

Article

Effects of Seed Biological Characteristics and Environmental Factors on Seed Germination of the Critically Endangered Species *Hopea chinensis* (Merr.) Hand.-Mazz. in China

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Abstract: *Hopea chinensis* (Merr.) Hand.-Mazz. is a Class II national key protected plant and Plant Species with Extremely Small Populations in China. In order to further analyze why *H. chinensis* is endangered and optimize conservation techniques, we carried out a study on the effects of seed biological characteristics and environmental factors on the seed germination and seedling growth of *H. chinensis*. The results show that there were no significant differences in seed morphology between four populations in southern China, but there were significant differences in calyx lobe morphology and seed germination. The removal or retention of the calyx lobes or the seed coat had no significant effect on seed germination. The weight of individual *H. chinensis* seeds was mainly >1.0 g, with small seeds (<0.5 g) not germinating, whereas seeds >2.0 g had the highest germination rate and fastest seedling growth. *H. chinensis* produces typical recalcitrant seeds, being sensitive to natural dehydration, with dehydration for 8 d reducing seed viability by 50%, whereas dehydration for 16 d resulted in seeds with zero viability. *H. chinensis* seeds are light-neutral and capable of germination at a temperature of 30 °C and on a substrate with good water retention and aeration. The seeds are not tolerant of drought, salinity, or flooding. In conclusion, the unique biological characteristics of *H. chinensis* seeds and the specific habitat which they inhabit contribute to a significant loss of viable seeds, which negatively affects the population's ability to regenerate and achieve maintenance.

Keywords: germination percentage; seedling growth; calyx lobe; dehydration; seed weight; temperature; drought; flooding; recalcitrant seed; endangered reasons



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1. Introduction

Due to over-exploitation and climate change, more and more plant species are becoming endangered, especially species with narrow ecological ranges [1,2]. In 2021, Botanic Gardens Conservation International released a report stating that, due to a series of factors such as deforestation, pests and diseases, and climate change, the world's trees are facing a major crisis, with 30% (17,500 species) of tree species being endangered [3]. Studying the causes of population decline in endangered plant species and formulating effective measures to protect their germplasm resources and population sizes have become important issues in biodiversity conservation research all over the world [4,5]. For most threatened species, difficulties with renewal in the wild and slow population renewal are key causes of the increased risk of extinction [6–8]. Therefore, conservation and restoration

efforts for endangered plant species should pay close attention to the early stages of the life history of the species. Seed germination is a key part of plant life history and is the basis of natural population renewal. Renewal is related to the establishment of seedlings, individual seedling survival, and competitiveness, and affects vegetation distribution and biodiversity level [9,10]. However, this process is also one of the most vulnerable and high-mortality periods in the life history of plant populations [11]. Therefore, research on the seed germination characteristics of endangered plants is of great significance for understanding their life history countermeasures, ecological adaptability, and ability to escape threats, and for researchers to formulate science-based conservation measures [12].

Hopea chinensis (Merr.) Hand.-Mazz. belongs to the Dipterocarpaceae family and is endemic to southern China and Vietnam. It is an evergreen tree and a relic species from the Tertiary Period primarily found in “refuge” habitats formed by specific local terrains. Wood from this tree is durable, extremely corrosion-resistant, and can be used for military uses, machinery, and high-quality furniture, and so it is a high-value timber species for specific purposes. The resin produced by this tree is similar to dammar resin and can be used in lacquer manufacturing. Its by-products include aromatic oils, which can be used in high-grade cosmetics and traditional Chinese herbal medicines, and are of high commercial value. In addition, it is a suitable tree species for landscape gardening because of its strong resistance to pests, diseases, natural decay, and wind damage, as well as its excellent natural attributes such as high heat tolerance and tolerance of nutrient deficiency [13]. Originally distributed in tropical rainforests below 600 m altitude in the Shiwandashan and Daqing Mountains of Guangxi, China, as a consequence of human activities and climate change, *H. chinensis* has become extinct in the Daqing Mountains, and only a small population remains in the valleys and along the stream banks of the Ten Thousand Mountains (Shiwandashan). It has been listed as a Class II national key protected plant and Plant Species with Extremely Small Populations in China and is categorized as a critically endangered (CR) species by the International Union for Conservation of Nature (IUCN) [14].

Previous studies have indicated that population decline due to tree harvesting and habitat loss are the primary reasons for the risk of extinction of *H. chinensis*, whereas slow population renewal under natural conditions is the main constraint on population recovery [13]. At present, there are few studies on *H. chinensis* seeds and seedlings, and those that were carried out only found that *H. chinensis* seeds had a high water content, easy germination, no dormancy, short life, and other information. But the reasons and factors influencing its slow population renewal remain unclear. In light of this, the present study was conducted to investigate the seed germination characteristics of *H. chinensis* from the earliest stage of its life history, with the aim of exploring (I) the biological traits and ecological adaptability of *H. chinensis* seeds, (II) the relationship between seed germination and population renewal, as well as the threat to *H. chinensis*, and (III) the propagation techniques for seed sowing and the selection of *H. chinensis*. This study aims to provide a theoretical foundation for the conservation, population restoration, and artificial propagation of *H. chinensis*.

2. Materials and Methods

2.1. Experimental Materials

In December 2019, a survey was conducted on the fruiting status of wild *H. chinensis* populations. Only four wild populations located in Hongqi Forest Farm in Guangxi Shiwan were normally fruitful: Dashan National Forest Park (HQ); Nanshan Station in Guangxi Fangcheng Golden Camellias National Nature Reserve (NS); Tongzhong Forest Farm in Fangchenggang City, Guangxi (DZ); and Pingfeng Rainforest Park in Dongxing City, Guangxi (PF) (Table 1). In order to ensure the maturity of the seeds, the seeds were collected from the mother trees during the natural shedding period. On 7 January 2020, mature fruits from the HQ, NS, DZ, and PF populations were collected (by gently shaking the tree trunk and collecting the fallen fruits). Fruits from different mother trees in the same

population were collected and then mixed, put in a wet cloth bag, and taken to the laboratory for immediate measurement of fruit morphology and weight. All collection activities were approved by local authorities and the competent authorities. All four populations are distributed in the area of Fangchenggang City, Guangxi; therefore, the climate and soil conditions are similar. In January 2020, the average temperature in Fangchenggang City, Guangxi, was 14.5 °C, with a maximum temperature of 26.0 °C and a minimum temperature of 9.0 °C, with an air humidity of 55%~78% (data from the National Meteorological Information Center (<http://www.nmic.cn/>), accessed on 10 August 2023). The soils of the four populations are all brown soil.

