Effects of Carbon Source and Temperature Treatments on Pollen Germination of *Camellia lawii*

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Abstract

The effects of carbon source in the medium and temperature treatments on the germination of pollen from *Camellia lawii* were determined. The overall aim of this research is to provide technical support for cross breeding of *Camellia* species. The effects of different carbon sources (sucrose, lactose, glucose, maltose, and sorbitol) at different concentrations (0.146–0.876 mol/L) in the medium, low temperature (5℃–20℃) or high temperature (30℃-35℃) treatments, and low temperature storage (at −80℃) on pollen germination on culture medium were determined.
Key words: *Camellia lawii*; pollen; viability; storage capacity

INTRODUCTION

Camellia lawii is in the *Camellia* group (family Theaceae), and originated in the Jinyun Mountains of Beipei, Sichuan, China. It is a shrub with brown pubescent young branches, leathery oval-shaped leaves with a hairy petiole, and flowers that are produced at the apex and axils during a 2–3 month flowering phase. The flowers have a white corolla, five petals, and a silky ovary (Zhang and Ren, 1998). It can be used for flower mirrors and flower beds, and has high ornamental and garden application value. There are 14 species in the *C. lawii* group (Sect. Eriandria Coh. St.). They are mainly distributed in China, and the distribution of some species extends to the south central peninsula and the south Himalayan slope.

Thus, this group of plants has high breeding value. However, although *C. lawii* has been introduced and domesticated, there are few reports on its cross breeding, indicating that it has not been systematically used in the artificial breeding of camellias. It would be advantageous to use *C.lawii* resources to crossbreed with other *Camellia* species to improve traditional camellias, and create new varieties with unique flower appearance.

Previous studies have focused on the pollen culture and low temperature storage of certain *Camellia* plants, such as those in Sect. Thea (L.) Dyer in Hook., Sect. Camellia (L.) Dyer in Hook., Sect. Oleifera Chang Tax., Sect. Theopsis Coh. St. in Meded., and St. Chrysan Chang, but none of those studies included members of Sect. Eriandria Coh. St.

In this study, the effects of five common carbon sources and their concentrations on the germination of *C.lawii* pollen were analyzed. According to the characteristics of diurnal temperature differences in winter and spring in South China, the low temperatures at night (5 ℃–25 ℃) or high temperatures at noon (30 ℃–35 ℃) were simulated. We also determined the effects

of long-term low temperature (−80 ℃) storage on pollen viability.

The overall aim of these analyses was to establish methods for the determination of pollen viability, and the storage and treatment of *C.lawii* pollen for optimum germination. These results provide technical support for the future development of interspecific hybridization of *Camellia* using Sect. Eriandria Coh. St. plants, and will allow breeders to use stored pollen rather than relying on plants at the flowering phase.

1 Materials and Methods

1.1 Material

The experimental material was *C.lawii*, which was collected from the International Camellia Species Park in Jinhua, Zhejiang, in February 2019. The flower buds of *C.lawii*, which were about to open, were collected and placed in a dry place for 12 h at 25±1 ℃. When the flowers opened, pollen was released and collected. First, the anthers with loose pollen were gently removed with tweezers and placed on paper sheets, and then placed in a dry place for 24 h at 25±1 ℃. Then, the pollen and the anther shell were separated and placed in a 1.5-mL tube. The cap was tightened and the tube was stored at −80 ℃.

1.2 Determination of pollen viability

1.2.1 Determination of suitable carbon source for pollen germination

Five solid culture media (8 g/L agar, pH 5.5-6.0) containing 0.292 mol/L sucrose (100.0 g/L), maltose (105.2 g/L), glucose (52.6 g/L), lactose (100.0 g/L) and sorbitol (52.6 g/L) were dripped on glass slide, and fresh pollen was applied to the surface of culture medium with an aseptic toothpick. The glass slide was placed in a culture box with wet filter paper, and then the culture box was sealed in a plastic self-sealing bag and kept for 24 h at 25±1℃. Then, the pollen grains were observed under a microscope, photographed, and counted. The suitable carbon source for pollen germination was determined on the basis of the germination rate.

1.2.2 Determination of suitable sucrose concentration for pollen germination

Five sucrose concentrations were tested: 0, 0.146, 0.292, 0.584, 0.876 mol/L. All media contained 8 g/L agar and had a pH of 5.5–6.0. After 24 h of culture, pollen grains were photographed under a microscope and the germination rate was calculated.

