Effects of Storage Methods on Germination and Vigor of Different-Colored Ammopiptanthus nanus Seeds Postprint

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Abstract

Ammopiptanthus nanus is an endangered evergreen shrub endemic to the desert regions of Central Asia, possessing extremely strong cold and drought resistance. It serves as a model species for investigating plant adaptation mechanisms to extreme environments and is a key focus of research on desert biodiversity conservation. This study investigated the germination and viability of newly harvested seeds and seeds subjected to three storage methods, using black and green colored seeds of Ammopiptanthus nanus as research materials. The results showed that the germination rate of newly harvested seeds increased with rising incubation temperature, and the viability of newly harvested seeds reached 100%. Significant differences in germination were observed between the two seed colors under dry-cold and dry-heat storage conditions, with green seeds showing significantly higher germination rates than black seeds ($P < 0.05$); however, no significant difference was found under wet-cold storage. Wet-cold storage promoted seed germination, whereas dry-cold and dry-heat storage inhibited germination. The three storage methods had minimal impact on the viability of both seed colors, with post-storage viability exceeding 95%. Temperature and humidity are important ecological factors affecting seed germination and viability in Ammopiptanthus nanus. Differences in seed germination represent an evolutionary strategy for adapting to harsh habitats, which is beneficial for enhancing its survival and reproductive capacity. Wet-cold storage effectively improved seed germination rates and maintained viability, providing technical guidance for germplasm resource conservation and nursery breeding.

Full Text

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Effects of Storage Methods on Seed Germination and Viability of *Ammopiptanthus nanus* with Different Colors

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Abstract

Ammopiptanthus nanus is an endangered evergreen shrub endemic to the desert regions of Central Asia. It exhibits strong cold and drought resistance, serves as a model species for investigating plant adaptation mechanisms to extreme environments, and is a key focus of desert biodiversity conservation research. This study examined black and green seeds of *Ammopiptanthus nanus* to investigate germination and viability of newly harvested seeds and seeds after three storage methods. The results showed that the germination rate of newly harvested seeds increased with increasing culture temperature, and the viability of newly harvested seeds reached 100%. Seeds of the two colors showed significant differences in germination under dry-cold and dry-hot storage conditions, with green seeds exhibiting significantly higher germination rates compared with black seeds ($P<0.05$). However, no significant difference was observed under wet-cold storage. Wet-cold storage promoted seed germination, whereas dry-cold and dry-hot storage inhibited it. The three storage methods had minimal impact on the viability of either seed color, with viability after storage reaching above 95%. Temperature and humidity are important ecological factors affecting seed germination and viability in *Ammopiptanthus nanus*. The difference in seed germination represents an evolutionary strategy for adapting to harsh habitats, which is conducive to enhancing its survival and reproductive capacity. Wet-cold storage effectively improves seed germination rates and maintains viability, providing valuable technical guidance for germplasm resource conservation and nursery breeding.

Keywords: *Ammopiptanthus nanus*; seed germination; seed viability; storage method

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Introduction

Seed germination and viability are critical factors for plant population reproduction and renewal. Seeds with high viability and germination rates possess

significant growth advantages, indicating strong survival and reproductive capacity of plant populations [1,2]. For endangered plants, seed germination rates and viability are often insufficient, with only a tiny fraction of seeds successfully germinating and developing into healthy adult plants in natural environments, which limits population survival and self-renewal [3,4].

Seed storage is a key method for protecting species germplasm resources [5], and storage conditions and methods significantly affect seed viability and germination rates [6,7]. Among these, storage temperature and humidity are important factors influencing seed viability and germination [8,9]. Appropriate low-temperature storage can reduce seed metabolic activity, decrease material consumption, thereby slowing viability decline and effectively extending longevity [10,11]. Storage humidity is also crucial, as both excessively high and low humidity are detrimental to seed germination and viability maintenance [12,13]. High humidity reduces seed viability and increases susceptibility to mold, while excessively low humidity causes seeds to become overly dehydrated, inhibiting metabolic activity and thus reducing seed viability and germination rates [14,18]. Therefore, temperature and humidity during storage significantly affect seed quality and life activities [19,20]. However, different seeds respond differently to storage methods [21]. For example, *Carex comosa* seeds exhibited the highest germination rate and viability after wet-cold storage, while dry-cold storage resulted in the lowest viability [22]; *Salsola arbuscula* seeds showed decay after short-term indoor and field storage, leading to loss of germination ability and viability [23]. Thus, selecting scientifically reasonable storage methods is crucial for long-term seed preservation, not only effectively extending seed longevity but also ensuring seeds maintain high viability and germination rates at planting time [24,25].

