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# EFFECTS OF LOW TEMPERATURE IN WINTER ON THE GERMINATION OF *CAMELLIA JAPONICA* SEEDS

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*Keywords*: *Camellia japonica*, Seeds, Winter temperature, Germination characteristics

**Abstract**

The effects of temperature on seed germination of Naidong *Camellia* species were observed under the simulated natural winter temperature. The results show that the seeds of Naidong *Camellia* also got the characteristic of winter dormancy to adapt to the winter low temperature, and the effective chilling temperature of releasing the dormancy is from 10 to 15°C, the suitable temperature range for seed germination in Naidong is 10 - 20°C, the most suitable germination temperature is 15°C, and this winter dormancy should belong to the southern winter dormancy. More than 50% of the seeds could bear under 10°C low temperature in winter and live through the winter.

## Introduction

From low- to high latitudes, the temperature gets lower and lower in winter, and the cold temperature and the drying restrict the distribution of many tree species. Evergreen broad-leaved species is the typical subtropical zone vegetation, which is generally and rarely distributed in temperate and warm temperate zone. The species located in the north temperate zone resist the cold and dry winter through winter dormancy, ensuring timely germination in spring. Dormancy is a kind of ecological adaptation for the perennial trees to the winter cold and dry climate, including the seed dormancy and bud dormancy (Vegis 1964, Roseann *et al.* 1983). The seeds of *Idesia polycarpa* and *Paulownia tomentosa* (Thunb.) Steud, which are deciduous broad leaved tree species distributed across the tropical and warm temperate zone, have dormancy characteristics to adapt to cold winter. However, the evergreen broad leaved tree species *Machilus thunbergii*, which is distributed in the same zone, has dormancy and no-dormancy ecotypes, one distributed in subtropical zone has no winter dormancy characteristic and the other distributed in warm temperate zone has shallow winter dormancy characteristics (Liu *et al.* 2000, Liu *et al.* 1999, Nagata *et al.* 1994, Nagata *et al.* 1990). *Camellia japonica*, also known as *Camellia* or *Naidong*, is theaceae plant, such as *Camellia* sect. nitidissima Chang is a subgroup occurring in a narrow range between 20°32' and 23°53'N and 104°00' and 108°56'E, and grows along shady and moist valleys in evergreen, broadleaf forests at altitudes of 50 - 650 m in south-west China and north Vietnam (The Editiorial Committee of Chinese Academy of Sciences China Flora. 1998). *Camellia* forms flowers 5 - 10 cm wide, golden-yellow in color, and it is honored as "the Queen of *Camellia*" and "the Giant Panda of the Plant Kingdom" (Liang 1993). In recent years this golden-yellow *Camellia* has received increasing attention due to its polyphenolic antioxidants and free radical scavenging activity (Song *et al.* 2011, Wan *et al.* 2011), therefore it has a high ornamental value. Natural distribution scope of *Camellia* is only in subtropical region which is not too cold in winter and can release dormancy in low temperature, because leaf bud has a characteristic of winter dormancy, but the flower bud does not have the characteristic. Also due to this, cultivation scope has expanded to warm temperate regions (Nagata *et al.* 1983). *Camellia* distributes in warm temperate zone of Laoshan Mountain in Qingdao, Shangdong province which

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sustains natural regeneration. In natural environments, *C. nitidissima* is able to produce only by seed (Yang *et al.* 2008). Due to increasing anthropogenic pressure, deforestation and destructive collection of seedlings, its natural population size has declined greatly in recent decades, and it is now classified as a first grade endangered plant species in China (Xing 2005). The genetic diversity and relevant endangered mechanisms of this plant have been extensively studied (Wei *et al.* 2008,Wei *et al.* 2008, Tang and Bin, 2006, Chen *et al.* 2010, Wei *et al.* 2010). In view of this, we need to use *Camellia* seed for plant breeding effectively. Do the *Camellia* seeds distribute in Qingdao get the characteristic of the winter dormancy? How to adapt to the local cold in winter? For this purpose, to study the effect of low temperature in winter on the germination of seeds in *Camellia japonica* has the important theoretical and practical significance. Yang reported that seed germination rate was more than 85% after warm water 25°C, and germination rate was between 20 and 70% under 10 to 20°C (Yang *et al.* 2011). Li found that whether through 5°C low temperature processing or not, it is difficult to germinate under 25°C (Li 2013). However, the result is inconsistent with Yang *et al*. The influence of low temperature on seed germination is rarely reported, and the characteristics of *Camellia* seeds' adaptation to low temperature in winter of Qingdao are unclear. Therefore, this article discusses the effect of low temperature in winter on the germination of seeds in *Camellia japonica*.

