Effects of Heat Shock on Seed Germination of Three Pinaceae Species in the Greater Khingan Mountains (Postprint)

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Abstract

This study investigated the effects of heat shock on seed germination of Larix gmelinii, Pinus sylvestris var. mongolica, and Picea koraiensis from the Greater Khingan Range region. Heat shock temperatures were set at 80°C, 100°C, 120°C, and 150°C, with exposure durations of 1 min, 3 min, 5 min, and 10 min at each temperature. The results indicated that: for Larix gmelinii seeds, germination percentage significantly decreased compared with the control under treatments of 100°C for 10 min, 120°C for 5 min and 10 min, and 150°C for 5 min ($P < 0.05$); except for treatments of 80°C for 1 min, 3 min, and 5 min, 120°C for 1 min, and 150°C for 1 min, germination speed index significantly decreased under all other treatments compared with the control ($P < 0.05$). For Pinus sylvestris var. mongolica seeds, germination percentage significantly decreased under all heat shock treatments above 80°C compared with the control ($P < 0.05$), with no germination observed in some cases; except for the treatment of 80°C for 1 min, germination speed index significantly decreased under all treatments compared with the control. For Picea koraiensis seeds, germination percentage significantly increased compared with the control under treatments of 80°C for 5 min and 100°C for 1 min ($P < 0.05$); germination speed index significantly increased compared with the control under treatments of 80°C for 1 min, 3 min, and 5 min, and 100°C for 1 min ($P < 0.05$). Larix gmelinii seeds could maintain germination percentage under short-duration high-intensity (150°C) heat shock, but could not tolerate prolonged heat shock. Pinus sylvestris var. mongolica seeds were sensitive to heat shock above 80°C, and heat shock reduced the germination capacity of Pinus sylvestris var. mongolica seeds. Heat shock could enhance the germination capacity of Picea koraiensis seeds; short-duration (1 min, 3 min, 5 min) low-intensity (80°C) heat shock (including 100°C for 1 min) increased both germination percentage and germination speed index of

Picea koraiensis seeds, and Picea koraiensis seeds could also withstand high-intensity (150°C) instantaneous (1 min) heat shock.

Full Text

Effect of Heat Shock on Seed Germination of Three Pinaceae Species in the Greater Khingan Mountains

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Abstract

This study examined the effects of heat shock on seed germination of *Larix gmelinii*, *Pinus sylvestris* var. *mongolica*, and *Picea koraiensis* from fire-prone forests in the Greater Khingan Mountains. Seeds of the three species were exposed to temperatures of 80°C, 100°C, 120°C, and 150°C for exposure times of 1, 3, 5, and 10 minutes. The results showed that the germination rate of *L. gmelinii* seeds decreased significantly ($P < 0.05$) relative to the control when subjected to 100°C for 10 min, 120°C for 5 min and 10 min, and 150°C for 5 min. With the exception of exposures at 80°C for 1, 3, and 5 min; 120°C for 1 min; and 150°C for 1 min, the germination velocity index of *L. gmelinii* seeds showed a significant ($P < 0.05$) decrease for all other heat shock treatments.

For *P. sylvestris* var. *mongolica*, the germination rate subsequent to all heat shock treatments above 80°C decreased significantly ($P < 0.05$) relative to the control, even to the point where no germination occurred at all. At the same time, the germination velocity index for every heat shock treatment higher than 80°C decreased significantly at 1 min exposure. For *Picea koraiensis*, the germination rate increased significantly ($P < 0.05$) relative to the control when subjected to heat shock treatments of 80°C for 5 min and 100°C for 1 min. Moreover, the germination velocity index increased significantly ($P < 0.05$) for heat shock treatments of 80°C for 1, 3, and 5 min and 100°C for 1 min.

Overall, *L. gmelinii* seeds were able to maintain standard germination rate after a transitory high-intensity heat shock of 150°C for 1 min; however, they cannot tolerate longer heat shock. The influence of transitory high-intensity heat on the germination rate was more significant than that on the germination velocity index. *P. sylvestris* var. *mongolica* seeds were quite sensitive to heat shock above 80°C: the germination rate sharply decreased with a 100°C heat shock and almost no seeds germinated above 100°C. *Picea koraiensis* seeds are capable of tolerating an instantaneous (1 min) high-intense heat shock (150°C), and a light heat shock over short time periods (1, 3, 5 min) for 80°C (100°C for 1 min included) stimulated seed germination of *P. koraiensis*.