1.2.3 Determination of the effects of low or high temperature on pollen germination

Pollen was subjected to temperatures based on low temperatures at night (5 ℃–20 ℃) and high temperatures at noon (25 ℃–35 ℃) in winter and spring in South China.

The experiment had two stages. In the first stage, pollen was exposed to 5, 10, 15, 20, 25, 30 or 35 (℃) for 6 h. In the second stage, the pollen was allowed to recover at 25±1 ℃ for 18 h before testing germination on culture medium containing 0.292 mol/L sucrose and 8 g/L agar (pH 5.5–6.0).

Each treatment was repeated three times, and more than 100 pollen grains were observed in each treatment. The criterion to judge pollination was that the pollen tube length was greater than the pollen grain diameter. The pollen germination rate per field of vision was calculated and the average value was taken as the pollen germination rate. The pollen germination rate (%) per field of vision was calculated as follows = (number of pollen grains germinated ÷ total number of

pollen grains) × 100.

1.3 Determination of pollen viability after low-temperature storage

Pollen stored at −80 ℃ was sampled after 0, 3, 6, 9, and 12 months to test viability by monitoring germination on culture medium containing 0.292 mol/L sucrose and 8 g/L agar (pH5.5–6.0).

1.4 Microscopy analyses

Pollen germination was observed under a ZEISS Upright Microscope (Axio Scope A1, Carl Zeiss, Jena, Germany). Axio Vision software was used to capture and save images.

1.5 Statistical analyses

Each experimental treatment was repeated three times, and the data were analyzed by SAS v. 8.02 software. Duncan's multiple comparison method was used to test the significance of differences.

2 Results and Analysis

2.1 Suitable carbon source

As shown in Fig. 1 and Fig. 2, the pollen germination rates were highest when sucrose or lactose was used as the carbon source (34.25% and 33.46%, respectively; no significant difference). The germination rates were lower on media containing glucose (24.07%); no sugars (15.21%); maltose (10.12%), and sorbitol (4.02%).

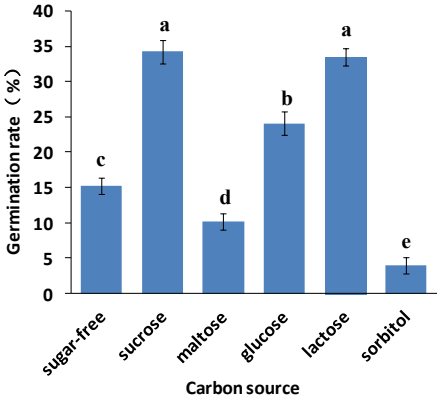
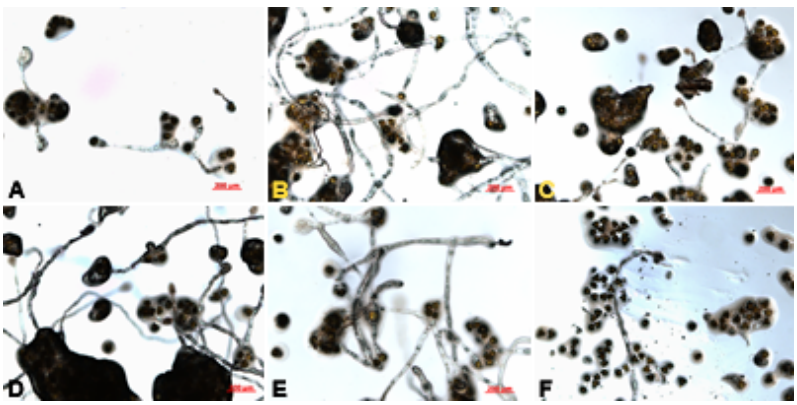


Fig.1 Effects of carbon source on germination of *Camellia lawii* pollen.

Different letters indicate significant difference ($P < 0.05$), the same below.



A. No sugars; B. Sucrose; C. Maltose; D. Glucose; E. Lactose; F. Sorbitol

Fig. 2 Germination of *Camellia lawii* pollen after 24 hours of culture on media containing different carbon sources.

2.2 Appropriate sugar concentration

As shown in Fig. 3 and Fig. 4, the highest pollen germination rate (34.25%) was on medium containing sucrose at a concentration of 0.292 mol/L. The pollen germination rates were lower on media containing higher or lower concentrations of sucrose: 0.146 mol/L (germination rate, 29.35%); 0.586 mol/L (16.46%); 0.876 mol/L, (3.40%).

