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Thermodormancy and Germination Response to Temperature of *Pyrus ussuriensis* Seeds

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Abstract: To determine the optimal germination temperature for *Pyrus ussuriensis* seeds and whether they experienced the phenomenon of thermodormancy and its inciting factors, several germination tests were conducted using non-dormant *P. ussuriensis* seeds for comparison. The results showed that the highest germination rate of *P. ussuriensis* seeds was reached at a constant temperature of 5 °C and variable temperature (night/day) of 5 °C/10 °C. Constant temperatures of 25 °C for three days induced thermodormancy, triggering significant drops in seeding emergence. Thermodormancy was related to the inhibitory effect of endogenous substances in the seed coat and an elevated abscisic acid concentration. The embryo, by contrast, remained non-dormant. Thermodormant and non-dormant seed embryos showed higher germination rates than dormant seed embryos when applied exogenous abscisic acid and gibberellic acid. We found that *P. ussuriensis* seeds showed thermodormancy; thus, during early spring sowing, high temperatures should be avoided to prevent low seed germination capacity. Additionally, applying exogenous gibberellic acid, shading and increasing soil moisture can be helpful to enhance the species seed germination.

Keywords: *Pyrus ussuriensis*; germination temperature; embryo; inhibitors; thermodormancy

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Citation: Liu, Z.; Li, M.; Zhu, M.; López, R.; Salomón, R.L.; Zhang, P. Thermodormancy and Germination Response to Temperature of *Pyrus ussuriensis* Seeds. *Agronomy* **2024**, *14*, 475. https://doi.org/10.3390/agronomy14030475

Academic Editors: Yong Ha Rhie, Seung Youn Lee and Duhyun Kim

Received: 8 February 2024
Revised: 23 February 2024
Accepted: 25 February 2024
Published: 27 February 2024

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1. Introduction

Dormancy is the physiological adjustment and adaptation of seeds to survive unfavourable conditions [1]. Seeds minimise their metabolic activity during dormancy, delaying germination until favourable conditions are reached. This strategy may lead to seed damage, reducing germination efficiency and eventually resulting in the misuse of forest production resources. After primary dormancy, occurring during seed maturation in the mother plant, unsuitable environmental conditions following seed dispersion, such as extreme temperatures (cold or hot), low water availability and insufficient oxygen, can induce seeds to enter a stage of secondary dormancy [2]. Among them, high temperature-induced secondary dormancy, known as thermodormancy, is a common phenomenon characteristic of species from cold regions. For instance, when *Cyclocarya paliurus* seeds that have overcome primary dormancy are subjected to an interruption of cold stratification by rising temperatures, seeds re-enter into a secondary thermodormancy state [3]. The phenomenon of thermodormancy gains ecologic and economic relevance with climate change, as increasing temperatures are expected in temperate and boreal regions [4]. Heat spells or prolonged warm periods in regions where species with thermodormant seeds are frequent may reduce their seed germination efficiency, seedling emergence and overall yield, causing significant economic losses to agriculture and forestry [5].

Dormancy can be classified as exogenous or endogenous. Exogenous dormancy is caused by the conditions in the endosperm surrounding the seed's embryo, commonly explained by physical mechanisms. The endosperm can inhibit the water uptake of the embryo or mechanically impede the embryo from expanding and the radicle from protruding. Endogenous dormancy is commonly driven by chemical and physiological cues directly affecting the seed embryo. Therefore, the presence or absence of endosperm in the seed may lead to differences in the mechanism of thermodormancy induction. For instance, in endosperm-seeded *Fraxinus mandshurica*, the germination rate of non-dormant seeds was significantly reduced by 15 days of dark incubation at 25 °C [6], with such thermodormancy induction being associated with reduced cellulase and hemicellulase enzymatic activities in the endosperm [7]. Accordingly, a polyethylene glycol (PEG) osmotic treatment was effective in preventing thermodormancy in *F. mandshurica* seeds, associated with a rise in cellulase and hemicellulase activities in the endosperm, simultaneous with an increment of gibberellin content in the seed embryo [8]. These observations denote that the induction of thermodormancy in seeds with endosperm can be related to (exogenous) endosperm weakening rather than endogenous drivers. In the case of endospermless species, some studies have found that thermodormancy can be induced in tomato (*Solanum lycopersicum*) seeds at 37 °C [9] and apple (*Malus pumila*) seeds at 30 °C [10]. However, the role of the seed embryo and seed coat in thermodormancy induction remains comparatively unexplored in endospermless species.

Seed dormancy can originate from the activity of hormonal repressors [11]. Most dormant seeds have inhibitors, which can be found in different seed tissues [12]. In *Euscaphis japonica,* the concentration of inhibitors was higher in the seed shells than in the embryo [13]. By contrast, the seed dormancy of Korean pine (*Pinus koraiensis*) was mainly related to endogenous inhibitors in the seed kernel [14]. Studies on thermodormancy mechanisms have predominantly focused on the induction conditions and synthesis and metabolic pathways of hormones involved in its regulation. Studies have shown that upon exposure to high temperatures, seed germination of sunflower (*Helianthus annuus*), carrot (*Daucus carota*), Arabidopsis (*Arabidopsis thaliana*) and lettuce (*Lactuca sativa*), among others, is affected by the overexpression of specific genes [15–18]. For instance, in Arabidopsis, high temperatures led to the accumulation of abscisic acid (ABA) and the depletion of gibberellic acid (GA) by regulating the expression of the corresponding biosynthetic genes [17]. Similarly, genes involved in ABA biosynthesis were overexpressed at high temperatures in lettuce (*Nasturtium officinale*) [19].