Keywords: heat shock; seed germination; Pinaceae; forest fire

Introduction

Forest fire is a common disturbance factor in ecosystems that affects natural regeneration of many plant species [1]. Low-intensity forest fires can maintain forest structure and even become a means of improving forestry and agricultural production in some regions [2]. In fire-prone areas, reproductive structures, particularly seeds, are closely related to fire for their own regrowth [3]. Many plant seeds have developed mechanisms where dormancy precedes germination, allowing themselves sufficient time for dormancy to better disperse seeds. In some fire-prone areas, fire can break such dormancy [4,5]. Among various fire products, high temperature is the main factor stimulating seed germination [1,2]. The mechanism by which high temperature promotes seed germination is currently widely believed to be that the heat from forest fires causes premature seed coat rupture [6] or increases seed coat water permeability after disturbance, thereby advancing the seed germination process [1,4]. Low-intensity fires may create certain temperature environments that stimulate germination of small seeds near the soil surface and large seeds in lower soil layers [7].

Heat shock is the primary method for simulating high temperatures from forest fires in laboratory settings. Scholars have conducted extensive research on seed germination responses to heat shock, with treatment temperatures typically ranging between 60°C and 150°C [9-12]. This temperature range is suitable for temperatures experienced by seeds on the soil surface and in subsurface soil layers during fire [13]. Heat shock can alleviate dormancy of herbaceous seeds in arid regions and promote germination of some legume seeds [14-15]. Seed germination responses to heat shock temperature vary among species in soil [4,7,8]. In studies of western Australian species, maximum germination rates occurred between 80°C and 120°C [16]. Exposure time to fire and temperature reached are the most important factors determining fire intensity, both of which have crucial impacts on plant community recovery capacity [12].

The Greater Khingan Mountains region is in the cold temperate zone, covered with vast virgin forests and rich in animal and plant resources. It serves as an important water conservation area and natural barrier for windbreak and sand fixation in northern China, playing a vital ecological function [17]. The Greater Khingan Mountains is a region with severe forest fires, with the shortest fire return interval being 15-20 years [18], making it a typical fire-prone area with many similarities to boreal coniferous forests in northern Europe [19]. The development and succession of its forests are closely related to forest fire [19]. Among the main dominant Pinaceae species composing the coniferous forests of the Greater Khingan Mountains, *Larix gmelinii*, *Pinus sylvestris* var. *mongolica*, and *Picea koraiensis* are the most important. Research on the effects of heat shock on tree seed germination in the Greater Khingan Mountains region is relatively scarce domestically. Therefore, conducting this study has important significance and can provide scientific basis for predicting post-fire vegetation recovery in the Greater Khingan Mountains and for conducting prescribed burning to improve forest community species diversity.

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1. Seed Collection

Seeds of *Larix gmelinii*, *Pinus sylvestris* var. *mongolica*, and *Picea koraiensis* used in the experiment were collected in September from the Nanweng River Wetland National Nature Reserve in the Greater Khingan Mountains (125°07 N, 125°50 E). This reserve preserves a relatively intact cold temperate coniferous forest ecological community and is representative. Seeds were separated from fruits and stored in a refrigerator at 4°C until the experiment.

2. Heat Shock Treatment

The temperature gradient for heat shock was set at 80°C, 100°C, 120°C, and 150°C. At each temperature, heat shock times were set at 1 min, 3 min, 5 min, and 10 min. Healthy and plump seeds were selected and placed in open glass petri dishes after disinfection in 0.5% potassium permanganate solution for 10 min, rinsed with distilled water, and air-dried. Three replicates of 50 seeds each constituted one heat shock treatment group. The 12 heat shock treatment groups (4 temperatures × 3 time intervals, excluding control) were placed separately into ovens preheated to 80°C, 100°C, 120°C, and 150°C. At each temperature, one replicate was removed at 1 min, 3 min, 5 min, and 10 min, respectively, to obtain one heat shock treatment. The remaining replicate served as the control.