Numerous studies have reported on the effects of environmental temperature and humidity on seed germination and viability of endangered plants, including *Rhododendron changii* [26], *Castanopsis kawakamii* [27], *Horsfieldia hainanensis* [28], and *Castanopsis sclerophylla* [29]. These studies reveal the importance of environmental factors for endangered plant seed germination and viability. Investigating the effects of external environmental conditions on seed germination and viability of endangered plants can provide theoretical foundations for selecting appropriate storage methods, artificial propagation, and population restoration [30,31].

Ammopiptanthus nanus is a rare relict species belonging to the Fabaceae family [32] and an ancient species originating from the Tertiary period Mediterranean coast [33]. *Ammopiptanthus nanus* is not only a valuable plant resource for landscaping but also serves functions in maintaining desert ecological balance, windbreak and sand fixation, and soil and water conservation [34]. Additionally, it has medicinal value for treating chronic rheumatoid arthritis [35]. Due to its small population size, *Ammopiptanthus nanus* has been included in the "Xinjiang National Key Protected Wild Plant List" (http://www.forestry.gov.cn/main/146/20220329/110510176303775.html). Pre-

vious studies on *Ammopiptanthus nanus* seed germination and viability have shown that concentrated sulfuric acid corrosion can break physical dormancy and promote germination [36]; seed water content and storage temperature significantly affect germination rates [37]; and low salt concentrations significantly affect seed viability [38]. Notably, correlations between seed coat color and seed viability have been reported in legumes. For example, germination rate and viability of *Astragalus membranaceus* seeds increase with darker seed coat color, while the opposite is true for *Glycyrrhiza uralensis* [39,40]. *Ammopiptanthus nanus* seeds exhibit black and green phenotypes, but systematic comparative studies on viability differences between the two color types are rare.

Seeds of desert plants in northwestern China typically mature in summer and autumn. Since direct germination makes it difficult to survive winter, seeds often require storage until spring to ensure successful growth [41]. *Ammopiptanthus nanus* inhabits harsh environments and faces severe survival challenges, with extremely low germination rates under natural conditions and difficult natural population renewal, leading to extinction risk [42]. Ex-situ conservation and establishment of germplasm resource banks are effective measures for population restoration of *Ammopiptanthus nanus*. However, research is urgently needed on which storage methods can promote germination and maintain seed viability. With long winters in Xinjiang, if *Ammopiptanthus nanus* seeds are planned for spring sowing, they require 4-5 months of storage. Based on Xinjiang's winter regional characteristics, this study employed three economical and practical storage methods: wet-cold storage simulating field conditions, dry-cold storage simulating unheated cold rooms, and dry-hot storage simulating heated indoor environments. Based on this background, this study aimed to answer the following questions: What are the differences in germination rates and viability between different colored seeds of *Ammopiptanthus nanus*? What are the differences in germination rates and viability between the two seed colors under different storage methods? How can storage conditions be optimized to maintain seed viability and improve germination rates? This study explored effective storage methods to enhance germination rates and viability of *Ammopiptanthus nanus* seeds, providing technical support for artificial propagation and population restoration of this species [43].

1.1 Seed Collection Site Overview

Seeds of *Ammopiptanthus nanus* were collected in September 2019 from natural distribution communities in Wuqia County, Kizilsu Kirghiz Autonomous Prefecture, Xinjiang (39°28 ~39°49 N, 74°54 ~75°35 E). Wuqia County is located at the western end of the southern Tianshan Mountains in the north and adjacent to the Pamir Plateau and northern Kunlun Mountains in the south, with an elevation of 2032~2434 m [44]. The region belongs to a temperate arid climate zone, with an average annual temperature of 7.3°C, extreme maximum temperature of 34.7°C, extreme minimum temperature of -29.9°C, average annual precipitation of 172 mm, average annual sunshine duration of 2797.2 h, and

frost-free period of 135 d [45].

1.2.1 Seed Morphological Characteristics

One hundred seeds of different sizes were randomly selected for each color type. Visual observation was used to assess seed size, shape, and color characteristics, and seeds were classified using a balance and vernier caliper. A 1/10,000 balance measured the weight of each seed type, with 10 replicates of 100 seeds each to calculate average values. Vernier calipers measured length, width, and thickness, with average values calculated.

1.2.2 Water Absorption Rate Measurement of Newly Harvested Seeds

Water absorption rate was measured following the method of Ren Yongxia et al. [46]: Two filter papers were placed in a 100 mm diameter petri dish, moistened with distilled water, and maintained at room temperature (25$±$2)°C. One hundred intact seeds were selected, with three replicates of 30 seeds each for each color. Seeds were placed in the petri dish, and water absorption rate was measured at 2 h intervals until seed germination began. During weighing, surface water was first absorbed with filter paper before weighing on a 1/10,000 electronic balance. After each weighing, appropriate water was added to maintain filter paper moisture. Water absorption rate was calculated as: Water absorption rate (%) = [(mass after absorption - mass before absorption) / mass before absorption] × 100%.