## Materials and Methods

The test materials *Camellia japonica* seeds were collected from Qingdao botanical garden on October 20, 2013. After stripping out the seeds from the fruits, and removing impurities, empty grain and the pest seeds, the full solid particles were collected in a paper bag, ready for the test. The ten-day average temperature change in autumn, winter and spring of the place where the seeds were collected is shown in Fig. 1.

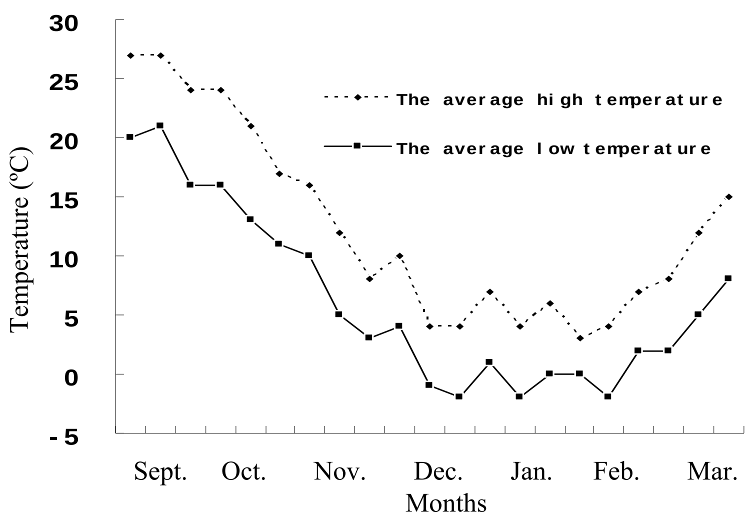


Fig.1. Change of mean air temperature during 10 days at Qingdao from September, 2013 to March, 2014.

The seeds were soaked and disinfected by using potassium permanganate of 0.5% concentration for 2 hrs treatment on October 29, 2013 and then were rinsed clean with sterile water and put in Petri dishes which have already put in cotton wool and filter paper, with a moderate amount of distilled water in 121°C high pressure sterilization for half an hour. Ten seeds were put in every Petri dish, and the treatment was repeated for three times. The Petri dishes were filled with seeds in 5, 10, 15, 20 and 25°C full exposure incubator, respectively, observed once every 7 days, and then picked out the moldy seeds and rinsed with sterile water. To observe the germination situation, respectively and terminate the experiment until there was no germination after 133 days, then calculation was done to determinate the germination rate. Second, the wet seeds were put in Petri dishes in 10°C low temperature processing, respectively 0, 20, 40, 60 and then put them into 20 and 25°C and observed the germination and calculated as the above.

*The calculation methods of indicators:*

Germination rate (%) = Germination number/ the tested number of seed*100%

Germination potential (%) = Germination number of peak-hour/the tested number of seed*100%

The average germination time = ∑D*n/∑n

D - the number of days since seed set bed, n - germination number of each day accordingly.

## Results and Discussion

The germination rate, germination potential and average germination time under different germination temperatures of *Camellia* seeds are shown in Table 1. From Table 1 it can be seen that *Camellia* seed germination rate is below 40% under 5 and 25°C, germination rate can reach above 87% under 10 - 20°C (Table 1). The germination rate and the germination potential are the largest under 15°C, reaching 97 and 73%, respectively and the average germination time is 48 days, under 15°C. In addition to germination rate and average germination time are not significantly different with 20°C, differences with the germination potential of temperature 20°C and the germination potential, germination rate and the average germination time under other temperatures all reach significant level. This result suggests that 15 °C is the most suitable temperature for *Camellia* seed germination.

