

Effects of Different Environmental Factors and Seed Coat Scarification on Seed Germination Characteristics of *Castanea henryi* (Postprint)

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Abstract

Castanea henryi Rehd. & Wils. is an important woody grain tree species and characteristic fruit tree resource in southern China, possessing high edible and medicinal value. To elucidate the optimal conditions for *C. henryi* seed germination and provide a theoretical foundation for seedling propagation, this experiment utilized freshly harvested *C. henryi* seeds as material to investigate the effects of temperature, light, substrate, substrate moisture content, and shell-breaking treatment on seed germination. The results demonstrated that the optimal temperature for *C. henryi* seed germination was 25°C, with a final germination rate of $92.67 \pm 2.67\%$; although germination rates decreased under other temperature conditions, no significant differences were observed compared with the 25°C treatment. Dark conditions shortened the germination time of *C. henryi* seeds and significantly increased both germination rate and germination potential; however, radicle length under dark conditions was significantly shorter than that under periodic illumination. The germination rate and germination potential of seeds in peat soil were superior to those in river sand, with radicle length in peat soil also being significantly greater. Substrate moisture content exerted no significant effect on germination rate or germination potential. The mechanical barrier imposed by the seed coat represents a limiting factor for *C. henryi* seed germination; shell-breaking treatment markedly improved germination rate, with significant differences observed in germination potential and radicle length compared to intact seeds. In practical production, cutting the seed shell can accelerate the germination process.

Full Text

Effects of Environmental Factors and Shell-Breaking Treatment on Germination Characteristics of *Castanea henryi* Seeds

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Abstract

Castanea henryi Rehd. & Wils is an important woody grain crop and characteristic fruit tree resource in southern China with high edible and medicinal value. To determine the optimal conditions for *C. henryi* seed germination and provide a theoretical basis for seedling propagation, this study investigated the effects of temperature, light, substrate, substrate moisture content, and shell-breaking treatment on seed germination using freshly harvested seeds. The results showed that the optimal germination temperature was 25°C, achieving a final germination rate of (92.67±2.67)%. Although germination rates decreased under other temperature conditions, no significant differences were observed compared with the 25°C treatment. Dark conditions shortened germination time and significantly improved both germination rate and germination potential; however, radicle length under dark conditions was significantly shorter than under periodic light exposure. Germination rate and germination potential were higher in peat soil than in river sand, with significantly longer radicles observed in peat soil. Substrate moisture content had no significant effect on germination rate or germination potential. The mechanical barrier of the seed coat was identified as a limiting factor for germination; shell-breaking treatment significantly improved germination rate, germination potential, and radicle length compared with intact seeds. In practical production, cutting the seed shell can accelerate the germination process.

Keywords: *Castanea henryi*; seed; environmental factors; shell-breaking treatment; germination

Introduction

Castanea henryi Rehd. & Wils, also known as chinquapin, pearl chestnut, or Henry's chestnut, belongs to the family Fagaceae and genus *Castanea*. It is a renowned dry fruit and woody grain tree species in southern China and represents a characteristic deciduous fruit tree resource in the country. *C. henryi* possesses high edible and medicinal value. The nuts are rich in starch, soluble sugars, unsaturated fatty acids, riboflavin, B vitamins, vitamin C, and essential

trace elements such as calcium, iron, magnesium, and manganese. The protein and fat contents are comparable to wheat and 2-3 times higher than rice, while carbohydrate content exceeds that of wheat. The unsaturated fatty acids and various vitamins in *C. henryi* demonstrate preventive and therapeutic effects against hypertension, coronary heart disease, and arteriosclerosis. The inner pellicle has anti-wrinkle and cosmetic properties; the shell can treat nausea, thirst, and diarrhea; the catkins can treat cervical lymph node tuberculosis; the bur and bark have detoxifying and anti-swelling effects; and the roots can treat partial kidney qi deficiency. The bur and bark contain abundant tannins for tannin extraction.

C. henryi is a deciduous tree that can reach 30 m in height, with a straighter trunk than Chinese chestnut and dwarf chestnut. Its wood is solid and water-resistant, making it a high-quality timber for railroad ties, construction, furniture, and shipbuilding. With well-developed root systems, it can consolidate and improve soil structure, prevent water loss and soil erosion, and serves as an excellent species for increasing forest coverage and improving ecological environments.

