Seed Germination Characteristics of the Precious Tree Species Keteleeria calcarea in Karst Regions (Postprint)

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Abstract

Keteleeria davidiana var. calcarea is a precious tree species in mid-subtropical karst regions, characterized by a narrow distribution range and scarcity of wild seedlings, which severely constrains its natural regeneration. To investigate the causes of its endangerment and provide a reference for its conservation and utilization, this study measured the cone and seed traits of K. davidiana var. calcarea and examined the influences of temperature, light, water, substrate, storage temperature and duration, and different geographical provenances on seed germination.

The results showed that: (1) The average cone seed rate of K. davidiana var. calcarea was 7.45%±6.54%, with seeds possessing long wings (2.27±0.32 cm). The average seed length was 1.55±0.15 cm (excluding wings), average width was 0.62±0.05 cm, average thickness was 0.46±0.04 cm, average 1000-seed weight was 214.81±14.76 g, and average moisture content of air-dried seeds was 15.28%±1.66%. (2) The suitable temperature for seed germination was 25 ℃, with germination rates decreasing significantly ($P<0.05$) at 20 ℃ and 30 ℃; seed germination did not require light, but germination rates under periodic light were significantly ($P<0.05$) higher than under continuous light or continuous darkness; seeds could germinate at soil water contents of 10%~30% and could tolerate a certain degree of drought; suitable germination substrates were loose, well-aerated peat soil and perlite; seeds were not storage-tolerant and should be sown promptly after collection; among three different geographical provenances, the population from Sanjiang Township, Gongcheng County, Guilin exhibited the highest seed germination rate. The low cone seed rate, strict temperature requirements for germination, and rapid loss of seed viability in K. davidiana var. calcarea may be strongly associated with its endangered status.

Full Text

Seed Germination Characteristics of *Keteleeria calcarea*, a Precious Tree Species in Karst Areas

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Abstract: *Keteleeria calcarea* is a precious tree species in mid-subtropical karst regions with a narrow distribution range and very few seedlings in wild populations, which severely limits natural regeneration. To explore the causes of its endangerment and provide references for its conservation and utilization, this study measured the morphological characteristics of cones and seeds and investigated the effects of temperature, light, water, substrate, storage temperature and duration, and different geographical provenances on seed germination. The results showed that: (1) The average seed setting rate of *K. calcarea* cones was 7.45% ± 6.54%. Seeds had long wings (2.27 ± 0.32 cm), with an average length of 1.55 ± 0.15 cm (excluding wings), width of 0.62 ± 0.05 cm, thickness of 0.46 ± 0.04 cm, 1000-seed weight of 214.81 ± 14.76 g, and average water content of air-dried seeds of 15.28% ± 1.66%. (2) The optimal temperature for seed germination was 25 °C, with germination rates decreasing significantly at 20 °C and 30 °C ($P < 0.05$). Seeds did not require light for germination, but germination rates under periodic light were significantly higher than under continuous light or continuous darkness ($P < 0.05$). Seeds could germinate at soil water contents of 10%–30% and could tolerate a certain degree of drought. Suitable germination substrates were loose, well-aerated peat soil and perlite. Seeds were not tolerant to storage and should be sown promptly after collection. Among the three geographical provenances, the Gongcheng County Sanjiang Township population had the highest germination rate.

The low cone seed setting rate, strict temperature requirements for germination, and rapid loss of seed viability likely contribute significantly to the species' endangerment.

Keywords: *Keteleeria calcarea*, seed, morphology, germination, storage

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Introduction

Seed germination is a critical stage in the plant life cycle, essential for population expansion, colonization of new habitats, and population regeneration. Studying seed germination can elucidate how species adapt to their habitats and how environmental factors regulate germination. As the most vulnerable stage in a plant’ s life, germination is influenced by environmental factors such as temperature, light, and soil moisture, as well as by physiological characteristics. These factors shape biological traits that enable plants to adapt to their environment and maintain reproductive success, holding significant ecological importance. Seed germination and establishment constraints have become key factors limiting the survival and distribution of many endangered species.

