

# Seed germination characteristics of *Phoebe sheareri*(Hemsl.)Gamble

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## Abstract

The germination characteristics of fresh seeds of *Phoebe sheareri* (Hemsl.) Gamble was investigated under three different treatments (gibberellin treatment, stratification treatment, seed removal treatment). Observations: after soaking seeds with different concentrations of gibberellin solution (0mg/L, 500mg/L, 1000mg/L, 1500mg/L) for 24h, the seed germination experiment showed that 1500mg/L gibberellin treatment resulted in the best germination for *P. sheareri* (Hemsl.) Gamble seeds, with germination percentage and germination potential (average of three replicates) reaching 82% and 71% respectively, among the highest across all treatments. Among the three stratification treatments (room temperature stratification, 4°C low temperature stratification, and 25°C to 5°C temperature stratification), the 25°C to 5°C temperature stratification treatment significantly promoted *P. sheareri* (Hemsl.) Gamble seed germination, with a seed germination percentage reaching 34% within half a month, far higher than the germination percentages of other stratification treatments. The seeds of *P. sheareri* (Hemsl.) Gamble without seed shells did not germinate on the 20th day of the germination experiment, and the experiment also indicated that the water permeability of the seeds was better, so the seed shell had no effect on the germination of the seeds.

## Keywords

*Phoebe sheareri*; Seed germination; Seed dormancy; Gibberellic acid; Cold stratification

## 1. Introduction

*P. sheareri* (Hemsl.) Gamble, also known as golden nanmu or purple golden nanmu, is a large shrub to tree in the Lauraceae family, genus *Phoebe* Nees. There are 34 species and 3 varieties of *Phoebe* plants in China, most of which grow in the Yangtze River Basin and regions south of it, with the highest distribution found in Hubei, Chongqing, Sichuan, Yunnan, Guizhou, Guangdong, and Guangxi. Plants of this genus are commonly referred to as nanmu. Most *Phoebe* species are tall trees with fine, solid wood that is not prone to cracking or deformation, making them high-quality

timber widely used in construction, furniture, and other applications [1]. In addition to their excellent material quality and high economic value, *Phoebe* species also possess aesthetic properties, making them suitable for landscaping and serving as excellent greening trees. Meanwhile, their ecological benefits cannot be overlooked; while beautifying the environment, *Phoebe* species also function to purify the air and conserve water resources[2].

Although *Phoebe* species are excellent precious timber trees, current resource cultivation, development, and utilization of these precious tree species are quite limited both domestically and internationally[3]. According to a review of relevant literature, existing research on *Phoebe* species is minimal, with most studies focusing on the growth characteristics and ecological properties of only a few species within the genus [4]. Research specifically using *P. sheareri* as an experimental subject is particularly scarce, and studies on the seed germination characteristics of *P. sheareri* can be counted on one's fingers. *P. sheareri* exhibits strong resistance to diseases, windbreak capabilities, and fire resistance, and is rarely disturbed by pests. With its tall stature, elegant form, and abundant foliage, it is an excellent species for landscaping, protection, and ornamental purposes in gardens and courtyards. Both its leaves and roots can be used as medicinal materials—the leaves warm the middle burner and regulate qi, while the roots remove blood stasis and reduce swelling. As an evergreen species, the roots and leaves of *P. sheareri* can be harvested throughout the year. In addition, *P. sheareri* has straight grain, hard material with a silky luster; its kernel can be pressed for oil, and its leaves can be used to extract essential oils, making it a precious timber species in China [5].

Research on *Zinnia* has primarily focused on the growth characteristics of *Zinnia* seedlings, *Zinnia* seedling cultivation experiments, the introduction and cultivation of *Zinnia*, and the spatial community structure analysis of *Zinnia* landscape forests. Among these, specialized research on the germination characteristics of *Zinnia* seeds is limited to a few cases. Li Zhen et al. [6] used *Zinnia* and Zhejiang *Zinnia* seeds as experimental materials, employing different seed storage methods, temperatures, and light conditions to study their germination characteristics. Experimental results showed that the germination effect of *Zinnia* and Zhejiang *Zinnia* seeds treated with constant-temperature moist sand layering was far inferior to that of seeds treated with low-temperature (4°C) moist sand layering. The germination percentage of seeds subjected to low-temperature moist sand layering was significantly higher than that of seeds subjected to constant-temperature layering. Both excessively high or low temperatures had adverse effects on the germination of *Zinnia* and Zhejiang *Zinnia* seeds. At the temperatures of 15°C, 20°C, 25°C, and 30°C, the germination potential and germination percentage of *Zinnia* and Zhejiang *Zinnia* reached their maximum values at 25°C. Under three light conditions—darkness, 12 hours of illumination, and 24 hours of illumination—the germination time of *Zinnia* seeds treated with 12 and 24 hours of illumination was advanced, indicating that