**Table 1. Effects of temperature on seed germination.**

| Sl. No. | Treatment temperature (°C) | Germination rate (%) | Germination potential (%) | The average germination time (days) |
|---|---|---|---|---|
| 1 | 5 | 20cdC | 17Cc | 102Aa |
| 2 | 10 | 87bB | 60Bb | 90Bb |
| 3 | 15 | 97aA | 73Aa | 48Cc |
| 4 | 20 | 90aA | 57Bb | 48Cc |
| 5 | 25 | 40cC | 20Cc | 28Dd |

In the same row, values with different small letters mean significant difference ($p < 0.5$).

As can be seen from the Fig. 2, after 5°C low temperature processing *Camellia* seed germination quickens significantly in 20°C continuous light conditions. After 20 days of 5°C low temperature processing, the difference of the *Camellia* seed germination rate between with and without low temperature treatment is not significant, and the germination rate is above 80%. But after 40 and 60 days treatment in 5°C low temperature, the germination rate is significantly lower, and the highest germination rate can only reach more than 50%, which is significantly lower than the germination rate with 0 and 20 days of 5°C low temperature processing. Fig. 3 illustrates that the seed germination speed is slightly faster after 5ºC low temperature processing, but not significant, and the final germination rate only reaches 40% and the difference is not significant.

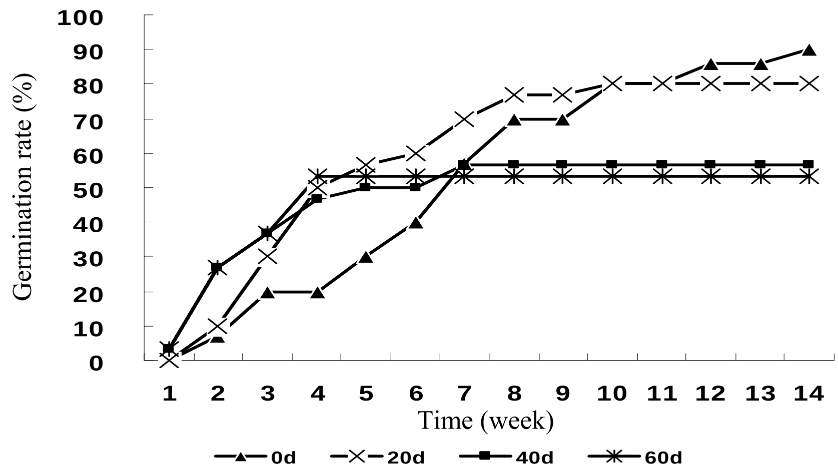


Fig. 2. Effects of pre-chilling periods at 5ºC on the germination process of seeds in Naidong (*Camellia japonica*) under conditions of continuous illumination at 20ºC.

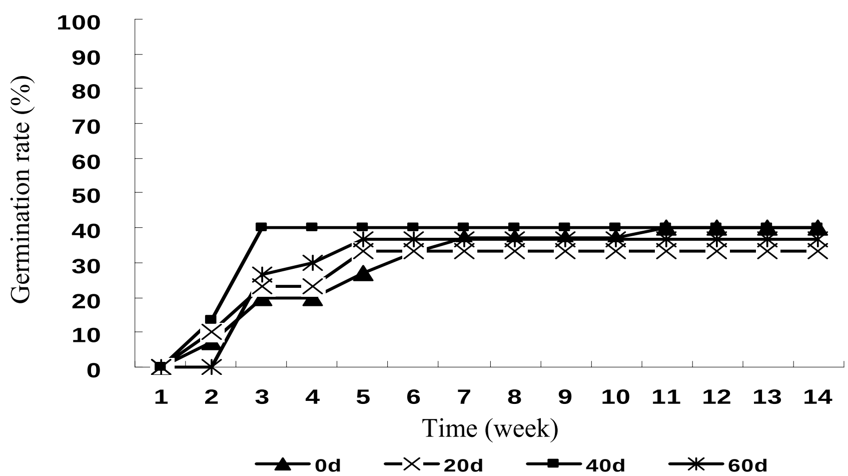


Fig. 3. Effects of pre-chilling periods at 5ºC on the germination process of seeds in Naidong (*Camellia japonica*) under conditions of continuous illumination at 25 ºC.

