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Seed Dormancy Class and Germination Characteristics of *Berberis amurensis* var. *latifolia* Nakai, Native to Korea

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Abstract: *Berberis amurensis* var. *latifolia* Nakai is a plant native to the Ulleung Island in Korea. In this study, we aimed to identify seed dormancy-breaking and germination requirements of this species using water imbibition experiments, gibberellic acid (GA_3) treatment (0, 10, 100, or 1000 mg/L), cold stratification (0, 2, 4, 8, or 12 weeks at 5 °C), move-along experiments, and phenological studies. In the water imbibition experiment, the seed weight increased by more than 120% after 24 h. Analysis of the internal morphological characteristics of the seeds revealed that the embryo in freshly matured seeds was fully grown and did not grow thereafter. The final germination percentages after 12 weeks of cold stratification at 5 °C were 49 ± 6.4% and 63 ± 3.4% under light and dark conditions, respectively. In move-along and phenological studies, a longer cold stratification treatment period resulted in a higher germination percentage; however, the warm stratification treatment did not affect germination significantly. The GA_3 treatment had little effect on seed germination. Therefore, we concluded that *B. amurensis* var. *latifolia* seeds have intermediate physiological dormancy, and pre-treatment with cold stratification for 12 weeks and incubation in the dark are required for effective seed propagation.

Keywords: cold stratification; phenology; seed dormancy; barberry; germination

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1. Introduction

The plant genus *Berberis* has about 500 species worldwide, and they are found in most regions of South Asia, central and southern Europe, northeastern America, and northern Pakistan [1–4]. *Berberis* plants have been reported as native to Korea: *B. koreana* Palib., *B. koreana* var. *angustifolia* Nakai, *B. koreana* var. *ellipsoidea* Nakai, *B. amurensis* Rupr., *B. amurensis* var. *latifolia* Nakai, *B. amurensis* var. *quelpaertensis* (Nakai) Nakai and *B. poiretii* C.K. Schneid [5]. Among these, *B. amurensis* var. *latifolia* is restricted to Ulleung Island in Korea and is undergoing speciation in the isolated environment of the island [6].

The main chemical compounds found in *Berberis* are berberine and berbamine, along with tannins, phenolic compounds, sterols, and triterpenes [7–15]. These plants have been reported to exhibit several biological effects, such as tonics, antibacterial, antiemetic, antipyretic, antipruritic, antioxidant, anti-inflammatory, antihypertensive, antivaricose, sedative, antinociceptive, anticholinergic, and cholagogue, and they have shown activity against cholecystitis, cholelithiasis, jaundice, dysentery, leishmaniasis, malaria, gallstone, hypertension, ischemic heart disease (IHDS), cardiac arrhythmia, and cardiomyopathy [15–17]. They have also been shown to alleviate diarrhea, fever, and indigestion and stimulate appetite [12].

Different types of seed dormancy exist, influenced by the plant's life cycle, environmental conditions, and geographical distribution [18]. Lang [19] classified seed dormancy into three kinds: eco-dormancy, para-dormancy, and endo-dormancy, whereas Baskin and Baskin [20,21] classified seed dormancy into five kinds by comprehensively considering

physiological and morphological factors: physiological dormancy (PD)—inhibitory compounds inside the embryo prevent germination; morphological dormancy (MD)—the seed has an immature embryo; morphophysiological dormancy (MPD)—a combination of PD and MD; physical dormancy (PY)—inhibition of water absorption by the seed; and combinational dormancy (PY + PD)—a combination of PY and PD. Baskin and Baskin [18] also reported that the seeds of ten species of the *Berberis* genus, namely *B. vulgaris*, *B. buxifolia*, *B. thunbergii*, *B. lycium*, *B. repens*, *B. aristata*, *B. dicrophyla*, *B. dubia*, *B. kansuensis*, and *B. vernae*, have PD. Their dormancy can be broken by cold stratification followed by incubations at 20 °C [22,23].

PD can be further divided into three levels: non-deep, intermediate, and deep. Non-deep PD can be broken with GA_3 treatment. Intermediate PD can be broken by cold stratification treatment for 2–3 months and deep PD can be broken by cold or warm stratification treatment for more than 3–4 months [24].

For the development of varieties and the mass production of plants of the genus *Berberis*, which have high value as medicinal forest resources, various propagation and cultivation studies on wild *Berberis* are necessary. In particular, the propagation method using seeds offers several advantages: it secures a large number of plants at once, reduces labor, and requires fewer auxiliary facilities compared to other methods [25]. However, information regarding the kind of seed dormancy in *Berberis* plants is limited [25–28], and this information is necessary for plant propagation. Therefore, this study aimed to identify the kind of seed dormancy in *B. amurensis* var. *latifolia*, which is native to Korea, to determine the most effective dormancy-breaking conditions.

2. Materials and Methods

2.1. Experimental Materials

Seeds of *B. amurensis* var. *latifolia* used in this study were collected from Namyang-ri, Ulleung-do, Republic of Korea, on 29 October 2019 (Table 1). The collected fruits (about 10,000) were de-pulped by hand, and healthy seeds were collected by eye, ensuring they were free from damage such as scratches on the seed surface, pest damage, or disease. The cleaned seeds were dried for 7 days in a well-ventilated place in the shade, away from direct sunlight. The seeds were stored in a refrigerator (5 °C) until the start of the experiments on 19 December 2019.

Table 1. Location information of *B. amurensis* var. *latifolia* seeds.