appropriate light treatment can promote the germination of Zinnia seeds. Li Tiehua et al. [7] also found that temperature is one of the factors affecting the germination of Zinnia seeds, and the germination speed is fastest at 25°C. However, the germination effect is best under alternating temperature treatment (30°C (day)/15°C (night)). Additionally, Cheng Xiang [8] conducted comparative experiments over two consecutive years, comparing winter sowing with spring sowing (moist sand storage, water storage, and storage in bags after drying), and concluded that winter sowing yielded the best results, followed by spring sowing with moist sand storage, while water storage and storage in bags after drying resulted in the poorest germination effects.

Research on *P. sheareri* is quite limited, therefore, it is necessary to intensify research efforts on this high-quality tree species in order to lay a solid foundation for its efficient large-scale production and resource expansion. This experiment used fresh *P. sheareri* seeds as experimental material to study their germination characteristics, providing a reference for their promotion and cultivation.

## 2. Materials and Methods

### 2.1. Experimental Materials

Healthy and plump seeds were selected from fresh *P. sheareri* seeds purchased online in November 2017 as experimental materials.

### 2.2. Experimental Methods

In this experiment, germination was defined as the radicle emerging 5mm through the seed coat. During the experimental period, seed germination was observed and recorded daily. For each treatment, 50 seeds were used, with three replications, and the experimental results were expressed as the mean of the three replications. Germination percentage and germination potential were calculated using the following formulas: Germination percentage = (Number of normally germinated seeds / Total number of test seeds) × 100%. Germination potential = (Number of germinated seeds from the start of germination to the germination peak period / Total number of test seeds) × 100%.

The participating *P. sheareri* seeds were soaked in a 50g/L carbendazim solution for one hour for sterilization.

Cut the gauze to an appropriate size, ensuring it remains moist but not dripping, then spread the gauze on the prepared culture tray.

The disinfected and pre-treated seeds were evenly placed on moist gauze in culture dishes, which were then covered with an additional layer of moist gauze to maintain humidity.

Place the seeds in an artificial intelligence incubator/constant temperature room, observe them daily, spray water appropriately to maintain moisture, and promptly

clean moldy seeds (seeds that have become moldy and necrotic need to be removed in a timely manner).

### 2.2.1. Study of Seed Physical Characteristics

- (1) Determination of 1000-seed weight: The seed 100-grain method was used to determine the 1000-seed weight. One hundred seeds were selected from healthy and plump seeds, with three repetitions. The mass was weighed using an electronic balance with precision to 0.001g, and the average value was taken.
- (2) Seed water permeability determination: Air-dried seeds were placed in a 30°C constant temperature chamber for imbibition and weighed at different time intervals (3, 6, 9, 12, and 24h). Seeds were removed and weighed every three hours, with surface moisture carefully blotted dry using absorbent paper before each weighing, and all measurements were recorded.

### 2.2.2. Seed soaking with gibberellin solution

P. sheareri seeds were divided into four treatments, with 50 seeds per treatment and three replicates.

- (1) P. sheareri seeds were soaked in a 0mg/L gibberellin solution for 2h, followed by a seed germination experiment.

P. sheareri seeds were soaked in a 500mg/L gibberellic acid solution for 2h before conducting the seed germination experiment.

- (2) P. sheareri seeds were soaked in a 1000mg/L gibberellic acid solution for 2h before conducting the seed germination experiment.

- (3) Soak the P. sheareri seeds in a 1500mg/L gibberellin solution for 2h, then proceed with the seed germination experiment after soaking.

### 2.2.3. Stratification treatment

P. sheareri seeds were divided into three treatments, with 50 seeds per treatment and three replicates. The seeds were subjected to stratification treatment for one month before conducting the seed germination experiment. Wrap the sterilized P. sheareri seeds in gauze, then enclose the gauze-wrapped seeds in pre-soaked moss to ensure moisture retention in the subsequent period. Next, place this moss-wrapped seed bundle into a sealed plastic bag, squeeze out the air inside the bag, seal the opening tightly, and label it clearly. Finally, lay the sealed plastic bag flat in a paper box.