P. ussuriensis is a common rootstock used in pear cultivation in cold regions of China due to its high resistance to cold and drought and its barrenness tolerance [20]. Despite its economic and ecologic relevance, the optimal germination conditions after lifting primary dormancy have not been systematically screened, and we are not sure whether unsuitable temperatures will reinduce dormancy. Moreover, *P. ussuriensis* seeds lack the endosperm [21], which makes the species suitable for exploring thermodormancy drivers in endospermless species, for which the role of the seed embryo and seed coat is not fully understood. To fill this gap, this study investigated the germination response of non-dormant *P. ussuriensis* seeds to constant and (sub-daily) variable temperature gradients to determine their optimal germination conditions and the time required at high temperatures to reinduce thermodormancy. We also assessed the causes of thermodormancy by multiple assays, considering the effect of the dormant state on isolated embryos, the role of different hormones (ABA and GA) in different tissues (seed embryo and coat) and the thermal interactive effects. The potential insights gained from this study can be helpful for guiding the seed germination protocols and practices in forest nurseries, aiming to improve production efficiency. We performed these assays under the following expectations: (i) The further the germination temperature from the species' ecological requirements, the lower the germination efficiency; (ii) the longer the incubation time at high temperatures, the more profound thermodormancy; (iii) the dormancy state strongly determines the germination efficiency of isolated embryos; (iv) the germination efficiency depends on the interactive effect be-

tween the chemical inhibitor and the seed tissue; and (v) a predominantly ABA-driven thermodormancy induction.

2. Materials and Methods

2.1. Plant Material

P. ussuriensis seeds were purchased from the Agroforestry Wood Flower and Lawn Seed Company (Changchun, China); the seed collecting area was Jilin Province. The weight of a thousand seeds was 55.16 g. To release incipient dormancy, seeds were soaked in 500 mg/L Gibberellic acid solution for two days and then naked stratified at low temperature (5 °C) for eight weeks.

For the thermodormancy study, seeds were released from dormancy and then incubated in the dark at 25 °C (thermodormancy temperature) for seven days. Seeds that did not germinate at the end of the incubation period were used as test material. In all tests, the germination percentage (G_P), the germination rate (G_R), the germination index (GI) and the mean daily germination (MDG) were calculated as follows [22]:

$$G_P\ (\%) = n/N \times 100, \tag{1}$$

$$G_R = \sum_1^T \left(\frac{ni}{Dt}\right), \tag{2}$$

$$GI = \sum_1^T \left(\frac{GR}{Dt}\right), \tag{3}$$

$$MDG = G_P/D, \tag{4}$$

where n is the number of germinated seeds, N is the number of total seeds, T is the final day of measurements, G_R is the germination rate per day corresponding to Dt, Dt is the number of days from the start of the experiment to the time when germinated seeds were recorded and D is the number of days to final germination. Germination tests detailed below were performed during the 2022 growing season (from April to September), followed by biochemical analyses (from October to December 2022).

2.2. Research Methodology

2.2.1. Determination of Suitable Germination Temperatures for Non-Dormant *P. ussuriensis* Seeds

Seeds released from dormancy by stratification were disinfected with 0.5% $KMnO_4$ for 30 min, rinsed with tap water, soaked in distilled water for 24 h and placed in plastic Petri dishes lined with a layer of moistened filter paper. They were placed in a climatic chamber (Panasonic MIR-254 Low-temperature constant-temperature laminar box) at different temperatures, and germination tests were conducted under dark conditions. A total of 28 germination temperature combinations were set. Seven treatments consisted of germination at constant temperatures: 5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C and 35 °C; in the other 21 treatments, we used variable sub-daily temperatures (16 dark hours/8 light hours): 5/10 °C, 5/15 °C, 5/20 °C, 5/25 °C, 5/30 °C, 5/35 °C, 10/15 °C 10/20 °C, 10/25 °C, 10/30 °C, 10/35 °C, 15/20 °C, 15/25 °C, 15/30 °C, 15/35 °C, 20/25 °C, 20/30 °C, 20/35 °C, 25/30 °C, 25/35 °C and 30/35 °C. Each treatment had four replications with 25 seeds per replicate.

Seed germination was observed and recorded once per day after the start of the germination test. We considered that a seed had germinated when the radicle extended out of the seed coat 2 mm. To prevent desiccation, distilled water was added to the Petri dishes every day, and long mould and rotting seeds were removed. The experiment lasted until no germinated seeds were observed for three consecutive days.

2.2.2. Effect of the Duration of High Temperature on the Germination of Non-Dormant Seeds

Non-dormant *P. ussuriensis* seeds were soaked in water for two days and then placed in a climatic chamber at 25 °C (thermodormancy temperature) for 1, 3, 5 and 7 days and then moved to 5 °C for germination. As in Section 2.2.1, each treatment had four replications with 25 seeds per replicate.

2.2.3. Effect of GA and ABA on the Germination Ability of Isolated Embryos at Different Dormant States

Initial dormant, non-dormant and thermodormant seeds were used to test the effect on germination of GA and ABA solutions at different concentrations. Isolated embryos of three seeds were taken and placed in 9 cm diameter Petri dishes with 5 mL of the following five solutions: (i) GA_3 10^{-6} mol/L (low [GA], hereafter), (ii) GA_3 10^{-5} mol/L (high [GA]), (iii) ABA10^{-6} mol/L (low [ABA]), (iv) ABA10^{-5} mol/L (high [ABA]) and (v) distilled water. Embryos were incubated in light at a constant temperature of 25 °C and 50% relative humidity for 28 days. Each treatment had 3 replications with 10 seeds per replicate.

2.2.4. Activity of the Inhibitors of the Seed Coat of *P. ussuriensis* Seeds at Different Dormant States

To test the activity of the inhibitors of the seed coat of *P. ussuriensis* seeds, we selected cabbage seeds with a germination rate of 90% as germination test material. Cabbage seeds have a very high germination rate and a short germination time, allowing for a quick determination of inhibitor activity.

Referring to the method of Zhang, P. [23], the concentration of the seed coat extraction solution was 0.7 g/mL. This concentration and the required seed coat fresh weight were calculated using the following equations:

$$n(\mathrm{g}) = \frac{DW}{FW - DW}, \tag{5}$$

$$C(\mathrm{g/mL}) = \frac{n}{1\ ml}, \tag{6}$$

$$m(\mathrm{g}) = V \times C, \tag{7}$$

where C denotes the concentration of the original seed coat extract; n is the coefficient; FW denotes the value of the fresh weight of the seed when saturated with water absorption; DW denotes the dry weight of the seed; and V denotes the volume of seed coat extraction solution required. m is the weight of the seed coat required.

