

A Study of Microsporangogenesis and Male Gametogenesis in *Camellia grijsii* Hamce

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Abstract: *Camellia grijsii* Hamce is used as a woody edible oil tree and wellknown for its commercial value in Southern China. The plants rarely set viable seeds and have little work on the reproduction biology. In order to verify whether there was any obstacle of male reproduction in the *C. grijsii*, microsporangogenesis and male gametogenesis in *C. grijsii* have been evaluated by paraffin section technique. The results are showed that the development of the anther wall belonged to a basic type and consisted of epidermis, endothecium, middle layers and tapetum. The tapetum conformed to the glandular type. Cytokinesis during meiosis of the microspore mother cell was simultaneous type. A majority of the microspores were arranged in tetrahedral tetrads. The mature pollen grains were 2-cell type and had three germ pores. Anthers were dehiscent and pollen grains shed on the early-February. Based our results, we did not find the abnormal male flower in the *C. grijsii*, suggesting that male gametes were fertile and male sterility was not the major cause of the low seed set in the *C. grijsii*.

Keywords: *Camellia grijsii* Hamce, male gametophyte, microsporangogenesis

INTRODUCTION

Camellia grijsii Hamce belongs to the genus *Camellia*, which has been placed in the family of Theaceae (Su *et al.*, 2004). In China, this species is distributed in relatively narrow ranges of Hunan, Zhengjiang, Jiangxi, Guangdong, Guangxi and Yunnan province (Rui-Lin, 1988). *C. grijsii* is the evergreen shrub species and is listed as the national second-grade protected plant species (Rui-Lin, 1988). It is suitable growth in the temperate-continental regions from sea level to 286 m, where the annual average temperature is 17-18°C and the average rainfall is about 1420 mm (Xiao and Lin, 1986). Flowering occurs from February to April and fruiting from September to October. It is an important non-wood forest for commercial tea oil production from their seeds (Qiu-fa *et al.*, 2008). As cooking oil, it compares favorably with olive oil. In addition, it is a very excellent breeding germplasm resources with resistance to anthracnose (Su-lan and Hu, 1981). However, this species has a very low seed rate limits its propagation.

The research of male reproductive processes of the plant is considered as the key factors responsible for generating seeds (María *et al.*, 2011). Thus, the development process of male gametophytes play a

prominent role in contributing to population maintenance and regeneration of important species (Yan *et al.*, 2010). In recent years the embryology of *Camellia* species has been received considerable attention (Tomo *et al.*, 1958; Wu, 1960; Kapil and Sethi, 1963; Hui-Juan, 1965; Sethi, 1965; Kato and Simura, 1970; Mathew, 1978; Cheng-Yun and Zhang, 1983; Liu *et al.*, 1983; Tian-Qing and Cao, 1986a, b; Chang-Jun and Zhen-heng, 1987; Yu-Ying and Yue-long, 1989; Tavares, 1995; Tsou, 1997; Shi-Xiong *et al.*, 2002; Cheng-Qi, 2004; Xiao-Ying *et al.*, 2010; De-Yi *et al.*, 2011; Ling-Feng, 2011; Ariyaratna *et al.*, 2011). Unfortunately, there was no concomitant description on anatomic characterization of this species. In general, many of the cultivated varieties are sterile or produce only a very few viable seeds, one of the possible causes being the morphological abnormalities observed in flowers. Therefore, detailed studies of the species' reproductive biology are necessary.

In this study, we aim at increasing our basic understanding of male gametophyte development in the *C. grijsii* by microscopy. Knowledge of reproductive biology is not only providing basic information in relation to the seed production, but also could be helpful in cross-breeding research.