- (1) Warm stratification: The packaged P. sheareri seeds were stored at room temperature for one month before conducting the seed germination experiment.

- (2) Combined stratification: First, the sealed P. sheareri seeds were placed in a 25°C environment for half a month, then transferred to a 5°C constant temperature incubator for another half month of stratification before conducting the seed germination experiment.

tion experiment.

(3) Cold stratification: The sealed *P. sheareri* seeds were stored in a refrigerator at a controlled temperature of 4°C, and the seed germination experiment was conducted one month later.

#### 2.2.4. Seed coat removal treatment

Healthy and plump *P. sheareri* seeds were selected and soaked in water for 10 minutes. After the seed coats had slightly softened, they were manually removed. The seeds were then disinfected with a disinfectant agent. One treatment consisted of 50 *P. sheareri* seeds, repeated three times, forming a control group with seeds that had not undergone seed coat removal.

### 2.3. Data Processing

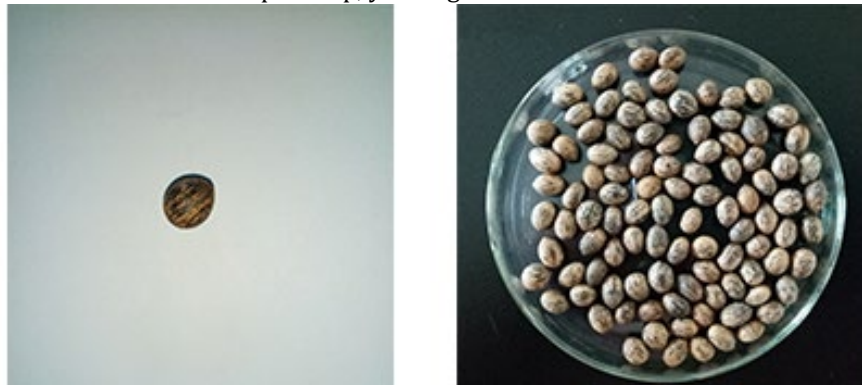
The data were statistically analyzed using Excel software.

## 3. Analysis Results

### 3.1. Seed Physical Characteristics

#### 3.1.1. Basic morphological characteristics of *P. sheareri* seeds

*P. sheareri* is an evergreen large shrub to tree with leathery leaves, conical inflorescences, and a flowering period from May to June. Its fruits mature from October to November, appearing as ovoid, purple-brown drupes approximately 1 cm in length and 7 mm in diameter. The fruits are soaked in clean water for a period of time, then rubbed to remove the pericarp, yielding clean *P. sheareri* seeds.

