Postprint of Research on Storage Conditions and Germination Characteristics of Medicinal Plant Erigeron breviscapus Seeds

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Abstract

By exploring the suitable storage conditions and germination characteristics of Erigeron breviscapus seeds, this study provides effective guidance for the rational storage and high-yield cultivation of this rare medicinal species. Using seeds of Erigeron breviscapus collected from the wild in Yunnan during spring as experimental material, this study investigated the effects of different storage humidity, storage temperature, storage duration, as well as different light, temperature, and imbibition conditions on their germination rate. The results showed that: (1) Reducing storage humidity (15% RH) and storage temperature (-20°C, 4°C) favored seed preservation, extending seed longevity to over two years with germination rates reaching above 80%; high temperatures (35°C, 45°C) and high humidity (60% RH) accelerated seed aging and were detrimental to preservation, indicating that these are short-lived seeds; (2) Whether light was present or not had no significant effect on seed germination rate, indicating that these are light-neutral seeds, but light was beneficial for seedling formation after germination; (3) 25°C was the optimal temperature for seed germination, with germination rates reaching 84.37%; (4) During sowing in production, avoiding low temperature (4°C) and high temperature (30°C, 35°C) is beneficial for improving seedling emergence rate; (5) These seeds were insensitive to imbibitional chilling injury, indicating that they are chilling-resistant seeds. Therefore, in seed research and medicinal material production, mature seeds should be promptly dried and sealed for storage at low temperature after harvest, and sown early in the following spring; in seedling production, appropriate light conditions should be provided, a separate constant temperature (25°C) seedling room should be established, and sowing during low-temperature seasons should be avoided.

Full Text

Preamble

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Study on the Seed Storage Conditions and Germination Characteristics of the Medicinal Plant *Erigeron breviscapus*

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Abstract: This study investigated suitable storage conditions and germination characteristics of *Erigeron breviscapus* seeds to provide effective guidance for the rational storage and high-yield cultivation of this rare medicinal species. Using seeds collected from Yunnan in spring, we examined the effects of storage humidity, temperature, duration, and various light, temperature, and imbibition conditions on germination rate. The results showed that: (1) Reduced storage humidity (15% RH) and low storage temperatures (−20 °C, 4 °C) were beneficial for seed preservation, extending seed longevity to over 2 years with germination rates exceeding 80%; high temperature (35 °C, 45 °C) and high humidity (60% RH) accelerated seed aging and were unfavorable for storage, indicating these are short-lived seeds. (2) Light conditions did not significantly affect germination rate, classifying the seeds as photoneutral, though light promoted seedling formation after germination. (3) 25 °C was the optimal temperature for seed germination, achieving a germination rate of 84.37%. (4) For field sowing, avoiding low temperature (4 °C) and high temperatures (30 °C, 35 °C) is recommended to improve seedling emergence. (5) The seeds were insensitive to imbibitional chilling damage, classifying them as chilling-resistant. Therefore, for seed research and medicinal material production, mature seeds should be promptly dried and sealed for low-temperature storage after harvest, with early spring sowing the following year; for seedling production, suitable lighting conditions should be provided, a dedicated constant-temperature (25 °C) growth room established, and sowing during cold seasons avoided.

Keywords: *Erigeron breviscapus*, seed, storage conditions, germination characteristics, germination rate, short-lived seed

Introduction

Erigeron breviscapus, also known as *Erigeron breviscapus* (Vant.) Hand.-Mazz., is a perennial plant in the Asteraceae family. Widely distributed in Yunnan, Sichuan, Guizhou, Guangxi, Hunan, and Tibet, it is a commonly used traditional Chinese medicine among ethnic groups (Zhao et al., 2013). First documented in *Dian Nan Ben Cao* and included in the 1977 edition of the Chinese

Pharmacopoeia (Ma et al., 2004), *E. breviscapus* contains flavonoids, phytosterols, volatile oils, pyrocatechol, amino acids, and trace elements (Liu et al., 2002). Its products include extracts, capsules, injections, tablets, granules, and oral liquids (Zhao et al., 2018). With cooling properties and a slightly bitter, sweet, and pungent taste, it is used for detoxification, dispelling wind and dampness, promoting blood circulation, removing blood stasis, unblocking meridians, and reducing inflammation and pain. Currently, *E. breviscapus* injection is clinically used primarily for cardiovascular and cerebrovascular diseases, with good efficacy also demonstrated in treating diabetes, nephropathy, cervical vertigo, and geriatric conditions (Zhang et al., 2012).