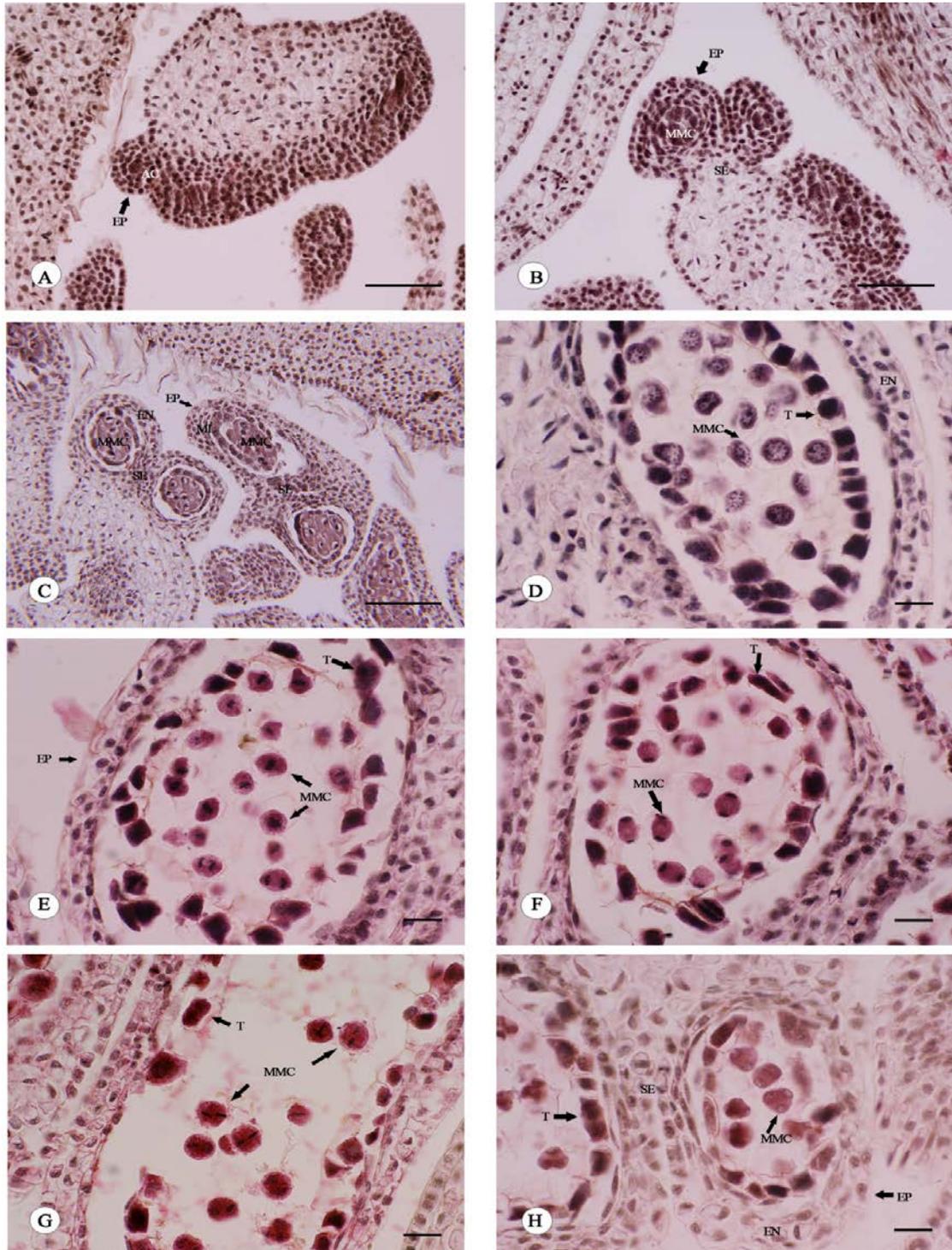


Fig. 1: Formation of microspores and development of male gametophyte in *Camellia grijsii* A. The archesporial cells appeared at the corners of the young anther; B, Detail of an early microspore mother cell wall showing the microspore mother cell coat and thin layer of callose (epidermis layer, endothecium, two middle layers and inner tapetum); C, Anthers showing microspore mother cell before meiosis; D, Detail of a late microspore mother cell wall at meiosis I prophase; E, Detail of a late microspore mother cell wall at meiosis I metaphase; F, Detail of a late microspore mother cell wall at meiosis telophase; G, Detail of a late microspore mother cell wall at meiosis II metaphase; H, Detail of a late microspore mother cell wall at meiosis II telophase. AC = Archesporial Cell; EN = endothecium; EP = epidermis; ML = Middle Layers; MMC = Microspore Mother Cell; SE = septum; T = Tapetum. Scale bars: A, B and C = 200 μ m; D, E, F, G, and H = 50 μ m