It can be seen from the Fig. 4 that 10 ºC low temperature treatment can significantly improve seed germination rate under the condition of continuous illumination in 20ºC, but the final germination rate achieves 90% and the difference is not significant. With the extension of 10ºC low temperature processing time, the seed germination rate gradually accelerates, and after more than 40 days germination rate reaches the fastest speed, but then does not continue accelerating with the extension of 10ºC low temperature time. Therefore, more than 40 days 10ºC low temperature processing can make the rate of seed germination the fastest. The Fig. 5 shows that 10ºC low temperature processing does not make seeds germinate in 25ºC continuous light speed up, but can obviously improve the germination rate; the seed germination rate in 10°C low temperature treatment for 20 days is slightly higher than that without low temperature treatment; 10°C low temperature treatment for more than 40 days can make the seed germination rate increase from 30 to nearly 70% . The seed germination rates in 10 °C low temperature processing for 40 and 60 days have no significant difference ($p > 0.5$).

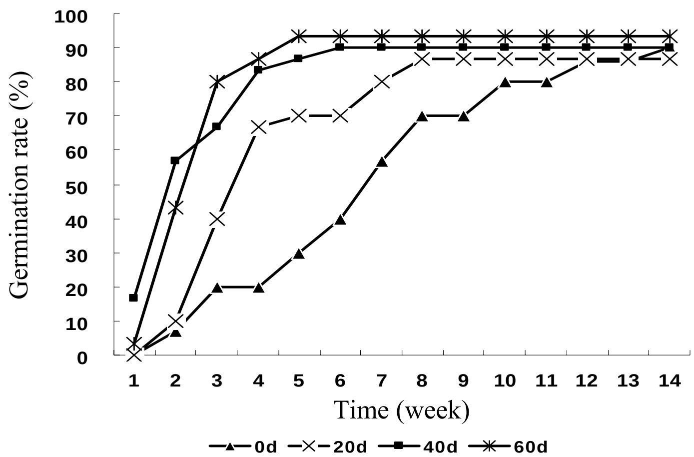


Fig. 4. Effects of pre-chilling periods at 10 °C on the germination process of seeds in Naidong (*Camellia japonica*) under conditions of continuous illumination at 20 °C.

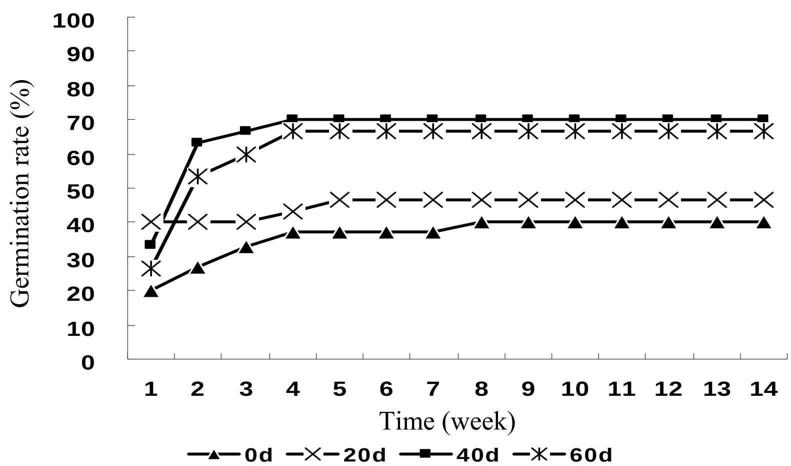


Fig. 5. Effects of pre-chilling periods at 10°C on the germination process of seeds in Naidong (*Camellia japonica*) under conditions of continuous illumination at 25°C.

The experimental results showed that the suitable temperature for the germination of *Camellia* seeds ranged from 10 to 20 °C and 15 °C was the most suitable temperature for the germination. These results are in conformity with that of Li (2013), but different from the results of Yang *et al.* (2011), which reported that the suitable temperature for the germination of *Camellia* seed varied between 20 and 25°C. In addition, the result that the germination rate is below 40% at 25°C and