1.3 Germination Test of Newly Harvested Seeds at Different Temperatures

In September 2019, seeds were collected from 10 *Ammopiptanthus nanus* plants in natural communities and thoroughly mixed. Collected seeds were air-dried indoors in a well-ventilated area for one week. After drying, healthy and plump seeds were selected and stored in kraft paper bags at room temperature in a dry environment. Germination experiments were conducted on black and green seeds one week after natural drying. All germination experiments used three replicates of 50 seeds each. Seeds were placed in 90 mm petri dishes lined with two layers of filter paper. Temperature treatments referenced the monthly average maximum temperature during the growing season in the native habitat, with three gradients selected: 15/5°C, 20/10°C, and 25/15°C. Seeds were germinated in an intelligent illumination incubator (12 h dark/12 h light, light intensity 8000 lx). During germination, filter paper was kept moist, and germination was defined as radicle emergence of 2 mm. Germinated seeds were counted daily for 4 weeks. After the experiment, ungerminated seeds were dissected with a scalpel to observe embryo color. Embryos showing red coloration were recorded as viable, and seed germination rate and viability were calculated: Germination rate = (number of germinated seeds / total seeds tested) × 100%; Seed viability = (number of viable seeds / total seeds tested) × 100%.

1.4 Effects of Storage Methods on Seed Germination and Viability

Storage treatments: In early November 2019, seeds of both colors were placed in nylon mesh bags, 200 seeds per bag, with 18 bags per color. The experiment consisted of three treatment groups, each with six bags (three black, three green). Group 1 was stored in a heated laboratory at Xinjiang Normal University, with environmental conditions approximating commonly used warehouses in production practice. This treatment served as dry-hot storage (15~25°C). Group 2 was placed in an empty iron-sheet warehouse on Taoshan at Xinjiang Normal University without heating supply, serving as dry-cold storage (-26~9°C). Group 3 was buried 2.5 cm deep in the soil of an experimental field at Xinjiang Normal University (with relatively consistent soil matrix and no surrounding vegetation) within a 50 cm × 50 cm square iron mesh frame (connected with wooden strips around the perimeter to prevent wind displacement), receiving natural snowfall until snow melted the following spring. This treatment served as outdoor wet-cold storage (-7~19°C). DS1923 button temperature loggers recorded environmental temperature for dry-hot and dry-cold storage, while TMS94246305 instruments recorded temperature for wet-cold storage.

Germination experiments: In early March 2020, stored seeds were retrieved for germination experiments. All experiments used three replicates of 50 seeds each. Seeds were placed in 90 mm petri dishes with two layers of filter paper, maintaining moisture. Three temperatures were set: 15/5°C, 20/10°C, and 25/15°C (12 h dark/12 h light, light intensity 8000 lx). Germination experiments were conducted in an intelligent illumination incubator, checked daily, with filter paper kept moist. Germination was defined as radicle emergence of 2 mm, with germinated seeds removed. Experiments lasted 4 weeks. After completion, ungerminated seeds were cut open. Based on previous TTC viability testing and embryo color observation, red embryos were considered viable, while black, brown, or rotten embryos were considered dead. Viable and dead seeds were counted to calculate germination rate and viability.

1.5 Statistical Analysis

Original data were preliminarily organized and integrated using Microsoft Excel 2021, and statistical analysis was performed using SPSS 26.0. Germination experiment results are expressed as mean values (± standard error). One-way analysis and independent samples t-tests were used to evaluate significant differences in seed germination and viability among different seed coat colors, temperatures, and storage methods. Multivariate analysis of variance was also conducted to assess significant differences in germination rates due to seed coat color, temperature, and storage method and their interactions. Origin 2021 software was used for data plotting.

2.1 Seed Morphology and Water Absorption Rate

Based on morphological characteristics including color, size, and weight, *Ammopiptanthus nanus* seeds were classified into black and green phenotypes. Statistical analysis of harvested seeds showed that black seeds accounted for approximately 60%. Both color types were flat and reniform with leathery, smooth seed coats. Black seeds were larger, without patterning, with length, width, and thickness of 6.14 mm, 4.96 mm, and 1.81 mm, respectively, and a 1000-seed weight of 3.87 g. Green seeds were smaller, with patterning, with length, width, and thickness of 5.42 mm, 4.20 mm, and 1.77 mm, respectively, and a 1000-seed weight of 3.12 g (Table 1). Water absorption experiments showed that seeds began germinating 12 h after placement in petri dishes. Water absorption rate increased with time during 0~12 h, with green seeds showing higher water absorption rates than black seeds, reaching 1.5 times the final absorption rate of black seeds (Fig. 2).

2.2 Germination Rate and Viability of Newly Harvested Seeds at Different Temperatures

Newly harvested seeds of both colors had a relatively wide suitable germination temperature range, germinating under all three temperature conditions. Black seed germination rates ranged from 11% to 79%, while green seed germination rates ranged from 17% to 75%. Culture temperature (i.e., germination temperature) significantly affected germination of both newly harvested seed colors ($P<0.05$), with germination rates increasing as culture temperature increased. The optimal germination temperature was 25/15°C, where germination rates exceeded 75% for both colors. Culture temperature and seed color had no significant effect on viability of newly harvested seeds ($P>0.05$), with viability maintained at 100% (Table 2).