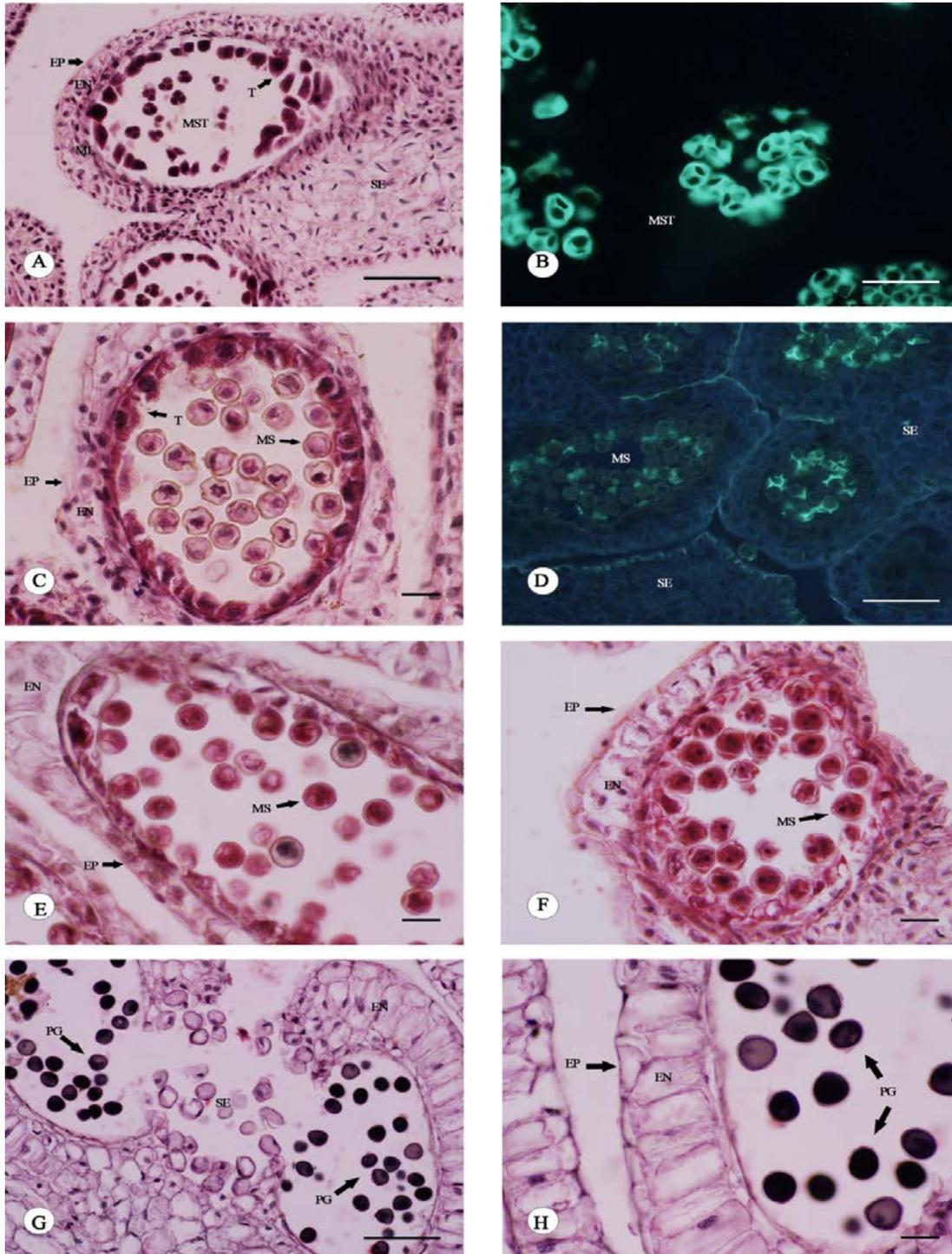


Fig. 2: Formation of microspores and development of male gametophyte in *Camellia grijsii* A, Details of microspores showing microspore terrads were tetrahedral and enclosed in callose; B, Details of anther showing microspore terrads were enclosed in the callose layer under the fluorescence microscope; C, Details of anther showing at microspores cell stage; D, Gradually dissolution of the callose walls surrounding the microsporogenous cells under the fluorescence microscope; E, Anthers at uninucleate microspore stage with stretched epidermis, fibrous thickened endothecium, relic of middle layers, degenerated tapetum; F, Anthers at binucleate microspore stage; G, Details of anthesis showing anther wall was composed of epidermis, endothecium and mature pollen grains. H; High magnification of G showing epidermis, endothecium and mature pollen grains; EN = endothecium; EP = epidermis; ML = Middle Layers; MMC = Microspore Mother Cell; MS = microspore; SE = septum; T = Tapetum; MST = Microspore Tetrad; PG = Pollen Grains; Scale bars: A, B, D and G = 200 μ m; C, E, F and H = 50 μ m

MATERIALS AND METHODS

Floral buds of *C. grijsii* at different developmental stages were collected every week from 2008 to 2011. The material was collected from *Camellia* orchard in Central South University of Forestry and Technology, Changsha city, Hunan province (28°11'49"N, 112°58'42"E). The samples were fixed in FAA (70% ethyl alcohol: formalin: glacial acetic acid = 90: 5: 5 v/v) and stored at 4°C prior to sectioning (Hsu *et al.*, 2002). The material was dehydrated in an ethyl alcohol series, embedded in paraffin with a 58-60°C melting point. Sections were cut to a thickness of 10 µm and then stained with haematoxylin-eosin Y (De-Yi *et al.*, 2011). Observation and photomicroscopy of sections were carried out using an Olympus BX-51 microscope. Some sections were stained with 0.5% decolorized aniline blue in 0.1 M K₃PO₄ to investigate callose deposition during the microspore processes by BX-51 fluorescence microscope (Akiko and Hiroshi, 2005).

RESULTS