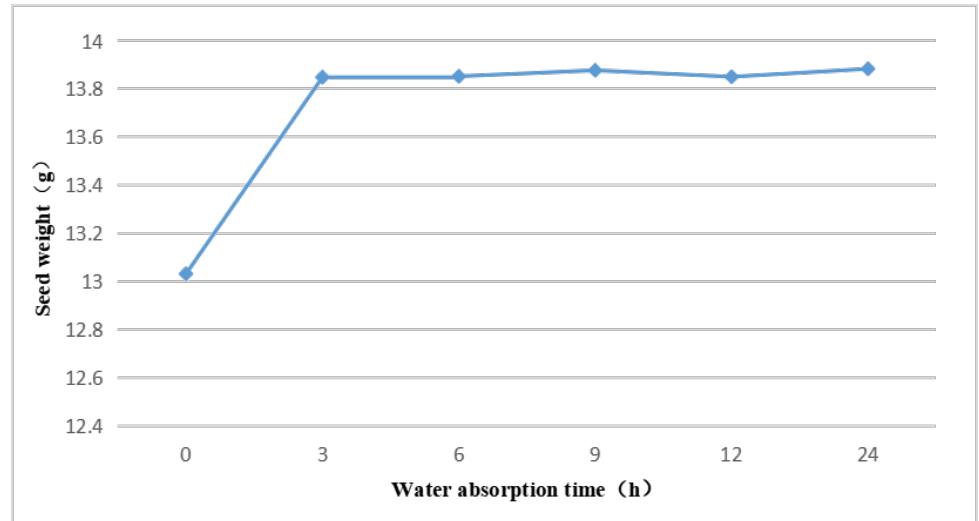


**Fig 1.** Morphological characteristics of *P. sheareri* (Hemsl.) Gamble seeds

*P. sheareri* seeds are oval-shaped with grayish-brown patterned seed coats, approximately 8mm in length and 6mm in diameter. The average of five randomly sampled groups of de-pulped *P. sheareri* seeds was calculated to determine the thousand-grain weight of de-hulled seeds, which was 262-269g (with an average of 26.615g/100 seeds, with individual group data ranging from 26.2-26.9g). The analysis indicates that *P. sheareri* seeds are of good quality, being moderately sized,

healthy and plump, with minimal individual variation, making them excellent experimental material.

### 3.1.2. Water Permeability of *P. sheareri* Seeds



**Fig 2.** Seed water absorption curve

As shown in Fig 2, the seeds of *P. sheareri* were in the imbibition phase during the first three hours, with the curve showing an upward trend. However, after three hours, water absorption almost ceased, indicating that the seeds of *P. sheareri* have good water permeability.

### 3.2. Gibberellin solution treatment

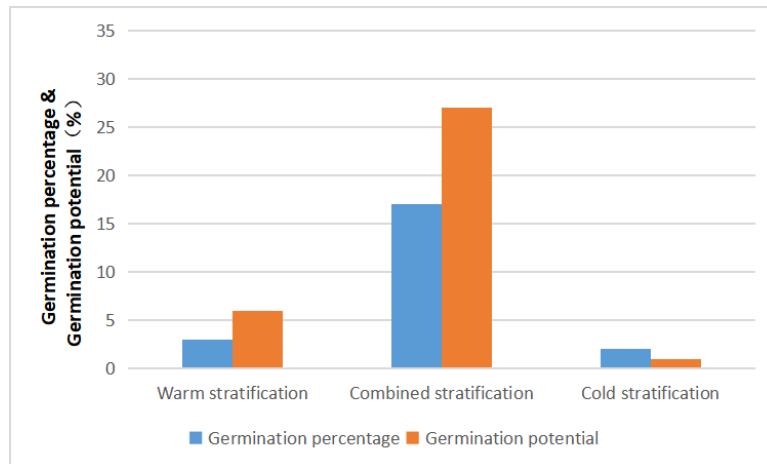
**Table 1.** Germination of seeds soaked in different concentrations of gibberellin solution

| GA3 concentration (mg/L)  | 0 mg/L | 500 mg/L | 1000 mg/L | 1500 mg/L |
|---------------------------|--------|----------|-----------|-----------|
| Germination percentage(%) | 71     | 72       | 72        | 82        |
| Germination potential(%)  | 33     | 53       | 37        | 71        |

In terms of seed germination percentage, soaking seeds in a 1500mg/L GA 3 (gibberellin) solution for 2 hours yielded the best results, with the highest total number of seed germination observed within one month. Moreover, the seed vigor of *P. sheareri* seeds under this treatment method was also the highest, with continuous and stable germination. The germination potential of all three replicate treatments was higher than that of seeds treated with other concentrations of GA3 soaking solution. Secondly, seeds treated with 1000mg/L GA 3 solution showed relatively high germination percentages, but their early germination performance was not optimal, and there were certain variations in germination percentages among the three replicate treatments, with inconsistent germination potential. The germination percentage of seeds treated with 500mg/L GA 3 gibberellin solution showed little difference compared to untreated seeds, but when comparing the germination potential of the two treatments, seeds soaked in GA 3 solution for 2 hours exhibited

significantly higher seed vigor than untreated seeds.

### 3.3. Stratification Treatment



**Fig 3.** Seed germination of different stratification treatments

After one month of seed stratification, the seeds were removed for germination experiments. The germination status of seeds subjected to room temperature, alternating temperature, and low temperature stratification treatments was observed over a period of half a month. The seeds of *P. sheareri* subjected to natural temperature stratification showed severe mold, and the gauze wrapping the seeds had also rotted when removed. Additionally, only two to three seeds germinated within the half-month period. The seeds under low temperature stratification showed no mold, but almost no seeds germinated within the half-month period. The seeds under alternating temperature stratification had only a small amount of mold and exhibited better germination performance within the half-month period, achieving the highest germination percentage.