The seed coat of each of the three seeds used in the previous experiment was cut into pieces and extracted with 80% methanol in an ice bath (0 °C) for 24 h and then filtered. The filtrate was stored at a low temperature (5 °C). The filtered seed coat fragments were then extracted under the same conditions for 24 h, followed by filtration, and the filtrates from both were mixed. The filtrate was concentrated under reduced pressure at 45 °C, and the concentrated solution was the crude extract of the seeds coat inhibitor. The crude extract was volume-determined to 16.5 mL and stored at low temperature (5 °C).

We used four different concentrations of the crude extract to carry out the bioassay of inhibitory substances: (i) 2-fold dilution of the standard concentration (C = 0.35 g/mL), (ii) 5-fold dilution of the standard concentration (C = 0.14 g/mL), (iii) 10-fold dilution of the standard concentration (C = 0.07 g/mL) and (iv) distilled water as a control. A layer of filter paper was placed inside a Petri dish, 3 mL of extraction solution was added and 50 cabbage seeds were placed in the Petri dish, with three replicates per treatment. The Petri dishes were placed in an incubator with light at 25 °C and 50% relative humidity for the germination test. We considered that a seed had germinated when the radicle grew larger than the seed length, and the cotyledons became larger. The G_P was calculated after 48 h.

2.2.5. Changes in Endogenous Hormone Content during Seed Germination at Different Temperatures

The Petri dishes containing non-dormant *P. ussuriensis* seeds were placed in an incubator in the dark at 5 °C (germination temperature) and 25 °C (thermodormancy temperature). Tissue samples were taken on days 0, 1, 3, 5, 7 and 14. On each sampling day, three seeds per treatment were randomly selected, and the seed embryo and seed coat were separated. For each tissue, 0.6 g was immediately frozen with liquid nitrogen and stored at −80 °C. The content of gibberellic acid (GA_1, GA_3, GA_4 and GA_7) and abscisic acid (ABA) was determined. The endogenous hormone content was determined by enzyme immunoassays [24] using the enzyme labelling analyser Infinite F50 (TECAN, Switzerland) and the kits "Plant gibberellin (GA) enzyme-linked immunoassay (ELISA)" and "Plant abscisic acid (ABA) enzyme-linked immunoassay (ELISA)".

2.3. Statistical Analysis

Data statistical analysis was performed using SPSS Statistics 19.0 software. The effect of temperature and time on germination rate and germination index were assessed by factorial analysis of variance (ANOVA) after homogeneity tests. Both factors were considered fixed factors. The effect of the duration of incubation on the germination capacity was assessed by one-way ANOVA with the number of incubation days as a factor. For the study of the effect of plant hormones in germination at different seed states, we first analysed the effect of ABA and GA at two concentrations in G_P by one-way ANOVA, and then, we performed another ANOVA for each treatment considering the fixed factor as the seed state: dormant, non-dormant or thermodormant. In the analysis of the effect of the inhibitors of the seed coat of *P. ussuriensis* at different dormant states and with three concentrations of G_P on cabbage seeds, we performed a factorial ANOVA with the dormant state, the extract concentration and their interaction as fixed factors. Finally, for the last test, changes in the concentration of ABA and GA for 14 days at 5 °C and 25 °C were assessed by factorial ANOVA with time and incubating temperature as factors. Differences among treatment groups were tested by the Duncan test, and an α threshold of 0.05 was considered to report significant differences. Graphs were made using Sigmaplot 12.5.

3. Results

3.1. Effect of Temperature on Germination of Lifted Dormant Seeds

The germination performance of non-dormant *P. ussuriensis* seeds varied with thermal conditions. Under constant temperature regimes (during dark and light conditions), G_P progressively decreased with increasing temperature. The G_P at 5 and 10 °C was 60% and 43%, respectively, higher than at warmer temperatures between 15 and 35 °C ($p < 0.05$), with minimum rates of 2% at 35 °C. This inverse relation between G_P and temperature was maintained when diurnal and nocturnal temperatures varied. Overall, G_P decreased with increasing diurnal temperature for each level of nocturnal temperature (Figure 1a). The GI showed a similar trend, with the highest GI at constant 5 °C, although differences became not significant when compared to constant temperatures between 10 °C and 25 °C. When temperature varied sub-daily, the GI usually decreased with increasing diurnal temperature (Figure 1b). The mean daily germination rate (MDG; Figure 1c) and the germination rate (GR; Figure 1d) showed consistent results, illustrating the worse germination performance of seeds as nocturnal and diurnal temperatures increased. For instance, MDG at lower (and constant) temperatures (5 °C and 10 °C) was significantly higher than above 15 °C ($p < 0.05$). The G_R behaved similarly to the G_I.

