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Megasporogensis and female gametophyte development of *Camellia grijsii* Hance

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ABSTRACT

Camellia grijsii Hance is used as a woody edible oil tree in the South of China. The oil contains tea polyphenols and camellia glucoside, which can effectively improve cardiovascular and cerebrovascular disease, lower cholesterol and glucose. However, it is particularly prone to erratic fruit set showing very low and have little work on the reproduction biology. In order to verify whether there was any obstacle of sexual reproduction in C. grijsii, the megasporogensis and development of the female gametophyte of C. grijsii were observed by a paraffin section technique. The results are showed that the development of embryo sac conformed to Allium type. Archespore under the nucellar epidermis directly developed a megaspore mother cell. The megaspore tetrad were arranged into a T shape, the megaspore of chalazal end was functional megaspore. The functional megaspore formed 7-celled or 8-nucleate embryo sac following its three times division successively. The ovules were anatropous, bitegminous and tenuinucellate. Abnormal embryo sacs or abortive ovules were observed in the ovary. Based on our results, the abortive rate of the examined ovules was 37.9%, which were likely the cause of the low seed set in C. grijsii.

Key words: *Camellia grijsii* Hance; female gametophyte; megasporogensis; abortive ovule

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INTRODUCTION

Camellia grijsii Hance belongs to the genus *Camellia* (Theaceae) which contains about 119 species widely distributed in tropical and subtropical regions of East and Southeastern Asia [1-2]. *C. grijsii* was firstly found in Youxian County, Hunan province, China. *C. grijsii* is the evergreen shrub species and is listed as the national second-grade protected plant species in China [3]. These endangered trees are scattered across a narrow range from 25°45′-27°32′N and 112°30′-113°30′E, and from sea level to 286m, where the annual average temperature is 17-18℃, and the average rainfall is about 1420mm [4]. It is an important non-wood forest tree for commercial tea oil production from their seeds [5]. As cooking oil, it compares favorably with olive oil and has health therapeutic value. In addition, it is used in the manufacture of soap, margarine, lubricants, hair oil, paint, rustproof oil and other compounds with a high-molecular weight as well as in cosmetology and dermopharmacy [6]. This plants mainly propagate by seed. However, this species has a very low seed reproduction limits its propagation.

The research of reproductive biology of the plant is considered as the key factors responsible for generating seeds [7]. The development process of male and female gametophyte play a prominent role in contributing to population maintenance and regeneration of important species [8]. In recent years considerable attention has been paid to the embryology of *Camellia* species [9-25]. Unfortunately, the causes of the low seed set of sexual reproduction in this species were still unclear, largely because many aspects of the reproductive processes, especially the female reproductive development remain elusive. Therefore, detailed studies of the species' embryology are necessary.

In this paper, we aims at increasing our basic understanding of the megasporogensis and female gametopyte development in the *C. grijsii* by a paraffin section technique. Knowledge of reproductive biology is not only providing embryological information for the low fruit set, but also important in the conservation and systematic analysis of this family.

EXPERIMENTAL SECTION

2.1 Sampling

Seedlings of *C. grijsii* grown in the Central South University of Forestry and Technology, Changsha city, Hunan province (28°11′49″N, 112°58′42″E), approximately 70 m above sea level were used in this study. This site is located in a typical subtropics moist climate, with a mean annual precipitation of 1392 mm and a mean annual temperature of 17.5°C. More than 10 floral buds or flowers of *C. grijsii* at different developmental stages were collected each time every week from 2008 to 2011. All flower materials were obtained from three 10-yr-old trees selected for good yielding ability and being representative of the *C. grijsii* population from the *Camellia* orchard.

2.2 Fixation

Collection materials were fixed for 24h in FAA (formalin: glacial acetic acid: 70% ethyl alcohol=5: 5: 90, v/v), then transferred to 70% ethanol, and were stored at 4°C prior to sectioning [26].

2.3 Pre-infiltration, infiltration, and embedding

The material was dehydrated in an ethyl alcohol series, embedded in paraffin wax [26].

2.4 Sectioning

Sections were cut to a thickenss of 10μm by leica RM2265(Germany).

2.5 Staining

Sections were stained with haematoxylin-eosin Y [24].

2.6 Photography

Observation and photomicroscopy of sections were carried out using an Olympus BX-51 microscope (Japan). We also investigated the aborted ovules during the flowering season by microscopy. The abortive ovules was judged by the presence of darkly stain with shrunken embryo sac cells or empty embryo sac [27].