For example, the endangered mangrove *Lumnitzera littorea* faces endangerment partly due to its narrow adaptability to microenvironmental factors including pericarp, temperature, salinity, and light during germination. *Dracaena cambodiana* shows strong dependence on microenvironmental conditions such as pericarp, temperature, and light intensity, and habitat destruction leading to microenvironmental changes may be a primary cause of its endangerment. *Sauvagesia rhodoleuca* exhibits slow, irregular germination and seedling growth, with narrow temperature adaptation ranges for germination, compounded by insufficient understory light that limits germination, making natural regeneration difficult. Both *Platycrater arguta* and *Garcinia paucinervis* have high requirements for temperature and moisture during germination, which is closely related to their endangered status. Investigating seed germination characteristics of endangered plants to identify limiting factors can provide crucial evidence for understanding endangerment mechanisms and developing conservation strategies.

Keteleeria calcarea is a large evergreen tree in the Pinaceae family and a valuable plant resource in China. It is primarily distributed in localized karst mountain areas at the intersection of Guangxi, Hunan, and Guizhou provinces. The species features attractive form, straight trunks, and robust stature, making it suitable for landscaping; it also demonstrates strong drought resistance, making it an excellent species for afforestation on limestone mountains. Its wood is hard, fine-grained, and straight-textured, ideal for construction, furniture, and water conservancy applications. However, *K. calcarea* seedlings grow slowly, mother trees produce few seeds, and natural regeneration is weak. Combined with severe illegal logging in recent years, wild populations have declined rapidly, leading to its inclusion in the *List of National Key Protected Wild Plants* (National Forestry and Grassland Administration, Ministry of Agriculture and Rural Affairs, 2021).

Our preliminary surveys of wild populations revealed that in small populations, even in mast years, the number of fruiting plants and cone yield are low, likely contributing to endangerment. In larger populations, 10%–20% of adult plants may bear cones during mast years, with some individuals producing over 500 cones, yet seedling numbers remain extremely low. This may result from both low numbers of viable seeds in cones and environmental constraints such as temperature, light, moisture, and soil that limit seed-to-seedling transition. Therefore, studying cone and seed traits and germination characteristics can help clarify endangerment mechanisms. Previous research on *K. calcarea* has focused on primer development, community characteristics, genetic diversity, photosynthetic traits, propagation techniques, and chemical composition, but seed morphological and germination characteristics remain unreported. This study aims to investigate seed morphological features and seed setting rates, examine the effects of temperature, light, soil moisture, substrate, storage conditions, and geographical provenance on germination, and analyze endangerment causes to inform conservation and utilization strategies.

Materials and Methods

1.1 Experimental Materials *Keteleeria calcarea* cones were collected in mid-October from two wild populations in Sanjiang Township, Gongcheng County (GC) and Chenqiao Village, Lingui District (LG), Guilin City, Guangxi, and from an ex situ conservation population at Guilin Botanical Garden (YS) (Table 1). Cones were air-dried naturally in the laboratory for one week. After all scales opened, seeds were extracted, and intact, plump seeds were selected for experiments.

Table 1. General profiles of sampling sites

Population	Location	Latitude	Longitude	Altitude	Habitat
Gongcheng, Guilin (GC)	Sanjiang Town, Gongcheng County	25°50 26 N	110°05 47 E	-	Karst mountain slope
Lingui, Guilin (LG)	Chenqiao Village, Lingui District	25°12 47 N	110°11 51 E	-	Karst mountain slope
Yanshan, Guilin (YS)	Guilin Botanical Garden	25°4 15 N	110°17 57 E	-	Acid soil flat

1.2 Measurement of Cone and Seed Morphological Characteristics Cones and seeds from all three populations were used for morphological measurements. Vernier calipers and electronic balances were used to measure length, diameter, and weight of 20 cones. Total seed number and plump seed number per cone were counted to calculate cone seed setting rate. For each cone, five plump seeds were selected to measure scale length and width, seed length, width, thickness, and weight (including wings). One hundred seeds were weighed in eight groups to calculate 1000-seed weight. Seed moisture content was determined using the oven-drying method: 100 seeds were dried at 105 °C to constant weight, with three replicates.