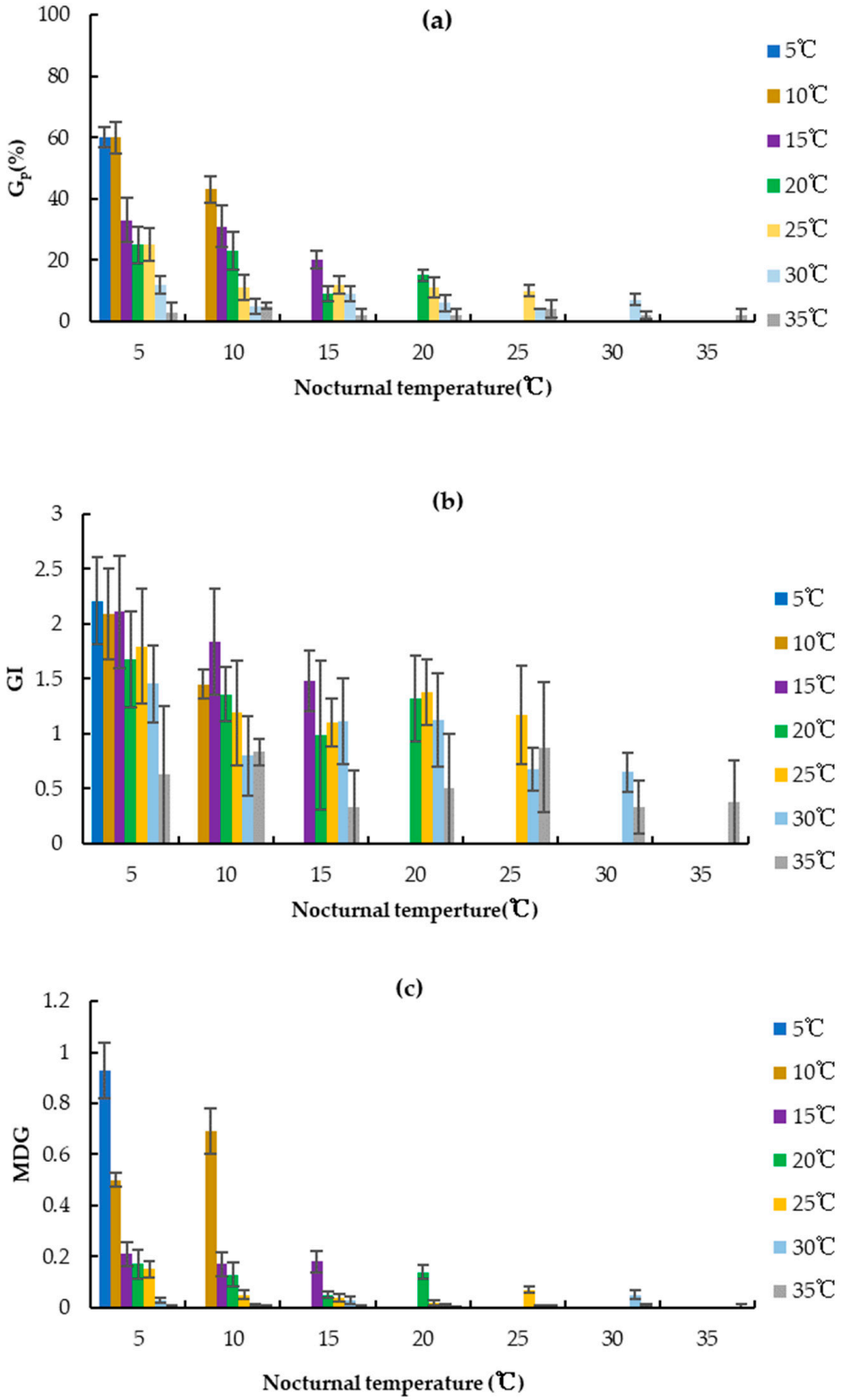


Figure 1. *Cont.*

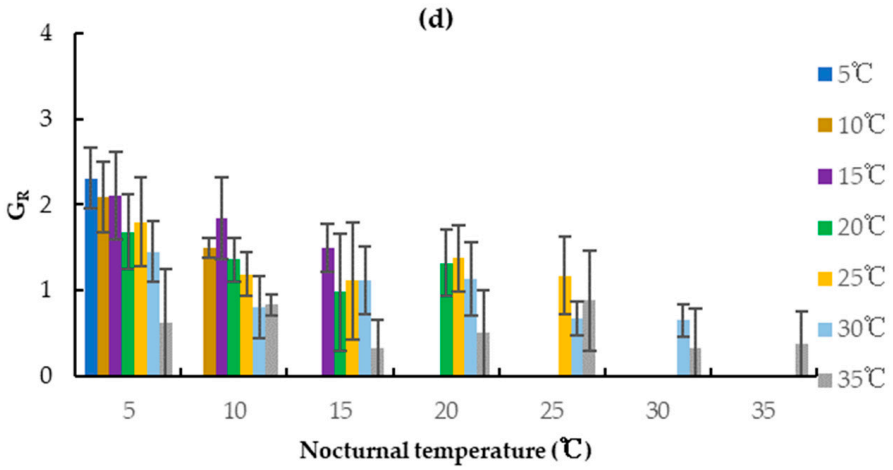


Figure 1. Germination percentage (G_P, (**a**)); germination index (*GI*, (**b**)); mean daily germination (*MDG*, (**c**)) and germination rate (G_R, (**d**)) of *P. ussuriensis* seeds after eight weeks of cold stratification at different nocturnal/diurnal temperature regimes (16/8 h). Note: Bars and intervals denote mean values and standard error. Different colours in the legend denote diurnal temperatures. Pairwise comparisons are not displayed for visual clarity.

3.2. Effect of the Duration of the High-Temperature Treatment on the Germination of Non-Dormant Seeds

The G_P of seeds without 25 °C incubation and seeds transferred to 5 °C after one day of 25 °C incubation was similar (40–60%; $p > 0.05$; Figure 2a). When 25 °C incubation lasted 3, 5 or 7 days, the G_P decreased to 5–15% ($p < 0.05$). The GI behaved slightly differently; the GI of seeds incubated at 25 °C progressively decreased with incubation time relative to control seeds not subjected to 25 °C incubation ($p < 0.05$; Figure 2b).

3.3. Effect of GA and ABA on the Germination of Isolated Embryos at Different Dormant States

We first analysed G_P differences among treatment solutions within each seed state (dormant, non-dormant and thermodormant) and then differences among seed states within each solution. In non-dormant seeds, the G_P rapidly increased (in three days) for seeds in water and GA solutions. At the end of the incubation period (12 days), the G_P for high and low [GA] solutions was slightly higher (ca. 80%) than the control treatment (G_P = 74%), although differences were not statistically significant. The inhibitory effect of ABA was apparent, with decreasing G_P with increasing [ABA], with percentages of 64 and 34% for low [ABA] and high [ABA] solutions, the latter being significantly lower than the control group ($p < 0.05$; Figure 3a). In dormant seeds, the G_P were overall lower (Figure 3b). The G_P reached the highest values in six days and remained below 45% for any treatment solution afterwards. Again, GA solutions promoted G_P relative to the control group, while ABA solutions inhibited germination; however, none of the treatment solutions significantly differed from that of the control group. Thermodormant seeds behave similarly to non-dormant ones (Figure 3c), rapidly reaching stable G_P. The G_P of the control group was 84%, and no apparent promotion of G_P was observed under GA treatment solutions at the end of the incubation period. Under high [ABA], a substantial inhibition of germination was observed (G_P = 37%; $p < 0.05$). Unexpectedly, low [ABA] did not inhibit germination ($p > 0.05$).