Table 1. Sample locations of *H. chinensis* collection data.

| Site | GPS Coordinates | Altitude (m) | Slope Aspect | Slope Degree (°) | Rock Exposed Degree (%) | Distribution Type | No. of Fruiting Plants |
|------|---------------------------|--------------|--------------|------------------|-------------------------|-------------------|------------------------|
| HQ | 21.6675° N 107.4997° E | 286 | Northwest | 35 | 50 | Contagious | 8 |
| NS | 21.7261° N 108.0425° E | 196 | South | 10 | 80 | Random | 3 |
| DZ | 22.0303° N 107.8944° E | 324 | Southwest | 25 | 85 | Contagious | 4 |
| PF | 21.7061° N 107.9767° E | 188 | Southeast | 20 | 70 | Random | 4 |

2.2. Determination of Morphological Characteristics of Fruits and Seeds

Fifty intact and healthy fruits were randomly selected from each *H. chinensis* populations three times to produce three biological replicates. The length (CLL) and width (CLW) of the two calyx lobes per fruit, seed length (SL), and seed width (SW) of each fruit were measured with Vernier calipers with a precision of 0.01 mm, and the ratios of calyx lobe length/width (CLLW) and seed length/width (SLW) were calculated. Because *H. chinensis* seed is round or oval, the length and width of the seed are measured at the longest and widest part of the seed, respectively. One hundred fruits were randomly selected from the population and, after removing the calyx lobes, the seeds were weighed to a precision of 0.01 g. These 100 seeds were then put back into the population and mixed evenly. Once again, 100 fruits were randomly selected from the population, the calyx lobes of the fruits were removed to obtain the seeds, and then each seed was weighed. This procedure was replicated five times to calculate the thousand seed weight (TSW).

To determine seed moisture content, fifty healthy seeds were randomly selected from each population three times to generate three biological replicates. Fresh seed weight was measured with an accuracy of ± 0.001 g, and then the seeds were dried in an oven at 103 °C for 24 h, and the seed dry weight was determined to calculate the seed moisture content (MC) [15].

The coefficient of variation (CV) was calculated according to the average and standard deviation of each index.

2.3. Experimental Design for Seed Germination Studies

2.3.1. General Seed Germination Experiments

Unless otherwise stated, all germination experiments were carried out according to the following procedure. Before sowing, the seeds were surface-sterilized with 0.2% (*w/v*) potassium permanganate solution for 5 min, and then rinsed with water. Three replicates were set up for each treatment, with 30 healthy seeds being randomly selected for each replicate, were placed in a 120 mm diameter Petri dish containing two disks of moistened filter paper, and germinated in an artificial climate incubator (Ningbo Yanghui RDN-1000; Ningbo Yanghui Instrument Co., Ltd., Ningbo, China) with a temperature of 20 °C, 3000 Lux light intensity with a 12 h/day photoperiod, and 80% relative humidity. Germination was considered to have occurred when the radicle had broken through the

seed coat and grown to one half of the seed length. Seed germination was observed and recorded daily, and appropriate water supplementation was provided. The experiment ended after 20 days. The seeds of four populations were used in the two experiments, which tested the seed germination of different populations and the effect of seed weight on seed germination. The seeds of the NS population were used in the rest of the seed germination experiments.

2.3.2. The Effect of Seed Biological Characteristics on Seed Germination

To compare seed germination among different populations, seeds from the HQ, NS, DZ, and PF populations were used.

To study the effect of calyx lobes and seed coat on seed germination, three treatments were designed, namely S1 (control), seeds with calyx lobes and seed coat intact; S2, seeds with calyx lobes removed but the seed coat retained; and S3, seeds with calyx lobes and seed coat removed.

To study the effect of seed weight on germination, calyx lobes were removed and each seed was weighed to within 0.1 g. Based on seed weight, the seeds were divided into five groups: <0.5 g, 0.5–1.0 g, 1.1–1.5 g, 1.6–2.0 g, and >2.0 g.

To study the effect of seed moisture content on seed germination, the seeds were spread out and placed in a cool indoor place to allow them to naturally dehydrate for 20 days (during which the daily average temperature was 17.0–19.0 °C and the air humidity was 70%–85% RH). Subsamples were taken every two days and divided into two, with one part being used to measure seed moisture content (three replicates were set up, with 20 healthy seeds each replicate) and the other part being used to measure seed germination characteristics (three replicates were set up, with 30 healthy seeds in each replicate). Fresh seeds (water loss rate 0%) were used as controls.

2.3.3. The Effect of Environmental Factors on Seed Germination

In order to understand the suitable temperature or temperature range for the seed germination of *H. chinensis*, seven constant temperatures, namely 10, 15, 20, 25, 30, 35, and 40 °C, and two variable day/night temperature conditions, namely 20/10 and 30/15 °C, were set up (the latter two were chosen to reflect the temperature at the time of dispersal of the *H. chinensis* seeds by the NS population). The 20 °C conditions were used as the controls.

To study the effects of light on seed germination, three light patterns, namely continuous light (3000 Lux, 24/0 h light/dark), continuous darkness (0/24 h light/dark), and periodic light (3000 Lux, 12/12 h dark/light) (control), were set up. To ensure continuous darkness, the germination situation was recorded twice, once during the 7th day and again at the end of the study.

To study the effects of substrate on seed germination, four treatments were set up with filter paper, sand, clay loam, and 1:1 sand: clay loam (by volume) used as substrates in the experiments. Before sowing, all substrates were autoclaved. A 5 cm thick substrate was spread in a plastic box (length × width × height = 28 cm × 17.5 cm × 8 cm), and then 30 seeds were sowed evenly on the surface of the substrate. Three plastic boxes were set up for each treatment. The boxes were placed into an artificial climate incubator (Ningbo Yanghui RDN-1000, Ningbo Yanghui Instrument Co., Ltd., Ningbo, China) at a temperature of 20 °C (24 h), periodic light (3000 Lux 12 h/day), and 80% relative humidity.

To study the effects of burial depth on seed germination, sand was used as the substrate, and a 6 cm thick layer was spread in a plastic box (length × width × height = 28 cm × 17.5 cm × 8 cm). Five treatments were set up at seeding depths of 0 (control), 0.5, 1.0, 3.0, and 5.0 cm. Three plastic boxes were set up for each treatment, and 30 seeds were evenly sown in each plastic box. The boxes were placed into an artificial climate incubator (Ningbo Yanghui RDN-1000, Ningbo Yanghui Instrument Co., Ltd., Ningbo, China) at a temperature of 20 °C (24 h), periodic light (3000 Lux 12 h/day), and 80% relative humidity. The germ breaking through the soil layer was regarded as germination, and the experiment was completed 25 days later.

To study the effects of drought and salinity stress on seed germination, polyethyleneglycol-6000 (PEG-6000) was used to simulate the effect of drought stress on seed germination [16] and NaCl was used to achieve salinity [17]. PEG-6000 solutions at concentrations of 0 (distilled water was used as a control), 10%, 15%, 25%, and 35% (*w/v*) were prepared to produce five treatments, and NaCl solutions at concentrations of 0 (distilled water was used as a control), 100, 200, 300, and 400 mM were prepared to produce five treatments. Two disks of filter paper were laid in 120 mm diameter Petri dishes and moistened with 10 mL of the treatment solution. The seeds were then sown on the filter paper and placed in an incubator. The filter paper and treatment solution were changed every two days.

To study the effect of flooding on seed germination, the seeds were submerged in distilled water for 1 d, 3 d, 5 d, and 7 d, and fresh seeds without flooding were used as a control (0 d), resulting in five treatments. The water was changed once a day during the experiment.