3. Germination Experiment

Fifty plump seeds were placed between two layers of filter paper as substrate in petri dishes 12 cm in diameter, with sufficient distilled water added to moisten the filter paper. This constituted one replicate. All treatment replicates were placed in an artificial climate chamber (BIC-300, Shanghai Boxun Industrial Co., Ltd.) with conditions set at 25°C, relative humidity 60%, and 12 h light/12 h dark photoperiod. Germination was counted daily for 4 weeks [12]. Seeds with radicles protruding through the seed coat were considered germinated [12,23]. Germinated seeds were removed from the petri dishes and distilled water was replenished.

4. Data Processing and Analysis

After data collection, germination rate and germination velocity index of *Larix gmelinii*, *Pinus sylvestris* var. *mongolica*, and *Picea koraiensis* were calculated using EXCEL 2003. Differences were analyzed for significance using SPSS 19.0 Duncan' s method. Formulas for germination rate and germination velocity index are as follows:

Germination Rate (GR) = (n/N) × 100%

Germination Velocity Index (GVI) = Σ(ni/ti)

Where n is the total number of germinated seeds, N is the total number of seeds in each replicate, ni is the number of seeds germinated on day i, and ti is day i. A higher GVI indicates more seeds germinating in the shortest time, reflecting faster germination speed and stronger seed activity.

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1. Effects of Heat Shock on Germination Rate of *Larix gmelinii* Seeds

As shown in , compared with the control, the germination rate of *L. gmelinii* seeds showed no significant change after 80°C heat shock for 1, 3, and 5 min, with an increasing trend at 1 min and 3 min. At 100°C for 1 min and 3 min, germination rate showed no significant change, but decreased significantly at 5 min and 10 min ($P < 0.05$). At 120°C, germination rate showed no significant change at 1 min, but decreased significantly at 3 min, 5 min, and 10 min ($P < 0.05$). At 150°C, germination rate showed no significant change at 1 min, but decreased significantly at 3 min, 5 min, and 10 min ($P < 0.05$).

Two-way ANOVA [25,26] showed that temperature, time, and their interaction all had significant effects on the germination rate of *L. gmelinii* seeds ($P < 0.05$). Among these, the time factor had the greatest influence ($F = 116.073$), followed by temperature ($F = 67.667$), and their interaction ($F = 25.797$). This indicates that *L. gmelinii* seeds have difficulty adapting to prolonged heat shock at extremely high temperatures, with exposure time being the most critical factor.

2. Effects of Heat Shock on Germination Rate of *Pinus sylvestris* var. *mongolica* Seeds

Compared with the control, 80°C heat shock for 1 min and 3 min did not significantly affect the germination rate of *P. sylvestris* var. *mongolica* seeds, but all other treatments caused significant decreases ($P < 0.05$). Germination rate decreased to 13.334% and 6.667% at 100°C for 5 min and 10 min, respectively. At 120°C, germination rates were 5.000% (1 min), 6.667% (3 min), 2.500% (5 min), and 4.167% (10 min). At 150°C, germination rates were 2.500% (1 min), 0% (3 min), 0% (5 min), and 0% (10 min).

Two-way ANOVA showed that temperature, time, and their interaction all had significant effects on germination rate ($P < 0.05$), with temperature having the greatest influence ($F = 279.595$), followed by time ($F = 13.457$), and their interaction ($F = 8.853$). This indicates that the limiting temperature that *P. sylvestris* var. *mongolica* seeds can withstand is above 80°C, with temperature being the most influential factor.

3. Effects of Heat Shock on Germination Rate of *Picea koraiensis* Seeds

Compared with the control, heat shock helped improve the germination rate of *P. koraiensis* seeds. At 80°C, germination rate showed an obvious increasing trend but did not reach significant levels. At 100°C for 1 min, germination rate increased significantly ($P < 0.05$), but showed no significant difference from the control at 3 min, 5 min, and 10 min. At 120°C, germination rate showed no significant difference from the control, but decreased with longer heat shock times (34.167% at 3 min, 27.5% at 5 min). At 150°C, germination rate showed no significant difference from the control.