2.3.1 Effects of Different Storage Methods on Seed Germination Rate

At the same culture temperature, germination rates of *Ammopiptanthus nanus* seeds under wet-cold storage were significantly different from those under dry-cold and dry-hot storage ($P<0.05$). Across the three temperatures, germination rates under wet-cold storage were generally high, mostly exceeding 70%, while germination rates under dry-hot and dry-cold storage were relatively low, at 11%~54% and 11%~39%, respectively. For seeds under dry-hot and dry-cold storage, the two colors showed significant differences in germination rate ($P<0.05$). Under dry-hot storage at 20/10°C and 25/15°C, green seed germination rates were significantly higher than black seed germination rates ($P<0.05$), being 54% and 39% higher, respectively. Under dry-cold storage at 15/5°C and 20/10°C, green seed germination rates were significantly higher than black seed germination rates ($P<0.05$), being 17% and 21% higher, respectively. However, under wet-cold storage, the difference in germination rate between the two colors was not significant ($P>0.05$). Additionally, compared with newly harvested

seed germination rates at the same culture temperature, germination rates under dry-hot and dry-cold storage were lower, while those under wet-cold storage were higher (Fig. 3).

Multivariate analysis of variance showed that seed coat color, temperature, storage method, and their pairwise interactions had significant effects on *Ammopiptanthus nanus* seed germination rate ($P<0.001$), but the three-way interaction had no significant effect ($P=0.320>0.05$) (Table 3).

2.3.2 Effects of Different Storage Methods on Seed Viability

Short-term storage (4-5 months) had little effect on *Ammopiptanthus nanus* seed viability, with neither culture temperature nor storage method significantly affecting viability ($P>0.05$). Both dry-hot and dry-cold storage maintained seed viability above 95%. In contrast, wet-cold storage caused a slight decrease in viability, but still maintained high levels above 90%. As culture temperature increased, viability of wet-cold stored seeds gradually decreased. At the same culture temperature and storage method, no significant difference in viability was observed between the two seed colors ($P>0.05$) (Fig. 4).

3 Discussion

This study found that germination rates of newly harvested *Ammopiptanthus nanus* seeds increased with increasing culture temperature. At higher temperatures (25/15°C), enhanced cellular metabolism and enzyme activity promoted rapid and extensive germination [47]. This is consistent with studies by Yang Qihe et al. [36] and Ma Miao et al. [37], both indicating that higher temperatures promote *Ammopiptanthus nanus* seed germination. At lower temperatures (15/5°C), newly harvested seeds showed lower germination rates, possibly due to insufficient accumulated heat for germination [48]. Research indicates that seeds of temperate species typically require higher accumulated temperatures for successful germination to avoid premature germination during brief warm periods that could lead to subsequent seedling mortality [49]. Therefore, appropriate germination temperature is crucial for newly harvested *Ammopiptanthus nanus* seeds.

Significant differences in germination rates between the two seed colors were observed after dry-cold and dry-hot storage, with green seeds showing significantly higher germination rates than black seeds (Fig. 3). This may be due to changes in seed coat hardness during these storage processes. Under both storage methods, seeds dehydrated in dry environments, increasing seed coat hardness [50]. Black seeds have harder seed coats with lower water permeability, severely hindering imbibition and reducing germination rates [51]. However, green seeds have softer seed coats, where small amounts of moisture can trigger imbibition, softening the seed coat while the waxy layer gradually thins or detaches, thereby increasing permeability and promoting germination [52]. Additionally, the two seed colors may differ in sensitivity to storage conditions

(temperature, humidity, etc.), leading to germination rate differences [14,45]. Due to different environmental sensitivities and adaptabilities, the two seed colors adopted a "bet-hedging" germination strategy after storage [6,45]. Green seeds employed an "opportunistic" strategy, being less sensitive to environmental conditions and maintaining relatively high germination rates across a wide temperature range and different storage methods. Black seeds adopted a "cautious" strategy, being more sensitive to environmental conditions with deeper dormancy, forming persistent seed banks under unfavorable conditions to reduce germination and benefit population renewal and reproduction [6,45,47].

However, under wet-cold storage, no significant difference in germination rate was observed between the two seed colors. This may result from adequate moisture provided by spring temperature rise and snowmelt. Although black seeds have harder seed coats that hinder germination to some extent, they can absorb sufficient moisture to soften the seed coat, allowing the embryo to break through constraints [15,21]. Simultaneously, rising temperatures enhance seed respiration and metabolism, providing sufficient energy for germination and effectively increasing germination rates [53]. Green seeds, with softer seed coats and better permeability, more easily imbibe and soften, promoting embryo development and achieving higher germination rates [14,43]. Under wet-cold storage, the combined effects of adequate moisture, suitable temperature, and seed characteristics enabled both seed colors to obtain resources needed for germination, overcoming obstacles to successful germination. Therefore, no significant difference in germination rate existed between the two colors under wet-cold storage.