1.3 Seed Germination Experiments Plump, uniformly sized seeds from the Gongcheng County Sanjiang Township population were used (except for the provenance experiment) in LRH-250-G illumination incubators. Before experiments, seeds were disinfected with 0.3% KMnO solution for 30 minutes, then rinsed with clean water. For germination tests, seeds were sown on the surface of a 2 cm thick substrate layer in small plastic boxes. After sowing, 0.5% carbendazim solution was sprayed for disinfection, repeated every two weeks, with water supplemented as needed. Each treatment had three replicates of 50 seeds. After germination began, counts were made every 4 days. Germination was defined as radicle length reaching half of seed length. Experiments ended when no seeds germinated for 20 consecutive days.

1.3.1 Effect of Temperature on Seed Germination

Conditions: 3,000 lx light, 12 h/d photoperiod, sandy soil substrate (1/2 river sand + 1/2 clay soil, sieved through 2 mm mesh and mixed in equal volumes, sterilized by high temperature). Three temperature gradients were set: 30 °C, 25 °C, and 20 °C.

1.3.2 Effect of Light on Seed Germination

Conditions: 25 °C temperature, sandy soil substrate. Light treatments were: continuous light (3,000 lx, 24 h/d), periodic light (3,000 lx, 12 h/d), and continuous darkness (0 lx, 24 h/d).

1.3.3 Effect of Soil Water Content on Seed Germination

Conditions: 25 °C temperature, 3,000 lx light (12 h/d), sandy soil substrate. Five soil water content treatments were set: 30%, 25%, 20%, 15%, and 10%. Substrate weight was measured every 4 days to replenish evaporated water.

1.3.4 Effect of Substrate on Seed Germination

Conditions: 25 °C temperature, 3,000 lx light (12 h/d). Six substrate types were tested: river sand, clay soil, sandy soil, peat soil, perlite, and karst mountain soil.

1.3.5 Effect of Storage Temperature and Duration on Seed Germination

Conditions: 25 °C temperature, 3,000 lx light (12 h/d), sandy soil substrate. Seeds stored at -20 °C, 4 °C, and room temperature (15-25 °C) for 1, 3, and 6 months were used for germination tests. Seeds at -20 °C were sealed in plastic bags, those at 4 °C were stored in kraft paper bags, and those at room temperature were stored in fiber bags.

1.3.6 Comparison of Seed Germination Rates Among Different Geographical Provenances

Conditions: 25 °C temperature, 3,000 lx light (12 h/d), sandy soil substrate. Seeds from GC, LG, and YS populations were tested to determine differences in germination rates among provenances.

1.4 Data Analysis Germination initiation time (days from sowing to first germination), total germinated seeds, and germination duration were recorded. Germination rate was calculated as the percentage of germinated seeds to total sown seeds. Germination duration was the period from germination start to end. SPSS 25.0 was used to test for significant differences among treatments with Duncan' s multiple comparisons. Origin 2015 was used for graphing.

Results

2.1 Morphological Characteristics of Cones and Seeds *Keteleeria calcarea* cones matured in mid-to-late October, cylindrical in shape, with average length of 13.90 ± 1.43 cm and diameter of 3.41 ± 0.28 cm. Scale length averaged 2.80 ± 0.25 cm and width 2.60 ± 0.17 cm. Seeds were light brown, smooth, with long wings (average 2.27 ± 0.32 cm). Seed dimensions (excluding wings) were: length 1.55 ± 0.15 cm, width 0.62 ± 0.05 cm, thickness 0.46 ± 0.04 cm, and weight (including wings) 0.21 ± 0.04 g (Table 2). The average 1000-seed weight was 214.81 ± 14.76 g, with average moisture content of air-dried seeds at 15.28% ± 1.66%. The average cone seed setting rate across three populations was 7.45% ± 6.54%, with the Gongcheng population highest at 14.89% ± 1.82%, followed by Lingui, and Yanshan lowest at 2.58% ± 1.10%.