2.3.4. Germination Indicator Statistics

Four parameters, namely germination percentage (GP), germination energy (GE), mean germination time (MGT), and vitality index (VI), were selected to evaluate seed vigor [18] and calculated as follows:

$$GP (\%) = \frac{\text{total number of germinated seeds}}{\text{number of test seeds}} \times 100 \quad (1)$$

$$GE (\%) = \frac{\text{number of seeds germinated within 7 days}}{\text{number of test seeds}} \times 100 \quad (2)$$

$$MGT = \sum (n_i \times t_i) / \sum n_i \quad (3)$$

where n_i is the number of seeds that germinated on day i after sowing and t_i is the number of days after sowing.

$$VI = \sum (n_i / t_i) \times (LR + SH) \quad (4)$$

where LR and SH refer to the length of the root (cm) and seedling height (cm) at the end of the germination test, respectively (in this study, LR is the total length of the radicle and hypocotyl). At the end of the seed germination period in each study, ten seedlings were selected at random from each plastic box for the measurement of LR and SH; in boxes with fewer than 10 seedlings, all seedlings were measured.

2.4. Data Analysis

Microsoft Excel 2016 was used to carry out statistical analyses and data mapping of the biological characteristics of *H. chinensis* seeds. SPSS 19.0 (IBM, Armonk, NY, USA) was used to carry out analysis of variance (ANOVA) and multiple pairwise comparisons of germination indices of seeds under different treatments (Duncan's new multiple range test). The significance levels of the above statistical analyses were set at $p < 0.05$.

3. Results

3.1. Biological Traits of the *H. chinensis* Seeds

The fruit of *H. chinensis* is a nut, connected to 1- or 2-year-old branches via a slender carpodium at the base (Figure 1A,B). The fruit is ovoid and approximately 1.4 cm long and 1.0 cm wide, with a pointed tip. It is covered by five elongated calyx lobes, of which two lobes enlarge into lanceolate or elliptic wings approximately 8.0 cm long and 2.0 cm wide, with 11–12 longitudinal veins. The remaining three lobes do not expand and are approximately 0.5–1.0 cm long (Figure 1C). The fruits of *H. chinensis* ripen from December to January, turning from green to yellowish-green when ripe. The calyx lobes change from green to brown (Figure 1C). Each fruit contains one seed enclosed by a seed coat. The seed coat can be easily separated from the seed, which consists of a cylindrical red embryo,

with a green endosperm. The endosperm splits into four petals, and the bottom of the endosperm is connected to the embryo (Figure 1D).



Figure 1. Fruit and seed morphology of *H. chinensis*: (A) parent plant of *H. chinensis*; (B) *H. chinensis* plant bearing fruit; (C) fruit morphology of *H. chinensis*; and (D) internal morphology of *H. chinensis* seed.

The differences in SL, SW, and SLW among the four *H. chinensis* populations were not significant ($p > 0.05$), whereas CLL, CLW, CLLW, TSW, and MC showed significant differences ($p < 0.05$) (Table 2). Specifically, the DZ and PF populations had a larger CLL, measuring 9.0 cm and 8.7 cm, respectively, which are significantly greater values than those found for the HQ and NS populations. The CLW of the DZ population was the largest (2.6 cm), being significantly greater than that of the other three populations. Additionally, the PF population exhibited a significantly larger CLLW, TSW, and MC than the other three populations (Table 2).

Table 2. Biological characteristics (mean \pm SD) and coefficient of variation (%) of *H. chinensis* fruits from different populations.

| Trait | | Population | | | | Mean |
|------------|----------|----------------|----------------|----------------|----------------|-------|
| | | DZ | HQ | PF | NS | |
| Phenotypic | SL (mm) | 15.0 ± 1.3 a | 13.9 ± 1.3 a | 14.6 ± 1.1 a | 13.7 ± 1.1 a | 14.3 |
| | | 8.8% | 9.2% | 7.6% | 7.8% | 8.2% |
| | SW (mm) | 10.4 ± 1.2 a | 9.2 ± 0.9 a | 10.7 ± 0.7 a | 10.2 ± 0.8 a | 10.1 |
| | | 11.1% | 9.8% | 6.9% | 8.1% | 9.7% |
| | SLW | 1.5 ± 0.1 a | 1.5 ± 0.1 a | 1.4 ± 0.2 a | 1.4 ± 0.0 a | 1.4 |
| | | 9.3% | 7.2% | 16.2% | 2.9% | 10.1% |
| | CLL (cm) | 9.0 ± 0.3 a | 7.2 ± 0.6 c | 8.7 ± 0.9 ab | 7.5 ± 0.8 bc | 8.1 |
| | | 3.6% | 8.3% | 10.3% | 10.3% | 12.3% |
| | CLW (cm) | 2.6 ± 0.2 a | 1.7 ± 0.2 b | 1.8 ± 0.1 b | 1.9 ± 0.0 b | 2.0 |
| | | 7.2% | 9.9% | 5.6% | 2.2% | 20.5% |
| | CLLW | 3.5 ± 0.3 c | 4.3 ± 0.1 b | 5.0 ± 0.6 a | 4.0 ± 0.2 bc | 4.2 |
| | | 7.2% | 3.3% | 13.0% | 4.2% | 15.4% |
| | TSW (g) | 598.8 ± 32.6 b | 584.0 ± 29.1 b | 719.5 ± 30.6 a | 470.7 ± 25.6 c | 593.3 |
| | | 5.4% | 5.0% | 4.3% | 5.4% | 16.1% |
| | MC (%) | 46.7 ± 2.2 b | 46.8 ± 1.6 b | 56.3 ± 2.7 a | 42.6 ± 0.9 c | 48.1 |
| | | 4.8% | 3.4% | 4.8% | 2.1% | 11.5% |

Table 2. Cont.

| Trait | | Population | | | | Mean |
|--------------------|---------|--------------|--------------|--------------|--------------|-------|
| | | DZ | HQ | PF | NS | |
| Germination traits | GP (%) | 73.3 ± 7.6 b | 91.7 ± 2.9 a | 76.7 ± 7.6 b | 90.0 ± 5.0 a | 82.9 |
| | | 10.4% | 3.2% | 10.0% | 5.6% | 11.9% |
| | GE (%) | 36.7 ± 5.8 a | 40.0 ± 5.0 a | 31.7 ± 2.9 a | 31.7 ± 5.8 a | 35.0 |
| | | 15.8% | 12.5% | 9.1% | 18.2% | 16.1% |
| | MGT (d) | 8.5 ± 0.8 b | 9.8 ± 0.1 a | 9.7 ± 0.4 a | 10.2 ± 0.3 a | 9.6 |
| | | 8.8% | 1.4% | 4.0% | 2.7% | 8.0% |
| | LR (cm) | 5.9 ± 0.3 b | 6.6 ± 0.3 a | 6.1 ± 0.3 ab | 6.2 ± 0.2 ab | 6.2 |
| | | 5.8% | 5.1% | 4.3% | 2.6% | 6.2% |
| | SH (cm) | 5.7 ± 0.4 c | 7.7 ± 0.4 a | 5.3 ± 0.4 c | 6.4 ± 0.2 b | 6.3 |
| | | 7.8% | 4.5% | 7.9% | 3.5% | 16.2% |
| | VI | 24.2 ± 2.3 c | 35.9 ± 2.3 a | 21.3 ± 2.5 c | 29.2 ± 0.3 b | 27.6 |
| | | 9.7% | 6.4% | 11.6% | 1.0% | 22.0% |

Note: SL: seed length; SW: seed width; CLL: calyx lobe length; CLW: calyx lobe width; SLW: seed length/seed width; CLLW: calyx lobe length/calyx lobe width; TSW: thousand seed weight; MC: moisture content; GP: germination percentage; GE: germinative energy; MGT: mean germination time; LR: length of root; SH: seedling length; VI: vitality index; DZ: Dongzhong Forest Farm; HQ: Hongqi Forest Farm of Shiwandashan National Nature Reserve; PF: Dongxing Pingfeng Rainforest Park; NS: Nanshan Station of Guangxi Fangcheng Golden Camellias National Nature Reserve. Percentage represents coefficient of variation. The row data for the same trait with different lowercase letters indicate significant differences between populations.