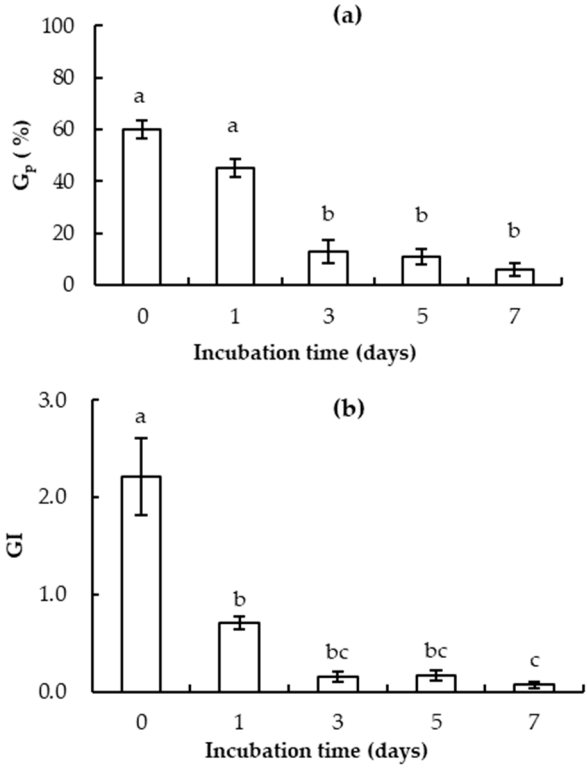


Figure 2. Germination percentage (G_P, (**a**)) and index (GI, (**b**)) of non-dormant *P. ussuriensis* seeds at 5 °C after incubation at a high temperature (25 °C). Note: Bars and intervals denote mean values and standard error. Different letters denote treatment differences ($p < 0.05$).

Differences in G_P among seed states (dormant, non-dormant and thermodormant) within the same treatment solution are shown in Figure 4. Differences in G_P of isolated embryos of *P. ussuriensis* seeds in different dormant states depended on the seed dormancy state ($p < 0.001$) and the solution ($p < 0.001$), denoting their interaction as not significant ($p > 0.05$) (Figure 4; Table S1). The G_P of dormant seeds was consistently lower than that of non-dormant and thermodormant seeds in water, ABA solutions and the GA solution at high concentrations. By contrast, no differences were observed in the low [GA] solution. Photographs of the germination behaviour of monitored seeds in different dormant states and GA and ABA concentrations are shown in Figure S1.

3.4. Activity of Inhibitors in the Seed Coat of Thermodormant P. ussuriensis Seeds

Differences in G_P of cabbage seeds in different extracts of *P. ussuriensis* coat seeds depended on the seed state ($p < 0.01$) and the solution concentration ($p < 0.001$), denoting their interaction as not significant ($p > 0.05$) (Figure 5; Table S2). When the concentration of seed coat extract was 0.35 g/mL, the G_P in non-dormant seed coats (G_P = 37%) was lower than the control group (G_P = 86%) ($p < 0.01$). The G_P further decreased for thermodormant and dormant seed coats (G_P = 6% and G_P = 0%). When the concentration of seed coat extract was 0.14 g/mL, the G_P was uniquely reduced for dormant seeds down to 42% relative to control values ($p < 0.05$). When the concentration of seed coat extract was 0.07 g/mL, there were no differences in G_P among control, dormant, non-dormant and thermodormant groups ($p > 0.05$), with values consistently above 86%.

