

**SPOROGENESIS AND GAMETOGENESIS IN DOUBLE-FLOWERED
JASMINUM MESNYI HANCE (OLEACEAE)**

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Abstract

Results on sporogenesis and gametogenesis in double-flowered *Jasminum mesnyi* showed that the development of anther wall follows the dicotyledonous type which is composed of epidermis, endothecium, middle layer and secretory tapetum. The cytokinesis of microspores follows the successive type. Microspores tetrads are tetragonal. Pollen grains are spheroidal, reticulate and tricolporate. The ovary that contains 2 ovules is anatropous, unitegmic and tenuinucellate on the axial placentation. Beneath the epidermis of the ovule, archesporial cells immediately turn to be the megaspore mother cells. Since the megaspore mother cells had dissolved, the megasporogenesis aborted. The horticultural interest and systematic significance of embryological features of double-flowered *J. mesnyi* is discussed.

Introduction

Jasminum mesnyi Hance belongs to Oleaceae, is cultivated in worldwide (Chang *et al.* 1996). Two subspecies of *J. mesnyi* (double- and single-flowered subspecies) are often used as ornamental plants for park arrangement and they are also Chinese tradition medicinal materials. The double-flowered *J. mesnyi* is more popular than single-flowered subspecies, in regarding to floral morphology.

New varieties of double-flowered ornamental plants become one of the main ornamental floral characters and important breeding objects in flower breeding (Reynolds and Tampion 1983). Understanding the reproductive process on the double flower species is key to breeding success. Several limiting traits on biology and reproduction had already been identified during evaluation, justifying breeding attempts (Saviadan *et al.* 1989). Embryo abortion at different developmental stages (preembryo to cotyledon embryo and main globular embryo) is a breeding barrier in 4x-2x crosses in *Dianthus caryophyllus* (Zhou XH *et al.* 2013). Three types of plant sterility have been observed including complete sterility, male sterility and environmentally induced male sterility, based on information on microgametogenesis in garlic (*Allium sativum* L.) (Einat *et al.* 2013). In investigating microspore development and pollen tube growth, it was found that Jeju old local citrus species are male fertile and have the ability to produce enough viable pollens for self- and cross-pollination (Panha *et al.* 2015).

Embryological features are also useful evidence for plant systematics. Gametophyte features have distinct characters in the genus *Onobrychis* Mill (Chehregani and Nayerch 2011). The developmental process of flower and fruit formation was studied in *Quercus schottkyana* and other related Fagaceae. The data on *Q. schottkyana* supported the hypothesis that evolutionary novelty in the development and anatomy of *Quercus* flowers is mostly found in terminal clades (Deng *et al.* 2008). Zhang *et al.* (2010) observed the sporogenesis and gametogenesis in *Agapanthus*, and

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compared with out-groups. Their view of the evolutionary sequence might be: Agapanthaceae→Amaryllidaceae→Alliaceae. The embryology of *Xanthoceras* was investigated and compared with related taxa by Zhou and Liu (2012). They suggested that *Xanthoceras* is closely related to other members of Sapindaceae, and proposed that *Xanthoceras* merits recognition at the subfamily level as Xanthoceroideae. Yamamoto *et al.* (2014) compared *Biebersteinia* embryology with those of eight other families of Sapindales, as well as with those of related orders. Considering the presence of anther tapetal cells with polyploidy nuclear, seed coats, number of autapomorphies and other features, *Biebersteinia* fits in Sapindales. Study by Tobe (2015) investigating the embryology of *Phyllonoma tenuidens* and compared its characteristics with other Aquifoliales species, supported the distinctness of *Phyllonoma* as a family and its sister-group relationship to East-Asian Helwingiaceae.

There have been no reported embryological studies on the double-flowered *J. mesnyi*. Most studies have been on plants of horticultural interest and systematic significance, with few being focused on single-flowered species. In this work microsporogenesis and male gametogenesis, and megasporogenesis are studied in double-flowered *J. mesnyi*.