The coefficient of variation averaged over the four populations for the eight phenotypic traits of the *H. chinensis* seeds ranged from 8.2% (SL) to 20.5% (CLW).

The differences in GP, MGT, VI, and SH among the four *H. chinensis* populations were all significant ($p < 0.05$). The GP of the HQ and NS populations was significantly greater than that of the DZ and PF populations. The SH and VI of the HQ population were significantly greater than those of the other three populations, whereas the MGT of the DZ population was significantly smaller than that of the other three populations. The average coefficient of variation of the six germination traits of *H. chinensis* ranged from 6.2% (LR) to 22.0% (VI) (Table 2).

3.2. Effects of Biological Traits of *H. chinensis* Seeds on Their Germination

3.2.1. Effect of Calyx Lobes and Seed Coat on Seed Germination

The differences in GP among the S1, S2, and S3 treatments were not significant (Table 3). The S3 treatment showed a significantly higher GE and VI compared with S1 and S2, but MGT and LR were significantly smaller than those of S1 and S2, and the SH of the S1 treatment was significantly larger than that of the S2 and S3 treatments (Figure 2). These results indicate that the presence or absence of calyx lobes and seed coat does not significantly affect the seed germination rate. Retaining the calyx lobes can promote seedling height growth, whereas removing the calyx lobes and the seed coat can improve seed germination uniformity and vitality index, as well as shortening the germination time.

Table 3. Effects of biological traits on mean ± SD seed germination parameters of *H. chinensis*.

| Study Object | Treatment | GP (%) | GE (%) | MGT (d) | VI |
|---------------------------|-----------|--------------|--------------|--------------|--------------|
| Calyx lobes and seed coat | S1 | 85.0 ± 5.0 a | 33.3 ± 2.9 b | 9.4 ± 0.3 b | 31.3 ± 0.7 b |
| | S2 | 90.0 ± 5.0 a | 31.7 ± 5.8 b | 10.2 ± 0.3 a | 29.2 ± 0.3 b |
| | S3 | 93.3 ± 2.9 a | 65.0 ± 5.0 a | 6.8 ± 0.2 c | 41.5 ± 3.1 a |

Table 3. Cont.

| Study Object | Treatment | GP (%) | GE (%) | MGT (d) | VI |
|-----------------------------|-----------|---------------|---------------|--------------|---------------|
| Seed weight | <0.5 g | 0.0 | – | – | – |
| | 0.5–1.0 g | 25.0 ± 5.0 b | 13.3 ± 2.9 d | 8.0 ± 0.4 ab | 6.6 ± 0.8 d |
| | 1.1–1.5 g | 93.3 ± 5.8 a | 41.7 ± 2.9 c | 8.3 ± 0.3 a | 31.3 ± 2.3 c |
| | 1.6–2.0 g | 96.7 ± 2.9 a | 48.3 ± 2.9 b | 7.6 ± 0.2 b | 44.6 ± 2.9 b |
| | >2.0 g | 95.0 ± 5.0 a | 73.3 ± 2.9 a | 6.0 ± 0.4 c | 49.3 ± 1.4 a |
| Dehydration period duration | 0 d | 90.0 ± 5.0 a | 31.7 ± 5.77 a | 10.2 ± 0.3 b | 29.2 ± 0.3 a |
| | 2 d | 86.7 ± 2.9 ab | 30.0 ± 0.0 ab | 10.1 ± 0.3 b | 25.7 ± 1.6 ab |
| | 4 d | 83.3 ± 2.9 b | 25.0 ± 5.0 b | 10.1 ± 0.3 b | 22.7 ± 2.7 b |
| | 6 d | 71.7 ± 2.9 c | 8.3 ± 2.9 c | 10.7 ± 0.4 b | 16.5 ± 1.1 d |
| | 8 d | 51.7 ± 2.9 d | 5.0 ± 5.0 cd | 10.4 ± 0.3 b | 10.8 ± 1.1 e |
| | 10 d | 38.3 ± 2.9 e | 0.0 ± 0.0 d | 10.3 ± 0.5 b | 6.1 ± 0.5 f |
| | 12 d | 28.3 ± 2.9 f | 0.0 ± 0.0 d | 11.5 ± 0.4 a | 3.3 ± 0.4 g |
| | 14 d | 13.3 ± 2.9 g | 0.0 ± 0.0 d | 12.1 ± 0.5 a | 1.4 ± 0.3 g |
| | 16 d | 0.0 | – | – | – |

Note: S1: seeds with calyx lobes and seed coat intact; S2: seeds without calyx lobes but with seed coat; S3: seeds with both calyx lobes and seed coat removed. Different lowercase letters in the same column and study object indicate significant differences at $p = 0.05$. – indicates no data.

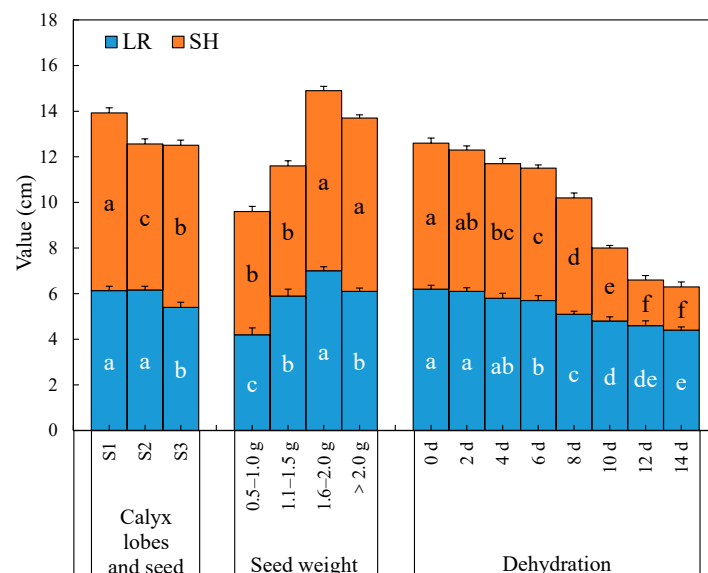


Figure 2. Effects of biological traits on root length and seedling height of *H. chinensis* seedlings. LR: length of root; SH: seedling height; S1: seeds with calyx lobes and seed coat intact; S2: seeds without calyx lobes but with the seed coat; S3: seeds with both calyx lobes and seed coat removed. Significant differences within the same trait ($p < 0.05$, using Duncan's new multiple range test) are indicated by different lowercase letters. The error bars represent SD.

3.2.2. Effect of Seed Weight on Seed Germination

The distribution of seeds among the five weight grades showed significant differences ($p < 0.05$). Among them, seeds weighing between 1.6 and 2.0 g and between 1.1 and 1.5 g made up a high proportion of all the seeds, accounting for 27.78% and 23.50%, respectively, whereas seeds weighing between 0.6 and 1.0 g had the lowest proportion at 11.97%. Seeds weighing more than 1.0 g accounted for 69.23% of all seeds (Figure 3).