Table 2. Morphological characteristics of cones and seeds of *Keteleeria calcarea*

Population	Cone		Scale		Seed Wing	Seed				Seed Setting Rate (%)
	Length (cm)	Diameter (cm)	Length (cm)	Width (cm)	Length (cm)	Length (cm)	Width (cm)	Thickness (cm)	Weight (g)	
Gongcheng	14.50±[illegible]	[illegible]	[illegible]	[illegible]	[illegible]±0.20	1.54±[illegible]	[illegible]	[illegible]	[illegible]	[illegible]±1.82
Lingui	12.73±[illegible]	[illegible]	[illegible]	[illegible]	[illegible]±0.15	1.50±[illegible]	[illegible]	[illegible]	[illegible]	[illegible]±2.67
Yanshan	14.47±[illegible]	[illegible]	[illegible]	[illegible]	[illegible]±0.32	1.60±[illegible]	[illegible]	[illegible]	[illegible]	[illegible]±1.10
Mean	13.90±[illegible]	[illegible]	[illegible]	[illegible]	[illegible]±0.32	1.55±[illegible]	[illegible]	[illegible]	[illegible]	[illegible]±6.54

A. Cones; B. Scales; C. Seeds

Fig. 1. Cones and seed morphology of *Keteleeria calcarea*

2.2 Effect of Temperature on Seed Germination Germination rates differed significantly among temperature treatments ($P < 0.05$). The 25 °C treatment showed significantly higher germination than 20 °C and 30 °C ($P < 0.05$) (Fig. 2). At 30 °C, both germination initiation and duration were shorter. *Keteleeria calcarea* seeds have strict temperature requirements for germination, with both excessively high and low temperatures inhibiting germination.

Different lowercase letters indicate significant differences ($P < 0.05$); the same below.

Fig. 2. Seed germination process of *Keteleeria calcarea* under different temperatures

2.3 Effect of Light Conditions on Seed Germination Germination rates differed significantly among light treatments ($P < 0.05$). Periodic light (3,000 lx, 12 h/d) produced significantly higher germination than continuous light (3,000 lx, 24 h/d) or continuous darkness (0 lx, 24 h/d) ($P < 0.05$) (Fig. 3). Germination duration was shorter under both continuous light and continuous darkness. Seedlings under continuous darkness appeared pale green or white, while those under continuous light showed poor growth, indicating these conditions are unfavorable for germination and seedling development.

Fig. 3. Seed germination process of *Keteleeria calcarea* under different light conditions

2.4 Effect of Soil Water Content on Seed Germination No significant differences in germination rates were observed among soil water content treatments ($P > 0.05$). All treatments initiated germination at 28 days, with durations of 65–88 days. Germination was faster at 30%, 25%, and 20% water content than at 15% and 10% (Fig. 4). The species does not have strict soil moisture requirements and can tolerate some degree of drought.

Fig. 4. Seed germination process of *Keteleeria calcarea* under different soil water contents

2.5 Effect of Substrate on Seed Germination Substrate type significantly affected germination rates ($P < 0.05$). Peat soil and perlite showed significantly higher germination than river sand, sandy soil, karst mountain soil, and clay soil ($P < 0.05$). Clay soil had the lowest germination rate, significantly below all other treatments ($P < 0.05$) (Fig. 5). Germination duration was notably longer in peat soil and perlite. *Keteleeria calcarea* seeds have strict substrate requirements for germination.