RESULTS AND DISCUSSION

3.1 Megasporogensis and Megagametogensis

In the tenuinucellate ovule, an archesporial cell functions directly as a megaspore mother cell (Figure 1, B). The megaspore tetrad were arranged into a T shape after meiosis (Figure 1, C). Three megaspores of the tetrad eventually degenerated, While the chalazal one became functional (Figure 1, D). The first division produced two megaspore nuclei, which, respectively, moving to each pole (Figure 1, E) and underwent the second meiotic division, giving rise to a four-nucleate female gametophyte (Figure 1, F-G). An additional division of these nuclei resulted in an eight-nucleate megagametophyte (Figure 1, H-J). Two polar nuclei (positioned close to each other) moved to the centre of the embryo sac, and a synergid cell was at the micropylar end (Figure 1, H). Two synergid cells and an egg cell that composed the egg apparatus were at the micropylar end (Figure 1, I). The three ephemeral antipodal cells were at the chalazal end (Figure 1, J). Thus, the mode of embryo sac formation was of the *Allium* type [28].

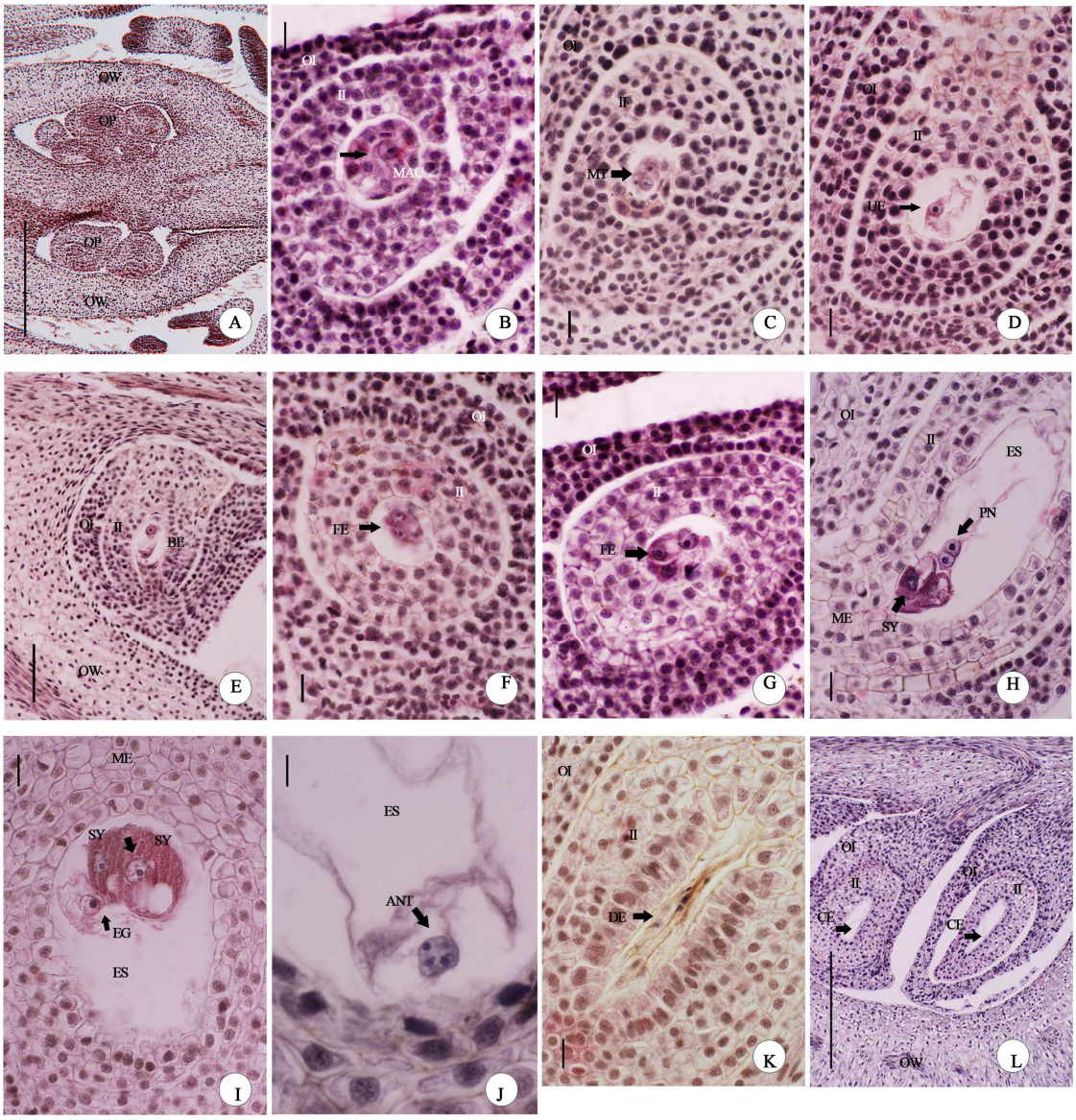
3.2 Ovule Development

The periclinal division of some cells under the placental epidermis led to the formation of ovule primordial (Figure 1, A). The inner integument was initiated from dermal cells at the base of the ovule primordial earlier than the outer one. The outer integument always grew more slowly than the inner (Figure 1, A). The inner integument soon enclosed the nucellus and formed the micropyle. As a hypodermal archesporial cell enlarged directly as a megaspore mother cell, the ovule becomes fully anatropous (Figure 1, B). Therefore, the ovule were anatripous, bitegmic and crassinucellate.

