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The temperature and length for the release of primary and induction of secondary physiological dormancy in Korean pine (*Pinus koraiensis* Sieb. et Zucc.) seeds

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Abstract
Primary physiological dormancy and secondary physiological dormancy of Korean pine seeds restrict the regeneration of broad-leaved Korean pine (*Pinus koraiensis*) mixed forest. Dry and imbibed seeds were stratified at 1 °C and 5 °C for 1, 2, 4 and 6 months. Germination percentage, mean germination time (MGT) and germination rate index (GRI) were measured to determine the optimal low temperature and its duration for the release of primary physiological dormancy. Once primary physiological dormancy was released through cold stratification, seeds were stored in an environment in which the temperature progressively increased from 5 to 25 °C. After one month of storage at each storage temperature, the germination percentage, MGT and GRI were measured to determine the threshold temperature for the induction of secondary physiological dormancy. Both dry and imbibed seeds not only exhibited a high germination percentage (approximately 80%) but also germinated rapidly (MGT and GRI were 17 days and 2.36, respectively) after 6 months of storage at either 1 °C or 5 °C. The germination percentage of cold stratified seeds gradually decreased from 78% (5 °C) to 72% (10 °C), 55% (15 °C), 10% (20 °C) and 8% (25 °C). The results of this study suggest that stratifying seeds at 1 °C or 5 °C for 6 months releases primary physiological dormancy. The induction of secondary physiological dormancy occurs at temperatures above 15 °C.

Keywords Korean pine · Primary physiological dormancy · Secondary physiological dormancy · Threshold temperature

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Introduction

The broad-leaved Korean pine (*Pinus koraiensis*) mixed forest (BKPF) was once the dominant forest type from Northeast China to the Far East region of Russia (Ishikawa et al. 1999). However, large-scale industrial deforestation led to a major reduction in the distribution of BKPF (Zhu et al. 2007). Korean pine is the dominant tree species in BKPF. Korean pine seeds have morphophysiological dormancy (MPD) at dispersal (Qi et al. 1993). In seeds with MPD, the embryos are not only underdeveloped or undifferentiated but also exhibit primary physiological dormancy (Baskin and Baskin 2004). Studies have previously shown that after fresh Korean pine seeds were buried between litter-fall and soil for 6 months (from the first late-autumn to the first early-spring season after seed dispersal), the embryo-length to megagametophyte-length was only approximately 0.64, but more than 80% of those seeds could germinate at 25/16 °C (Song et al. 2018). It can be concluded that primary physiological dormancy is released in the first early-spring season after seed dispersal. Since an incubation temperature of 25/16 °C promotes the growth of the embryo, primary morphological dormancy is also released. Therefore, seeds have a high germination percentage under laboratory incubation conditions. However, because the field temperature in early spring is low, this morphological dormancy is still maintained. This characteristic dormancy leads to poor seed germination in the first spring after dispersal.

If environmental conditions are insufficient to trigger full germination, the remaining viable seeds can enter secondary physiological dormancy (Bewley et al. 2013). Korean pine seeds that fail to germinate in the first spring after dispersal gradually enter secondary physiological dormancy (Song et al. 2018). The secondary physiological dormancy in seeds of Korean pine may be released during the second winter after dispersal. Once Korean pine seeds have their primary and secondary physiological dormancy released, they rapidly germinate during the second spring after dispersal. Normally, only a few Korean pine seeds germinate during the first spring after dispersal, and the majority of seeds germinate during the second spring following dispersal. During this long germination duration (approximately 20 months), Korean pine seeds fall prey to birds and rodents or are infected by fungal pathogens, leading to a reduction in seed numbers (Li et al. 2012). Regeneration of Korean pine is a key step in the restoration of BKPF. Therefore, it is critical to understand the mechanisms of their germination pattern.

Temperature is a major environmental factor that influences changes in seed dormancy states in temperate forests (Baskin and Baskin 1998; Benech-Arnold et al. 2000; Brändel 2005). Cold stratification simulates low temperature conditions in winter and is an effective method for releasing dormancy and obtaining fast, vigorous and uniform germination (Leadem 1997). For example, Hawkins (2018) found that 12 weeks of cold stratification at 5/1 °C (simulating winter air temperature) was required for *Quercus pagoda* (Raf.) acorns to break dormancy. For many temperate tree species, exposure of imbibed seeds to low temperatures (0–10 °C) can release primary physiological dormancy (Yamauchi et al. 2004; Koyuncu 2005; Einali and Sadeghipour 2007). Although primary physiological dormancy can be released over a wide range of low temperatures, the effect is modulated by the availability of water (Baskin and Baskin 1998; Wang et al. 2009). Temperature and moisture are important in both inducing seed dormancy and breaking physiological dormancy (Dey et al. 2019). Moreover, the effect of temperature on primary physiological dormancy also depends on the period of a seed's exposure to the low temperatures (Miura and Araki 1996; Corbineau et al. 2002; Hu et al. 2012; Daneshvar et al. 2016).

Temperature releases primary physiological dormancy and affects the relief and induction of secondary physiological dormancy. The threshold temperature for the relief and induction of seed secondary physiological dormancy in some herbaceous plants is approximately 15 °C (Bewley and Black 1994; Brändel and Schütz 2003; Brändel 2004). However, there has been little research conducted to estimate the effects of temperature on secondary physiological dormancy of the seeds of woody species, especially pine seeds. Additionally, to obtain secondary dormant seeds, researchers incubated nondormant seeds in a high temperature environment that disfavors germination (Miura and Araki 1996; Jones et al. 1997; Brändel 2004). However, the soil temperature that induces secondary physiological dormancy gradually changes after seeds are dispersed in soil under natural conditions. Therefore, the results of these studies cannot accurately identify the process of induction and release of secondary physiological dormancy affected by natural temperature.