Materials and Methods

The materials were continuously collected during November, 2014 to January, 2015 in school experimental garden at Guizhou University, Guizhou province, China. Fresh flowers at all ages (tiny buds to mature flowers) were fixed immediately in FAA (formalin: acetic acid: 90 parts 50% alcohol = 5 : 5 : 90, v/v) for at least 24 hrs. Then dehydrated in alcohol, embedded in paraffin, and sectioned (at a thickness of 8 μ m cross-sections and longitudinal-section) using a rotary microtome. Staining and embedding was done according to the protocol suggested by Li ZR (2016). Several sections for each developmental stage were observed and photographed under Olympus BX51. Pollen at maturation stage were naturally dehydrated and then critical point dried with CO₂. Pollen grains were then mounted on aluminum stubs and coated with gold-palladium before they were observed under the Hitachi S-3400SEM. For each stage, at least 15 flowers were surveyed and best pictures were selected.

Results and Discussion

Primary sporogenous cells underwent mitosis, and primary sporogenous cells formed an un-differentiated wall and a mass of peripheral cells (Fig. 1a). At first stage spore mother cells were well differentiated and large in size with high density of cytoplasm (Fig. 1b). The microsporocytes increased in size and formed a special wall with callosic nature. After the first meiotic division of microsporocytes, a cell wall was developed and a dyad occurred (Fig. 1c). At the second meiosis, tetragonal tetrads were formed which were surrounded with special callose wall (Fig. 1d). The cytokinesis of microspores followed the successive type.

Microspores that just released from the callose wall had no vacuole and were somewhat irregular in shape, with a dense cytoplasm and a centrally placed nucleus (Fig. 1e-g). With the large vacuole being formed, cytoplasm and nucleus were pushed towards microspore wall margin (Fig. 1h-j). As result of an un-equal mitosis, microspore nucleus divided into two cells, a larger vegetative cell and a smaller reproductive cell (Fig. 1k). Then the reproductive cell divided into two sperms. Thus, pollen grain belongs to two cells (Fig. 1l). The shape of pollen grain is spheroidal, reticulate or tricolporate as viewed by SEM (Fig. 1m-n).

Prior to spore cell maturation, the anther wall was composed of four cell layers: an epidermis, an endothecium, a middle layer and a tapetum (Fig. 2a). The epidermis of anther primordia was divided into two cell layers by periclinal and anticlinal division (Fig. 2b). The outer cells were

divided again into endothecium and middle layer. The inner cells were differentiated in tapetum. Thus, the anther wall formation was conformed to follow the dicotyledonous type. During spore mother cell stage, tapetum was differentiated mostly in binucleate or some in uninucleate. Tapetal cells began to degenerate *in situ* during the spore tetrads stage (Fig. 2c). By the time of mature pollen, the tapetum had been completely consumed for the development of pollen grains (Fig. 2d-e). Tapetum layer is secretory type. During the stage of 2-cell pollen grain, one-layered middle layer was completely disintegrated. At the stage of anther dehiscence, anther wall was composed of two cell layers: an epidermis and an endothecium (Fig. 2f).