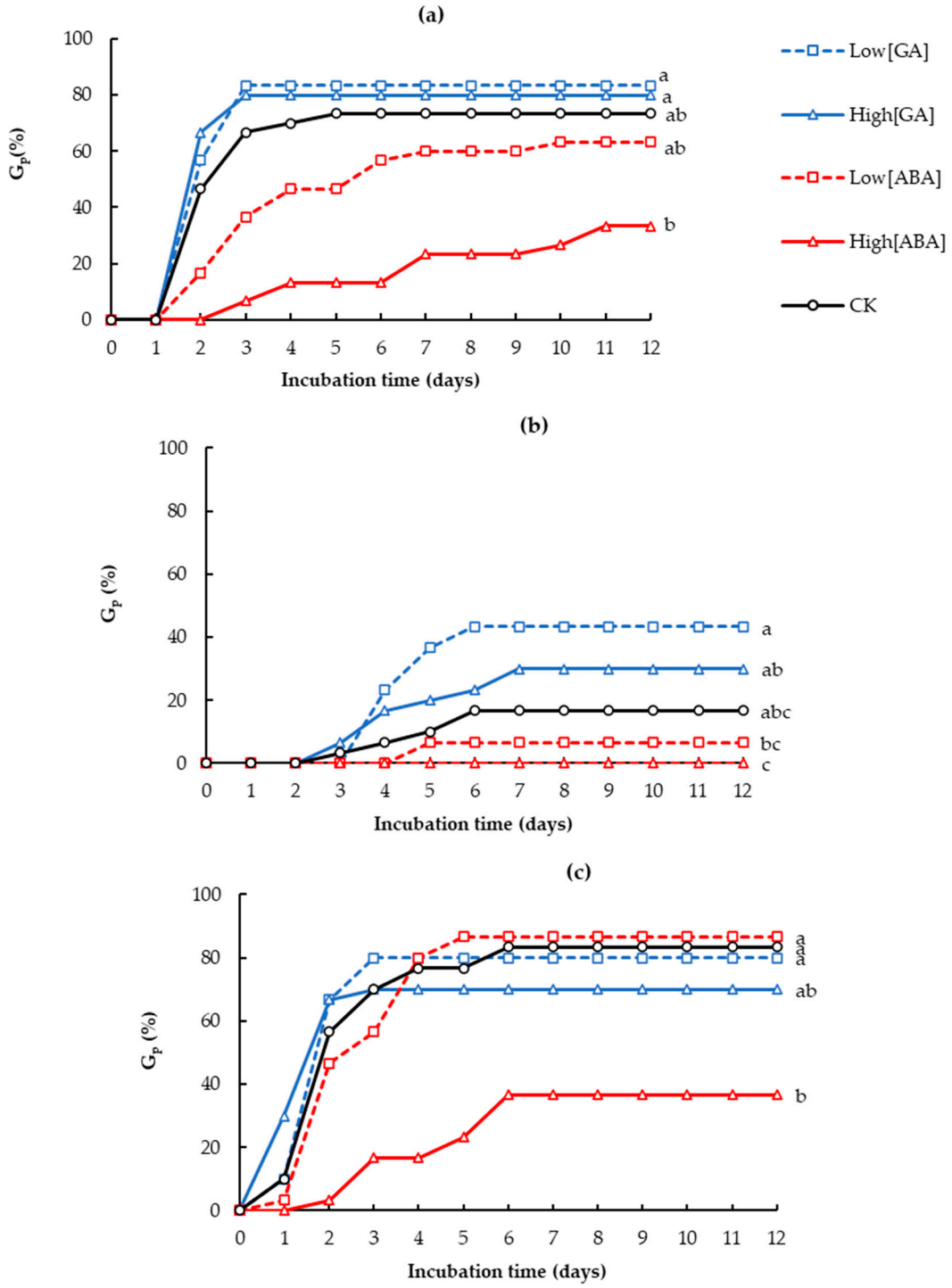


Figure 3. Temporal evolution of the in vitro germination percentage (G_P) of isolated embryos of *P. ussuriensis* seeds in non-dormant (**a**), dormant (**b**), and thermodormant (**c**) seeds. Seeds were placed in treatment solutions containing gibberellic acid (GA) and abscisic acid (ABA) at high and low concentrations. Note: Mean ± standard error are shown. Different lowercase letters indicate differences among treatment solutions at the end of the incubation period ($p < 0.05$). The five treatments consisted of distilled water (CK), 10^{-6} and 10^{-5} mol/L GA solutions (low and high [GA]) and 10^{-6} and 10^{-5} mol/L ABA solutions (low and high [ABA]).

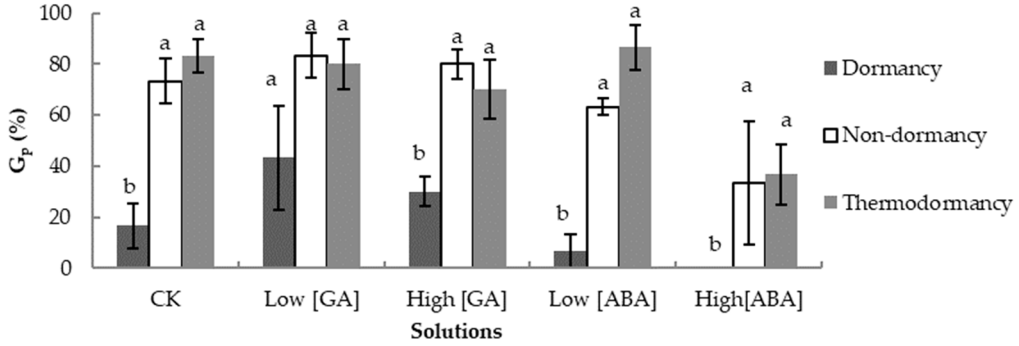


Figure 4. Germination percentages (G_P) of isolated embryos of *P. ussuriensis* seeds in different dormant states. Seeds were placed in treatment solutions containing gibberellic acid (GA) and abscisic acid (ABA) at high and low concentrations. Note: Mean ± standard error are shown. Different lowercase letters indicate differences among seed states within each treatment solution ($p < 0.05$). The five treatments consisted of distilled water (CK), 10^{-6} and 10^{-5} mol/L GA solutions (low and high [GA]) and 10^{-6} and 10^{-5} mol/L ABA solutions (low and high [ABA]).

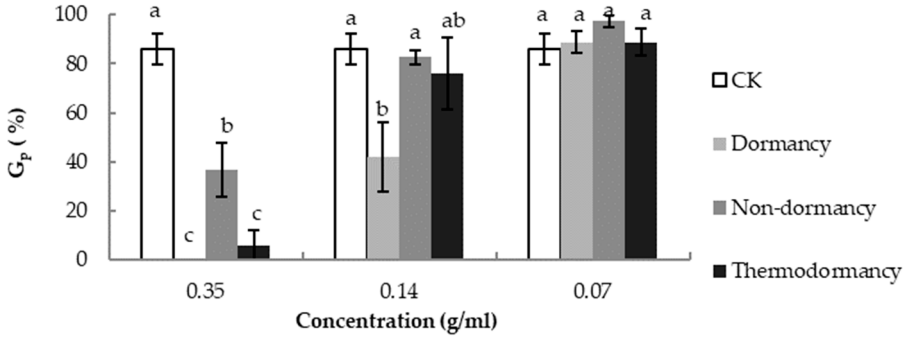


Figure 5. Germination of cabbage seeds in seed coat extracts of *P. ussuriensis* seeds in different dormancy states. Note: Bars and intervals denote mean and standard error. Lowercase letters indicate significant differences among seed coat states within each concentration level ($p < 0.05$).

3.5. Changes in Endogenous Hormone Content during Seed Germination at Different Temperatures

3.5.1. Changes in GA Content

The trend of [GA] in the seed embryo varied with germination temperature, with [GA] being consistently higher at 5 °C than at 25 °C. The [GA] at 5 °C progressively increased 2-fold during the whole incubation period, while irregular temporal fluctuations were observed at 25 °C (Figure 6a). In the seed coat, [GA] did not show a regular temporal pattern at 5 or 25 °C, with differences between temperature groups being irregularly found during the incubation period (Figure 6b).

3.5.2. Changes in ABA Content

At the beginning of the incubation (1–5 days), [ABA] in seed embryos at 5 °C were lower than at 25 °C, showing M-type fluctuations (Figure 6c). After one and two weeks of incubation, [ABA] at 5 °C levelled off, while [ABA] at 25 °C tended to decrease. Throughout the germination process (from day 0 to day 14), [ABA] increased 1.6-fold at 5 °C, and there was no apparent change in the [ABA] at 25 °C.

The [ABA] in the seed coat (Figure 6d) was overall higher than in the embryo. During the first three days, [ABA] followed similar patterns in both temperature groups, declining sharply on day three. Afterwards, [ABA] remained stable at 5 °C while temporarily increasing at 25 °C on day 5 to return to similar values of the 25 °C treatment. Overall (from day 0 to day 14), [ABA] decreased for both temperature groups by 44%.