In the forest nursery industry, cold stratification is a common method used to release the primary physiological dormancy of Korean pine seeds. The specific procedures are as follows. At the beginning of winter, the freshly harvested seeds are soaked in water for 7 days. These imbibed seeds are then mixed with wet sand and buried in a cellar ($3 \times 1.0 \times 1.0$ m length × height × width) for approximately 6 months (Zuo 2011). However, the optimal cold stratification temperature and the length of time required to completely release primary physiological dormancy have not been accurately assessed. In the present study, the complete release of physiological dormancy refers to the high percent of germination (> 80%) and fast germinate rate. To effectively break the primary physiological dormancy of Korean pine seeds, it is necessary to determine the optimal low temperature and its duration during cold stratification treatment. In addition, to prevent Korean pine seeds in natural conditions from completely entering secondary physiological dormancy in the second late-summer season following dispersal, we can apply embryo growth-promoting measures (e.g., preincubating seeds at 25 °C, application of gibberellin acids to seeds) to release primary morphological dormancy in the second spring and early summer seasons. However, the temperature required for the induction of secondary physiological dormancy in seeds of Korean pine has not been determined quantitatively. It seems that this information is important for successful regeneration of Korean pine. However, to the best of our knowledge, no studies have addressed these questions to date.

To determine the optimal low temperature and the length of cold stratification time, both dry and imbibed seeds were stored at 1 °C and 5 °C for 1, 2, 4 or 6 months under laboratory conditions. These two temperatures were selected because natural soil temperature above-zero varies between 0 and 5 °C in winter (Song and Zhu 2016), and higher soil temperatures in winter may result in faster primary physiological dormancy release compared lower soil temperatures. These two seed water content levels were selected for the following two reasons: first, high seed water content contributes to the release of physiological dormancy during cold stratification (Baskin and Baskin 2014). Thus, we used imbibed seeds in the present study. Second, rainfall that occurs in autumn following seed dispersal may also lead to imbibition. For example, the primary dormant Korean pine seeds are completely imbibed following long-term heavy rainfall. If freshly dry seeds can absorb enough water from the surrounding moist soil environment to release the physiological dormancy component of MPD, then dry seeds can be utilized directly during cold stratification without needing to be presoaked. Furthermore, we may also directly sow freshly dried seed in the forest to promote regeneration, reducing the cost of raising seedlings in forestry production. Thus, dry seeds were also selected in the present study. Cold stratification duration is a key factor influencing the release of dormancy (Kildisheva et al. 2019). In addition, the duration of cold stratification that is

applied in forestry to release the primary physiological dormancy of Korean pine seeds is approximately 6 months (Yao 1966). Therefore, we selected four different lengths of cold stratification time to accurately investigate the dynamics of primary physiological dormancy release.

Previous studies showed that the soil temperature gradually increased from late spring to late summer (April to August) and then decreased in the following autumn (September and October) (Song et al. 2018). Korean pine seeds also gradually enter secondary physiological dormancy during the period from April to August, and then are progressively released from secondary physiological dormancy during September and October. It can be inferred that the induction of secondary physiological dormancy is related to the increasing summer soil temperature. Therefore, MPD seeds released from primary physiological dormancy were incubated under gradually increasing temperature conditions in the laboratory to determine the threshold temperature needed for the induction of secondary physiological dormancy of Korean pine seeds.

Materials and methods

Seed collections

In September 2013 and 2014, fresh seeds were gathered from a Korean pine plantation forest in Qingyuan Forest CERN at the Chinese Academy of Sciences (CAS) in Liaoning Province, northeastern China (41°51.102′N, 124°54.543′E, 456–1116 m a.s.l.). The water content of seeds collected in 2013 and 2014 were 12% and 10%, respectively. Seeds were stored at − 20 °C. Seeds collected in 2013 were used to determine the threshold temperature for the induction of secondary physiological dormancy. Seeds collected in 2014 were used to determine the optimal low temperature and duration for the release of primary physiological dormancy.

The release of primary physiological dormancy following different cold stratification conditions

Two seed water content levels (dry and imbibed), two cold stratification temperatures (5 °C and 1 °C) and four durations of cold stratification (1, 2, 4 and 6 months) were used for these experiments. In April 2015, seeds collected in 2014 and stored at − 20 °C were taken out of storage and cold stratified either dry or after imbibition (soaking dry seeds in water for 7 days). To stratify, seeds were mixed with moist sand and incubated at either 5 °C or 1 °C for different durations (1, 2, 4 or 6 months). Specifically, one hundred seeds were mixed with saturated moist sand and placed in a box. For a duration of 6 months at each cold stratification temperature, three sample boxes containing the seeds were exhumed after 1, 2, 4 or 6 months of cold stratification for evaluation of germination in the laboratory. Each exhumed box corresponded to one replicate at the tested temperature. These seeds in boxes were used for the germination test. Distilled water was added as required during cold stratification to ensure that moisture was not a limiting factor for the release of primary physiological dormancy.