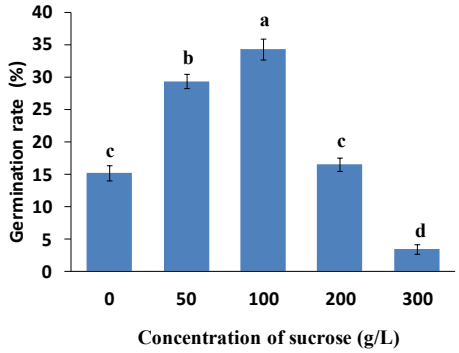


Fig. 3 Effect of sucrose concentration on germination of *Camellia lawii* pollen.

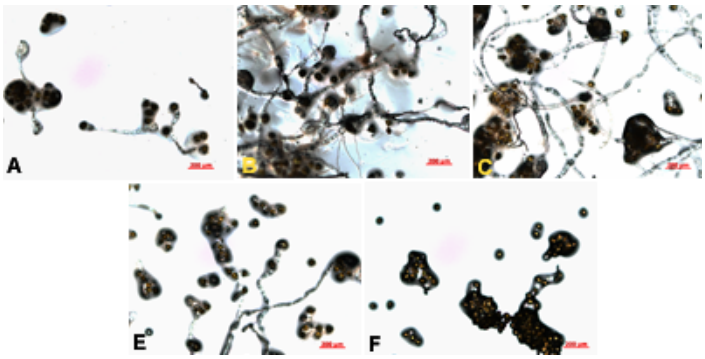


Fig. 4 Germination of *Camellia lawii* pollen on culture media containing different concentrations of sucrose.
A. No sucrose; B. 0.146 mol/L; C.0.292 mol/L; D.0.584 mol/L; E.0.876 mol/L

2.3 Effects of low or high temperature on pollen germination

To test pollen viability after exposure to high and low temperatures, the pollen was subjected to a temperature treatment for 6 h followed by a recovery period of 18 h at 25 ℃. As shown in Fig. 5 and Fig. 6, the pollen germination rates were 33.77%, 34.08%, 34.87%, and 34.25% after treatment at 5 ℃, 10 ℃, 15 ℃, and 25 ℃, respectively. These values were not significantly different. However, the pollen germination rate decreased significantly after 6 h of heat treatment at 30 ℃ (26.48%) and 35 ℃ (1.78%).

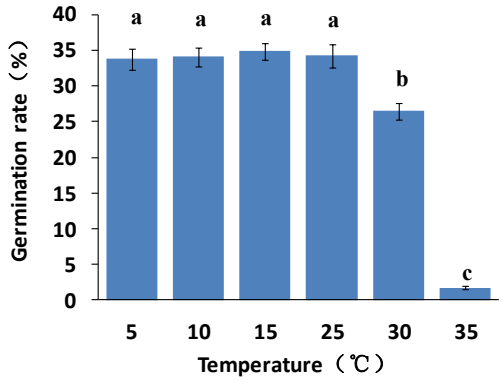


Fig. 5 Effect of temperature on germination of *Camellia lawii* pollen.

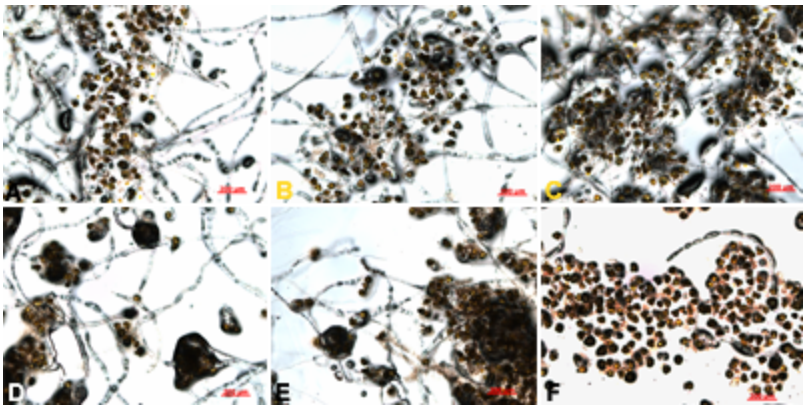


Fig. 6 Germination of *Camellia lawii* pollen after culture at different temperatures.

A. 5 ℃ culture; B. 10 ℃ culture; C. 15 ℃ culture; D. 25 ℃ culture; E. 30 ℃ culture; F. 35 ℃ culture;

2.4 Determination of pollen viability after storage

The results of a pollen germination test after storage at −80 ℃ are shown in Fig. 7. The germination rate of pollen before preservation was 34.25%. After storage for 3, 6, 9, and 12 months, the germination rates were 33.84%, 34.14%, 33.92% and 33.51%, respectively. These values were not significantly different from that of fresh pollen.