Cardiovascular diseases, the primary indication for *E. breviscapus*, rank as the fourth leading cause of disease worldwide. Annual global sales of cardiovascular drugs reach approximately 80 billion USD, while the domestic market exceeds 30 billion RMB, with traditional Chinese and Western medicines each occupying about half of the market. Demand continues to increase at an annual rate of 15–20% (Zhang et al., 2013). Recent resource surveys in the original distribution areas have revealed that *E. breviscapus* populations are no longer found in large patches, with wild distribution frequency approaching rarity and depletion (Xiong et al., 2012). As market demand gradually increases, high-quality wild germplasm has become a bottleneck for the development of the *E. breviscapus* pharmaceutical industry.

In production, *E. breviscapus* is primarily propagated through seeds. Under natural conditions, seed viability is maintained for a very short period, approximately 3–5 months. When stored at room temperature, seed vigor decreases with storage time, with an average lifespan of about 6 months (Li et al., 2005). This creates significant difficulties for germplasm resource research and medicinal material production. If this species could be stored long-term, it would enable efficient, low-cost preservation of *E. breviscapus* germplasm resources while providing high-quality seeds for medicinal production. Since seed storage longevity is determined by two critical factors—storage temperature and humidity—determining the optimal storage temperature and humidity is key to effective storage of this species.

Seed germination and normal seedling production are influenced by comprehensive effects of external ecological conditions such as water, temperature, oxygen, and light. However, adequate moisture, suitable temperature, and sufficient oxygen are indispensable for seed germination, with light also being a necessary factor for certain seeds (Yu, 2012). This study primarily investigated the effects of light, temperature, and imbibition treatment on *E. breviscapus* seed germination. Seed germination can be divided into four stages: imbibition, germination initiation, sprouting, and seedling establishment. Imbibition is the first stage of seed germination. Many cultivated plant seeds suffer imbibitional chilling injury when absorbing water rapidly at low temperatures, occurring not only in alpine and cold, humid regions but also during early spring irrigation sowing in arid areas, particularly in sensitive seeds. Imbibitional chilling injury

is damage caused by the combined effects of low temperature and rapid water absorption, which can affect seedling establishment rates or even cause seed viability loss, resulting in large-scale seedling deficiency and severe economic losses (Yu, 2012). Therefore, determining the light requirements, optimal germination temperature, and presence of imbibitional chilling injury in *E. breviscapus* seeds has important guiding significance for production.

In recent years, numerous studies have been conducted on *E. breviscapus* cultivation, tissue culture, pharmacology, and extraction (Zhang et al., 2012; Lin et al., 2009; Ke et al., 2017; Zhao et al., 2018), but few reports have addressed seed-related research (Li et al., 2005; Cao et al., 2012; Lin et al., 2008). Systematic studies on seed storage conditions, light requirements, optimal germination temperature, and imbibitional chilling characteristics have not been conducted. These research topics hold not only important theoretical significance but also practical guiding value for *E. breviscapus* production.

Materials and Methods

1.1 Materials

Wild mature *E. breviscapus* seeds collected from Dali, Yunnan in May 2015 were used as experimental material. Seeds were stored in the "double fifteen" (15% relative humidity, 15 °C) seed drying room at the Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences. After manual rubbing to remove pappus, seeds were cleaned using a gravity separator (Selecta Gravity Separator ZigZag-1, Machinefabriek BV Enkhuizen, Holland) to remove impurities and pappus. Plump, pest-free, undamaged high-quality seeds were selected for experiments.

1.2 Seed Morphological Observation and Quality Testing

From the cleaned seeds, partial samples were taken for observation and photography under a dissecting microscope. Morphological characteristics were observed to determine seed plumpness, and seed length and width were measured to determine seed size. The experimental seeds were confirmed to be plump, undamaged, and free from insect damage. Thousand-seed weight was measured (Cao et al., 2012), and initial germination rate was tested: after surface sterilization, seeds were placed on MS medium, 36–40 seeds per dish with six replicates. Dishes were sealed with parafilm and placed in a 25 °C constant-temperature walk-in growth room for germination. Light conditions: light intensity 15 $mol \cdot m^{-2} \cdot s^{-1}$ with a 12 h/12 h day/night photoperiod. The total number of germinated seeds was recorded after one month, and germination rate was calculated (Cao et al., 2012). The same method was used for subsequent germination rate tests.