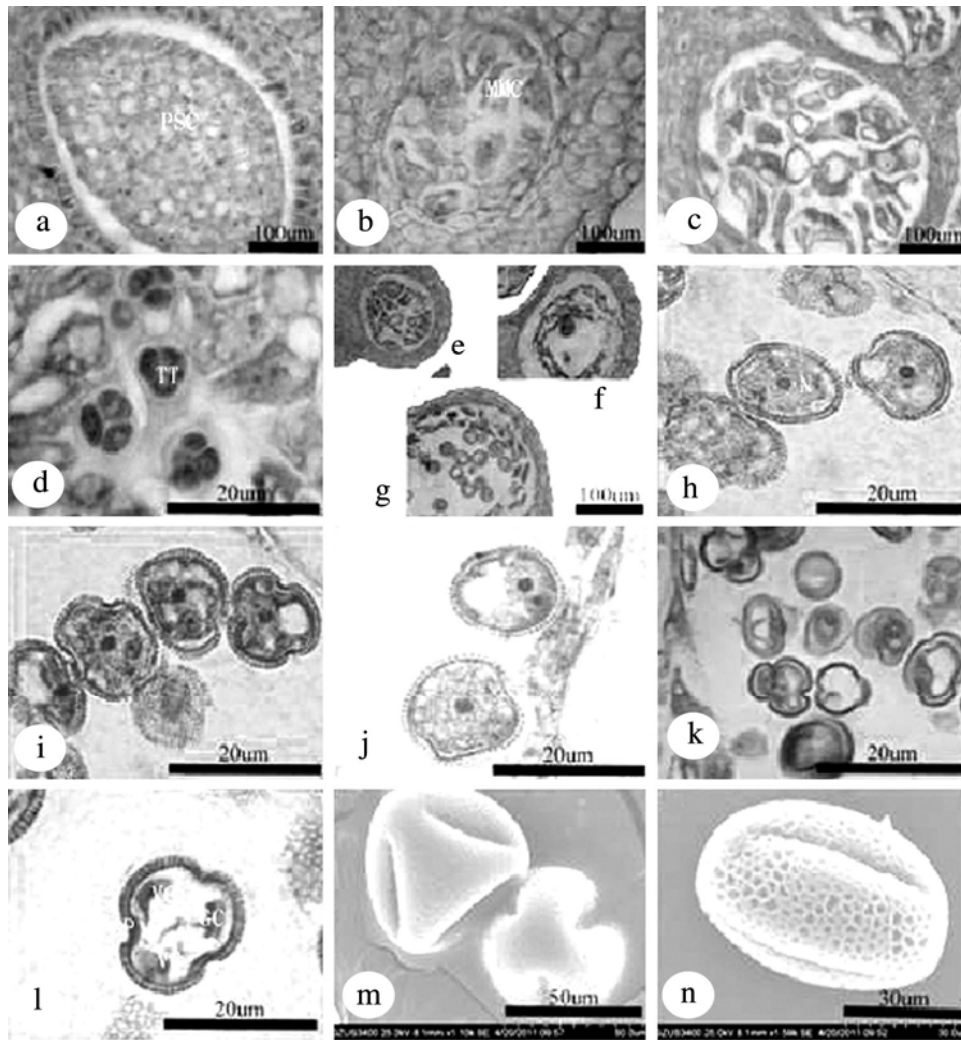


Fig. 1. Microsporogenesis and male gametogenesis of double-flowered *Jasminum mesnyi*. a: primary sporogenous cells, b: microspore mother cells, c: the first meiotic division of microsporocytes, d: the second meiosis, tetragonal tetrads, e-f: microspores just release from the callose wall, h-j: nucleus at microspore wall margin, k: microspore first meiotic division, l: microspore second mitotic division, m-n: shape of pollen grain in SEM, PSC: primary sporogenous cells, MMC: microspore mother cells, TT: tetragonal tetrads, N: nucleus, GP: germination pore, GC: germ cell, VC: vegetative cell.

Comparative embryology has been an useful method in revealing systematic problems in plant taxonomy (Tobe and Takahashi 2009). The systematic position of Oleaceae has been controversial in the past for a long time. Hutchinson (1960) placed Oleaceae in Loganiales. Takhtajan (1997) regarded the Oleaceae should be a single order of Oleales, close to the Gentianales. Croquist (1981) and The APG III (2009) considered that Oleaceae belongs to Scrophulariales. From the above four taxonomic views, we know Oleaceae should be close to the Scrophulariales and Gentianales. Comparative data are summarized in Table 1 based on our results and literature for Oleaceae (Zhang 1982), Scrophulariaceae (Gopalra and Govindappa 1987), Gesneriaceae (Wang *et al.* 2001) and Gentianaceae (Akhalkatsi and Wagner 1997).