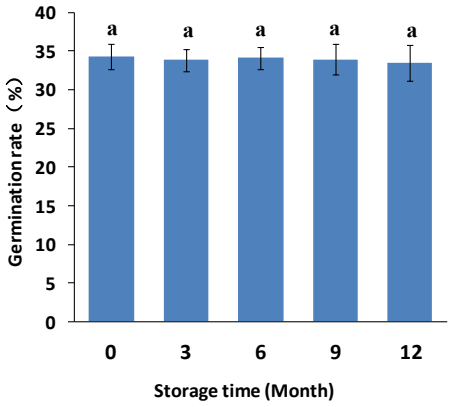


Fig. 7 Germination rates of *Camellia lawii* after storage at −80 °C

3 Conclusion

The results showed that sucrose and lactose were suitable carbon sources for pollen culture of *C.lawii*. High temperature (≥30 ℃) was not conducive to pollen germination. Low temperature storage (−80 ℃) could maintain pollen viability for 1 year.

4 Discussion

Pollen requires a carbon source for germination. Some carbohydrates, such as starch, are stored in pollen, and can promote pollen germination under suitable conditions. Exogenous sugars affect the pollen tube as it elongates. Sucrose is a widely used carbon source in pollen culture. The results of this study showed that both sucrose and lactose could promote pollen germination of *C.lawii,* and their promoting effects were similar. The other sugar sources could be ranked, from strongest ability to promote pollen germination to weakest, as follows: glucose > no sugars > maltose > sorbitol.

Some previous studies have reported similar results. For example, sucrose was more effective than fructose or mannitol for promoting the germination of cherry pollen. For wild tarragon pollen, sucrose was better than maltose and glucose. However, some studies obtained different results. For example, maltose was superior to sucrose as the carbon source for tea pollen germination, and glucose was superior to sucrose for peach pollen germination. For pollen of *Lonicera japonica*, lactose was superior to glucose, sucrose, and fructose as the carbon source for germination.

Therefore, to culture the pollen of a given species, it is necessary to determine the appropriate carbon source experimentally. Notably, the pollen germination rate on 0.292 mol/L maltose or sorbitol medium was lower than that on sugar-free medium, indicating that these two sugars not only did not promote pollen germination, they seriously inhibited it.

Sugars not only provide energy for pollen germination and pollen tube growth, but also maintain the osmotic pressure balance inside and outside cells at appropriate concentrations. Previous studies have shown that the suitable concentration of sucrose for *in vitro* pollen germination of *Camellia* species is 0.146–0.586 mol/L. In our study, the optimal concentration of sucrose for pollen germination *in vitro* was 0.292 mol/L. Therefore, for each *Camellia* species, it is important to determine the suitable concentration of sucrose for pollen germination by conducting appropriate experiments.

Temperature is a critical factor in pollen germination. For many plants, the optimum temperature for pollen germination is 20–28 ℃ , but it can be as high as 30 ℃ or as low as 10 ℃. Temperatures outside the optimum temperature range will inhibit pollen germination to varying degrees. In this study, we tested temperatures based on those in winter and spring in South China.

To test the effects of temperatures within this range on the germination of *C.lawii* pollen, a two-stage experiment was conducted. In the first stage, pollen was exposed to seven different temperatures for 6 hours. In the second stage, the pollen was allowed to recover at 25 ℃ for 18 h before evaluating the germination rate. The results showed that temperatures of 5 ℃–25 ℃ did not affect the germination rate, but those of 30 ℃–35 ℃ had severe negative effects on pollen

germination. These results suggest that low temperatures at night will not affect pollen germination when *Camellia* hybrid pollination is conducted in winter and spring in South China, but the effect of high temperature at noon should be fully considered.

Pollen preservation is usually divided into cold storage (4 ℃ to 5 ℃), low-temperature storage (−80 ℃ to −20 ℃), and ultra-low- temperature storage (−196 ℃ in liquid nitrogen) methods. In general, cold storage can maintain pollen viability for a short time; low-temperature preservation can maintain pollen viability for a long time; and ultra-low-temperature preservation can maintain pollen viability for a long time, but the cost is high. In this study, the pollen germination rate of *C.lawii* remained above 30% after storage at −80 ℃ for 1 year. The ability to store pollen for a long time without a reduced viability means that breeders do not have to wait to obtain flowering plants for cross-pollination. This will facilitate the generation of new *Camellia* varieties with desirable characteristics.

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