Anther wall development: The archesporial cells appeared at the corners of the young anther (Fig. 1A). The archesporial cells divided by periclinal divisions to form an outer layer of primary parietal cells and inner primary sporogenous cells (Fig. 1B). The outer secondary parietal layer developed directly into an endothecium, whereas the inner produces two middle layer and a tapetum (Fig. 1B). From the section, we saw that the anther wall was composed of four cell layers, including an epidermis, two middle layer and a tapetum (Fig. 1C). Therefore, the anther wall formation conformed to the basic type (Davis, 1966).

As the microspore mother cells began to undergo meiotic division, the endothelial cells enlarged gradually and acquired fibrous thickening at the time of anthesis (Fig. 2G and H). At the tetrad stage, tapetal cells elongated and lost close contact but still remained in their original position (Fig. 2A and B). They began to degenerate at the stage of uninucleate microspore and disintegrate at the binucleate pollen stage (Fig. 2F). Thus, the tapetal was of the glandular type as defined by Davis (1966).

The middle layers had a common histogenetic origin with the endothecium and it persisted until the tetrad stage and degenerated before forming two-celled pollen grains (Fig. 2F). As a result, the mature anther wall was constituted of an epidermis and an endothecium (Fig. 2G and H).

Microsporogenesis and microgametogenesis: A row of sporogenous cells deriving from archesporial cells, gave rise to a mass of microspore mother cells by several mitotic divisions (Fig. 1B). Microsporocytes were recognizable by their large volume, dense

cytoplasm and conspicuous nuclei (Fig. 1C). The microsporocyte underwent meiosis which involved two cell divisions. Meiosis I includes the prophase (Fig. 1D), metaphase (Fig. 1E), anaphase (Fig. 1F) and telophase. After the first meiotic division of the microspore mother cell, a cell wall developed and a dyad was formed. A microspore tetrad was formed after meiosis II, such as metaphase II (Fig. 1G) and telophase II (Fig. 1H). Most of the microspores were arranged in tetrahedral tetrads (Fig. 2A). Callose deposition occurred at the onset of meiosis of pollen mother cells, reached a peak at metaphase II or anaphase II by enveloping the pollen mother cells or microtetrads (Fig. 2B) and disappeared at end of meiosis (Fig. 2D). Instead, simultaneous cytokinesis took place at the end of meiosis II of pollen mother cells.