Seed germination represents the most vulnerable stage in a plant's life cycle, influenced by comprehensive factors including oxygen, temperature, light, water, and intrinsic seed viability. As a biological characteristic formed through adaptation to environmental conditions for reproductive success, germination holds significant ecological importance for population dispersal, colonization of new habitats, and population renewal. This study investigated the effects of various factors (temperature, light, substrate type, substrate moisture content, and shell-breaking treatment) on *C. henryi* seed germination to determine optimal conditions and provide theoretical guidance for seed propagation.

Materials and Methods

1.1 Materials

C. henryi seeds were collected in October 2016 from wild populations in Xiaoping Village, Haiyang Township, Lingchuan County, Guangxi. After cleaning and removal of impurities, thousand-seed weight and seed diameter were measured. Seeds were stored in sand at 4°C with approximately 4% moisture content for preservation.

1.2 Methods

Thousand-seed weight was determined by weighing three replicates of 1000 clean seeds each. Seed diameter was measured by randomly selecting 30 seeds for longitudinal and transverse diameter measurements. Germination experiments were conducted from November 2015 to April 2016 under room temperature conditions and in an LRH-250-G illumination incubator (Guangdong Medical Equipment Factory).

Prior to experiments, seeds were washed with water, and plump seeds were selected by water flotation. Seeds were disinfected with 0.5% KMnO₄ for 30 minutes, rinsed with clean water, and drained. Single-factor experiments were designed to investigate five treatment factors: temperature, light, substrate, substrate moisture content, and shell-breaking treatment. All treatments included three replicates of 50 seeds each. Seeds were placed in petri dishes for germination. For substrate treatments, two substrates were used: river sand and peat soil. For other factors, river sand served as the culture substrate. For substrate moisture treatments, three moisture gradients were established: 5%, 10%, and 20%. Other treatments maintained approximately 10% substrate moisture, with weekly water supplementation based on weight loss to maintain respective moisture levels. For light treatments, seeds were sown with half the nut exposed above the substrate; for other treatments, seeds were completely submerged. Germinated and moldy seeds were removed every 30 days, and germinated seeds (those with radicles extending ≥ 5 mm) were counted. During each count, radicle length and thickness of all newly germinated seeds were measured, with final averages calculated to assess treatment effects on radicle development.

Experimental treatments:

1. **Temperature:** Four temperature gradients were established: 15, 20, 25, and 30°C, with room temperature as control.
2. **Light:** (i) Under room temperature: periodic indoor light (placing petri dishes near windows) and continuous darkness (0 lx, 24 h · d⁻¹, covered with opaque lids); (ii) Under 25°C incubator conditions: periodic light (3000 lx, 12 h · d⁻¹) and continuous darkness (0 lx, 24 h · d⁻¹, covered with opaque lids).
3. **Substrate:** Under both incubator (25°C, periodic light 3000 lx, 12 h · d⁻¹) and room temperature (periodic indoor light) conditions, two substrates were tested: river sand and peat soil.
4. **Substrate moisture content:** Under both incubator (25°C, periodic light 3000 lx, 12 h · d⁻¹) and room temperature (periodic indoor light) conditions, three moisture gradients were established: 5%, 10%, and 20%.
5. **Shell-breaking treatment:** Under both incubator (25°C, periodic light 3000 lx, 12 h · d⁻¹) and room temperature (periodic indoor light) conditions, seeds were either shell-broken (a small hole cut at the seed tip) or left intact.

1.2.2 Data Statistics and Processing

Germination percentage (GP) and germination energy (GE) were calculated as indices. GE was determined at 60 days, while final GP was calculated at experiment termination (150 days). Formulas were as follows:

- Germination percentage (GP) = (Number of normally germinated seeds / Total number of tested seeds) × 100%
- Germination energy (GE) = (Number of germinated seeds within specified

$$\text{time (60 d) / Number of tested seeds) } \times 100\%$$

Data were analyzed using SPSS 13.0 software for one-way ANOVA, with Duncan's multiple comparison test. For non-homogeneous variances, Tamhane's T2 test was applied. Figures were generated using Sigmaplot 10.0.

Results

2.1 Seed Morphological Characteristics

C. henryi nuts are conical or ovate, with transverse diameter of (0.65 ± 0.01) cm and longitudinal diameter of (0.83 ± 0.02) cm. The seed coat is reddish-brown, with a thousand-seed fresh weight of (3300 ± 84.98) g.