1.3.1 Low-Humidity Storage at Different Temperatures

Seeds were stored in the double fifteen drying room. Using a sealed benchtop water activity meter (Hygrolab3-set40, Rotronic, Switzerland) placed in a constant-temperature room (15 °C), humidity was measured. When humidity reached 15%, seeds were aliquoted into 30 mL small sealed bottles containing silica gel bags (pre-equilibrated at 15% relative air humidity), then distributed into five 1 L sealed jars and stored at −20, 4, 25, 35, and 45 °C. The germination rate at the time of storage was set as the control (CK). Seeds stored at −20 and 4 °C were tested every 3 months for 30 months; seeds at −20 °C were removed to the double fifteen room for temperature equilibration one day before germination testing. Seeds at 35 °C and 45 °C were tested at 2, 5, 8, 13, and 21 weeks.

1.3.2 High-Humidity Storage at Different Temperatures

At 20 °C, 30% anhydrous lithium chloride solution was used for humidity equilibrium (Hay et al., 2008). Using the sealed benchtop water activity meter, when seeds reached equilibrium with the surrounding humidity at 60%, they were aliquoted and stored at −20, 4, 20, 35, and 45 °C. The germination rate at the time of storage was set as CK. Seeds at −20, 4, and 20 °C were tested at weeks 1-6, 12, 16, 20, and 24; seeds at −20 °C were removed to the double fifteen room for temperature equilibration one day before testing. Seeds at 35 °C and 45 °C were tested weekly for 12 weeks.

1.4 Determination of Seed Light Requirements for Germination

Two treatments were established: a 12 h/12 h day/night photoperiod and complete 24 h darkness. For the dark treatment, inoculated dishes were wrapped in aluminum foil, placed in sealed paper boxes, and incubated under the 12 h/12 h photoperiod for germination rate testing.

1.5.1 Temperature Gradient Treatments on MS Medium

Six temperature treatments were established: 10, 15, 20, 25, 30, and 35 °C. Seeds were placed on MS medium, 36 seeds per dish with six dishes per temperature. Germination occurred under warm white fluorescent light (light intensity 15 $\mathrm{mol \cdot m^{-2} \cdot s^{-1}}$, 12 h/12 h photoperiod) at each temperature.

1.5.2 Temperature Treatments in Soil

Four temperature treatments were established: 4, 25, 30, and 35 °C. Seeds were sown in soil (humus:red soil = 1:1), 50 seeds per pot with six replicates per temperature. Pots were placed in illumination incubators at the respective temperatures with a 12 h/12 h photoperiod and light intensity of 15 $\mathrm{mol \cdot m^{-2} \cdot s^{-1}}$. Germination rates were recorded after one month.

1.6 Determination of Imbibitional Chilling Injury in Seeds

After surface sterilization, seeds underwent four imbibition treatments (Yu, 2012), with 36–40 seeds per treatment and six replicates: (1) Warm soaking: placed in sterile 1.5 mL centrifuge tubes and soaked in deionized water at 25 °C; (2) Cold soaking: placed in sterile 1.5 mL centrifuge tubes and soaked in deionized water at 4 °C; (3) Warm wet imbibition: imbibed on 1% agar medium at 25 °C; (4) Cold wet imbibition: imbibed on 1% agar medium at 4 °C. After 24 h of imbibition, all treatments were transferred to MS medium for germination at 25 °C under warm white fluorescent light.

1.7 Seed Surface Sterilization

The seed surface sterilization method followed Yu (2012): seeds were placed in 1.5 mL centrifuge tubes with 1 mL 75% (v/v) ethanol, vortexed for 4 min, and centrifuged; the supernatant was discarded. Seeds were then treated with 1 mL 5% (v/v) sodium hypochlorite solution, vortexed for 4 min, and centrifuged; the supernatant was discarded. Seeds were washed with 1 mL sterile water, vortexed for 2 min, and centrifuged; the supernatant was discarded. This sterile water rinse was repeated three times, with 1 mL sterile water added to the final centrifuge tube.