Fig. 5. Seed germination process of *Keteleeria calcarea* under different substrates

2.6 Effect of Storage Temperature and Duration on Seed Germination Storage temperature and duration significantly affected germination rates ($P < 0.05$). Seeds stored at -20 °C showed 0% germination. Seeds stored at 4 °C and room temperature (15–25 °C) for 1 month had germination rates of 38.00% and 36.00%, respectively, with no significant difference ($P > 0.05$). After 3 months, germination rates dropped significantly to 20.00% at 4 °C and 12.00% at room temperature ($P < 0.05$). After 6 months, rates were only 6.00% and

2.00%, respectively. While above-freezing low temperature storage was superior, germination capacity declined rapidly with extended storage time.

Fig. 6. Seed germination process of *Keteleeria calcarea* under different storage temperatures and durations

2.7 Effect of Geographical Provenance on Seed Germination Significant differences in germination rates were observed among geographical provenances ($P < 0.05$). The Gongcheng population (GC) showed significantly higher germination than Lingui Chenqiao (LG) and Guilin Yanshan (YS) populations ($P < 0.05$) (Fig. 7). Germination initiation time and duration were similar across all three provenances.

Fig. 7. Seed germination process of *Keteleeria calcarea* from different geographical provenances

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Discussion

3.1 Morphological Characteristics of *Keteleeria calcarea* Cones and Seeds Seeds represent a crucial stage in the life cycle of seed plants, ensuring successful establishment and regeneration while allowing temporal and spatial escape from unfavorable conditions. Seed size affects offspring fitness and determines seedling establishment and vigor. Generally, larger seeds store more nutrients, benefiting germination and seedling growth. Seed size variation arises from genetic factors, resource competition, and environmental conditions. Plants adopt two reproductive strategies: producing few large seeds for competitive advantage or many small seeds to occupy more niches.

In this study, *K. calcarea* seeds averaged 1.54 cm in length (excluding wings), 0.61 cm in width, 0.47 cm in thickness, and 0.21 g in weight—slightly lower than the average 0.328 g for tree species but larger than congeneric *Keteleeria evelyniana* (0.099 g) and *Keteleeria cyclolepis* (0.107 g), and substantially heavier than related species *Abies chensiensis* (33.92 g/1000 seeds) and *Abies yuanbaoshanensis* (16.96 g/1000 seeds). The Gongcheng population had a seed setting rate of 14.89%, while Lingui had only 4.90%, both far below the widespread congener *Keteleeria cyclolepis* (49.35%). This suggests *K. calcarea* adopts a "few large seeds" strategy. The Gongcheng population is the largest known, with over 2,000 individuals including many large-diameter trees, while Lingui has fewer than 400 individuals dominated by small- to medium-sized trees. These differences in population size and structure likely explain the variation in seed setting rates. With only five fruiting individuals in Lingui producing dozens to 200 cones each, low seed production represents a major barrier to population regeneration. In Gongcheng, about 10% of plants produce cones, with some individuals bearing over 500 cones, indicating that seed quantity is not the primary obstacle to regeneration. Instead, environmental constraints during seed-to-seedling transition likely play a major role in the species' endangerment.

3.2 Effects of Environmental Factors on *Keteleeria calcarea* Seed Germination Temperature is a primary determinant of seed germination, with extremes inhibiting germination, inducing dormancy, or causing seed death. This relates to temperature-dependent enzyme activity required for germination. Our results show *K. calcarea* germination was significantly higher at 25 °C than at 20 °C or 30 °C, indicating strict temperature requirements. Low temperatures provide insufficient accumulated heat, leading to reduced viability, rot, and mold, while high temperatures affect enzyme stability, membrane permeability, and membrane-bound activity. These findings align with studies on *Keteleeria cyclolepis*.

