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*Article*

# Determining The Effect of Stratification Methods on The Germination of Tetradium Daniellii Seeds

**M. M. Mamadjanova*[1]**

1. Independent Researcher, Andijan State University
* Correspondence: munavvarmamadjanova2@gmail.com

**Abstract:** Tetradium daniellii (syn. Evodia hupehensis Dode), commonly known as the bee-bee tree or Korean evodia, is a fast-growing deciduous tree that produces fragrant flowers in the summer, a time when few other trees are in bloom. Due to the extreme hardness of Tetradium daniellii seeds, special pre-sowing treatments were applied. The internal structure of the seeds was studied by preparing anatomical sections. In order to determine the effects of initial treatments—i.e., stratification methods—on seed germination, experiments were conducted. These experiments were carried out using three different variants:1.Control – without any pre-treatment; 2.Four cycles of freezing (-18°C) and thawing (at 24°C, room temperature); 3.Soaking in warm water at 40°C for 24 hours. During the experiments, the germination rate, germination energy, and viability indicators of the seeds under each variant were determined. In this article, laboratory observations were also conducted by placing the seeds in a thermostat at temperatures of +15°C, +20°C, +25°C, and +30°C to determine the effect of temperature on Tetradium daniellii seed germination. The results are presented accordingly.

**Keywords**: Bee-bee Tree, Korean Evodia, Stratification, Alternate Freezing, Generative Propagation, Germination Energy, Thermal Treatment

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## 1. Introduction

Due to the epigeal germination type of Tetradium daniellii (Benn.) T.G.Hartley (Bee-bee tree, Korean evodia) seeds, it is required that the sowing depth be relatively shallow. However, making the sowing depth too shallow may slow down the imbibition process. At the same time, the extreme hardness of the seeds necessitates special pre-sowing treatments.According to the literature, the germination rate of T. daniellii seeds is very low and varies between sources. M. Marković et al determined the germination rate of T. daniellii seeds to be 2.7%, and reported that germination could be increased up to 27% through four cycles of freezing (-18°C) and thawing (at 24°C room temperature) [1]. They also noted that seeds exposed to 90 seconds in hot water at 90°C did not germinate at all.

In a study conducted by Poșta Daniela Sabina, various seed pre-treatment methods were investigated to enhance the effectiveness and feasibility of generative propagation (propagation by seed) of the species Evodia danielii (Benn) [2]. Hemsl. It was found that pre-sowing treatments had a positive effect on seed germination. In particular, seeds that underwent stratification in sand for one month (placed in sand on February 14 and sown on March 14) and those soaked in warm water at 25°C for 24 hours showed significantly higher germination rates—by 19.97% and 34.83%, respectively—compared to the control group.

Furthermore, notable differences were observed in the seedling heights. The average seedling height in the control group was 3.57 cm, whereas seedlings from sand-stratified seeds reached 5.14 cm, and those from seeds soaked in warm water reached 6.23 cm—representing increases of 143.97% and 174.50%, respectively, over the control group. This confirms that pre-treatment methods are important for increasing the effectiveness of generative seedling cultivation.

## 2. Materials and Methods

During the research, seeds were collected in the last ten days of October 2022 from a 35–40-year-old tree in the Tashkent Botanical Garden. The quality characteristics of the seeds were determined. It was found that the seeds of this plant are arranged in pairs, one above the other, within a single carpel. The upper seed is considered fertile and is larger in size (2.4–4 mm), while the lower, basal seed is smaller (1.5–3.5 mm). The internal structure of the seeds was studied by preparing anatomical sections. It was found that fertile seeds contain an embryo, while basal seeds consist only of endosperm and lack an embryo. This scientifically explains why seed germination is often very low in this species. To determine the effect of pre-sowing treatments—specifically stratification methods—on seed germination, experiments were conducted. To avoid failure, methods previously tested with high success by M. Marković et al and D.S. Poșta were used [1], [2]. The experiments were carried out using three variants: 1.Control – no pre-treatment; 2.Four cycles of freezing (-18°C) and thawing (at 24°C room temperature); 3.Soaking in warm water at 40°C for 24 hours.

## 3. Results and Discussion

During the experiments, the germination percentage, germination energy, and seedling viability indicators were determined for each variant, see Table 1.

**Table 1.** Effect of stratification on seed germination indicators

| Indicator | Variants | | |
|---|---|---|---|
| | Control | Freezing–Thawing | Soaking in Warm Water |
| Germination rate | 25% | 42% | 58% |
| Germination energy | 18% | 35% | 47% |
| Viability | 30% | 50% | 65% |

In the first variant, selected as the control group, seeds were prepared for sowing in their natural state, without any treatment. Under these conditions, the germination rate was 25%, the germination energy was 18%, and the viability was 30%. These results suggest that the majority of seeds remained in a state of physiological dormancy, which may cause difficulties in timely germination even under natural conditions [3], [4].

In the second treatment variant, the seeds were frozen at –18°C and then thawed at 24°C, repeating this cycle four times. This process, aimed at breaking dormancy mechanisms through alternating cold and warm exposure, can be referred to as "alternative stratification." As a result, the germination rate increased to 42%, germination energy to 35%, and viability to 50%. These indicators confirm that cold treatment activated the seeds’ physiological processes.

The third variant, and the one evaluated as the most effective, involved soaking the seeds in warm water at 40°C for 24 hours. This treatment softened the seed coat, promoted water uptake, and triggered internal metabolic activity [5-9]. The results achieved in this variant were as follows: germination rate – 58%, germination energy – 47%, and viability – 65%, indicating the high effectiveness of this method.