1.8 MS Medium Preparation

MS medium was prepared following Murashige and Skoog (1962): macronutrient stock solution (40×, 25 mL · L ^{1}): MgSO (7.22 g · L ^{1}), NH NO (66 g · L ^{1}), KH PO (6.8 g · L ^{1}), KNO (76 g · L ^{1}), CaCl (13.29 g · L ^{1}); micronutrient stock solution (200×, 5 mL · L ^{1}): MnSO · H O (3.3804 g · L ^{1}), ZnSO · 7H O (1.72 g · L ^{1}), H BO (1.24 g · L ^{1}), KI (0.166 g · L ^{1}), Na MoO · 2H O (0.05 g · L ^{1}), CuSO · 5H O (0.005 g · L ^{1}), CoCl · 6H O (0.005 g · L ^{1}); iron salt stock solution (100×, 10 mL · L ^{1}): Na -EDTA · 2H O (3.725 g · L ^{1}), FeSO · 7H O (2.78 g · L ^{1}); organic element stock solution (200×, 5 mL · L ^{1}): nicotinic acid (0.1 g · L ^{1}), thiamine (0.02 g · L ^{1}), pyridoxine (0.1 g · L ^{1}), glycine (0.4 g · L ^{1}); inositol stock solution (100×, 10 mL · L ^{1}): inositol (10 g · L ^{1}). After mixing all stock solutions, sucrose (30 g · L ^{1}) was added, the volume was adjusted with deionized water, pH was adjusted to 5.85, and phytagel (3.5 g · L ^{1}) was added. The medium was sterilized at 121 °C, 260 kPa for 25 min.

1.9 Relative Humidity Detection and Germination Rate Calculation

A sealed benchtop water activity meter placed in a constant-temperature room (15 °C) was used to detect relative humidity during seed storage (Cheng et al., 2011).

Germination rate is a common indicator for detecting seed vigor (Cao et al., 2012). Seeds were considered germinated when fresh radicles protruded from the seed coat. After germination initiation, the number of normal seedlings was

recorded daily until no further germination occurred. The calculation formula was: Germination Rate GR = (n/N) × 100% (where n is the final number of normally germinated seeds and N is the total number of test seeds).

1.10 Data Analysis

The Q-test was used to remove outliers. SPSS 17.0 software and t-tests were used for significance analysis. GraphPad Prism 5.0 and Adobe Photoshop CS6 were used for figure preparation.

Results

2.1 Seed Morphology and Quality

The achenes were narrowly oblong, approximately 1.5–2.0 mm long, compressed, often with one rib on the dorsal side, covered with fine hairs throughout, with white pappus at the apex that was bristle-like. Mature seeds were plump with a bright luster [Figure 1: see original paper]. The thousand-seed weight reached 0.2 g, and the initial germination rate was 90.71%.

2.2.1 Effects of Different Storage Temperatures on Seed Germination Rate Under Low Humidity

Under low humidity (15% RH) conditions, germination rates at −20 °C showed no significant difference from the control at 3, 6, 15, 18, 21, 24, and 30 months, but were significantly different at 9, 12, and 27 months. At 4 °C, germination rates showed no significant difference from the control at 3, 6, 12, 18, 21, 27, and 30 months, but were significantly different at 9, 15, and 24 months. At 25 °C, germination rates showed no significant difference from the control at 3, 6, 9, and 18 months, but were significantly different at 12, 15, 21, 24, 27, and 30 months. Overall, during the 30-month storage period, no significant differences in germination rates were observed among different temperatures. Germination rates at −20 °C and 4 °C showed no significant difference from the control during most storage periods, while 25 °C was significantly lower than the control during most periods. After 30 months of storage, germination rates at −20 °C and 4 °C reached 86.90% and 84.84%, respectively, while 25 °C only reached 79.25%. These results indicate that low temperature (−20 °C, 4 °C) and low humidity (15% RH) were beneficial for extending storage longevity . If seeds are stored at room temperature for no more than two years, low humidity (15% RH) sealed storage can reduce production costs while extending seed viability.

Under low humidity (15% RH) conditions, germination rates at 35 °C (2–21 weeks) and 45 °C (2–13 weeks) showed no significant difference from the control. However, after 21 weeks at 45 °C, a significant difference was observed. Overall, during the 21-week storage period, except for 45 °C at 21 weeks, which was significantly lower than the control, other storage periods at 35 °C and 45 °C showed no significant difference from the control. After 21 weeks, germination

rates at 35 °C and 45 °C were 75.46% and 68.98%, respectively . These results indicate that short-term storage under high temperature (35 °C, 45 °C) and low humidity (15% RH) did not significantly reduce germination rates, but with continued storage, germination rates declined slowly, with higher storage temperatures causing faster declines.