### 3.4. Seed coat removal treatment

The dehulled *P. sheareri* seeds showed signs of mold on the third day of the germination experiment. After treating the moldy seeds (washing off the mold), the seeds became moldy again after a few days. Although efforts were continuously made to inhibit mold growth, some seeds eventually became necrotic. By the twentieth day of the germination experiment, no seed germination was observed, which was no different from the germination status of untreated *P. sheareri* seeds. It is therefore evident that the seed coat of *P. sheareri* does not inhibit its own germination.

## 4. Discussion

Many healthy and viable seeds fail to germinate promptly even when placed in environments that meet all germination conditions (such as suitable temperature, humidity, and oxygen). This is a characteristic gradually formed by plants for survival

and adaptation to complex environments [9]. This biological characteristic enables seeds to control their germination at the most appropriate time and conditions. *P. sheareri* seeds also possess dormancy characteristics. For seeds in unfavorable environments, dormancy can maintain their viability until the optimal period for germination, which is of significant importance for the seeds [10]. However, only by promptly breaking this dormancy can greater convenience be brought to their production and research.

Research on seed dormancy is common both domestically and internationally, with Liang Ming [11] and others demonstrating that the causes and states of seed dormancy are diverse. The factors that induce seed dormancy generally include: whether the embryo is morphologically mature, whether the seed coat is permeable to water and air, and whether it presents mechanical barriers to embryo growth, as well as induced secondary dormancy. Chen Wei [12] and others, in their research on seed dormancy types and breaking methods, have also shown that factors such as seed structure, germination-inhibiting substances within the seed, hormonal and phytochrome interactions, and the developmental stage of the embryo can all cause seeds to enter a dormant state, inhibiting seed germination.

#### **4.1. Effect of Seed Coat and Shell on the Germination of *P. sheareri* Seeds**

The dormancy of many plant seeds is caused by their seed coats, such as poor gas and water permeability, mechanical barriers of the seed coat, or the presence of inhibitors in the seed coat [13]. These conditions can be directly overcome by artificially breaking the seed coat to promote seed germination. The dormancy of snake gourd (*Trichosathes kirilowii* Maxim.) seeds is related to its seed coat, and breaking the seed coat can eliminate the mechanical obstruction to the embryo's growth and development, thereby significantly increasing the germination percentage of snake gourd seeds [14]. Related studies have shown that cutting the seed coat of dormant soapwort (*Saponaria officinalis* Linn) seeds can effectively break their dormancy [15]. The research by Shi Xiaohua et al. [16] indicates that the seed coat of *P. sheareri* has poor water permeability and contains germination-inhibiting substances, and removing the seed coat can significantly increase the germination speed and percentage of *P. sheareri* seeds.

While our results and those of Shi Xiaohua et al. [16] suggest that the seed coat may contain inhibitors, our seed coat removal treatment did not promote germination, likely due to severe microbial contamination. This highlights a significant practical challenge: manual testa removal is inefficient and can increase the risk of infection, rendering it unsuitable for large-scale application. In fact, for a large number of plant species, seed dormancy is not merely due to inhibition by the seed coat, as the seed shell also plays a significant role in promoting dormancy. Chen Qinhuo et al. [17] also indicated that the seed coat of *P. sheareri* seeds is an important factor

affecting their germination, but through experimental verification, breaking the seed shell proved to be the most effective treatment for germination. They proposed a seed-breaking method that is easily applicable to production (mixing with 3-4 times the amount of sand and gently rolling to break through the seed coat and shell).

#### 4.2. Stratification treatment to break dormancy

The cells of fresh seeds are arranged relatively tightly. After stratification treatment, the seed coat can be softened, and the softened seed coat has improved air and water permeability, thereby promoting seed germination [18]. Research by Zhao et al. [19] shows that low-temperature stratification also helps maintain seed moisture and promotes post-maturation development of the embryo, increasing seed germination percentages. Shen et al. [20] found that low-temperature sand storage could double the germination percentage of *Macropanax rosthornii* (Harms) C.Y. Wu ex Hoo seeds, resulting in more uniform germination and better seedling quality. In Li et al.'s [6] study on the germination characteristics of *P. shearerii* seeds, it was concluded that low-temperature stratification treatment can effectively improve the germination percentage of *P. shearerii* seeds. However, Chen et al.'s [21] research indicates that while low-temperature stratification does promote seed germination, its effect is far less than that of alternating temperature stratification.