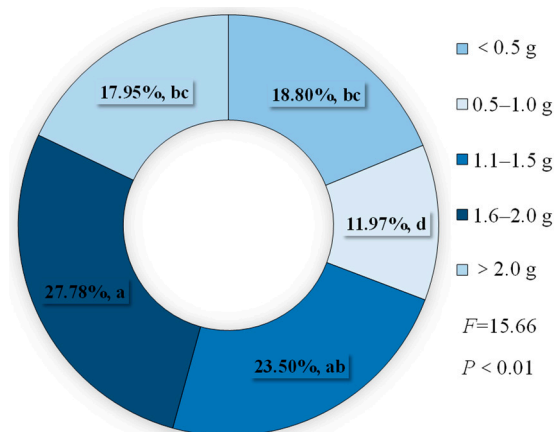


Figure 3. Percentage of seed quality grading in *H. chinensis*. Significant differences ($p < 0.05$ using Duncan's new multiple range test) are indicated by different lowercase letters.

The differences in GP, GE, MGT, VI, LR, and SH among the four seed weight grades were all significant ($p < 0.05$). The GP of seeds weighing between 1.1 and 1.5 g, between 1.6 and 2.0 g, and >2.0 g did not differ significantly, with percentages of 93.3%, 96.7%, and 95.0%, respectively. All of them had a significantly higher GP than seeds weighing between 0.6 and 1.0 g (25.0%). With increasing seed weight, seed GE and VI increased gradually, whereas MGT showed a decreasing trend in response to increasing seed weight (Table 3). Among the four larger seed weight grades, seeds weighing between 1.6 and 2.0 g and >2.0 g had a larger seed SH, and seeds weighing between 1.6 and 2.0 g had the longest seed LR (Figure 2).

3.2.3. Effect of Water Content on Seed Germination

The initial water content of *H. chinensis* seeds in the NS population was 42.6%. As the period of indoor dehydration increased, the seed water content gradually decreased, showing a binomial pattern. The seed water content decreased faster from 0 to 6 days, and then slowed from the 6th to 14th days, with the seed water content decreasing faster from the 14th to 18th days. By the 18th day, the seed water content was 18.91%, representing a 55.6% decrease relative to the initial water content (Figure 4).

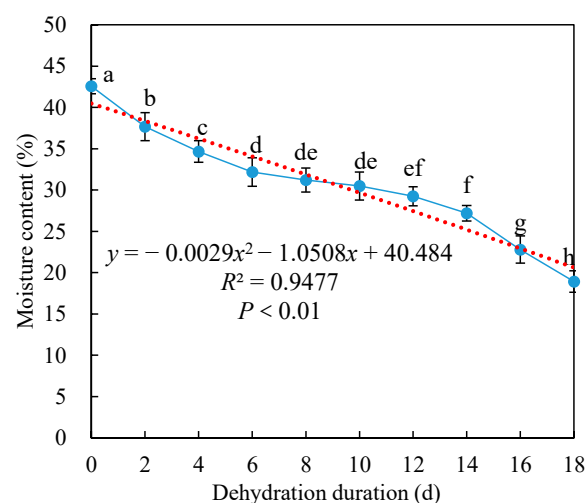


Figure 4. Moisture content of *H. chinensis* seeds in response to the duration of the dehydration period. Significant differences ($p < 0.05$, using Duncan's new multiple range test) are indicated by different lowercase letters. The error bars represent SD.

There were significant differences in GP, GE, MGT, VI, LR, and SH among the different dehydration duration treatments ($p < 0.05$). As the dehydration period increased, the GP, GE, VI, LR, and SH of the seeds showed a gradual decreasing trend. There was no significant change in seed MGT when the dehydration period was less than 10 days. However, seed MGT increased significantly when the dehydration period was more than 10 days. During dehydration from 0 to 4 days, the decrease in GP of the seeds was slow. The seed GP was 90.0% at 0 d, and seed germination was 83.3% at 4 d of dehydration, being 7.4% lower than that of fresh seeds (i.e., at 0 d). From 4 to 16 days of dehydration, the germination rate of the seeds decreased rapidly. By the 8th day, the germination rate had decreased to 51.67%, which was close to seed semi-lethality. By the 16th day, the seeds had completely lost their viability and could not germinate (Table 3, Figure 2).

3.3. Effects of Environmental Factors on Seed Germination of *H. chinensis*

3.3.1. Effect of Temperature on Seed Germination

There were significant differences in GP, GE, MGT, VI, LR, and SH under different temperature conditions ($p < 0.05$). However, the GP of seeds at 20 °C, 25 °C, and 30 °C did not differ significantly from one another, with values of 88.3%, 90.0%, and 83.3%, respectively, which were significantly higher than the GP of seeds incubated under other temperature conditions. The GE, VI, LR, and SH of seeds at 30 °C were the highest. The MGT of seeds at 35 °C and 40 °C was shorter, with values of 4.3 d and 4.2 d, respectively (Table 4, Figure 5).

Table 4. Effects of environmental factors on mean \pm SD seed germination parameters of *H. chinensis*.