2.2.2 Effects of Different Storage Temperatures on Seed Germination Rate Under High Humidity

Under high humidity conditions (60% RH), germination rates at −20 °C showed significant differences from the control at weeks 1, 2, and 5, but not at weeks 2, 3, 4, 6, 12, 16, 20, and 24. At 4 °C, germination rates showed significant differences from the control at weeks 1, 4, 5, 6, 12, 16, 20, and 24, but not at weeks 2 and 3. At 20 °C, germination rates showed significant differences from the control at weeks 1, 3, 4, 5, 6, 12, 16, 20, and 24, but not at week 2. Overall, during the 24-week storage period, germination rate declines appeared in the first week of storage. At −20 °C, seed germination rates showed essentially no significant difference from the control during weeks 3-24. However, at 4 °C and 20 °C, germination rates declined significantly faster than at −20 °C during the 24-week storage period. After 24 weeks, germination rates at −20, 4, and 20 °C were 76.39%, 66.27%, and 62.96%, respectively. These results indicate that short-term storage under low temperature (−20 °C) and high humidity (60% RH) did not significantly change seed germination rates, but with extended storage, germination rates gradually declined, with 4 °C and 20 °C showing significantly faster declines than −20 °C . Therefore, high humidity (60% RH) is not conducive to storage; if conditions are limited, short-term storage under high humidity (60% RH) should use sealed storage at −20 °C.

Under high humidity (60% RH) conditions, germination rates at 35 °C and 45 °C showed significant differences from the control throughout the 12-week storage period, with both declining significantly over time. After 12 weeks at 35 °C, germination rates decreased from 83.8% to 6.02%; after 6 weeks at 45 °C, rates decreased from 83.8% to 0% . Overall, during the 12-week storage period, germination rates began to decline after one week at both 35 °C and 45 °C, with 45 °C showing a significantly faster decline than 35 °C.

2.3 Effects of Light on Seed Germination Rate

The germination rate under light conditions was 84.37%, while under dark conditions it was 81.48%. Light conditions did not significantly affect germination rate, classifying the seeds as photoneutral [Figure 2: see original paper] and [Figure 3: see original paper]. However, as shown in [Figure 3: see original paper], seedlings under dark conditions were etiolated and weak. Therefore, appropriate light should be provided during seed germination to promote seedling growth and development.

2.4.1 Effects of Different Temperatures on Seed Germination Rate on MS Medium

Germination rates at 10, 15, 20, 25, 30, and 35 °C were 64.81%, 70.56%, 82.11%, 84.37%, 76.11%, and 69.91%, respectively. The germination rate at 25 °C was significantly higher than at 10, 15, 30, and 35 °C. Although not significantly different from the rate at 20 °C [Figure 4: see original paper], seedlings at 25 °C were more uniform and robust than at 20 °C [Figure 5: see original paper], indicating that 25 °C is the optimal germination temperature.

2.4.2 Effects of Different Temperatures on Seed Germination Rate in Soil

At low temperature (4 °C), the seed germination rate was 0%; at 25 °C it was 79.44%; and at high temperatures of 30 °C and 35 °C, rates were 58.33% and 31.11%, respectively. These results indicate that germination rates under low temperature (4 °C) and high temperatures (30 °C, 35 °C) were significantly lower than at 25 °C, with 35 °C significantly lower than 30 °C, and 4 °C significantly lower than 35 °C [Figure 6: see original paper]. As shown in [Figure 7: see original paper], seedlings essentially died after one month at 35 °C, indicating that high temperature not only affects seed germination but also seedling survival. Therefore, 25 °C is the optimal germination temperature for soil sowing.

To verify whether seeds that failed to germinate after one month at 4 °C under high humidity (80–90% RH) remained viable, seeds from the 4 °C treatment were returned to 25 °C for continued germination. After one month, the germination rate was 77.78%. This rate was significantly higher than that of seeds maintained at 4 °C for one month. As shown in [Figure 6: see original paper] and [Figure 7: see original paper], the germination rate of seeds transferred from 4 °C to 25 °C showed no significant difference from that of seeds germinated at 25 °C continuously. Therefore, although one month of low temperature and high humidity inhibited germination and extended germination time, it did not damage the seeds. When returned to room temperature, seeds could germinate normally and form healthy seedlings [Figure 8: see original paper] and [Figure 9: see original paper].