Wet-cold storage was more favorable for *Ammopiptanthus nanus* seed germination. In addition to appropriate low-temperature storage, environmental moisture content during storage is an important factor affecting germination [14,56]. Compared with dry-cold and dry-hot storage, wet-cold storage provided conditions closer to the natural field environment for *Ammopiptanthus nanus* seeds, including higher moisture content. Seeds under wet-cold storage entered dormancy after experiencing extreme winter low temperatures and snow cover. Spring snowmelt and frozen soil thawing provided adequate moisture. *Ammopiptanthus nanus* seeds absorbed sufficient moisture to soften seed coats for rapid germination and completed seedling growth before the arrival of dry summer conditions, which helps improve seedling survival rates [14,46]. In contrast, seeds under dry-cold and dry-hot storage lacked snow cover, remaining in dry environments prone to dehydration and increased seed coat hardness, thereby hindering germination [54].

This study showed that viability of both seed colors remained at high levels before and after short-term storage. Previous research indicates that legume seeds in China's arid regions typically have high viability [57], consistent with our findings. Additionally, Jiao Peipei [42] reported that short-term storage had little effect on *Ammopiptanthus* seed viability, which could be maintained at high levels, matching our results. The insignificant effect of short-term storage on

seed viability and the ability to maintain high viability have also been demonstrated in other desert plants. For example, *Bassia dasyphylla* and *Ceratoides latens* maintained viability unaffected after 4-5 months of snow storage, while *Plantago minuta* and *Camelina sativa* seeds retained their viability [58]. For 13 desert plant species from the Gurbantunggut Desert, short-term dry storage had minimal impact on viability, which remained as high as 90% [59]. Wang Juhong et al. [14] reported that wet-cold storage effectively promoted seed germination while dry-hot storage inhibited it in eight desert plant species. Liu Liping et al. [60] reached similar conclusions in their study on *Lycium ruthenicum* seeds, finding that wet-cold storage benefited germination while dry-cold storage had the opposite effect. Collectively, these results confirm that wet-cold storage promotes *Ammopiptanthus nanus* seed germination, while dry-cold and dry-hot storage inhibit it.

The results indicate that appropriate short-term storage conditions can effectively maintain seed viability, which is significant for seed preservation and subsequent germination. Among the three storage methods, wet-cold storage caused a slight decrease in viability but still maintained levels above 90%. During wet-cold storage, snowmelt, temperature fluctuations, and microbial activity may soften seed coats, reduce defense against external damage, and damage membrane structures, leading to decreased viability and mold in a small number of seeds [14,52]. Additionally, black seeds showed higher viability under wet-cold storage, possibly due to genetic characteristics such as larger mass and lower water absorption rate that enable them to maintain higher viability under stress [14,47]. Overall, differences in seed viability among the three storage treatments were minor, all effectively maintaining viability and playing positive roles in germplasm resource conservation.

Compared with newly harvested seed germination, wet-cold storage promoted *Ammopiptanthus nanus* seed germination, while dry-cold and dry-hot storage inhibited it, indicating that suitable moisture and low-temperature storage conditions can promote germination. This may be because temperature and humidity in the storage environment significantly affect seed germination [14,53]. The main difference between dry-cold and wet-cold storage regarding low temperature is snow cover. Without snow cover in dry-cold storage, seeds suffer damage from thermal expansion and contraction due to dramatic temperature changes, prompting seed coat hardening to resist external damage but hindering imbibition and embryo breakthrough [54]. Wang Juhong et al. [14] also reported that wet-cold storage effectively promoted seed germination while dry-hot storage inhibited it in eight desert plant species. Liu Liping et al. [60] reached similar conclusions in their study on *Lycium ruthenicum* seeds.

In species conservation and reproduction work, control of seed storage and germination conditions is crucial. For *Ammopiptanthus nanus* seeds, wet-cold storage demonstrates unique advantages compared with dry-hot and dry-cold storage methods. After wet-cold storage, no significant difference in germination rate existed between the two seed colors, a characteristic that simplifies sowing pro-

cedures without requiring additional time and effort to select seeds, greatly improving operational convenience. Additionally, given the precious and scarce seed resources of *Ammopiptanthus nanus*, wet-cold storage can effectively maintain viability and improve germination rates, ensuring maximum utilization of seed resources and reducing waste. However, wet-cold storage is only suitable for short-term seed preservation; long-term wet-cold storage may increase risks of mold and decay due to high humidity from spring snowmelt, thereby reducing seed viability and longevity. Therefore, in conservation efforts for *Ammopiptanthus nanus*, short-term wet-cold storage is recommended, with temperature conditions controlled during the high germination phase to ensure effective utilization of seed resources and promote species preservation and sustainable reproduction.