Subsequently, the microspores were separated from the tetrads (Fig. 2E). They had a dense cytoplasm, conspicuous wall, inconspicuous vacuoles and a prominent central nucleus. During further development, uninucleate microspores gradually increased their volumes and had more vacuoles (Fig. 2C). The microspore soon experienced mitosis and divided unequally, resulting in two nuclei, which contained a large vegetative and a small generative cell (Fig. 2F). When shed on the early- February, the pollen grains were two-celled and tricolpate (Fig. 2H).

DISCUSSION

The method of embryogenesis in *C. grijsii* is very similar to those reported for other members of the *Camellia* in terms of the glandular tapetum, simultaneous occurrence of microsporocyte meiosis, two-celled pollen grains (Kapil and Sethi, 1963; Mathew, 1978; Cheng-Yun and Zhang, 1983; Liu *et al.*, 1983; Tian-Qing and Cao, 1986a; Yu-Ying and Yue-long 1989; Tsou, 1997; Shi-Xiong *et al.*, 2002; Cheng-Qi, 2004; Xiao-Ying *et al.*, 2010; De-Yi *et al.*, 2011; Ling-Feng, 2011; Ariyaratna *et al.*, 2011). The embryological characters in *Camellia* provide clearer factual basis for phylogenetic inferences of angiosperms.

Notably, the tapetum was of the glandular type, a feature also described from previous observations in *Camellia* (Kapil and Sethi, 1963; Mathew, 1978; Cheng-Yun and Zhang, 1983; Liu *et al.*, 1983; Tian-Qing and Cao, 1986a; Yu-Ying and Yue-long, 1989; Tsou, 1997; Shi-Xiong *et al.*, 2002; Cheng-Qi, 2004; Xiao-Ying *et al.*, 2010; De-Yi *et al.*, 2011; Ling-Feng, 2011; Ariyaratna *et al.*, 2011). At about the time of pollen tetrads, the walls of the tapetal cells became indistinct and the tapetal cells degenerated at their original site. The tapetal cells began to degenerate at the stage of one-nucleate pollen grains and the nucleus near the wall. The callose cycling (or cellulose synthesis) during microsporogenesis might play important roles in protecting the pollen mother cells from various

environmental stresses and release of microspores from the microtetrads (Scott *et al.*, 2004). In view of this its structure and function, this kind of tapetum is considered to be more evolutionally advanced than the normal glandular type (Yan *et al.*, 2010). Therefore, the tapetum structure might be one of the important characters at the population level in *C. grijsii*.

Male sterility might be an important factor influencing seed production (Yong-Zhong *et al.*, 2011). Male sterility in plants implied an inability to produce or to release functional pollen and is the result of failure of formation or development of functional stamens, microspores or gametes (Yan *et al.*, 2010). There were some instances of failure to develop normal pollen, probably caused by the abnormal development at three levels: the sporogenous tissue, tapetum layer and microspore (Kaul, 1988). Tapetum's main functions include producing pollen wall components, nutrients for pollen development and enzymes for microspore release from tetrads (Robert *et al.*, 1993). Male sterility are usually accompanied by abnormal callose deposition or anomalies in tapetum (Kaul, 1988). However, in our anatomical investigation, we did not find the abnormal callose deposition and anomalies in tapetum during microsporogenesis in the *C. grijsii*, indicating that male gametes were fertile and male sterility was not the major cause of the low seed set in the *C. grijsii*.

CONCLUSION

This study provided basic information on male reproduction aspects of *C. grijsii* and could shed light on the embryogenesis in this species. In *C. grijsii*, the microgametogenesis results in binucleate pollen as has been observed in other genera in *Camellia*. We did not find the abnormal male flower in the *C. grijsii*, suggesting that male gametes were fertile.

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