2.5 Effects of Different Imbibition Treatments on Seed Germination Rate

After 24 h of imbibition at 4 °C, germination rates were 86.57% (cold soaking) and 87.22% (cold wet imbibition). At 25 °C, rates were 88.42% (warm soaking) and 85.56% (warm wet imbibition). After rapid imbibition (warm soaking, cold soaking) at 4 °C and 25 °C, germination rates were 86.57% and 88.42%, respectively. After slow imbibition (warm wet imbibition, cold wet imbibition) at 4 °C and 25 °C, rates were 87.22% and 85.56%, respectively. No significant differences were observed among the four imbibition treatments [Figure 10: see original paper] and [Figure 11: see original paper]. Therefore, *E. breviscapus*

seeds do not suffer from imbibitional chilling injury and are insensitive to water absorption temperature and imbibition speed during early germination, classifying them as chilling-resistant.

Discussion and Conclusion

In seed storage research, 15% relative humidity is considered optimal for seed preservation in germplasm banks (Smith et al., 1992). Currently, 45 °C and 60% RH are standard environments for studying seed aging (Hay et al., 2008). Generally, temperature and humidity work together to affect seed longevity (Zhou et al., 2017). Our results showed that *E. breviscapus* seeds were generally tolerant to storage under low humidity, with −20 °C and 4 °C being optimal storage temperatures. The reason may be that at low temperatures, seed respiration weakens, reducing consumption rate of stored substances and maintaining seed viability, while low humidity results in lower metabolic rates than high humidity, better preserving membrane integrity and enzyme activities and maximizing seed longevity (Zhou et al., 2017). Under high humidity, seeds were generally intolerant to storage, with the fastest germination rate decline at 45 °C. This may be because high temperature strengthens respiration, accelerating consumption of stored substances and seed aging. As seed moisture content increases, electrolyte leakage intensifies, while activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) decrease, weakening the seed' s ability to cope with oxidative stress and shortening seed lifespan (Zhou et al., 2017). Under natural conditions, *E. breviscapus* seeds stored at room temperature after harvest are not tolerant to storage, possibly due to inadequate drying or improper storage. Therefore, for seed research, seeds should be promptly placed in the double fifteen drying room after harvest, then cleaned and stored in sealed jars at low temperature. For production, mature seeds should be harvested on sunny days, promptly dried, and stored in sealed bags at low temperature to ensure high-quality germplasm for the following year. Rainy-day harvesting and storage in warm, humid environments should be avoided to prevent viability loss and damage to high-quality germplasm.

Seed lifespan is defined as the time from full seed maturity to viability loss (Shen et al., 2004). The general concept of seed lifespan refers to the time from seed harvest to when germination rate declines to 50% (average lifespan or half-life), while the agricultural concept refers to the period during which seed germination rate can reach over 80% under certain conditions (Sun et al., 2016). Different plant species have varying seed lifespans, ranging from hours or days for short-lived seeds to thousands or tens of thousands of years for long-lived seeds (Zhou et al., 2017). Based on seed lifespan, seeds can be classified into three categories: short-lived (lifespan generally within 3 years), medium-lived (3–15 years, also called normal-lived), and long-lived (over 15 years) (Shen et al., 2004). Among reported short-lived seeds, *Sophora tonkinensis* seeds have a lifespan of over 6 months by general standards and 3 months by agricultural standards, with 5 °C low-temperature storage extending lifespan by 3 months

(Sun et al., 2016). *Kraschenninnikovia ceratoides* seeds showed reduced viability to 4% after one year of field storage, with high temperature and humidity significantly affecting seed vigor while low temperature and humidity significantly extending seed viability (He, 2015). *Salix linearistipularis* seeds showed 3% germination after approximately 45 days of storage at room temperature, while seeds stored at −80 °C maintained 50% germination after 1 year (Qian, 2015). These reports indicate that short-lived seeds are not tolerant to storage at room temperature and under natural conditions, with lifespans generally within 3 years, and that low temperature or dry storage can significantly increase the lifespan of short-lived seeds. *E. breviscapus* seeds have a short lifespan when stored at room temperature, with 53.3% germination after 6 months and only 17.7% after 12 months; 4 °C low-temperature storage can significantly extend lifespan to over 1 year (Li et al., 2005). In this study, low temperature and low humidity storage significantly extended lifespan to over 2 years, while high temperature and high humidity caused significant germination rate decline after just one week. Based on these findings, we conclude that *E. breviscapus* seeds are short-lived. Previous studies have shown that *E. breviscapus* seeds have no obvious dormancy characteristics (Li et al., 2005). Therefore, in actual seedling production, sowing immediately after seed collection is recommended when conditions permit. When this is not possible, seeds should be dried and sealed for storage at −20 °C or 4 °C, or stored in a seed drying cabinet after drying treatment, with early spring sowing the following year.