The induction of secondary physiological dormancy under gradually increasing temperature conditions

In late October 2013, seeds collected in 2013 and stored at − 20 °C were taken out of storage and cold stratified. Seeds were soaked in running water for 7 days and then kept moist for 6 months of cold stratification. After cold stratification, seeds were subjected sequentially to a set of temperature regimes that are widely used in research on seed dormancy: 5 °C, 10 °C, 15 °C, 20 °C and 25 °C (Miura and Araki 1996; Jones et al. 1997; Brändel 2004). Cold stratified seeds were moved from 5 to 10 °C, where they were kept for 1 month, then moved to 15 °C for 1 month and so forth. When seeds were moved from one temperature to the next in the sequence, some seeds were left at the lower temperature for monthly germination tests. The germination test was also conducted at the end of each month when seeds were moved to a higher temperature in the sequence (Fig. 1).

Germination tests

Germination tests were conducted in a growth chamber (MGC-450HP-2, Bluepard, Shanghai, China) with a regime of 25 °C/14 h light alternating with 10 °C/10 h dark. Three replicates of 20 seeds each were used for germination tests. Seeds were placed on filter paper moistened with deionized water in 9-cm Petri dishes. To ensure that adequate moisture was maintained inside the Petri dishes, deionized water was added as required, and dishes were sealed with Parafilm. The process of germination was monitored at two-day intervals for 56 days (incubation period). Seeds were considered germinated when the radicle protrusion was greater than 2 mm (Bai et al. 2004). Germination percentages, mean germination time (MGT) and the germination rate index (GRI) were calculated as follows.

$$\text{Germination percentage} = \text{number of germinated seeds/total number of viable seeds with white and firm embryos} \times 100\% \quad (1)$$

$$\text{MGT(days)} = \sum \left(\mathrm{n}_i \times \mathrm{d}_i\right)/\mathrm{N} \quad (2)$$

where n_i is the number of germinated seeds on day i, d_i is the number of days after beginning of the experiment, and N is the total number of seeds germinated (Ellis and Roberts 1981).

$$\mathrm{GRI} = \frac{G_{tot}}{P} \sum \frac{g_i}{t_i} \quad (3)$$

where G_{tot} is the total number of germinated seeds at the end of the germination test, p is the total number of seeds, and g_i is the number of seeds germinated between time t_{i-1} and t_i (h) (Steinmanus et al. 2000).

Statistical analyses

One-way ANOVA and LSD tests were used to test the differences in germination percentage, MGT and GRI among different storage temperatures and different storage periods ($P < 0.05$). When the data did not exhibit a normal distribution and the variance was

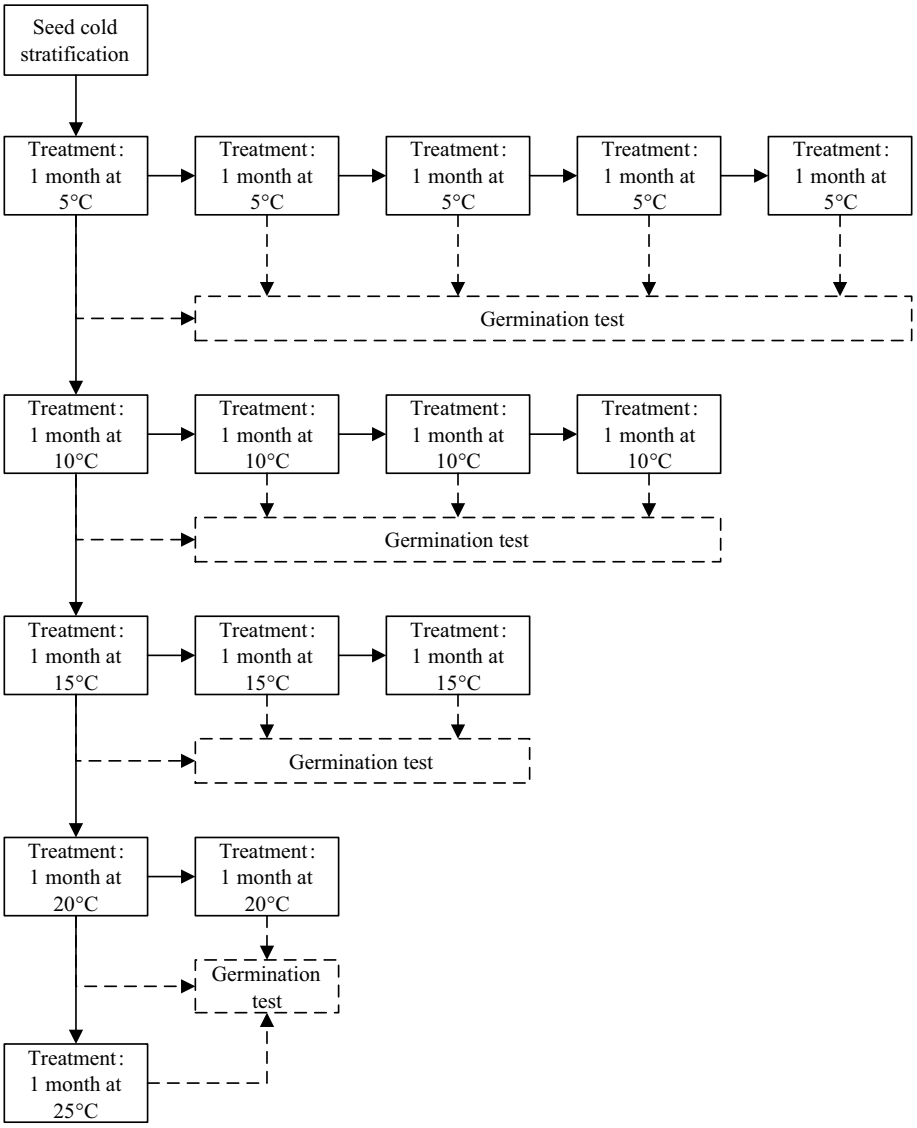

Fig. 1 Design scheme of the controlled laboratory experiment. Cold stratified seeds were stored under gradually increasing temperatures prior to being placed at 25/16 °C for germination test. Meanwhile, when seeds were moved to the next storage temperature, some of these seeds remained at this storage temperature and were also used for germination tests