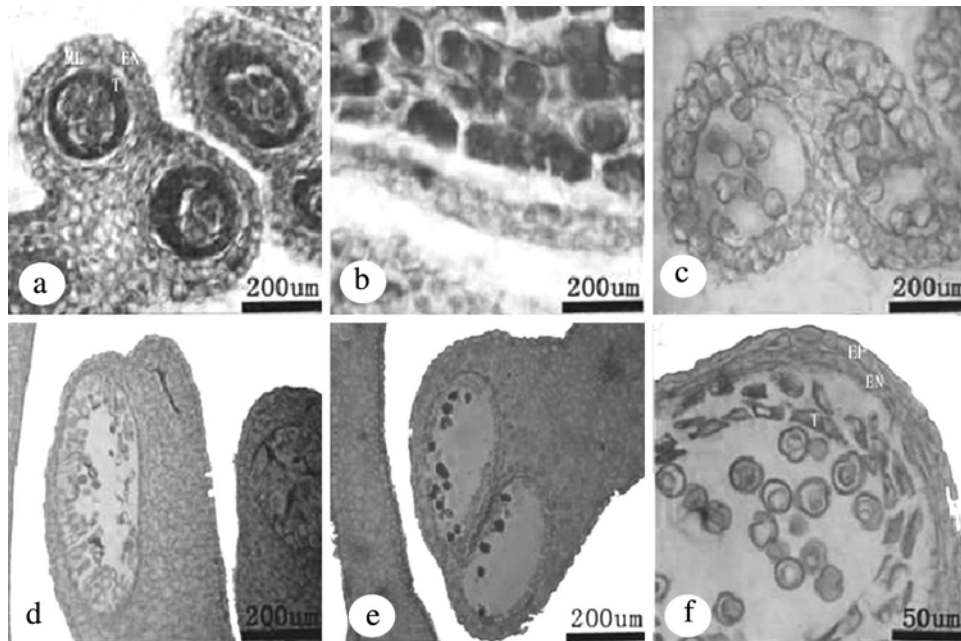


Fig. 2. Anther wall formation of double-flowered *Jasminum mesnyi*. a: young anther wall formation, b: anther primordia division, c: tapetal cells degenerate, d-e: tapetum consumed completely, f: mature anther wall, EP: epidermis, EN: endothecium, ML: middle layer, T: tapetum.

Table 1 shows the embryological comparisons among Oleaceae, Scrophulariaceae, Gesneriaceae, and Gentianaceae. Embryological features of Oleaceae are similar with Scrophulariaceae and Gesneriaceae, except tapetum cell nucleus and the placenta type. However, embryological characteristic of Oleaceae are higher distinct from those of Gentianaceae, including middle layer, tapetum, tapetum cell nucleus, mature pollen, pollen shape, pollen grains ornamentation, placenta type, and antipodal cells number. As a consequence, the higher similarity between Oleaceae and Scrophulariales support Oleaceae might belong to Scrophulariales, which agrees with viewpoint of Croquist (1981) and The APG III (2009). The systematic significance is no doubt useful, however, molecular biology should be used to infer systematic affinities.

The gynoecium is made up of two apocarpous ovaries arranged in a spiral manner on an elongate axis. Each ovary contains two ovules, which is anatropous, unitegmic and tenuinucellate on the axial placentation (Fig. 3a). The archesporial cells were beneath the epidermis of the ovule and easily recognized by its larger size and denser cytoplasm as compared with others (Fig. 3b).

Table 1. Embryological comparisons among Oleaceae, Scrophulariaceae, Gesneriaceae and Gentianaceae.

Characteristics	Oleaceae	Scrophulariaceae	Gesneriaceae	Gentianaceae
Sporangia	4	4	4	4
Anther wall formation	Dicoty/ledonous type	Dicoty/ledonous type	Dicoty/ledonous type	Dicoty/ledonous type
Anther wall epidermis	Persistent	Persistent	Persistent	Persistent
Endothecium thickenings	Fibrous	Fibrous	Fibrous	Fibrous
Middle layer	1	1	1	2
Tapetum type	Secretory	Secretory	Secretory	Secretory or glandular
Tapetum cell nucleus	2 or 4	2	2	1 or more
Cytokinesis microspore type	Successive	Successive	Successive	Successive
Tetrads microspore	Tetragonal	Tetragonal	Tetragonal	Tetragonal
Mature pollen	2-celled	2-celled	2-celled	2-celled
Pollen shape	Spheroidal	Spheroidal	Spheroidal	Ellipsoidal
Pollen grains aperture	Trisulcate	Trisulcate	Trisulcate	Trisulcate
Pollen grains ornamentation	Reticulate	Reticulate	Reticulate	Stripe-reticulate
Ovules type	Anatropous	Anatropous	Anatropous	Anatropous
Integuments number	Unitegmic	Unitegmic	Unitegmic	Unitegmic
Nucellus	Tenuinucellate	Tenuinucellate	Tenuinucellate	Tenuinucellate
Placentation type	Axial	Parietal	Parietal	Parietal
Functional megaspore location	Chalazal end	Chalazal end	Chalazal end	Chalazal end
Tetrads megaspore	Linear	Linear	Linear	Linear
Embryo sac type	Polygonum	Polygonum	Polygonum	Polygonum
Antipodal cells number	3	3	3	5-8