Light requirements vary among species. *Keteleeria calcarea* germination under continuous light or darkness was significantly lower than under periodic light, with poor seedling growth, indicating these conditions are unfavorable. Under continuous darkness, seedlings cannot photosynthesize and deplete stored reserves, preventing survival. Continuous light inhibits germination and causes desiccation, threatening seed viability and seedling growth.

Soil moisture significantly affects germination. *Keteleeria calcarea* showed no significant germination rate differences across 10%–30% water content, though germination was faster at higher moisture levels. This indicates the species has no strict moisture requirements and can tolerate karst drought conditions, contrasting with *Keteleeria cyclolepis*, which cannot germinate at 10% moisture and shows significantly lower germination at 15% and 20% than at 25% and 30%. This drought tolerance likely reflects long-term adaptation to karst habitats.

Substrate composition affects nutrient availability, microbial communities, and compaction, influencing germination and seedling survival. Peat soil and perlite produced significantly higher germination than other substrates, while clay soil had the lowest rate. The loose texture and good aeration of peat soil and perlite provide adequate oxygen and reduce mold, favoring germination. Clay soil' s small interparticle spaces limit aeration and water permeability, increasing mold and reducing germination.

Optimal storage temperature and duration vary among species. For *Liquidambar formosana* from subtropical China, seeds with 10.54% moisture showed no viability differences after one year at room temperature, 4 °C, -20 °C, or -70 °C, but -70 °C was optimal for storage beyond two years. In contrast, *Cathaya argyrophylla* seeds require timely sowing and are best preserved at 4 °C in moss. Temperature is the main factor affecting seed metabolism; low temperatures minimize respiration and resource consumption, preserving embryo viability. Our results show *K. calcarea* seeds stored best at 4 °C, but germination still declined by nearly half after three months, indicating poor storability and the need for prompt sowing. The inability to survive -20 °C storage may result from ice formation causing tissue damage.

Seed quality and viability vary among provenances due to maternal location and habitat conditions, affecting germination rates. The Gongcheng population' s superior germination likely reflects better seed quality. This population exceeds 2,000 individuals, including many large trees with the highest proportion of fruiting mothers, while Lingui Chenqiao has fewer fruiting individuals with poorer seed quality.

Our germination tests were conducted in relatively enclosed small plastic boxes (20 cm in all dimensions). Despite disinfection of seeds and substrates, the prolonged germination period led to substantial mold, resulting in relatively low germination rates. Improved sterilization or better ventilation and light conditions would likely enhance germination success.

3.3 Analysis of Endangerment Causes and Conservation Implications *Keteleeria calcarea* relies on wind pollination, with pollen maturing synchronously and having a short lifespan. The pollen dispersal period in early April coincides with frequent rainy weather, which can hinder pollen transfer and ovule fertilization, likely contributing to low seed setting rates. Cones mature in mid-to-late October to early November, releasing seeds that fall to the forest floor as temperatures decline. In Guilin, average temperatures drop below 18 °C in November and below 10 °C in December and January, inhibiting germination. Combined with poor seed storability, most seeds lose viability over winter, limiting seed-to-seedling transition.

Additionally, abundant understory litter reduces light availability, potentially affecting germination. Even when seeds germinate, slow initial growth prevents seedlings from competing with fast-growing shade-tolerant broadleaf trees, leading to elimination by shrubs or weeds. To promote natural regeneration, weeding and soil loosening around mother trees can facilitate seed-soil contact and increase light availability. In dense stands, selective thinning can improve understory light conditions and promote seedling growth. During cone maturation, artificial collection and indoor propagation under optimal conditions, followed by reintroduction to small populations at appropriate times, can support population renewal.

In conclusion, low cone seed setting rates, strict temperature requirements for germination, and poor seed storability contributing to rapid viability loss are closely related to the endangerment of *Keteleeria calcarea*. With few seedlings and difficult natural regeneration in wild populations, artificial tending and reintroduction should be implemented to promote population recovery.

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Note: Figure translations are in progress. See original paper for figures.