heterogeneous, the data were transformed. Specifically, the GRI of dry seeds stratified at 1 °C was $\log_{10}$ (1/GRI)–transformed. The variance was still heterogeneous after transformation; therefore, nonparametric tests (Mann–Whitney Test) were used to test the difference in MET of imbibed seeds stratified at 1 °C among different storage periods.

The differences in MET and GRI of MPD seeds released from primary physiological dormancy among different storage temperatures (5 °C, 10 °C, 15 °C, 20 °C and 25 °C) were also determined by the Mann–Whitney test.

Results

Germination of seeds at 25/10 °C following cold stratification

After imbibed seeds were cold stratified at either 1 °C or 5 °C for 1 month, their germination percentages reached approximately 85% at 25/10 °C (Fig. 2a). As stratification time was extended, imbibed seeds still displayed high germination percentages (87–91%) at 25/10 °C, but the MGT reached a minimum (17 days) only after 6 months of cold stratification (Fig. 2b). Moreover, regardless of whether imbibed seeds were cold stratified at 1 °C or 5 °C, the GRI did not show significant changes for the first 1–4 months of stratification but drastically increased to a maximum at 6 months (Fig. 2c).

As the cold stratification times of dry seeds at 1 °C increased, the germination percentages gradually increased from 57 to 88% (Fig. 2a). Dry seeds that were cold stratified at 5 °C for 4 months reached maximum germination (89%) (Fig. 2a). After 6 months of cold stratification at either 1 °C or 5 °C, dry seeds showed a minimum MGT and a maximum GRI (Fig. 2b, c).

Induction of secondary physiological dormancy following storage at gradually increasing temperatures

After 1 month of stratification at 5 °C, the germination percentage was 78% at 25/10 °C. As the storage temperatures increased, the germination percentage subsequently decreased to 72% (1 month of storage at 10 °C), 55% (1 month of storage at 15 °C), 10% (1 month of storage at 20 °C) and 8% (1 month of storage at 25 °C) (Fig. 3A). There were no significant changes ($P > 0.05$) in either MGT or GRI (Figs. 4A, 5A).

Although seeds still had a high germination percentage (72–85%) (Fig. 3B) as the storage duration at 5 °C was extended, GRI progressively increased (Fig. 5B). Moreover, MGT was also significantly ($P < 0.05$) reduced to 17 days (Fig. 4B). The germination percentage decreased to 52% after 2 months of storage at 10 °C, but significantly ($P < 0.05$) increased

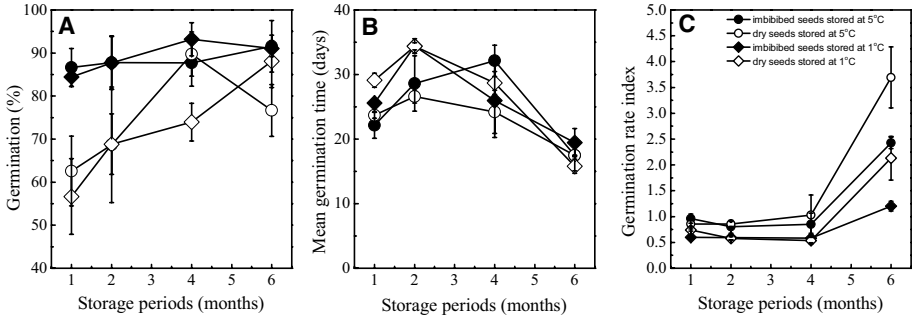

Fig. 2 Germination (**a**), mean germination time (**b**) and germination rate index (**c**) of seeds cold stratified for different durations at either 1 °C or 5 °C