2.2 Effects of Different Temperatures on Seed Germination

Germination processes differed among temperature treatments. During early germination (within 60 days), temperature significantly affected germination rate, with the steepest increase observed at 25°C, followed by 30°C, and the smallest increase under room temperature. The 25°C treatment showed higher germination rates than 15°C and 20°C treatments. After 60 days, germination rate increased substantially under room temperature, while other treatments gradually plateaued. At experiment termination, the highest germination rate occurred at 25°C, followed by 15°C and 20°C, with 30°C and room temperature showing the lowest rates (Figure 1 [Figure 1: see original paper]).

For germination energy, no significant difference was observed between 25°C and 30°C treatments; however, both were significantly higher than 15°C, 20°C, and room temperature treatments (Figure 2 [Figure 2: see original paper]A). Among the five temperature conditions, 25°C proved optimal for germination. Both germination rate and germination energy decreased with temperature deviation from 25°C, though 30°C showed higher values than 15°C, 20°C, and room temperature. No significant differences in radicle thickness were detected among temperature treatments ($P > 0.05$), but radicle length was significantly affected (Figure 2B). No significant differences were found between 15°C and 20°C or between 25°C and 30°C for radicle length ($P > 0.05$); however, radicles at 25°C and 30°C were significantly longer than those at 15°C, 20°C, and room temperature ($P < 0.05$).

2.3 Effects of Different Light Conditions on Seed Germination

Light conditions significantly affected germination rates. At 25°C under dark conditions, germination peaked at 92.67% after 60 days, while under periodic light, germination increased more gradually, reaching only 79.33% at experiment termination. Under room temperature, dark conditions produced substantially higher germination rates than periodic light during early and middle stages (before 120 days), with cumulative rates becoming comparable only at the final stage (120 days) (Figure 3 [Figure 3: see original paper]).

Germination energy under dark conditions was significantly higher than under periodic light at the same temperature ($P < 0.05$) (Figure 4 [Figure 4: see original paper]A). Overall, dark conditions produced significantly higher germination rates and germination energy than light conditions, indicating darkness favors *C. henryi* seed germination. No significant differences in radicle thickness were observed between light treatments ($P > 0.05$), but radicle length differed significantly (Figure 4B). Under identical temperature conditions, radicle length under dark conditions was significantly shorter than under periodic light ($P < 0.05$).

2.4 Effects of Different Substrates on Seed Germination

River sand and peat soil differentially affected germination rate and speed (Figure 5 [Figure 5: see original paper]). Under 25°C and periodic light, germination rates increased rapidly within 60 days in both substrates, with river sand showing slightly higher rates. Over time, rates increased slowly, peaking in the middle-to-late experimental stage (after 90 days), with peat soil showing marginally higher final rates. Under room temperature and periodic light, peat soil produced higher germination rates than river sand at all stages (Figure 5).

Under 25°C and periodic light, germination energy in river sand was slightly higher than in peat soil, though not significantly different ($P > 0.05$). However, under room temperature and periodic light, germination energy in river sand was significantly lower than in peat soil ($P < 0.05$) (Figure 6 [Figure 6: see original paper]A). Comprehensive analysis indicates peat soil is more suitable for seed germination, likely due to its light texture, good aeration, and superior fertility compared with river sand, which has smaller intergranular pores, poor permeability, and heavier texture, leading to increased seed mold. Substrate type did not significantly affect radicle thickness ($P > 0.05$) but significantly influenced radicle length ($P < 0.05$) (Figure 6B). Under identical temperature conditions, radicle length in river sand was significantly shorter than in peat soil ($P < 0.05$).

2.5 Effects of Substrate Moisture Content on Seed Germination

Substrate moisture content exhibited certain effects on germination. Under 25°C and periodic light, final germination rates in substrates with 5%, 10%, and 20% moisture content were 84.44%, 86.67%, and 81.11%, respectively. Under room temperature and periodic light, corresponding rates were 88.89%, 90%, and 84.44% (Figure 7 [Figure 7: see original paper]).