4 Conclusion

Analysis of the effects of different colors and storage methods on germination and viability of *Ammopiptanthus nanus* seeds yielded the following main conclusions:

1) Germination rates of newly harvested *Ammopiptanthus nanus* seeds increased with increasing culture temperature, and viability of all newly harvested seeds reached 100%.

2) After storage, green seeds under dry-cold and dry-hot storage showed significantly higher germination rates than black seeds, while no significant difference in germination rate existed between the two colors under wet-cold storage. Seed viability remained at high levels under all three storage methods.

3) Storage methods differentially affected seed germination: wet-cold storage promoted germination, while dry-cold and dry-hot storage inhibited it.

Therefore, in conservation efforts for *Ammopiptanthus nanus*, short-term wet-cold storage followed by germination at 25/15°C in the following spring is recommended to effectively maintain high seed viability and improve germination rates, promoting species preservation and sustainable reproduction.

References

[1] Baskin C C, Baskin J M. Seed: Ecology, Biogeography, and Evolution of Dormancy and Germination[M]. San Diego: Academic Press, 2014: 5-39.

[2] Wen D, Hou H, Meng A, et al. Rapid evaluation of seed vigor by the absolute content of protein in seed within the same crop[J]. Scientific Reports, 2018, 8(1): 5569.

[3] Li Sunling, Jing Yuebo, Li Shufang, et al. Seed viability determination of the rare and endangered plant *Cinnamomum chago*[J]. Journal of West China Forestry Science, 2021, 50(3): 131-134, 143.

[4] Chen Yuanzheng, Ma Xiangqing. Researches on the reproductive ecology of endangered plant populations[J]. Chinese Journal of Eco Agriculture, 2007, 15(1): 186-189.

[5] International Seed Testing Association. International Rules for Seed Testing 1996[M]. Beijing: China Agriculture Press, 1999.

[6] Yang Pengpeng. Analysis on meteorological factor variation in recent 60 years in Wuqia County[J]. Northwest Hydropower, 2018(6): 13-16.

[7] Nonogaki H, Bassel G W, Bewley J D. Germination still a mystery[J]. Plant Science, 2010, 179(6): 574-581.

[8] Upretee P, Bandara M S, Tanino K K. The role of seed characteristics on water uptake preceding germination[J]. Seeds, 2024, 3(4).

[9] Yang Yang, Wang Haiyang, Ma Lihui. Characteristics of fruiting and seed germination of endangered plant, *Rhododendron changii*[J]. Scientia Silvae Sinicae, 2020, 56(10): 173-183.

[10] Liu X, Xiao Y, Wang Y, et al. Seed germination ecology of endangered plant *Horsfieldia hainanensis* Merr. in China[J]. BMC Plant Biology, 2024, 24(1): 486.

[11] Le Xingui, Wang Zhengwei, Ning Xin, et al. The germination characteristics and dormancy type of the seed of *Castanopsis sclerophylla*[J]. Bulletin of Botanical Research, 2022, 42(4): 688-693.

[12] Chang Haiwen, Zhang Fenglan, Yang Zhongren, et al. Physiological and biochemical responses of *Allium mongolicum* seeds to storage aging[J]. Plant Physiology Journal, 2015, 51(7): 1075-1081.

[13] Budelsky R A, Galatowitsch S M. Effects of moisture, temperature, and time on seed germination of five wetland *Carices*: Implications for restoration[J]. Restoration Ecology, 1999, 7(1): 86-97.

[14] Wang Juhong, Ma Ruijun, Chen Wen. Effects of cold stratification and dry storage at room temperature on seed germination of eight desert species from the Hexi Corridor of China[J]. Chinese Journal of Plant Ecology, 2012, 36(8): 791-801.

[15] Omar S, Abd Ghani R, Khalid N, et al. Effects of seed quality and hybrid type on maize germination and yield in Hungary[J]. Agriculture, 2023, 13(9): 1836.

[16] Halmurat Paruza, Zhao Xiaoying, Cheng Caragay-Peng. Responses of seed viability and germination in four species when buried in soil under snow cover in Xinjiang[J]. Plant Science Journal, 2023, 41(4): 513-520.

[17] Wu Qinxia, Hu Yuchen, Chen Ying, et al. Responses of seed germination to low temperature storage in different cultivars of *Xanthoceras sorbifolium*[J]. Guihaia, 2024, 44(3): 541-553.

[18] Han Yuzhu, Zeng Bing, Wu Lian, et al. Effect of different storage conditions on Indian lettuce seed vigor[J]. Journal of Henan Agricultural Sciences, 2011, 40(7): 138-143.