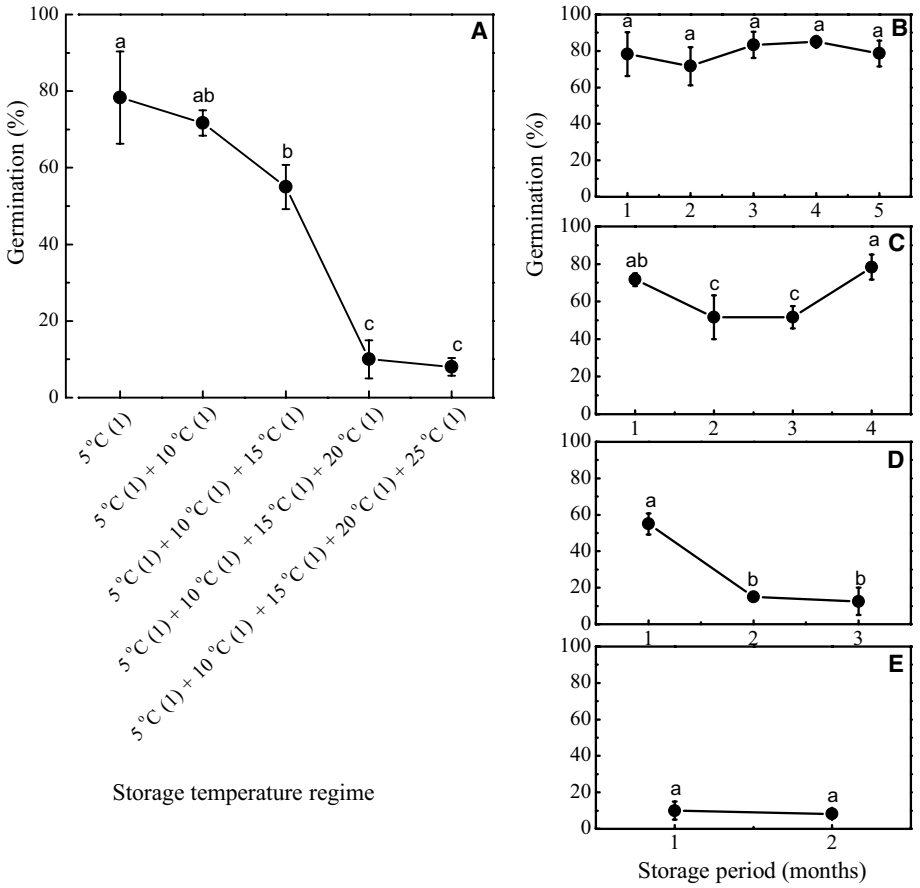

Fig. 3 Germination percentage of seeds following the five storage temperature regimes: the number in the parentheses behind the temperature legend indicates the number of months the seeds were stored at this temperature (**A**). Germination of seeds stored at 5 °C (**B**), 10 °C (**C**), 15 °C (**D**) and 20 °C (**E**). Bars represent mean ± standard deviation. Different lower-case letters indicate significant differences in germination percentage

to 78% following 4 months (Fig. 3C). Seeds stored at 10 °C for 2 months exhibited a maximum GRI (Fig. 5C) and a minimum MGT (Fig. 4C). The germination percentages of seeds stored at 15 °C significantly ($P < 0.05$) decreased to 13% (Fig. 3D) as storage duration was gradually extended. Furthermore, the MGT of these seeds progressively increased (Fig. 4D), and GRI gradually decreased (Fig. 5D). Seeds kept at 20 °C only exhibited 8% germination (Fig. 3E).

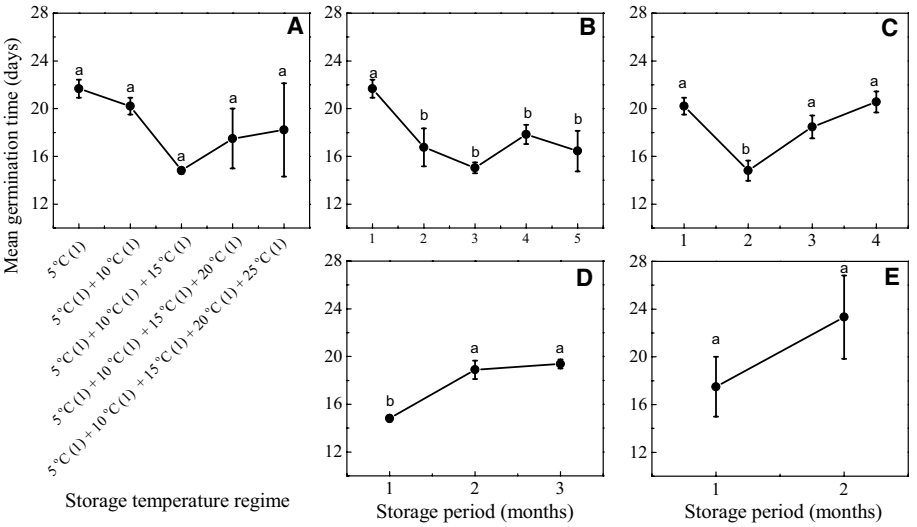

Fig. 4 Mean germination time of seeds following the five storage temperature regimes: the number in the parentheses behind the temperature legend indicates the number of months the seeds were stored at this temperature (**A**). Mean germination time of seeds stored at 5 °C (**B**), 10 °C (**C**), 15 °C (**D**) and 20 °C (**E**). Bars represent mean ± standard deviation. Different lower-case letters indicate significant differences in germination percentage

Discussion

The results of the present study suggested that after imbibed seeds were stratified at either 1 °C or 5 °C for 1 month, the germination percentage had already reached approximately 85%. However, those seeds germinated more slowly. In contrast, imbibed seeds stratified at 1 °C or 5 °C exhibited more than 90% germination at a range of incubation days from 18 to 20 only after 6 months of storage. These results indicate that stratifying the imbibed seeds at 1 °C or 5 °C for 6 months can completely release primary physiological dormancy in seeds of Korean pine. Our findings correspond with a previously documented study that found low soil temperature in winter (0–5 °C) effectively release primary physiological dormancy in seeds of Korean pine (Song and Zhu 2016). The germination rate of the imbibed seeds cold stratified at 5 °C (2.43) was two times that of the imbibed seeds cold stratified at 1 °C (1.20), indicating that stratifying the imbibed seeds at 5 °C was more effective. It has also been reported that cold stratification at 5 °C is favorable for the release of primary physiological dormancy in seeds of Douglas fir (*Pseudotsuga menziesii* var. menziesii [Mirb.] Franco) (Corbineau et al. 2002), smoke tree (*Cotinus coggygria* var. *Cinerea* Engler) (Deng et al. 2016) and the seeds of most annual and perennial herbs in southern Wisconsin (Struik 1965). The germination percentage of dry seeds stratified at 1 °C or 5 °C gradually increased with increasing storage duration. After 6 months of storage at 1 °C or 5 °C, these dry seeds also exhibited a high germination percentage (77–88%) within 16–18 days of incubation. Therefore, it can be concluded that stratifying dry seeds under cold and moist conditions for 6 months can also release primary physiological dormancy in seeds of Korean pine.