#### 4.3. Hormone treatment

To promote the germination of dormant seeds, in addition to physical methods such as seed coat removal and stratification, exogenous hormones can also be used to break seed dormancy and induce germination. Research has shown that plant growth regulators such as ethylene, cytokinin, and GA (gibberellin) can weaken germination-inhibiting substances in seeds, or even render them ineffective, while simultaneously enhancing seed activity and promoting germination [22]. GA is commonly used to break seed dormancy, and the higher the concentration of GA (below its optimal concentration), the greater the promotional effect on seed germination. GA has a significant impact on the growth and development of seeds [23]. According to the research by Liu Yanwen et al. [24], soaking seeds in GA solution can effectively improve the germination percentage of blue honeysuckle (*Lonicera caerulea* L. var. *edulis* Turcz. ex Herd.), with the optimal effect at a concentration of 100mg/L. However, if the GA solution concentration is too high, it will inhibit seed germination, while if it is too low, there will be no promotional effect. In the study on the germination characteristics of belladonna (*Atropa belladonna* L.) seeds by Fan Xue et al. [25], it was also shown that soaking seeds with an appropriate concentration of GA solution can effectively improve the germination percentage, germination potential, and germination index, but if the GA solution concentration exceeds the appropriate value, it will have the opposite effect and inhibit seed germination.

## 5. Conclusion

*P. sheareri* seeds possess dormancy characteristics, and to improve their germination percentages, the key lies in breaking the seed dormancy to promote germination. This experiment employed three treatment methods: stratification, gibberellin soaking, and seed coat removal. Through a series of comparative experiments and discussion analysis, this study sought to identify an effective method for breaking *P. sheareri* seed dormancy.

Many experiments employ the method of moist sand stratification, while the material used in this experiment was moist sphagnum moss, which provides better moisture retention. The stratification treatments were divided into room temperature stratification, alternating temperature stratification (first at 25°C, then at 5°C), and low-temperature stratification. The results indicate that alternating temperature stratification is highly effective in breaking the dormancy of *P. sheareri* seeds and is also convenient to operate, making it suitable for widespread application in production. The cold stratification and room temperature stratification treatments showed no significant effects, and the seeds under room temperature stratification severely mold.

GA (gibberellin) can reduce germination-inhibiting substances in the embryo and promote seed germination. In this experiment, *P. sheareri* seeds were soaked for 2 hours in gibberellin solutions of different concentrations (0mg/L, 500mg/L, 1000mg/L, 1500mg/L). The differences among the treatments were not significant, possibly due to the obstruction of the seed coat and the fact that seeds were only soaked in gibberellin solution for 2 hours, which weakened the influence of gibberellin on the seeds. If gibberellin solution were sprayed on the seeds regularly, its influence on *P. sheareri* seeds might be enhanced. However, overall, the treatment with 1500mg/L gibberellin solution resulted in the highest germination percentage, the most stable germination conditions, and the most uniform germination.

The germination effect of *P. sheareri* seeds with seed coat removal did not improve, showing no superiority compared to untreated seeds. However, Chen Qinhu's research indicates that the germination percentage of *P. sheareri* seeds significantly increases after seed coat removal, suggesting that the seed coat is an important factor inhibiting seed dormancy. The large discrepancy in experimental results may be due to repeated mold formation on the seeds during this experiment, which inhibited seed germination, or based on other unknown factors. The specific reasons require further investigation.

While suitable temperature and humidity in the artificial climate chamber promoted the germination of *P. sheareri* seeds, they also fostered mold growth. Although all experimental materials were disinfected, the seeds still developed mold multiple times during the experiment, which had a certain impact on germination. The experimental process still had many shortcomings, and disinfection and sterilization work should be strengthened. Additionally, when comparing different treatments,

the alternating temperature stratification treatment showed the best germination performance, followed by the treatment with 1500ml/L gibberellin solution for 2 hours. If these two treatments could be cleverly combined, better germination results might be achieved. In conclusion, the optimal treatment method for *P. sheareri* seeds is: alternating temperature stratification → gibberellin soaking (specific treatment parameters such as gibberellin concentration, soaking time, and stratification duration still need to be explored).

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