[19] He Zhongsheng, Liu Jinfu, Hong Wei, et al. Effects of different treatments on seed germination of *Castanopsis kawakamii*[J]. Journal of Beijing Forestry University, 2012, 34(2): 66-70.

[20] Yang Qihe, Ge Xuejun, Ye Wanhui, et al. Characteristics of *Ammopiptanthus nanus* seed and factors affecting its germination[J]. Acta Phytoecologica Sinica, 2004, 28(5): 651-656.

[21] Ji T F, Li J, Liang C H. The chemical constituents of the twigs of *Ammopiptanthus nanus*[J]. Journal of Asian Natural Products Research, 2013, 15: 332-336.

[22] Paruza Halmurat, Cui Guoying, Zhao Xiaoying, et al. Response of seed germination of four wild plants to short term dry storage at room temperature[J]. Chinese Wild Plant Resources, 2022, 41(12): 5-9.

[23] Jiao Peipei. Study on reproductive biology of endangered plant *Ammopiptanthus nanus*[D]. Alar: Tarim University, 2007.

[24] Editorial Committee of Flora of China, Chinese Academy of Sciences. Flora of China: 42(2)[M]. Beijing: Science Press, 1998: 388-397.

[25] Cao S, Wang Y, Li X, et al. Characterization of the AP2/ERF transcription factor family and expression profiling of DREB subfamily under cold and osmotic stresses in *Ammopiptanthus nanus*[J]. Plants, 2020, 9(4): 455.

[26] Li A, Ma M, Li H, et al. Genetic diversity and population differentiation of a Chinese endangered plant *Ammopiptanthus nanus* (Pop.) Cheng f.[J]. Genes, 2023, 14(5): 1020.

[27] Ren Yongxia, Guo Yupin, Liu Guihe, et al. Drought resistance of three forage species *Vicia* during germination period[J]. Crops, 2016(3): 158-162.

[28] Gao Zhihai, Liu Shenglong, Zhong Shujun, et al. Experiments on introduction and culture of *Ammopiptanthus nanus*[J]. Journal of Gansu Forestry Science and Technology, 1995(1): 28-31, 34.

[29] Liu Meiqin, Lu Cunfu, Yin Weilun. Research progress of biological characteristics and abiotic stress tolerant mechanisms of rare & endangered plant *Ammopiptanthus*[J]. Chinese Journal of Applied & Environmental Biology, 2004, 10(3): 384-388.

[30] Liu Wen, Liu Kun, Zhang Chunhui, et al. Effect of accumulated temperature on seed germination—a case study of 12 Compositae species on the eastern Qinghai Tibet Plateau of China[J]. Chinese Journal of Plant Ecology, 2011, 35(7): 751-758.

[31] Buhailiqiemu Abudureheman. Seed germination characteristics of 18 Leguminosae and ephemeroid species of *Carex physodes* in arid area of China[D]. Beijing: University of Chinese Academy of Sciences, 2015.

[32] Zhang Yulin, Yin Benfeng, Tao Ye, et al. Effects of freezing and thawing cycle on seed germination of desert ephemeral plants[J]. Chinese Journal of Ecology, 2021, 40(2): 301-312.

[33] Yang Han, Liu Hongfei, Yang Helong, et al. Effect of storage temperature and moisture content on the seed quality of *Agropyron cristatum*[J]. Pratacultural Science, 2016, 33(10): 2033-2040.

[34] Chang Hui, Cheng Qiuxiang, Li Yinping, et al. Study on the correlation between seed size, seed coat colour and seed vigor of *Astragalus membranaceus* mongholicus (Fisch.) Bge. var. (Bge.) Hsiao[J]. Seed, 2015, 34(8): 95-97, 104.

[35] Qi Xuefeng, Sun Qun, Yang Ligang, et al. Effect of seed coat color on seed quality of *Glycyrrhiza uralensis* Fisch.[J]. Seed, 2007, 26(7): 31-33.

[36] Guo Congcong, Shen Yongbao, Shi Fenghou. Effects of temperature on stored substance metabolism and enzyme activity during germination of *Pinus bungeana* seeds[J]. Journal of Nanjing Forestry University (Natural Science Edition), 2023, 47(6): 25-34.

[37] Wang Yanmei, Zhang Xiaoxue, Zhu Xiuzheng, et al. Research progress on dormancy and germination mechanism of forest seeds[J]. Scientia Silvae Sinicae, 2021, 57(10): 128-144.

[38] Li Xiaoling, Wen Haoran, Cheng Suihan, et al. Effect of submergence in water and different storage methods on germination and physiological characteristics of seeds and seedling growth vigor of *Distylium chinense*[J]. Acta Botanica Boreali Occidentalia Sinica, 2017, 37(10): 2033-2041.

[39] Li Mingyuan, Zhao Xiaoying, Ai Saiti, et al. Effects of appendages on seed germination and viability of four desert shrubs in China[J]. Grassland and Turf, 2021, 41(5): 93-98.

