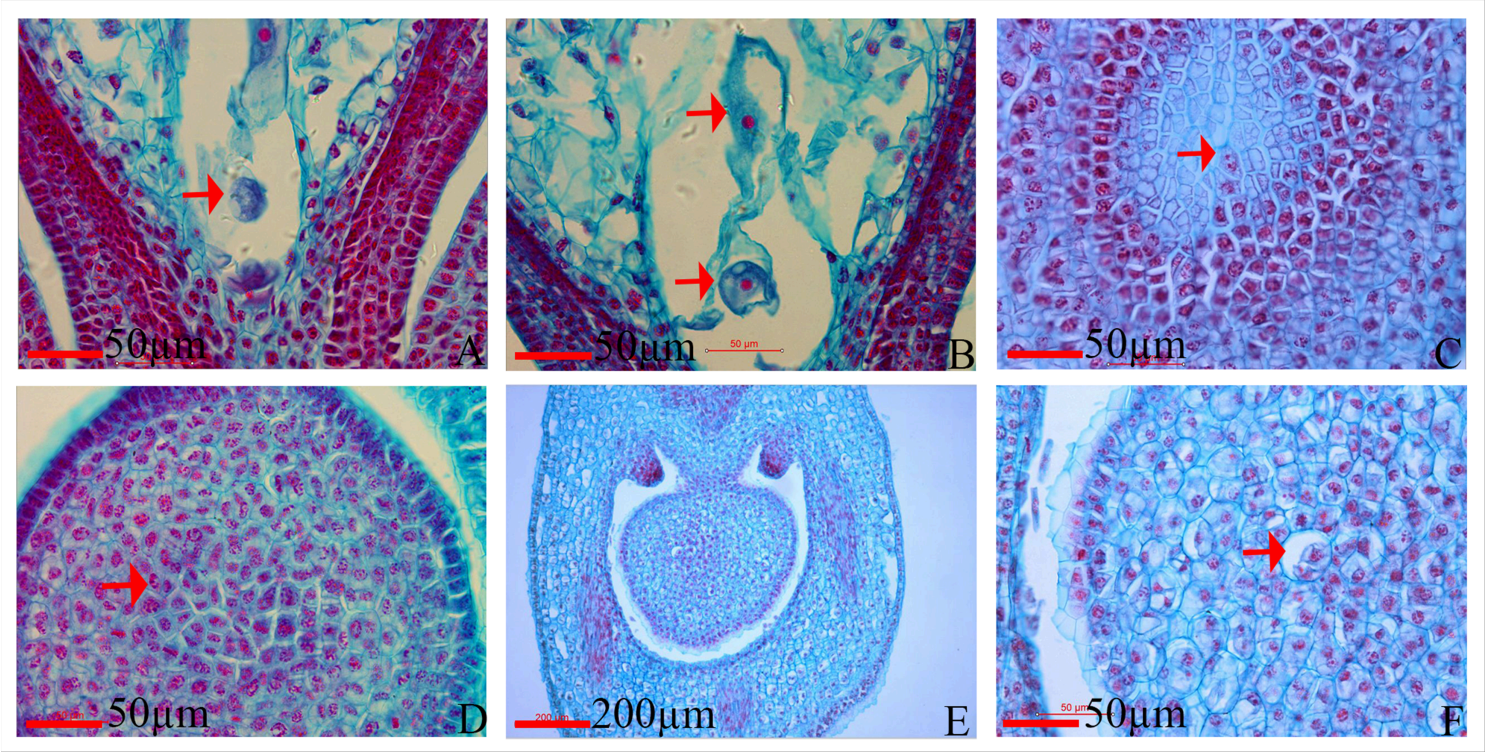


Figure 9

Illustrates the development process of the female gametophyte. they should be listed as:(A) Indicates a binucleate embryo sac (shown by the arrow); (B) Represents mitosis (as indicated by the arrows); (C) Depicts a tetranucleate embryo sac (shown by the arrow); (D) Shows the eight-nucleate stage of the

embryo sac (arrow); (E) Illustrates a mature embryo sac (shown by the arrow); (F) Also represents a mature embryo sac (shown by the arrow).