3.3 Abortive ovules during development

Abortion happened both before and after the development of the embryo sac. We examined about 37.9% abortive ovules in 2736 ovules. It was hard to discriminate between the normal and aborted ovules unless the larger embryo sac appeared in the ovules. The aborted ovules were darkly stained with shrunken embryo sac cells or cavity embryo sacs (Figure 1, K-L). Thus, It was reasonably to assume that these ovules were going to abort.

Figure 1 : Formation of megaspores and development of female gametophyte in *Camellia grijsii* A, Longitudinal section showing ovule primordium, septa and ovary wall. B, Longitudinal section showing megaspore mother cell. C, Longitudinal section showing a T shape tetrad of megaspores. D, Longitudinal section showing uninuclear embryo sac, outer integument and inner integument. E, Longitudinal section showing binuclear embryo sac, outer integument and inner integument. F-G, Longitudinal section showing four-nucleate embryo sac, outer integument and inner integument. H-J. Developmental stages of the mature embryo sac. H, Longitudinal section showing a synergid cell, two central polar nuclei, ovarian wall, inner integument and outer integument. I. Transverse section showing an egg cell, two synergid cell at the micropylar end. J, Enlarged embryo sac showing three antipodal cells at the chalazal end. K, Longitudinal section showing degenerated embryo sac. L, Longitudinal section showing cavity of embryo sac, ovarian wall, inner integument and outer integument



(II = inner integument; MAC = megaspore mother cell; OI = outer integument; OP = ovule primordial; OW = ovarian wall; SE = septum; ANT = antipodal cells; AO = abortive ovule; BE = binuclear embryo sac; CE = cavity embryo sac; DE = degenerate embryo sac; EG = egg cell; ES = embryo sac; FE = four-nucleate embryo sac; II = inner integument; L = locule; ME = micropylar end; MT = megaspore tetrad; OI = outer integument; OW = ovarian wall; PN = polar nucleus cells; SY = synergid; UE = uninuclear embryo sac). Scale bars: A and L = 400µm; B, C, D, F, G, H, I and K = 50µm; E = 200µm; J = 20µm.

The method of embryogenesis in *C. grijsii* was the same as those reported for other species of *Camellia* in terms of the tenuinucellate, unitegmental, and anatropous ovules, and the *Allium*-type embryo sac [11,13-14,16,18,20-25]. However, In other *Camellia* species, the development of embryo sac was of the *Adoxa* [29] or *Polygonum* type [17]. Thus, the embryological characters in *Camellia* provide clearer factual basis for phylogenetic inferences of angiosperms.

It is generally known that the normal ovule development is important for high fruit set in seed orchards [30]. Each ovule might be fertilized and be capable of producing a seed. It was proposed that all of the ovules that develop a

normal embryo sac are potential seeds [27,31]. In our experiment, although most of the female gametophyte developed normal, some ovules were abortive in *C. grijsii*. The observed anomalies in *Camellia* species also were consistent with previous observations [12,15-16,23]. To our knowledge, the ovule is the source of the megagametophyte and the progenitor of the seed [32]. Poor fruit set has been attributed to an undesirable environmental conditions or male sterility or female sterility [7,33]. However, in our study, we found *C. grijsii* not only grew under a good hydrothermal condition in orchards, but also exhibited male fertility. We examined about 37.9% abortive ovules in 2736 ovules at flowering stage. Cao Hui-juan [12], Li Tian-qing et al. [16] and Luo Xiao-ying et al. [23] also observed the abortion rate of ovules were 75.0%, 11.0%, 39.4%, respectively. The cause of female sterility appears to be due to postzygotic seed abortion. Essentially, abnormalities in embryo development lead to abortion and resultant female sterility [34]. Female sterility leads to low seed set in many seed plants [35]. Thus, our observations suggest that the low fruit set in *C. grijsii* could be partially explained by abortive ovules in the female gametophyte development [12,15-16,23], and the high percentage of abortive ovules were identified as the major factor causing female sterility and possiblely influenced the seed production in *C. grijsii* orchards. But further studies should be performed to delineate whether the abortive ovules were caused by resources limitation or fertilization failure in the future.

CONCLUSION

This study provided basic information on the sexual reproduction aspects of *C. grijsii* and could shed light on the embryogenesis in this species. In *C. grijsii* the macrogametogenesis belongs to the *Allium* type. Abnormal embryo sacs or abortive ovules were observed in the ovary, suggesting that the same cellular mechanisms might act in somatic as well as in embryo rescue and embryogenesis induction [19]. A large number of abortive ovules may influence yield in *C. grijsii*, and further studies are needed to corroborate these results.

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