[40] Wei Baorui. Effects of different storage treatments on the quality of split seeds of *Panax quinquefolius*[D]. Changchun: Jilin Agricultural University, 2021.

[41] Liu Liping, Zhang Dongzhi, Zhang Chong, et al. Research progress of stress tolerance and breeding research on *Lycium ruthenicum* Murr[J]. Biotechnology Bulletin, 2016, 32(10): 118-127.

[42] Jia Fengqin, Huang Mintao, Deng Li, et al. Effects of drought, salt, and acidity stresses on *Asclepias curassavica* and *Amaranthus tricolor* seed germination[J]. Arid Zone Research, 2025, 42(2): 312-320.

[43] Nerson H. Effects of seed maturity, extraction practices and storage duration on germinability in watermelon[J]. Scientia Horticulturae, 2002, 93(3-4): 245-256.

[44] Omar S, Abd Ghani R, Khalid N, et al. Effects of seed quality and hybrid type on maize germination and yield in Hungary[J]. Agriculture, 2023, 13(9): 1836.

[45] Yue Hongjuan, Tong Chuan, Zhu Jinmao, et al. Seed rain and soil seed bank of endangered *Taxus chinensis* mairei in Fujian, China[J]. Acta Ecologica Sinica, 2010, 30(16): 4389-4400.

[46] Qu Yan, Ou Zhi, Xia Ying, et al. Effects of different pretreatment on germination features of *Meconopsis racemosa* Maxim. seeds[J]. Seed, 2018, 37(2): 5-9, 13.

[47] Bai Shilin, Lyu Yaya, Shi Xiaojun. Effects of different habitats and substrates on seed germination and seedling growth of *Malus sieversii* after passing through digestive tract of cattle[J]. Arid Zone Research, 2024, 41(5): 821-829.

[48] Ma Wenbao, Tan Dunyan, Xue Jianhui, et al. Seed heteromorphism and its ecological significance[J]. Journal of Sichuan Forestry Science and Technology, 2011, 32(6): 34-43.

[49] Reyilamu Maimaituerxun, Halibunuer, Aysajan Abdusalam. Seed germination and dormancy traits of fruit heteromorphism species *Lycium ruthenicum* in an elevational heterogeneity environment[J]. Arid Zone Research, 2023, 40(7): 1152-1163.

[50] Gao Yue, Qin Qijuan, Yan Jiayue, et al. Germination and seedling growth characteristics of heterotypic seeds from *Polygonum aviculare*[J]. Rrata-cultural Science, 2024, 41(7): 1660-1667.

[51] Liu Fei, Pang Jingjing, Zhang Keliang. Seed dormancy and germination characteristics of *Cercis canadensis*[J]. Jiangsu Agricultural Sciences, 2023, 51(3): 161-165.

[52] Guo Huanhuan, Liu Yong, Wu Chengliang, et al. Research progress of forest seed dormancy and storage in foreign countries[J]. Journal of Northwest Forestry University, 2017, 32(4): 133-138.

[53] Liu H L, Zhang L W, Yin L K, et al. Effects of temperature, dry storage, and burial on dormancy and germination of seeds of 13 desert plant species from sand dunes in the Gurbantunggut Desert, Northwest China[J]. Arid Land Research and Management, 2013, 27(1): 65-78.

[54] Bai Mengjie, Tao Qibo, Han Yunhua, et al. Germination response to temperature of ten desert plant species[J]. Acta Prataculturae Sinica, 2019, 28(12): 53-62.

[55] Zhang Yingjuan, Wang Yushan, Li Shaoshun. Effects of different storage conditions and durations on seed viability and germination of five desert plants in West Erdos[J]. Scientia Silvae Sinicae, 2011, 47(1): 36-41.

[56] Li Dexin, Zhang Daoyuan, Zhang Liuwei, et al. Biological seed characteristics of spring *Erodium oxyrrhynchum* emergence and autumn emergence[J].

Arid Zone Research, 2020, 37(6): 1562-1568.

[57] Liu X, Xiao Y, Wang Y, et al. Seed germination ecology of endangered plant *Horsfieldia hainanensis* Merr. in China[J]. BMC Plant Biology, 2024, 24(1): 486.

[58] Yue Hongjuan, Tong Chuan, Zhu Jinmao, et al. Seed rain and soil seed bank of endangered *Taxus chinensis* mairei in Fujian, China[J]. Acta Ecologica Sinica, 2010, 30(16): 4389-4400.

[59] Qu Yan, Ou Zhi, Xia Ying, et al. Effects of different pretreatment on germination features of *Meconopsis racemosa* Maxim. seeds[J]. Seed, 2018, 37(2): 5-9, 13.

[60] Liu Liping, Zhang Dongzhi, Zhang Chong, et al. Research progress of stress tolerance and breeding research on *Lycium ruthenicum* Murr[J]. Biotechnology Bulletin, 2016, 32(10): 118-127.

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