| Study Object | Treatment | GP (%) | GE (%) | MGT (d) | VI |
|--------------|---------------------|-------------------|--------------------|-------------------|------------------|
| Temperature | 15 °C | 60.0 \pm 5.0 d | 16.7 \pm 2.9 e | 10.9 \pm 0.8 a | 5.4 \pm 0.6 f |
| | 20 °C | 88.3 \pm 5.8 a | 33.3 \pm 5.8 b | 9.2 \pm 0.4 b | 23.1 \pm 1.2 c |
| | 25 °C | 90.0 \pm 5.0 a | 31.7 \pm 5.8 bc | 10.2 \pm 0.3 a | 29.2 \pm 0.3 b |
| | 30 °C | 83.3 \pm 2.9 ab | 46.7 \pm 2.9 a | 7.4 \pm 0.6 c | 45.2 \pm 1.2 a |
| | 35 °C | 33.3 \pm 5.8 e | 30.0 \pm 5.0 bcd | 4.3 \pm 0.4 d | 9.4 \pm 1.7 e |
| | 40 °C | 26.7 \pm 2.9 e | 23.3 \pm 2.9 de | 4.2 \pm 0.5 d | 2.8 \pm 0.3 g |
| | 20/10 °C | 68.3 \pm 2.9 c | 25.0 \pm 0.0 cd | 9.4 \pm 0.2 b | 15.7 \pm 0.7 d |
| | 30/15 °C | 78.3 \pm 2.9 b | 36.7 \pm 2.9 b | 8.9 \pm 0.3 b | 30.2 \pm 3.4 b |
| Light | Continuous darkness | 86.7 \pm 2.9 a | 30.0 \pm 5.0 a | – | – |
| | Continuous light | 91.7 \pm 2.9 a | 33.3 \pm 2.9 a | – | – |
| | Periodic light | 88.3 \pm 7.6 a | 31.7 \pm 5.8 a | – | – |
| Substrate | Filter paper | 90.0 \pm 5.0 a | 31.7 \pm 5.8 a | 10.2 \pm 0.3 a | 29.2 \pm 0.3 b |
| | Sand | 88.3 \pm 2.9 a | 33.3 \pm 2.9 a | 10.0 \pm 0.4 a | 34.5 \pm 0.9 a |
| | Clay loam | 85.0 \pm 5.0 a | 30.0 \pm 5.0 a | 10.2 \pm 0.4 a | 25.7 \pm 1.3 c |
| | Sand + Clay loam | 85.0 \pm 5.0 a | 30.0 \pm 0.0 a | 9.8 \pm 0.3 a | 27.8 \pm 1.2 b |
| Burial depth | 0.0 cm | 86.7 \pm 2.9 a | 28.3 \pm 2.9 a | 9.7 \pm 0.2 d | 29.8 \pm 1.5 a |
| | 0.5 cm | 28.3 \pm 2.9 d | 0.0 \pm 0.0 b | 15.0 \pm 0.3 b | 3.6 \pm 0.4 d |
| | 1.0 cm | 38.3 \pm 2.9 c | 0.0 \pm 0.0 b | 15.0 \pm 0.1 b | 7.8 \pm 0.5 c |
| | 3.0 cm | 66.7 \pm 2.9 b | 0.0 \pm 0.0 b | 13.9 \pm 0.4 c | 17.3 \pm 0.4 b |
| | 5.0 cm | 65.0 \pm 5.0 b | 0.0 \pm 0.0 b | 16.1 \pm 0.3 a | 17.5 \pm 0.9 b |
| PEG6000 | 0% | 95.6 \pm 3.9 a | 46.7 \pm 13.3 a | 7.7 \pm 0.7 d | 20.6 \pm 1.4 a |
| | 5% | 82.2 \pm 3.9 b | 40.0 \pm 6.7 a | 7.9 \pm 0.4 d | 17.1 \pm 1.0 b |
| | 10% | 68.9 \pm 3.9 c | 24.4 \pm 3.9 b | 9.6 \pm 0.6 c | 8.8 \pm 1.0 c |
| | 15% | 48.9 \pm 7.7 d | 4.4 \pm 3.9 c | 11.8 \pm 0.9 b | 3.5 \pm 0.5 d |
| | 25% | 20.0 \pm 6.7 e | 0.0 \pm 0.0 c | 12.8 \pm 0.8 ab | 0.5 \pm 0.2 e |
| | 35% | 8.9 \pm 3.9 f | 0.0 \pm 0.0 c | 13.2 \pm 0.8 a | 0.2 \pm 0.1 e |

Table 4. Cont.

| Study Object | Treatment | GP (%) | GE (%) | MGT (d) | VI |
|--------------|-----------|---------------|---------------|--------------|--------------|
| NaCl | 0 mM | 95.6 ± 3.9 a | 46.7 ± 13.3 a | 7.7 ± 0.7 c | 20.6 ± 1.4 a |
| | 100 mM | 82.2 ± 7.7 b | 26.7 ± 17.6 b | 9.3 ± 1.0 bc | 14.3 ± 2.4 b |
| | 200 mM | 48.9 ± 10.2 c | 11.1 ± 3.9 bc | 10.2 ± 1.1 b | 5.0 ± 1.0 c |
| | 300 mM | 42.2 ± 7.7 c | 0.0 ± 0.0 c | 13.2 ± 0.8 a | 2.3 ± 0.5 d |
| | 400 mM | 6.7 ± 0.0 d | 0.0 ± 0.0 c | 11.0 ± 1.0 b | 0.1 ± 0.0 d |
| Waterlogging | 0 d | 90.0 ± 0.0 a | 33.3 ± 2.9 a | 9.8 ± 0.3 a | 30.5 ± 1.5 a |
| | 1 d | 86.7 ± 2.9 a | 33.3 ± 2.9 a | 9.4 ± 0.4 a | 29.5 ± 2.3 a |
| | 3 d | 65.0 ± 5.0 b | 30.0 ± 5.0 ab | 8.6 ± 0.1 b | 18.7 ± 2.3 b |
| | 5 d | 40.0 ± 5.0 c | 26.7 ± 2.9 b | 7.3 ± 0.3 c | 11.8 ± 1.8 c |
| | 7 d | 15.0 ± 5.0 d | 11.7 ± 2.9 c | 6.6 ± 0.5 d | 3.5 ± 1.1 d |

Note: GP: germination percentage; GE: germinative energy; MGT: mean germination time; VI: vitality index. Different lowercase letters in the same column and same study object indicate significant differences at $p = 0.05$. – indicates no data.

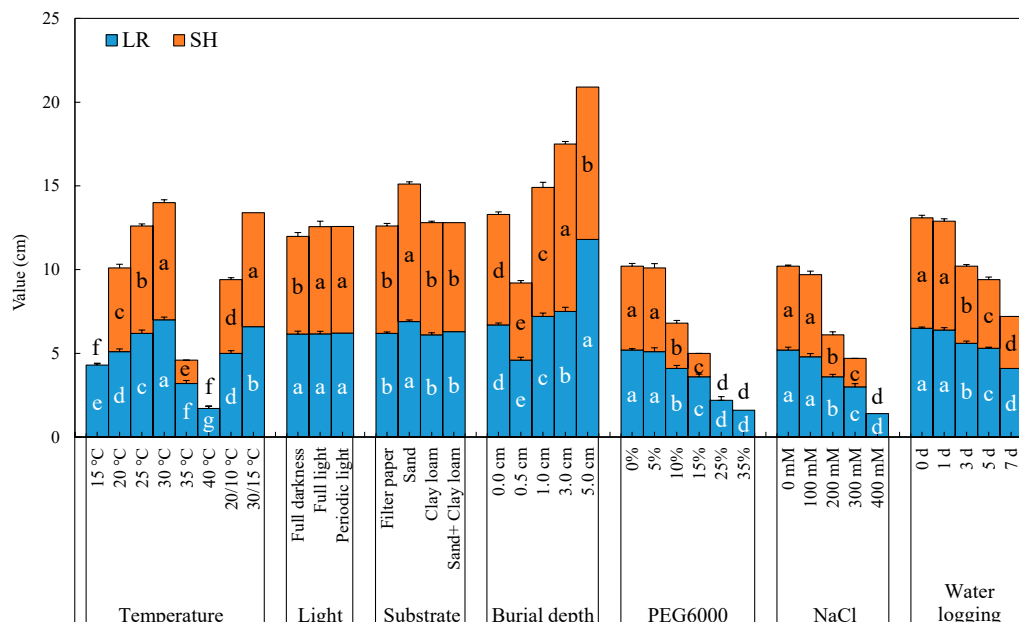


Figure 5. Effects of environmental factors on mean ± SD root length and seedling height of *H. chinensis* seedlings. LR: root length; SH: seedling height. Significant differences ($p < 0.05$ using Duncan's new multiple range test) are indicated by different lowercase letters. The error bars represent SD.

3.3.2. Effect of Light on Seed Germination

In this experiment, to ensure that the continuous dark treatment was not affected by light, germination was not recorded on a daily basis; therefore, only four indices, GP, GE, LR, and SH, were calculated. There were no significant differences in GP, GE, or LR ($p > 0.05$), but significant differences in SH ($p < 0.05$) existed among the three light treatments. Multiple comparison results show that SH was significantly smaller under continuous dark conditions than under periodic light and continuous light (Table 4, Figure 5). In conclusion, light had no effect on the germination of *H. chinensis* seeds but had some effect on seedling growth.