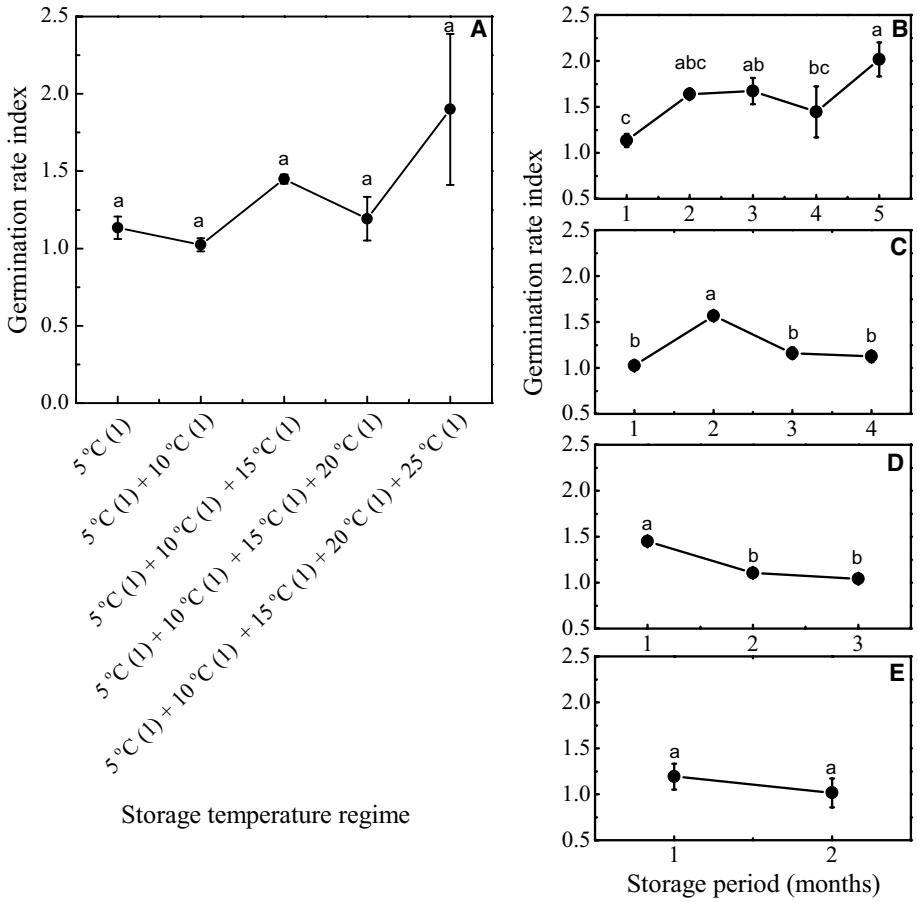

Fig. 5 Germination rate index of seeds following the five storage temperature regimes: the number in the parentheses behind the temperature legend indicates the number of months the seeds were stored at this temperature (**A**). Germination rate index of seeds stored at 5 °C (**B**), 10 °C (**C**), 15 °C (**D**) and 20 °C (**E**). Bars represent mean ± standard deviation. Different lower-case letters indicate significant differences in germination percentage

This finding may be observed in part because dry seeds absorb water from surrounding moist conditions, thereby leading to the release of primary physiological dormancy. Additional studies have also revealed that the germination percentage increases with increasing water content of cold stratified seeds of *Aesculus hippocastanum* L. and Douglas fir (Tompsett and Pritchard 1998; Gosling et al. 2003).

The germination percentage of seeds significantly decreased with increasing storage temperature, demonstrating that the gradually increasing temperature induced secondary physiological dormancy in seeds of Korean pine. Induction of secondary physiological dormancy by high temperature has also been reported for seeds of *Picea sitchensis* [Bong.] Carr. (Jones et al. 1997). In some species, secondary physiological dormancy was induced as seeds were incubated at high temperatures that disfavor germination for a certain period of time (Pekrun et al. 1997; Kępczyński and Bihun 2002). However, our research suggested that the secondary physiological dormancy in seeds of Korean pine was induced by gradually increasing

temperatures. Several studies on herbaceous plant seeds have reported that the threshold temperature for the induction of secondary physiological dormancy was approximately 15 °C (Brändel 2004). Nevertheless, little is known about the temperature threshold for the induction of secondary physiological dormancy in gymnosperm seeds. The germination percentage significantly decreased from 78 to 55% following the transfer of seeds from 10 to 15 °C for 1 month of storage. Approximately 10% of seeds germinated following their transfer from 15 to 20 °C for 1 month of storage. Moreover, when the seeds remained at 15 °C for 2 months and an additional 3 months, the germination percentage significantly decreased to 15% and 13%, respectively. In addition, the MGT significantly increased to 19 days, and the GRI significantly decreased to approximately 1.06. Thus, it seems likely that secondary physiological dormancy in seeds of Korean pine was induced by high temperatures ≥ 15 °C. Temperatures that vary between 15 and 20 °C appear to be critical to the induction of seed secondary physiological dormancy in many species (Steadman 2004). For example, moist seeds of Sitka spruce (*Picea sitchensis* [Bong.] Carr.) kept at 15 °C and 20 °C cycle between the secondary dormant state and the released secondary dormant state (Jones et al. 1997). The induction of secondary physiological dormancy in imbibed seeds of *Orobanche* spp. occurs at 15 °C (Kebreab and Murdoch 1999).