Light is essential for germination of some plant seeds, with different species having varying light requirements (Wei et al., 2012). Our results showed that *E. breviscapus* seeds could germinate under both light and dark conditions, but light was more conducive to seedling formation after germination and resulted in relatively higher germination rates. As a heliophyte, the light requirement for *E. breviscapus* seed germination may represent an adaptive strategy for environmental adaptation and resource utilization. Based on this light requirement characteristic, production should select sunny hillside grasslands for planting and avoid shaded areas. Sowing depth should not be excessive, and a thin layer of fine soil should be applied after sowing to facilitate seed germination and robust seedling formation.

Suitable temperature promotes water absorption rate, enhances enzymatic processes and respiration, and accelerates conversion of stored substances into usable soluble forms (Zhang et al., 2013), thereby promoting seed germination and seedling growth. Both excessively high and low temperatures significantly affect seed germination (Wang et al., 2016). Our study found that the suitable temperature for *E. breviscapus* seed germination is 25 °C, consistent with the results of Li et al. (2005). We also found that within the range of 20–35 °C, increasing temperature promoted earlier germination of *E. breviscapus* seeds, which may represent one of the survival strategies of this species. When temperature decreases, extended germination time helps the seed survive adverse environments; when temperature increases, *E. breviscapus* shortens germination time to occupy favorable conditions. Therefore, transferring seeds from 4 °C low temperature

and high humidity after one month (when germination rate was 0%) to 25 °C did not affect normal germination and seedling formation. For production, to obtain uniform and robust seedlings, a dedicated constant-temperature (25 °C) growth room should be established.

Imbibitional chilling injury is damage caused by low-temperature water absorption in seeds (Zheng et al., 2001). Water absorption rate and temperature are two important factors determining whether imbibitional chilling injury occurs (Tao and Zou, 2000). Our results showed that *E. breviscapus* seeds do not suffer from imbibitional chilling injury. This may be related to the initial water content before imbibition, as lower seed water content results in more severe imbibitional chilling injury (Tao and Zou, 2000), suggesting that *E. breviscapus* seed water content may be within the safe range for imbibitional chilling injury. Alternatively, it may be because during low temperature, seeds simultaneously undergo physical and biochemical repair processes of biological membranes, with relatively complete "dual repair" and a "sealed" persistent effect in cells (Zheng et al., 2001). Although *E. breviscapus* seeds do not suffer from imbibitional chilling injury, low-temperature season sowing should still be avoided in field production to increase planting yield.

As a medicinal herb with very large market demand, *E. breviscapus* requires large quantities of high-quality seeds and seedlings to meet production needs as artificial cultivation increases annually. This study employed seed separation technology and dissecting microscope observation to reduce empty seed rate, save storage space, and minimize experimental error, providing high-quality seeds for production. Through systematic investigation of storage conditions and germination characteristics, we have solved the problem of poor storability of *E. breviscapus* seeds and provided an economical and efficient storage solution for research and production. Using our storage method, *E. breviscapus* seeds can be stored for over 2 years while maintaining germination rates above 80%. We conclude that *E. breviscapus* seeds are short-lived, providing new evidence for the above research and important implications for further studies on Asteraceae seeds. This conclusion also provides a new clue for further research on this species. Determination of optimal germination conditions provides effective guidance for field sowing and seedling production, establishing a good foundation for high-quality, high-yield *E. breviscapus* production.

References

Cao Q, Wang KL, Shun WB, 2012. Study on seed quality grading standards of *Erigeron breviscapus* (Vant.) Hand.-Mazz.[J]. *Seed*, 31(1): 107-109.

Cheng HY, Shun WB, Li WQ, 2011. Seed storage behavior of *Formanodendron doichangensis* (Fagaceae), a rare and endangered plant[J]. *Plant Diversity and Resources*, 33(5): 540-546.

Hay FR, Adams J, Manger K, et al, 2008. The use of non-saturated lithium chloride solutions for experimental control of seed water content[J]. *Seed Science*

and Technology, 36(36): 737-746.

He QS, 2015. Responses of seed germination and seedling growth of *Kraschenin-nikovia arborescens* to environmental factors[D]. Zhengzhou: Henan Agricultural University.