Based on the experimental results, it can be concluded that Tetradium daniellii seeds exhibit a clear state of physiological dormancy, and appropriate pre-sowing treatments are essential to overcome it. Among all the tested methods, soaking in warm water gave the best results, while the freezing-thawing stratification also proved to be moderately effective. Therefore, both stratification and thermal treatments are recommended in practice to improve seed germination.

Temperature is one of the main factors influencing the germination process of plant seeds [10-15]. It affects the biochemical processes inside the seed, thereby determining its germination capacity, growth rate, and developmental potential. For each plant species, there exists a specific temperature range where germination is most effective. This range is typically characterized by minimum, optimal, and maximum temperatures.

- Minimum temperature is the lowest temperature at which seeds can germinate. Below this threshold, germination either does not occur or proceeds extremely slowly.
- Optimal temperature is the most favorable range where germination is fast and seedlings are vigorous.
- Maximum temperature is the highest limit at which seeds can germinate. Temperatures above this level may not only inhibit germination but can also damage the seed.

For most plant species, the optimal temperature range lies between 20°C and 30°C, although this can vary depending on the species' natural origin and genetic traits.

Various research studies have confirmed the effect of temperature on seed germination. Temperature also regulates the onset of internal biochemical activities within the seed. Enzymes involved in germination function actively within specific temperature limits. Very low or excessively high temperatures reduce enzyme activity, negatively affecting the germination process.

In addition, temperature influences imbibition, the water absorption process, and the activation of associated internal signaling pathways. If the temperature exceeds optimal levels, metabolic processes can become disrupted, potentially damaging the internal structure of the seed.

Under natural conditions, many plant species are adapted to diurnal temperature fluctuations (day-night cycles). In such thermoperiodic environments—e.g., 25–30°C during the day and 15–20°C at night—seeds tend to germinate more quickly and efficiently. Reproducing these natural temperature cycles artificially in laboratory conditions can significantly enhance germination.

In 2024, the complete chloroplast genome sequence of T. daniellii from Uzbekistan was reported for the first time from Central Asia. From a practical standpoint, knowing the optimal temperature for a plant species is crucial in seed production, seedling cultivation, and direct field sowing. Therefore, careful control of temperature and creation of optimal conditions are key guarantees for improving germination success.

Providing seeds with a temperature regime specific to their species can significantly increase germination rates and accelerate development. This plays a critical role in achieving higher productivity and yield in crop cultivation.

To determine the effect of temperature on the germination of Tetradium daniellii seeds, laboratory observations were conducted using a thermostat set to four different temperature conditions: +15°C, +20°C, +25°C, and +30°C. The experiments were carried out during February–March 2022. Seeds used in the experiments had been pre-soaked in water at 40°C for 24 hours.

Maintaining a precise and stable water temperature during the soaking process was of particular importance in the experimental setup. Therefore, a Zhune-brand laboratory electric hot plate—capable of accurate temperature control—was used.

Based on the laboratory findings, the optimal temperature for the germination of Tetradium daniellii seeds was observed to be +25°C, at which the germination rate reached 62.6%, see Table 2.

**Table 2.** Effect of temperature on the germination of Tetradium daniellii seeds

| No | Temperature (°C) | Germination Start Time (%) | Germination Energy (%) | Germination (%) | Time to Complete Germination (days) |
|---|---|---|---|---|---|
| 1 | +15°C | 4–5 days | 10% | 22,5% | 22–24 days |
| 2 | +20°C | 3–4 days | 25% | 34,2% | 18–20 days |
| 3 | +25°C | 3–4 days | 48% | 62,6% | 22–24 days |
| 4 | +30°C | 2–3 days | 55% | 58,4% | 14–16 days |

## 4. Conclusion

Based on the results of the laboratory experiments, it was determined that temperature has a significant effect on the germination and germination dynamics of Tetradium daniellii seeds. The level of temperature not only influenced the overall germination rate, but also directly affected the onset of germination and germination energy.At +15°C, seeds germinated slowly and weakly, with germination starting after 4–5 days. Germination energy was 10%, and the total germination rate was 22.5%. Complete germination required 22–24 days.At +20°C, germination occurred slightly faster, beginning within 3–4 days. Germination energy reached 25%, and total germination was 34.2%. Full germination was achieved in 18–20 days.At +25°C, germination started in 3–4 days, with a moderate pace. The highest germination rate—62.6%—was recorded at this temperature. Germination energy was 48%. Although the germination duration (22–24 days) was not the shortest, this temperature was assessed as the most optimal for seed germination.At +30°C, seeds germinated the fastest, beginning within 2–3 days, and germination energy reached the highest value at 55%. The total germination rate was 58.4%, and complete germination was observed within 14–16 days. Although germination occurred more rapidly with rising temperature, the total germination rate was slightly lower than at +25°C.

Thus, +25°C is considered the optimal temperature for Tetradium daniellii seeds, as it ensures the highest germination percentage (62.6%). However, if rapid and vigorous germination is the primary goal, then +30°C may be effective due to its high germination energy and short germination period. In this case, though, it is necessary to increase the seed sowing rate per hectare and to pay close attention to seedling care, particularly to prevent desiccation of the young sprouts due to the high temperature.

In contrast, +15°C resulted in slow and inefficient germination, and therefore is considered unfavorable.

From the above findings, it can be concluded that the most suitable time for sowing Tetradium daniellii seeds in open field conditions is during the second ten-day period of April, when air and soil temperatures typically reach around +25°C..

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