In addition, the induction of secondary physiological dormancy may also depend on the period during which imbibed seeds were kept in unfavorable germination conditions. Many studies focused on the effect of the duration of unfavorable germination conditions on the induction of secondary physiological dormancy in herbaceous plants seeds. For example, Larsen and Eriksen (2004) reported that a period of more than 5 days at 25 °C is required to induce the secondary physiological dormancy of *Berberis thunbergii* seeds. Secondary physiological dormancy in rice seeds was induced when the seeds were imbibed at 10 °C and 15 °C for more than 10 days (Miura and Araki 1996). Brändel and Schütz (2003) also observed that the secondary physiological dormancy in seeds of *Rumex* species was induced after seeds were stratified at 10 °C and 15 °C for 2 weeks. However, the effect of the duration of unfavorable germination conditions on the induction of secondary physiological dormancy has not been reported in conifer trees seeds. In our study, the germination percentage decreased from 55 to 13–15% after seeds were stored at 15 °C for 2 to 3 months. Therefore, it appears that the longer the duration of the high-temperature period, the deeper the secondary physiological dormancy (the germination percentage, MGT and GRI were used to indicate dormancy depth in the present study).

Breaking seed physiological dormancy in many species is achieved by incubating imbibed seeds in moist and cold (0–10 °C) conditions (i.e., cold stratification) (Baskin and Baskin 1998; Yamauchi et al. 2004; Einali and Sadeghipour 2007). Although cold stratification is widely used to release the primary physiological dormancy, little is known about whether it was also effective in the release of secondary physiological dormancy. The induction of secondary physiological dormancy in many species occurs in early summer (May–July) and is released in November/December (Benech-Arnold et al. 2000; Brändel 2004). For all species mentioned above, low temperatures are also effective at releasing secondary physiological dormancy. To the best of our knowledge, the range of effective temperature suitable for secondary physiological dormancy release had not been determined as accurately as that for the primary physiological dormancy. In the present study, for the seeds stored at 10 °C, 72% of seeds germinated after 1 month of storage, but their germination percentage decreased to 52% after 2 to 3 months of storage. However, as the storage duration was extended to 4 months at 10 °C, the germination percentage significantly increased from 52 to 78% once more, but the MGT and GRI did not change significantly. We suggest that storage for 2 months or more at 10 °C can be used for the release of secondary physiological dormancy in Korean pine seeds.

Conclusion

Cold stratification of imbibed or dry seeds at 1 °C or 5 °C for 6 months can completely release primary physiological dormancy in Korean pine seeds. Once primary physiological dormancy is released, gradually increasing temperature can induce Korean pine seeds into secondary physiological dormancy. The induction of secondary physiological dormancy in Korean pine seeds occurs at temperatures above 15 °C. Secondary physiological dormancy in Korean pine seeds can be prevented by wetting stratified seeds at 10 °C for 2 months or more.

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References

Bai Y, Thompson D, Broersma K (2004) Douglas fir and ponderosa pine seed dormancy as regulated by grassland seedbed conditions. J Range Manag 57:661–667

Baskin JM, Baskin CC (1998) Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego

Baskin CC, Baskin CC (2004) A classification system for seed dormancy. Seed Sci Res 14:1–16

Baskin CC, Baskin JM (2014) Seeds: ecology, biogeography, and evolution of dormancy and germination, 2nd edn. Elsevier/Academic Press, San Diego

Benech-Arnold RL, Sánchez RA, Forcella F, Kruk BC, Ghersa CM (2000) Environmental control of dormancy in weed seed banks in soil. Field Crop Res 67:105–122

Bewley JD, Black M (1994) Seeds: physiology of development and germination. Plenum Press, New York

Bewley JD, Bradford K, Hilhorst H, Nonogaki H (2013) Seeds: physiology of development, germination and dormancy. Springer, New York

Brändel M (2004) The role of temperature in the regulation of dormancy and germination of two related summer-annual mudflat species. Aquat Bot 79:15–32

Brändel M (2005) The effect of stratification temperatures on the level of dormancy in primary and secondary dormant seeds of two Carex species. Plant Ecol 178:163–169

Brändel M, Schütz W (2003) Seasonal dormancy patterns and stratification requirements in seeds of *Verbena officinalis* L. Basic Appl Ecol 4:329–337

Corbineau F, Bianco J, Garello G, Côme D (2002) Breakage of *Pseudotsuga menziesii* seed dormancy by cold treatment as related to changes in seed ABA sensitivity and ABA levels. Physiol Plant 114:313–319

Daneshvar A, Tigabu M, Karimidoost A, Odén PC (2016) Stimulation of germination in dormant seeds of *Juniperus polycarpos* by stratification and hormone treatments. N For 47:751–761

Deng ZJ, Hu XF, Ai XR, Yao L, Deng SM, Pu X, Song SQ (2016) Dormancy release of *Cotinus coggygria*, seeds under a pre-cold moist stratification: an endogenous abscisic acid/gibberellic acid and comparative proteomic analysis. N For 47:105–118