Two-way ANOVA showed that temperature ($F = 23.462$) and heat shock time ($F = 18.139$) both had significant effects on germination rate ($P < 0.05$), with temperature having slightly greater influence than time, and their interaction also being significant ($F = 2.827$, $P < 0.05$). This indicates that *P. koraiensis* seeds can withstand longer periods of heat shock.

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1. Effects of Heat Shock on Germination Velocity Index of *Larix gmelinii* Seeds

Compared with the control, the germination velocity index of *L. gmelinii* seeds showed an increasing trend at 80°C for 1, 3, and 5 min, but did not reach significant levels. At 100°C, the index decreased significantly at 10 min ($P < 0.05$). At 120°C, the index decreased significantly at 3 min, 5 min, and 10 min ($P < 0.05$). At 150°C, the index decreased significantly at 3 min and 5 min ($P < 0.05$).

Two-way ANOVA showed that temperature, time, and their interaction all had significant effects on the germination velocity index ($P < 0.05$), with time having greater influence ($F = 206.512$) than temperature ($F = 108.780$). This indicates that heat shock time has a larger effect on the germination velocity index of *L. gmelinii* seeds.

2. Effects of Heat Shock on Germination Velocity Index of *Pinus sylvestris* var. *mongolica* Seeds

Compared with the control, the germination velocity index of *P. sylvestris* var. *mongolica* seeds changed significantly at 80°C, decreasing significantly at 10 min

($P < 0.05$), while the germination rate at these three treatments did not decrease significantly. All other treatments caused significant decreases in the index ($P < 0.05$).

Two-way ANOVA showed that temperature, time, and their interaction all had significant effects on the germination velocity index ($P < 0.05$), with temperature having the greatest influence ($F = 242.021$), followed by time ($F = 16.171$), and their interaction ($F = 4.949$). This indicates that heat shock temperature has the greatest impact on the germination velocity index of *P. sylvestris* var. *mongolica* seeds.

3. Effects of Heat Shock on Germination Velocity Index of *Picea koraiensis* Seeds

Heat shock could improve the germination velocity index of *P. koraiensis* seeds. At 80°C for 1, 3, and 5 min, the index increased significantly ($P < 0.05$), reaching the highest value of 0.862 at 5 min. At 100°C for 1 min, the index increased significantly ($P < 0.05$). At 120°C, the index showed no significant change, but decreased with longer heat shock times. At 150°C, the index showed an increasing trend but no significant difference from the control.

Two-way ANOVA showed that temperature, time, and their interaction all had significant effects on the germination velocity index ($P < 0.05$), with temperature ($F = 24.537$) having slightly greater influence than time ($F = 16.601$), and their interaction being significant ($F = 2.888$, $P < 0.05$). The effects of heat shock temperature and time on *P. koraiensis* seed germination velocity index were not substantially different.

Conclusion and Discussion

The results indicate that heat shock at 80°C for 1, 3, and 5 min can slightly improve the germination rate and germination velocity index of *L. gmelinii* seeds. Heat shock had a greater effect on the germination velocity index than on the germination rate of *L. gmelinii* seeds. Longer heat shock treatments caused significant decreases in both germination rate and germination velocity index, leading to extended germination time. However, short-duration high-intensity heat shock (150°C for 1 min) could maintain germination rate without significant difference from the control, though the germination velocity index decreased significantly ($P < 0.05$), resulting in slower germination speed. After forest fires, *L. gmelinii* seeds in lower soil layers and some upper layer seeds in fast-burning areas can survive in large numbers. While a small portion of seeds showed improved germination rate, most seeds had extended germination times.

The study also showed that heat shock had no significant effect on the germination rate of *P. sylvestris* var. *mongolica* seeds at 80°C for 1 and 3 min, but caused significant decreases at other treatments, with germination rate and velocity index both decreasing significantly under heat shock above 100°C, and zero germination in four treatments above 120°C. Heat shock did not promote germination of *P. sylvestris* var. *mongolica* seeds. Many foreign scholars have obtained the same conclusion in studies of its close relative *Pinus sylvestris* [12,23,26]. *P. sylvestris* var. *mongolica* seeds cannot withstand high-intensity and long-duration heat shock. After forest fires, only extremely small amounts of seeds in lower and upper soil layers can survive, with germination capacity significantly inhibited and germination time extended.