Ke XT, Zhang YF, Zhu ZY, et al, 2017. Research progress of prevention and cure effects of caffeoylquinic acids from *Erigeron breviscapus* on ischemic stroke[J]. *Chinese Traditional and Herbal Drugs*, 48(3): 609-615.

Li P, Dang CL, 2005. Seed germination characteristics of *Erigeron breviscapus*[J]. *Journal of Chinese Medicinal Materials*, 28(11): 975-976.

Lin LF, Tao FQ, Hu XQ, et al, 2009. Rapid propagation and plant regeneration of *Erigeron breviscapus* (Vaniot) Hand.- Mazz.[J]. *Journal of Anhui Agricultural Sciences*, 37(4): 1580-1581.

Lin LF, Liu CG, Tao HZ, 2008. Effects of several reagents on germination of *Erigeron breviscapus* seed[J]. *Journal of Anhui Agricultural Sciences*, 36(21): 9118-9119.

Liu H, Yang XL, Xu HB, 2002. Advances in studies on *Erigeron breviscapus*[J]. *Chinese Traditional and Herbal Drugs*, 33(6): 566-568.

Ma YH, Luo GA, Wang YM, 2004. Research status of *Erigeron breviscapus*[J]. *Chinese Traditional Patent Medicine*, 26(1): 63-65.

Murashige T, Skoog F, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures[J]. *Physiologia Plantarum*, 15(3): 473–497.

Qian L, 2015. Influence of environmental factors on seed germination of *Salix linearistipularis*[D]. Harbin: Northeast Forestry University.

Shen LX, Wang P, Zhang RB, 2004. The relation between seed life and ultra-dry preservation[J]. *Seed*, 23(2): 45-47.

Sun CS, Zhu H, Long XY, et al, 2016. Study on the storage and longevity of *Sophora tonkinensis* Gapnep seeds[J]. *Seed*, 35(1): 90-92.

Smith RD, 1992. Seed storage, temperature and relative humidity[J]. *Seed Science Research*, 2(2): 113-116.

Tao ZY, Zou Q, 2000. Imbibitional chilling injury and imbibition damage in seeds[J]. *Plant Physiology Journal*, 36(4): 368-376.

Wang JJ, Ma AW, Wang ZG, et al, 2016. Effects of different temperature and moisture conditions on seed germination of *Festuca sinensis*[J]. *Acta Prataculturae Sinica*, 25(4): 73-80.

Wei CQ, Liu MC, Tang SC, et al, 2012. Effects of light and temperature on seed germination of *Eupatorium odoratum*[J]. *Guihaia*, 32(4): 527-530.

Xiong Y, Du RH, Ma JL, 2012. Construction of DNA fingerprinting and analysis of genetic diversity with ISSR for *Erigeron breviscapus*[J]. *Biotechnology*, 22(5): 45-48.

Yu XM, 2012. Lipidomic analysis of the effect of low temperature and water stress on seed germination and storage[D]. Beijing: University of Chinese Academy of Sciences.

Zhang DN, Luo AY, Xu GP, et al, 2013. Influence of temperature and soil moisture on seed germination of *Cyclobalanopsis*[J]. *Guihaia*, 33(3): 306-312.

Zhang L, Yu LN, Ma YL, 2012. Relevant patent information analysis on cultivation techniques and compatibility of whole herb of *Erigeron breviscapus*[J]. *Chinese Traditional and Herbal Drugs*, (6): 1241-1244.

Zhang W, Yang SC, Zhang GH, et al, 2013. Present situation and countermeasures of *Erigeron breviscapus* planting[J]. *China Journal of Chinese Materia Medica*, 38(14): 2227-2230.

Zhao M, Wang PF, Wang XM, et al, 2018. Structural identification of related substances in breviscapine by UPLC-QTOF-MS[J]. *China Journal of Chinese Materia Medica*, (14): 2872-2777.

Zhao Z, Wang DK, Yang JW, et al, 2013. Identification and anther development cytological studies of the male sterile individuals in *Erigeron breviscapus*[J]. *Acta Botanica Boreali-Occidentalia Sinica*, 33(2): 287-294.

Zheng GH, Liang Z, Lin J, 2001. Studies on the imbibitional chilling injury in seeds and osmoconditioning[J]. *Bulletin of the Chinese Academy of Sciences*, 16(3): 182-187.

Zhou WG, Meng YJ, Chen F, et al, 2017. Advances in studies of physiological and molecular mechanisms of plant seed longevity[J]. *Acta Botanica Boreali-Occidentalia Sinica*, 37(2): 408-418.

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