Dey DC, Knapp BO, Battaglia MA, Deal RL, Hart JL, O'Hara KL, Schweitzer CJ, Schuler TM (2019) Barriers to natural regeneration in temperate forests across the USA. N For 50:11–40

Einali AR, Sadeghipour HR (2007) Alleviation of dormancy in walnut kernels by moist chilling is independent from storage protein mobilization. Tree Physiol 27:519–525

Ellis RA, Roberts EH (1981) The quantification of ageing andsurvival in orthodox seeds. Seed Sci Technol 9:373–409

Gosling PG, Samuel Y, Peacea A (2003) The effect of moisture content and prechill durationon dormancy breakage of Douglas fir seeds (*Pseudotsuga menziesii* var. menziesii [Mirb.] Franco). Seed Sci Res 13:239–246

Hawkins TS (2018) Regulating acorn germination and seedling emergence in *Quercus pagoda* (Raf.) as it relates to natural and artificial regeneration. N For. https://doi.org/10.1007/s11056-018-9667-z

Hu XW, Huang XH, Wang YR (2012) Hormonal and temperature regulation of seed dormancy and germination in *Leymus chinensis*. Plant Growth Regul 67:199–207

Ishikawa Y, Krestov PV, Namikawa K (1999) Disturbance history and tree establishment in old-growth *Pinus koraiensis*-hardwood forests in the Russian Far East. J Veg Sci 10:439–448
Jones SK, Ellis RH, Gosling PG (1997) Loss and induction of conditional dormancy in seeds of Sitka spruce maintained moist at different temperatures. Seed Sci Res 7:351–358
Kebreab E, Murdoch A (1999) A quantitative model for loss of primary dormancy and induction of secondary dormancy in imbibed seeds of *Orobanche* spp. J Exp Bot 50:211–219
Kępczyński J, Bihun M (2002) Induction of secondary dormancy in *Amaranthus caudatus* seeds. Plant Growth Regu 38:135–140
Kildisheva OA, Erickson TE, Kramer AT, Zeldin J, Merritt DJ (2019) Optimizing physiological dormancy break of understudied cold desert perennials to improve seed-based restoration. J Acid Environ. https://doi.org/10.1016/j.jaridenv.2019.104001
Koyuncu F (2005) Breaking seed dormancy in black mulberry (*Morus nigra* L.) by cold stratification and exogenous application of gibberellic acid. Acta Biol Cracov Bot 47:23–26
Larsen SU, Eriksen EN (2004) Delayed release of primary dormancy and induction of secondary dormancy in seeds of woody taxa caused by temperature alternations. Acta Hortic 630:91–100
Leadem CL (1997) Dormancy-unlocking seed secrets. National Proceedings, Forest and Conservation Nursery Associations. Gen. Tech. Rep. PNW-G TR-419. Portland, OR: US Department of Agriculture, Forest Service, Pacific Northwest Research Station, pp 43–52
Li YB, Mou P, Wang TM, Ge J (2012) Evaluation of regeneration potential of *Pinus koraiensis* in mixed pine-hardwood forests in the Xiao Xing'an Mountains, China. J Forestry Res 23:543–551
Miura K, Araki H (1996) Low temperature treatment during the imbibition period for the induction of secondary dormancy in rice seeds (*Oryza sativa* L.). Breed Sci 46:235–239
Pekrun C, Lutman P, Baeumer K (1997) Induction of secondary dormancy in rape seeds (*Brassica napus* L.) by prolonged imbibition under conditions of water stress or oxygen deficiency in darkness. Eur J Agron 6:245–255
Qi Y, Bilan MV, Chin KL (1993) New method for breaking Korean pine seed dormancy. J Arboric 19:113–117
Song Y, Zhu JJ (2016) How does moist cold stratification under field conditions affect the dormancy release of Korean pine seed (*Pinus koraiensis*)? Seed Sci Technol 44:1–16
Song Y, Zhu JJ, Yan QL, Wang GC (2018) Korean pine seed: linking changes in dormancy to germination in the two years following dispersal. Forestry 91:98–109
Steadman KJ (2004) Dormancy release during hydrated storage in Lolium rigidum seeds is dependent on temperature, light quality, and hydration status. J Exp Bot 55:929–937
Struik GJ (1965) Growth patterns of some native annual and perennial herbs in southern Wisconsin. Ecology 46:401–420
Tompsett PB, Pritchard HW (1998) The effect of chilling and moisture status on the germination, desiccation tolerance and longevity of *Aesculus hippocastanum* L. seed. Ann Bot 82:249–261
Wang WQ, Song SQ, Li SH, Gan YY, Wu JH, Cheng HY (2009) Quantitative description of the effect of stratification on dormancy release of grape seeds in response to various temperatures and water contents. J Exp Bot 60:3397–3406
Yamauchi Y, Ogawa M, Kuwahara A, Hanada A, Kamiya Y, Yamaguchi S (2004) Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. Plant Cell 16:367–378
Yao GQ (1966) The methods to bury seeds of *Pinus koraiensis* Sieb. et Zucc. and *Fraxinus mandshurica* for short period. Forest Sci Technol 23:6 **(in Chinese)**
Zhu JJ, Mao ZH, Hu LL, Zhang JX (2007) Plant diversity of secondary forests in response to anthropogenic disturbance levels in montane regions of northeastern China. J Forestry Res 12:403–416
Zuo XC (2011) Experience introduction of pine seedling techniques. Forest Inves Des 2:68–69 **(in Chinese)**