*Camellia* seeds germination temperature range is close to the Silene secundiflora's 8 - 15°C (Thompson 1970), distributed in the Mediterranean coast shows that the *Camellia* seed after ripening in the fall will face the winter climate, and the winter climate is similar to the winter warm and humid Mediterranean climate. The Mediterranean subtropical climate in summer is high temperature and dry, while Chinese *Camellia* distributed mainly in warm and humid summer subtropical and warm temperate coastal islands. The two have distinctly different summer climate. The summer high temperature on seed germination and seedling does not pose a deadly threat but Mediterranean plant *S. secundiflora* at high temperature germination means that the seedling will face the dry summer climate threat. So, *Camellia* seed germination temperature range mainly distributed in the warm temperate coastal Qingdao is slightly higher than the plant distributed in the Mediterranean. Deciduous broad-leaved tree species, Paulownia, are widely distributed across the subtropical and warm temperate in China, and Paulownia fortune (Thompson 1970 and 2010) distributed in subtropical and Empress tree distributed in the warm temperate zone, the two seed germination's suitable temperature is from 20 - 30°C and Empress tree seed germination's suitable temperature is from 20 - 27°C (Liu 1999) distributed in the Japanese warm temperate marine climate, and the germination temperature range was significantly higher than that of *Camellia japonica*. Since *Paulownia* is strong positive species, and scattered in the forest or the forest margins, and some distribution is bounded on the north by up to 40 degrees, the north latitude cold and warm temperate regions, the winter cold and dry is the key to the survival of seedlings, and the temperature is relatively high in autumn when they ripe, and germination seedling at low temperature in winter will lead to death. Improving the germination temperature range is to adapt to the winter cold and dry climate, and *Camellia* distributed hardly in the higher latitude inland area is probably because they can't endure the cold and dry winter. Lychnis flos-cuculi except that distributed in the Mediterranean and *Silene viscosa* distributed in the central and East European Campion, the two's seed germination temperature ranges are 18 - 28 and 15 - 29°C, which shows that the distribution areas are colder and drier in winter and they have wider adapted temperature.

Most species of tree seeds distributed in the temperate zone acquire winter dormancy characteristics in order to avoid seedlings from frost damage as a result of autumn germination, winter death. General winter dormancy seeds experience winter low temperature, break dormancy and lower germination temperature range limit to ensure seeds are be able to germinate timely in the spring relatively low temperature to maintain species system. Without low temperature treatment, *Camellia* seed can also achieve higher germination rate under 15 - 20°C, but germination speed obviously accelerated after 40 d treated with 10ºC low temperature, and germination rate reduced instead after 40 d treated with 5°C low temperature. That is to say *Camellia* seed also possesses winter dormancy characteristic to adapt to the winter low temperature. But the most effective low temperature to break dormancy is in the range of 10 to 15 °C. It is higher than generally effective low temperature range of 0 to 10ºC to break temperate species dormancy, and similar to *Idesia polycarpa* seed dormancy which stretches across subtropical and warm temperate zone, also similar to *Machilus thunbergii* shallow dormancy characteristic which stretches across warm temperate zone, should belong to the south winter dormancy or *Machilus thunbergii* dormancy (Liu *et al.* 2004). Only by studying whether the *Camellia* seed has winter dormancy characteristic or not can we decide the type of *Camellia* dormancy.

The temperature characteristics of *Camellia* seeds germination just show its adaptation to the local climate. It originated in Qingdao, and the temperature in Qingdao from the late October to middle November is 10 - 15°C. Under suitable temperature range, its seed should be able to sprout. But in mid November after the temperature drops below10 degrees, it is difficult to germinate fast. They release dormancy, after the mature seeds experience effective low temperature from late

October to middle November for 40 days. Then they enter the winter temperature of 10 degrees below from December to February. In early March, 20% seeds can also begin to germinate regardless of the low temperature. More than 40% of the seeds cannott germinate because of the chilling injury of low temperature 10 degrees below in winter. As the temperature increases to 5-10 degrees, more than 30% other seeds that can stand the low temperature will germinate in succession, thus ensuring that more than 50% *Camellia* seeds can overwinter in the condition of Qingdao climate and that they can germinate and grow into seedling in spring for the sake of system maintenance.

The evergreen broad-leaves *Camellia* seeds distributed in Qingdao have the dormancy characteristics of adapting to the low temperature and dry climate in winter. The range of suitable temperature for germination is 10 - 20$^{o}$C, and the optimum germination temperature is 15$^{o}$C. The most effective low temperature for release of dormancy is 10 - 15$^{o}$C. This indicates that *Camellia* seed belongs to the Southern Department of winter dormancy types. More than 50% seeds can survive at 10 below in Qingdao. The seeds' dormancy particularity just shows the adaptability to the local climate. It is a genetic physiological and ecological adaptation in the process of long-term evolution (Ueno *et al.* 2002).

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