For germination energy, maximum values were observed at 10% moisture content under identical temperature and light conditions, followed by 20% and 5% moisture treatments; however, no significant differences were detected among moisture levels ($P > 0.05$) (Figure 8 [Figure 8: see original paper]A). These results demonstrate that *C. henryi* seeds have broad adaptability to substrate moisture content. Moisture content did not significantly affect radicle thickness

($P > 0.05$). Under 25°C and periodic light, no significant differences in radicle length were observed among moisture treatments ($P > 0.05$), whereas under room temperature and periodic light, significant differences were detected ($P < 0.05$). Under identical temperature and light conditions, maximum radicle length occurred at 20% moisture content, followed by 5% and 10% treatments (Figure 8B).

2.6 Effects of Shell-Breaking Treatment on Seed Germination

Under 25°C and periodic light, intact seeds began germinating after 30 days, later than shell-broken seeds. After 90 days, cumulative germination rates were equivalent between treatments, but germination energy of intact seeds was significantly lower than that of shell-broken seeds ($P < 0.05$). Under room temperature and periodic light, shell-broken seeds showed higher germination rates and significantly greater germination energy than intact seeds ($P < 0.05$) (Figure 9 [Figure 9: see original paper], Figure 10 [Figure 10: see original paper]A). These results indicate that shell-breaking affects both germination rate and germination energy under identical temperature and light conditions by overcoming mechanical constraints, facilitating water and oxygen absorption, and promoting germination.

Shell-breaking treatment did not significantly affect radicle thickness ($P > 0.05$) (Figure 10B). However, under identical conditions, radicle length of shell-broken seeds was significantly greater than that of intact seeds ($P < 0.05$).

Discussion and Conclusion

Seed germination requires adequate moisture, suitable temperature, sufficient oxygen, and appropriate light. However, primary limiting factors vary among species and habitats. The germination process is comprehensively influenced by environmental factors including temperature, light, substrate, substrate moisture content, and pre-sowing shell-breaking treatment.

Temperature is a critical factor affecting seed germination, with optimal temperatures enhancing germination rates and promoting seedling growth (Ojeifo et al., 2007; Zhang et al., 2014). Optimal germination temperatures vary among species. Some species such as *Triglochin maritima* (Khan & Ungar, 1999) and *Dodonaea viscosa* (Huo et al., 2016) show higher germination rates at elevated temperatures, whereas low temperatures enhance germination of *Haloxylon ammodendron* (Huang et al., 2001). Our results demonstrate that *C. henryi* seeds exhibit strong environmental adaptability with a broad temperature range for germination (Yang et al., 2005), with 25°C being optimal. Both excessively high and low temperatures negatively affected germination, similar to *Artemisia sphaerocephala* seeds (Huang et al., 2001).

Light requirements for seed germination vary among species (Yang et al., 2001). *C. henryi* seeds germinated under both light and dark conditions; however, dark conditions were more favorable regardless of temperature regime, consistent

with Yang et al. (2005) concluding that *C. henryi* seed germination is light-independent. This may be attributed to the relatively large seed size and abundant stored reserves sufficient to support germination and early seedling growth, similar to findings by Pamukov & Schneider (1978).

Different substrates contain varying nutrients, microorganisms, and physical structures, thereby affecting seed germination and seedling growth (Puerta-Pinero et al., 2006). Under relatively optimal light and temperature conditions, peat soil produced higher germination rates than river sand, likely due to its light texture, good aeration, and adequate oxygen supply, which reduce seed mold and promote germination. In contrast, river sand's small intergranular pores, poor permeability, and heavy texture increase mold incidence. Therefore, peat soil is more suitable for *C. henryi* seed germination.

Water is essential for seed germination and represents the most important controlling factor. Seeds absorb water from the substrate to dissolve stored reserves into usable nutrients for the embryo, and substrate moisture content affects germination (Lin et al., 2008). *C. henryi* seeds germinated across a moisture range of 5%-20% without significant differences in germination rates, indicating broad adaptability to substrate moisture content.

The hard, tough seed coat of *C. henryi* lacks sutures, resulting in poor air and water permeability that hinders water absorption and affects germination. Artificially breaking this mechanical barrier places seeds in a more favorable state for water absorption, activating protoplasm and stored substances to promote germination. Shell-breaking treatment significantly improved *C. henryi* germination rate and germination energy, similar to *Pinus dabeshanensis* seeds (Han et al., 2014).

In summary, the optimal germination temperature for *C. henryi* seeds is 25°C; dark conditions are more favorable; seeds exhibit broad adaptability to substrate moisture content; light-textured, well-aerated substrates such as peat soil are optimal; and shell-breaking can significantly enhance germination rates in practical production.

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