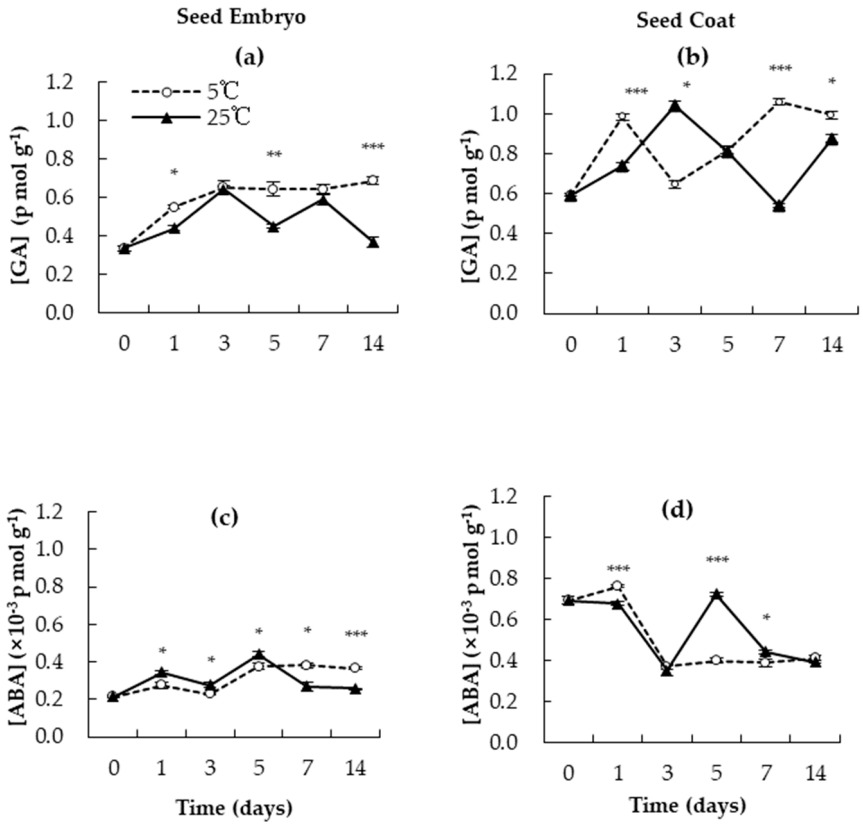


Figure 6. Changes in gibberellic (**a**) and abscisic acid (**c**) concentrations ([GA] and [ABA], respectively) in seed embryos and changes in gibberellic (**b**) and abscisic acid(**d**) concentrations in seed coats during seed germination when incubated at 5 and 25 °C. Note: Symbols and intervals show mean values ± standard error. Note that in some cases, SEs are small and overlap with the symbol. Asterisks denote significant differences between temperature groups for a given incubation period. (**a**) means the changes in GA concentrations in seed embryos; (**b**) means the changes in GA concentrations in seed coat; (**c**) means the changes in ABA concentrations in seed embryos; (**d**) means the changes in ABA concentrations in seed coat. Significance: * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

4. Discussion

The optimum germination temperature of *P. ussuriensis* seeds was ca. 5 °C and decreased as temperature increased, in accordance with the ecological requirements of the species, the most resistant to cold among the genus *Pyrus* [25]. In fact, incubation at 25 °C for more than three days induced thermodormancy in the seeds. Thermodormancy was associated with high ABA content in the seed coat. Conversely, by application of exogenous GA or by increasing the GA synthesis in seeds, dormancy was alleviated.

4.1. Temperature and Time Inducing Thermodormancy in P. ussuriensis Seeds

The optimum germination temperature for releasing dormancy of *P. ussuriensis* seeds was low, ca. 5–10 °C, while germination temperature over 15 °C induced thermodormancy. In many plants, seed germination rates increase with increasing temperature, as in Scots pine (*Pinus sylvestris*) or sand spruce (*Picea asperata*) [26], with an optimum germination temperature around 25 °C. and cold inhibition occurs if the temperature is too low, as in sugar beet (*Beta vulgaris*) [27]. However, in other species, germination is higher at lower temperatures, and high temperatures lead to thermodormancy, as occurs in seeds of *Sorbus pohuashanensis* [28], *H. annuus* [18], *L. sativa* [15], *S. lycopersicum* [9] and seeds of *P. ussuriensis* in the present study. In these seeds, temperature not only regulates the germination rate but also the dormancy level and dormancy termination [29]. The range of temperatures inducing thermodormancy of *P. ussuriensis* may be related to the geoclimatic origin of the

species [30], inhabiting a cold-temperate climate characterised by prolonged periods with low temperatures.

Thermodormancy in *P. ussuriensis* is time-dependent. We observed that short exposure periods, lower than three days, to high temperatures did not induce thermodormancy. However, at longer exposure times, germination was significantly reduced when germination temperatures returned to optimum (Figure 2a). This result is consistent with the thermodormancy of apple (*M. pumila*) [10] or tomato (*S. lycopersicum*) [9], where the induction of thermodormancy is also highly dependent on the duration of exposure to high temperatures.

4.2. Evidence of a Hormonal Control of Thermodormancy

Different mechanisms have been proposed for the induction of thermodormancy in seeds, including weakening of the endosperm, hormonal balance and changes in phytochrome [31–33]. Moreover, in some species, these factors seem to interact. In some cultivars of lettuce, for example, thermodormancy has been proposed to be morphological due to the prevention of radical expansion by the endosperm. Physiologically, changes in the levels of cytokinin, gibberellin and ABA occur at the same time, and changes in the phytochrome have also been reported [34].