Subsequently archesporial cell did not undergo meiosis and differentiates directly into megaspore mother cell during growth (Fig. 3c). Therefore, the ovule is tenuinucellate type. It is important to note that megaspore mother cells never undergo meiotic division to form dyad cell, hence the functional megaspore is not initiated. The megasporogenesis of the double-flowered *J. mesnyi* was abortive after the stage of megaspore mother cells. At subsequent stages, owing to the megaspore mother cells being dissolved, the embryo sac formed a cavity (Fig. 3d-f). All these results in female sterility of the double-flowered *J. mesnyi*. This sterility may be caused by the abnormal structure of the flower (Baer and Kho 1971).

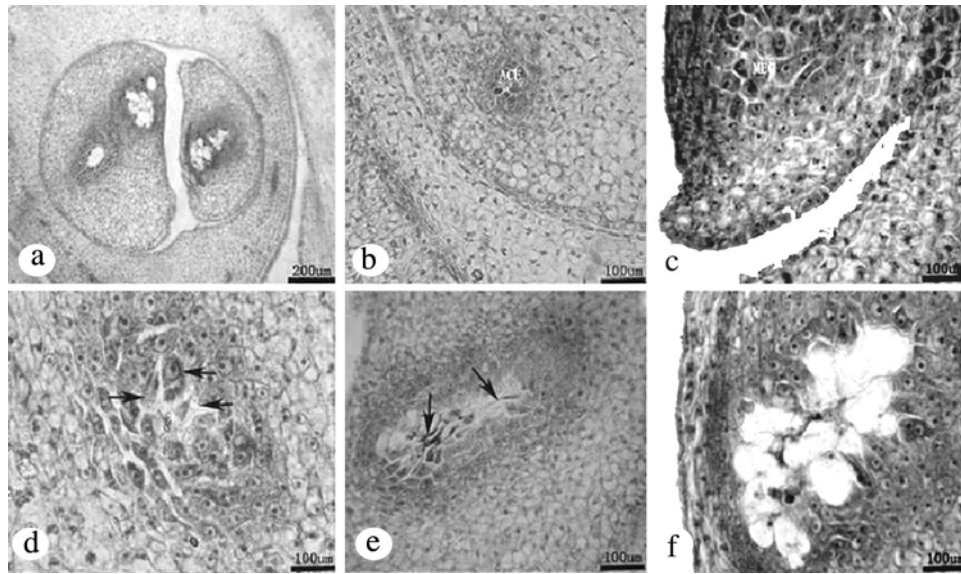


Fig. 3. Megasporogenesis abortion of double-flowered *Jasminum mesnyi*. a: ovule in apocarpous ovary, b: archesporial cells, c: megaspore mother cells, d-f: megaspore mother cells dissolve. ACE: archesporial cells, MEC: megaspore mother cells.

In breeding programs female sterility may play an important role in horticultural implications. In the last few years, female sterility has been regarded as a bad trait in breeding. Male sterility in plants has been widely recognized as a useful trait in breeding programs and in the commercial production of F_1 hybrid seed (Sawhney 1994). However, we consider that female sterility in double-flowered *J. mesnyi* is useful in horticultural breeding. As propagation of *J. mesnyi* is via cuttings, not seed propagation, so the female sterility could be used in horticultural breeding. Floral morphological characteristics is often representation of plant female sterility (Carapetian 1994). The double-flowered character *J. mesnyi* is the representation of its female sterility. The double-flowered subspecies of *J. mesnyi* is more beneficial and ornamental than single-flowered subspecies. If the representation is beneficial, female sterility can be useful in horticulture. Thus we consider that female sterility may be a useful trait in horticultural breeding if it results in beneficial traits.

From embryology of double-flowered *J. mesnyi*, we learnt that female sterility may be a useful trait in horticultural implications if it results in beneficial traits. The higher similarity of embryological features between Oleaceae and Scrophulariales support Oleaceae might belong to Scrophulariales, which supports the Croquist' (1981) and The APG III '(2009) viewpoint.

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