3.3.3. Effects of Substrate on Seed Germination

There were no significant differences in seed GP, GE, or MGT among the four substrates ($p > 0.05$), but significant differences in VI, LR, and SH occurred among the four substrates ($p < 0.01$). Among the four germination substrates, the seeds in the fine-sand substrate had the largest VI, LR, and SH, which were 34.5, 6.9 cm and 8.2 cm, respectively, and were significantly larger than those in the remaining three substrates (Table 4, Figure 5). The

results indicate that the substrate had no significant effect on seed germination but could significantly affect seedling growth.

3.3.4. Effect of Burial Depth on Seed Germination

With increasing burial depth, the seed GP and VI of the *H. chinensis* seeds showed a trend of decreasing first and then increasing. The GP, GE, and VI of 0 cm seeds were the highest, at 86.7%, 28.3%, and 29.8, respectively, which were significantly greater than the other four burial depths. The MGT of the seeds buried at 0 cm of depth was the smallest (9.7 d), being significantly lower than that at the other four burial depths (Table 4). Seeds buried at 3.0 cm had the highest SH (10.0 cm), and seeds buried at 5.0 cm had the largest LR (11.8 cm). The LR and SH values of the seeds buried at 1.0 cm, 3.0 cm, and 5.0 cm depths were significantly greater than those of the seeds at 0 cm depth (Figure 5).

3.3.5. Effects of Simulated Drought Stress on Seed Germination

There were significant differences in seed GP, GE, MGT, VI, LR, and SH among seeds exposed to different concentrations of PEG-6000-simulated drought stress ($p < 0.05$). As the concentration of PEG-6000 (drought stress) increased, the seed GP, GE, VI, LR, and SH showed a gradual decreasing trend, and MGT showed a gradual increasing trend in response to increasing stress. Seed GP and VI were significantly higher in the 0% PEG-6000 treatment than in the other concentrations. The seed GE, LR, and SH showed no significant difference between the 0% and 5% PEG-6000 treatments but were significantly higher in these treatments than in the other concentrations (Table 4, Figure 5). These results indicate that seeds of *H. chinensis* are sensitive to drought, whereas the seedlings could tolerate mild drought stress.

3.3.6. Effects of Salt on Seed Germination

With increasing NaCl concentration, seed GP, GE, VI, LR, and SH showed a gradually decreasing trend. Under the 0 mM NaCl treatment, seed GP, GE, VI, and LR were the highest of all the treatments, measuring 95.6%, 46.7%, 20.6, and 5.2 cm, respectively, and were significantly higher than the corresponding values for the other concentrations. The SH of seeds treated with 0 mM NaCl and 100 mM NaCl was significantly higher than that of seeds treated with the remaining concentrations. In response to the increase in NaCl concentration, seed MGT showed an increasing and then decreasing trend, reaching a maximum value of 13.2 d under the 300 mM NaCl treatment (Table 4, Figure 5).

3.3.7. Effects of Flooding on Seed Germination

With the increasing duration of flooding time, seed GP, GE, MGT, VI, LR, and SH all showed a gradual trend of decreased values. There were no significant differences in seed GP, GE, MGT, VI, LR, or SH between the 1 d and 0 d flooding treatments, whereas the GP, MGT, VI, LR, and SH of seeds flooded for 3 d, 5 d, and 7 d periods were significantly lower than those under the control (0 d) treatment (Table 4, Figure 5). These results indicate that short-term flooding has no significant impact on the germination of *H. chinensis* seeds. However, when the duration of flooding exceeded three days, the vigor of the *H. chinensis* seeds decreased significantly.

4. Discussion

4.1. Differences in Biological Seed Characteristics among Different Populations of *H. chinensis*

There were no significant differences in *H. chinensis* seed shape, whereas the calyx lobes showed significant variation, with the area of the lobes in the DZ population being the largest, favoring seed dispersion. Generally speaking, the larger the seed shape of a tree species, the higher the weight [19]. The seed shapes of these four populations were basically similar, but there were significant differences in the thousand-grain weight and moisture content of the seeds, which could have been caused by the genetics of the mother tree and the habitat during seed formation [20,21]. There was abundant variation

in seed germination parameters among the four populations. In these four *H. chinensis* populations, the coefficients of variation of the phenotypic and germinative characters of the NS population were the lowest, indicating that the seed variation of the NS population was stable and well adapted to the local environment.

There are two strategies for seed resources: one is to produce a small number of large seeds to gain a competitive advantage, and the other is to produce many small seeds to occupy more ecological niches [22]. The results show that the single-seed weight of *H. chinensis* seeds was more than 1.0 g, which is higher than the average seed weight for arboreal species (0.328 g) [23]. Therefore, *H. chinensis* prefers the reproductive strategy of producing a small number of large seeds to support rapid growth of early seedlings via a well-developed endosperm structure in order to gain a competitive advantage.

4.2. The Influence of Biological Characters on *H. chinensis* Seed Germination

Research has shown that the influence of the seed coat and its appendages on seed germination involved mostly mechanical obstruction or chemical inhibition [24,25]. In the current study, there was no significant effect of the calyx lobes and seed coat on the seed germination rate. Retaining the calyx lobes could clearly promote the early growth of the seedling, possibly because, after decomposition, the calyx lobes could provide some nutrition to power seedling growth. After removing the seed coat, the indices of seed germination potential and vigor were markedly improved, but the time taken to achieve germination was clearly reduced. This result indicates that removing the seed coat not only enhanced germination uniformity and vigor, but also shortened germination time. These effects may be because the seed could absorb water more quickly, promoting germination, when the seed coat was removed [26].

It has been noted that seed size is positively correlated with GP and seedling growth [27]. However, it was also reported that seed size is not significantly correlated with germination [28]. *H. chinensis* seeds weighing 0.5 g or less did not germinate, possibly because they were abortive or underdeveloped seeds. The GP of seeds weighing >1.0 g was significantly higher than that of seeds weighing <1.0 g, indicating that the GP of medium- and large-sized seeds was obviously greater than that of small-sized seeds. The GE and VI of >2.0 g seeds were larger, the MGT was smaller, and the LR and SH of >1.5 g seeds were larger; this finding, that large seeds resulted in faster germination and larger seedlings, is similar to the results reported for *Acacia catechu*, *Acacia nilotica* [29], *Castanopsis chinensis* [30], and *Polylepis tomentella* [31] seeds. Therefore, the seed size of *H. chinensis* is positively correlated with GP and seedling growth. The reason for this relationship could be that large seeds store more nutrients, enough to power rapid germination and rapid early growth of seedlings [31].

The water loss rate of *H. chinensis* seeds was fast under indoor conditions; with the decrease in water content, the germination rate of seed gradually decreased. When the seed moisture content was higher than 34.67% (natural dehydration for less than 4 days), the decreases in seed germination percentage, germination energy, and vitality index were smaller. However, after 4 days of dehydration, these three germination indices decreased sharply, and after 16 days of dehydration, seeds lost their vitality, showing that *H. chinensis* seeds were intolerant of water loss and were sensitive to dehydration; they could only tolerate mild dehydration. In the original habitat, if there are no suitable germination conditions after the seeds fall off from mother plants, they will soon lose their vitality due to dehydration. Furthermore, the research results show that *H. chinensis* seeds were sensitive to low temperature (seeds could not germinate at temperatures lower than 10 °C), indicating that *H. chinensis* seed behavior is similar to that of seeds from the rainforest, such as *Hopea mollissima* [32], *Butia capitata* [33], *Garcinia gummi-gutta* [34], and *Garcinia mangostana* [35]. These are recalcitrant seeds and belong to the highly recalcitrant type proposed by Farrant (1988) [36].