Plant hormones are the most important endogenous factor in the regulation of seed dormancy and germination. Both processes are affected by the absolute hormone content within the seed and also by the hormone sensitivity of the seed [35–37]. It has been reported that whether some seeds remain dormant or germinate does not depend directly on the content of ABA but on the sensitivity of the seed embryo to ABA [38]. ABA is indirectly involved in the regulation and maintenance of seed dormancy [39] and acts antagonistically with GA [40,41]. In the process of releasing dormancy, seeds would promote germination by increasing the sensitivity of seed embryos to GA and decreasing the sensitivity of ABA [42]. The sensitivity of seeds in different dormant states to hormones is obviously different. Seed embryos of primary dormant seeds undergoing low-temperature stratification have weakened sensitivity to GA, which is manifested as a weak germination promotion of isolated embryos, whereas thermodormant embryos showed higher sensitivity to GA and reached the same germination rates as non-dormant embryos (Figure 4). On the contrary, the sensitivity to ABA increased, which was shown by a strong inhibitory effect on germination of this hormone, even in the low concentration, in dormant seeds, higher than in non-dormant or thermodormant seeds (Figure 4). High concentrations of ABA had a strong inhibitory effect on the germination of embryos of the three types of seeds. It has been generally reported that high concentrations of ABA and GA will disrupt the hormonal balance within the seed embryo [36] and even produce toxic effects. Unexpectedly, in our study, low concentrations of ABA did not affect the germination of thermodormant embryos, and only high concentrations of ABA inhibited their germination capacity. However, the inhibitory germination effect of ABA on wild oat (Avena sativa) seed embryos at high temperatures was 1000-fold stronger than that at 10 °C [43], and the same was also found in barley (Hordeum vulgare) seeds [44].

When we followed the content of endogenous GA and ABA during germination, we found that the GA content in seed embryos increased and the ABA content in the seed coat decreased in a peak-like manner during germination at a higher temperature (25 °C) compared with more smooth changes at 5 °C. This suggested that GA metabolism in the seed embryo was active and has been maintained at a level sufficient to inhibit germination at high temperatures and that ABA was synthesised and accumulated at a certain level in the seed coat, which in turn leads to dormancy. It has been reported that GA and ABA are jointly involved in regulating the germination of lettuce seeds at high temperatures. The seed embryo perceives the unfavourable environment and maintains high levels of ABA within the seed embryo by decreasing the metabolism of ABA or enhancing the synthesis of ABA [45] and decreasing the GA synthesis. After thermodormancy was induced in barley seeds by high temperature, the ABA content and sensitivity also increased [44]. Similarly,

the tomato seed ABA biosynthesis pathway genes NCED1 and NCED9 were upregulated in expression at high temperatures, whereas the GA biosynthesis-regulated genes GA3ox1 and GA20ox1 were downregulated at high temperatures, both demonstrating the important role of ABA in the process of seed thermodormancy [9]. Therefore, the application of ABA synthesis inhibitors may prevent *P. ussuriensis* seeds from entering thermodormancy.

4.3. Role of the Seed Coat in Thermodormancy Induction

In some endospermous seeds, such as lettuce or tomato, it has been suggested that secondary dormancy might be related either to the endosperm or to the seed coat [46]. If the mechanisms for thermodormancy induction in endospermless seeds are similar, substances released by the seed coat should induce thermodormancy, as occurred in our study, where we investigated the germination rate of isolated embryos of *P. ussuriensis* seeds in different hormonal solutions. As highlighted above, we found that the seed embryos of thermodormant *P. ussuriensis* seeds performed similarly to those of non-dormant seeds, suggesting that the seed embryos of thermodormant *P. ussuriensis* seeds were not dormant. We also found that the germination capacity of cabbage seeds imbibed in a solution with extractives of the seed coat of dormant and thermodormant *P. ussuriensis* seeds significantly decreased, which indicated that there were endogenous inhibitors in the seed coat of *P. ussuriensis* seeds. Similarly, high endogenous inhibitor activities were found in the endocarp and exocarp of the seeds of Amyrgdalus mongolica [47]. The inhibitory effect of the seed coat was enhanced with the induction of thermodormancy. The inhibitory effect of the seed coat weakened during the release of dormancy in Fraxinus chinensis [48] seeds. In general, seed coat inhibitory activity is related to dormancy properties, and some studies on Taxus yunnanensis seeds [49] showed that there is a link between seed coat inhibitory activity and seed coat permeability and relative hormone content. Excessive inhibitor activity in the seed coat may cause the seed embryo to fail to germinate properly, but the signalling pathways involved have not been explored.

5. Conclusions

In conclusion, the optimum germination temperature of dormant *P. ussuriensis* seeds released by stratification is low (5–10 °C), and germination temperature over 15 °C will induce thermodormancy. The seed embryo of thermodormant *P. ussuriensis* seeds is not dormant, and thermodormancy was associated with elevated endogenous inhibitor activity in the seed coat. Thermodormancy of *P. ussuriensis* seeds in production sowing is an undesirable phenomenon, which will lead to a sudden drop in seedling emergence if the germination rate is reduced. Therefore, in early spring sowing, high temperatures should be avoided to prevent thermodormancy, resulting in unnecessary losses. We believe the following are worth trying: optimising sowing dates to minimise the risk of heat outbreaks; providing shade or cover to create microclimates more conducive to seed germination and early growth and improving soil moisture management, mulching or exploring alternative production methods such as greenhouse cultivation or laboratory germination for susceptible crops.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy14030475/s1. Figure S1. Photographs of the germination behaviour of dormant, non-dormant and thermodormant *P. ussuriensis* seeds in gibberellic acid at low (A) and high (B) concentrations and in abscisic acid at low (C) and high (D) concentrations; Table S1. Degrees of freedom and p values of the two-way analysis of variance of the effect of dormancy state, solution treatment, and its interaction on the germination percentage of isolated embryos of *P. ussuriensis* seeds; Table S2. Degrees of freedom and *p* values of the two-way analysis of variance of the effect of dormancy state, solution treatment, and its interaction treatment on the germination percentage of cabbage seeds.

Author Contributions: P.Z. and Z.L. conceived and designed the study; Z.L., M.L. and M.Z. performed the experiments; Z.L. collected the data and analysed the data; Z.L. drafted the manuscript;

P.Z., R.L. and R.L.S. revised various drafts of the manuscript; P.Z. supervised the study. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by the National Natural Science Foundation of China (Grant numbers 31870615, 31670639).

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Acknowledgments: Thanks to Liu Mingyu, Wang Zhe, Chen Li, Qu Zhiyi, Lv Jintao, Zhang Yipeng, Dong Bowen, Wang Jing, Yu Xue and Zhang Zheng for their help in completing the experiments.

Conflicts of Interest: The authors declare no conflicts of interest.

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