The results also demonstrated that heat shock at 80°C for 1, 3, and 5 min and at 100°C for 1 min significantly improved both germination rate and germination velocity index of *P. koraiensis* seeds ($P < 0.05$), reaching maximum values. Other treatments at 100°C and all treatments at 120°C showed no significant difference from the control in germination rate and velocity index, while treatments at 150°C showed decreasing trends. *P. koraiensis* seeds can withstand high-intensity instantaneous heat shock and show improved germination capacity under low-intensity moderate-duration heat shock. After forest fires, seed germination quantity in lower soil layers increases significantly compared with natural conditions, with faster germination speed and shorter germination time. Seeds in upper layers can maintain germination capacity after fire.

The development cycle of tree species and the length of regional fire return intervals affect species distribution in particular areas. Generally, if the fire return interval is longer than the species development cycle, the species can survive easily; if shorter, survival is difficult [27]. The development cycle of *L. gmelinii* is 30-40 years, *P. sylvestris* var. *mongolica* is 40-50 years, and *P. koraiensis* is 80-100 years. In the southern Greater Khingan Mountains where the fire return interval is only 15-20 years, even if some areas have weak fire intensity and soil seed banks of *P. koraiensis* receive light heat shock that promotes germination, *P. koraiensis* cannot complete regeneration before new fires occur because the fire return interval is much shorter than the development period. The same situation applies to *L. gmelinii* and *P. sylvestris* var. *mongolica*. Therefore, the three tree species are relatively less distributed in the southern Greater Khingan Mountains, being replaced by broadleaf trees such as birch [27] and shrubs with shorter development cycles [29].

In the northern Greater Khingan Mountains, the fire return interval reaches 110-120 years, much longer than the development cycles of *L. gmelinii* and *P. sylvestris* var. *mongolica*. After fire occurrence, *L. gmelinii* seeds show better germination status than *P. sylvestris* var. *mongolica* in lightly burned northern areas. The germination speed of *P. koraiensis* seeds increases substantially, but due to its long development cycle, it cannot become forested first. In the process of post-fire vegetation recovery in lightly burned northern areas, *L. gmelinii* regenerates best after fire, *P. sylvestris* var. *mongolica* ranks second, and *P. koraiensis* appears only in some areas. In severely burned northern areas, most seeds in soil layers experience high-intensity heat shock, while small amounts of seeds in lower layers experience light heat shock. Large numbers of *P. sylvestris* var. *mongolica* seeds in soil lose germination vitality or are killed, while lower layer seeds can maintain germination vitality. The mortality rate of *L. gmelinii* seeds is significantly lower than that of *P. sylvestris* var. *mongolica*. The germination capacity of lower layer *P. koraiensis* seeds is improved by light heat shock. Therefore, in severely burned northern areas, *L. gmelinii* shows the best post-fire regeneration, and *P. koraiensis* germination is better than *P. sylvestris* var. *mongolica*. However, because *P. sylvestris* var. *mongolica* cones have delayed opening characteristics [27] and fire causes cones to open and release seeds for rapid germination [28], and its seeds disperse over long distances [18], while *P. koraiensis* is constrained by its long growth cycle, *P. sylvestris* var. *mongolica* is more abundant than *P. koraiensis* in most areas, with *P. koraiensis* distributed only in some areas. This matches the distribution characteristics of *P. koraiensis* in the Greater Khingan Mountains [29].

The study results prove that heat shock effects on seed germination differ among the three Pinaceae species, with complex reasons. Torres and others believe this is related to the protection of conifer cones and their delayed opening characteristics [30,31]. Hu Haiqing et al. believe seed dispersal methods and seed production are related to fire adaptation, improving species fire resistance [32]. The mechanism of changes in soil seed bank germination after fire in fire-prone areas is important for ecosystem community composition [30]. However, high temperature is only one of many fire products, making it necessary to conduct research on effects of other fire products on seed germination.

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