4.3. The Influence of Environmental Factors on *H. chinensis* Seed Germination

Temperature plays a vital role in seed germination; suitable temperatures can enhance enzyme activity, promote the conversion of food reserves into metabolic building blocks and energy, and promote the seed germination rate [37]. The GP of *H. chinensis* seeds was high at 20 °C, 25 °C, and 30 °C, and at 30 °C, the GE, VI, LR and SH were the highest, which meant that the optimal temperature of *H. chinensis* seeds was approximately 30 °C. The seeds would not germinate when the temperature was lower than 10 °C. The seeds of *H. chinensis* mature in winter, when the temperature is low (the average temperature in January is 14.5 °C), which will inhibit seed germination. Therefore, the low temperature in winter is one of the factors affecting the regeneration of *H. chinensis* populations. There was no significant difference in *H. chinensis* germination rate and germination potential among the seeds of *H. chinensis* under continuous darkness, periodic, or continuous light. The results suggest that light is not essential for seed germination in *H. chinensis*: the seeds of *H. chinensis* are light-neutral seeds [38]. This is a strategy by which *H. chinensis* seeds can adapt to the changeable illumination in their natural habitat, under the forest canopy.

The results of the substrate experiment show that there was no significant effect of substrate type on the seed germination of *H. chinensis* but that it obviously influenced seedling growth. It may be that the seeds were less dependent on the outside environment to germinate, after meeting the necessary temperature and moisture conditions. Of the four substrates, filter paper and fine sand had the highest water retention and gas permeability; on the other hand, the water permeability of clay-based soil was poor. Under conditions of insufficient oxygen, the seeds would produce harmful metabolic products and too much heat because of respiration, leading to a decline in seed vigor [39]. This could be the reason why the seedling height and vigor index of *H. chinensis* were significantly lower in the clay-based soil substrate than in the other three substrates. The soil types of the four *H. chinensis* populations are all brown soil with a loam texture and good water permeability. Therefore, the soil in the habitat may not be the factor restricting population regeneration. The seed burial depth study results indicate that burial obviously reduced the germination rate and vigor index and prolonged the period of seed germination, but could significantly promote seedling growth if the depth reached 1.0–5.0 cm. It is possible that seed burial reduced the contact between seeds and air, making it difficult for seeds to germinate. However, seed burial was advantageous to the attachment growth of radicles, promoting seedling growth.

Seed germination and seedling growth are closely related to habitat conditions. Water and mineral salts are vital environmental factors affecting seed germination and seedling growth [40]. This study indicated that drought stress and salinity stress could significantly inhibit the seed germination and seedling growth of *H. chinensis*, the rate of seed germination being extremely low under severe drought stress and salinity stress. The results show that the adaptability of *H. chinensis* seeds to drought and salt was weak, a finding similar to that observed for the seeds of *Pinus pinea* [41], *Lathyrus sativus* [42], and *Citrullus colocynthis* [43]. Too much water also inhibited *H. chinensis* seed germination. The present research showed that seeds of *H. chinensis* could withstand short-term flooding, but when the flooding time continued for more than three days, seed vigor decreased significantly. This effect was due to the seeds not obtaining enough oxygen during long-term flooding, producing alcohol as a result of anaerobic respiration, and inhibiting the normal physiological metabolism activities of seeds [44].

4.4. The Relationship between Seed Germination and the Threat of Extinction of *H. chinensis*

The seed of *H. chinensis* has two enlarged calyx lobes; when mature seeds of *H. chinensis* fall, the enlarged calyx lobes may spin, slowing down the dropping speed of falling seeds and reducing any impact injury. In addition, enlarged calyx lobes can promote seed dispersal, with the effect of wind increasing the distance of spread. Nevertheless, seed spread is influenced by the effect of gravity. Because of the weight of individual seeds, most seeds spread under the canopy of the parent tree; this reduces the risk that the seeds will lose their vitality if they float into direct sunlight or dry habitats, but it also narrows the

dispersal range of the seeds at the same time, limiting the extent and speed of *H. chinensis* population spread [45]. The *H. chinensis* seed embryo is red, which may be caused by the presence of anthocyanins in the seed embryo [46]. Anthocyanins help plants resist the stress of reactive oxygen species caused by abiotic factors [47]. *H. chinensis* seeds mature in winter, which is a dry season, and low temperatures and drought can easily cause seed free radical accumulation and membrane lipid peroxidation damage to seeds [48]. *H. chinensis* seed embryos scavenge free radicals and resist the damage caused by peroxidation by secreting anthocyanins. This is the result of the long-term evolution of *H. chinensis* seeds to adapt to the environment.

The current study showed that *H. chinensis* seeds have a high moisture content, no dormant character, and limited tolerance to water loss, drought, and low temperatures. They act as typical recalcitrant seeds. The seeds of *H. chinensis* mature from December to January, the winter and dry season in the tropical monsoon climate zone of southern China, where the prevailing conditions of low temperatures and drought can easily cause seed viability decline or even death. If the population vegetation is preserved well, it could alleviate drought and increase the rate of seed survival. However, at present, due to humankind's destructive activities, there has been severe fragmentation in the habitat of *H. chinensis* trees, aggravating the influence of drought on seeds and resulting in *H. chinensis* being distributed only on the banks of gullies and streams with high humidity [13,14]. There are many bare rocks beside the banks of gullies and streams where the seeds ripen and fall on the rocks, in streams, and in water from rain between rocks. The seeds which fall on the rocks will lose viability as a result of air-drying dehydration, whereas the viability of seeds in the streams will decline after a long soak. Furthermore, no soil can be provided for seeds to take root, so they cannot become seedlings, which has a serious impact on population regeneration and maintenance. In the long run, it will lead to a threat to survival of the species and even extinction.

5. Conclusions

H. chinensis fruits are nuts, and there are significant differences in fruit phenotypic characters and seed germination characters among populations. The *H. chinensis* seed germination rate is high, there is no dormancy, no significant effect of calyx lobes and seed coat on germination percentage, and the seed germination effect and seedling growth of large seeds within the population are better. *H. chinensis* seeds are sensitive to dehydration and intolerant to drought, salinity, flooding, and low temperature, being typical recalcitrant seeds. The seeds are suitable for germination on a moist substrate surface with good water retention and breathability at 30 °C. The biological characteristics of the *H. chinensis* seeds and the ambient environment of the habitat where the tree population survives caused a significant loss of viable seeds and gravely impacted population regeneration and maintenance; this is the main reason why *H. chinensis* has become endangered. Therefore, to protect the surviving populations and enable populations to recover and spread, we should pay attention to protecting and recovering its plant community and habitat. At the same time, relocation and conservation can be carried out in areas with better-preserved vegetation, higher humidity, flatter terrain, and loose soil in their distribution areas, increasing the effectiveness of *H. chinensis* population restoration in southern China from seeds.

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