Sampling Site	Collection Date	Altitude (m)	Geographical Coordinates
10-12, Namyang 1-gil, Seo-myeon, Ulleung-gun, Gyeongsangbuk-do, Republic of Korea	29 October 2019	3	37°28′00.46″ N 130°50′22.67″ E

2.2. Morpho-Anatomical Characteristics of Seeds

Immediately after collecting the seeds, their surface morpho-anatomical characteristics were investigated by taking pictures using a scanning electron microscope (SEM; CX-200; COXEM, Daejeon, Republic of Korea). In addition, the inner morpho-anatomical features of the seeds were examined after slicing them in half with a double-edged razor blade (stainless steel; Dorco, Seoul, Republic of Korea). Images were captured using a digital microscope (DVM6; Leica, Land Hessen, Germany). We observed changes in the seed coat, embryo, and endosperm before and after seed germination using both SEM and digital microscope. Color analysis of the seed surface and interior was performed using the Royal Horticultural Society color chart (RHS colour chart six edition; Royal Horticultural Society, London, UK).

2.3. Seed Disinfection and Setting

Before the experiment, seeds were immersed in a disinfectant (Benomyl; FarmHannong, Seoul, Republic of Korea) at a concentration of 1000 mg/L for 24 h and then washed with distilled water at least three times. The disinfected seeds were sown in Petri dishes with two sheets of filter paper (Whatman No. 1; GE Healthcare, Buckinghamshire, UK) and 5 mL of distilled water, with 25 seeds per dish and four replicates per treatment.

For GA_3 and cold stratification experiments, the germination percentage was determined by incubating the seeds in a growth chamber (TGC-130H; Espec Mic Corp., Aichi, Japan) at a constant temperature of 20 °C after the GA_3 and low-temperature treatments. During the incubation, any microbial growths that occurred on the seed were removed by immersing the seeds in a disinfectant (Benomyl; Farmhannong, Seoul, Republic of Korea) at a concentration of 1000 mg/L for 24 h in each growth chamber, and distilled water was replenished before the filter paper dried out.

Germination was determined when the radicle pierced the seed coat and protruded by more than 2 mm, and the germination percentage was investigated at weekly intervals. Any seeds that decayed or died during the investigation were immediately removed and included in the germination percentage calculations.

2.4. Water Imbibition Test

To determine if seeds had physical dormancy, we measured water absorption. Two sheets of filter paper (Whatman No. 1; GE Healthcare) were placed in a Petri dish, and distilled water was added. Subsequently, 100 seeds of *B. amurensis* var. *latifolia* were sown in each dish, with three Petri dishes altogether. The initial weight before water absorption and the weights at 3, 6, 9, 12, 24, 36, and 48 h after sowing were measured. The water absorption was calculated using the formula $\%W_s = [(W_h - W_i)/W_i] \times 100$ [29], where W_s is the relative weight ratio of the seeds increased by water absorption, W_h is the weight of the seeds at each time point after water supply, and W_i is the initial weight of the seeds in the dried state.

2.5. A Move-along Experiment

According to Baskin and Baskin [30], the temperature requirements for breaking seed dormancy can be easily estimated by artificially applying temperature changes that are similar to those in the climate. Therefore, we investigated germination according to two temperature change conditions. The treatment temperatures were set as spring (15 °C), summer (25 °C), autumn (20 °C), and winter (5 °C) conditions according to the natural environmental conditions of the four seasons. The treatments were divided into two temperature change conditions: T1 (5→15→20→25 °C), which is the temperature change from winter to spring and summer, and T2 (25→20→15→5 °C), which is the temperature change from summer to autumn and winter. The time spent at each temperature in the T1 and T2 treatments was set to 12, 4, 4, and 12 weeks (Table 2). All treatments were performed with 25 seeds and four replicates, and measurements were performed at weekly intervals. In addition, we cut cross-sections of the seeds at monthly intervals and observed them with a digital microscope to observe changes in the embryo and endosperm according to temperature change.

Table 2. Outline for a modified move-along experiment [30].

		Weeks at Treatment Temperatures					
		4 Weeks	**4 Weeks**	**4 Weeks**	**4 Weeks**	**4 Weeks**	**4 Weeks**
Move along	T1	5 °C (winter)	5 °C (winter)	5 °C (winter)	15 °C (early spring)	20 °C (late spring)	25 °C (summer)
	T2	25 °C (summer)	25 °C (summer)	25 °C (summer)	20 °C (early autumn)	15 °C (early autumn)	5 °C (winter)

2.6. Effect of Cold Stratification on Germination

The seeds that were disinfected and sown in Petri dishes were placed at 5 °C in a growth chamber for 0, 2, 4, 8, and 12 weeks, respectively. After each period of cold stratification, seeds were moved to a growth chamber at 20 °C, and the germination was investigated while culturing them. Germination was monitored weekly for 30 weeks.

2.7. Effect of GA_3 on Germination

To determine the effects of GA_3, a plant growth hormone, on germination of *B. amurensis* var. *latifolia* seeds were immersed in GA_3 solutions at concentrations of 0 (distilled water, control), 10, 100, 500, and 1000 mg/L at room temperature for 24 h. The treated seeds were washed with distilled water more than three times to remove any hormone residue on the seed coat and then disinfected and sown in 25-seed lots with four replicates in a growth chamber at 20 °C. The germination percentage for each treatment was measured at weekly intervals for 30 weeks.

2.8. Effect of Light Conditions on Seed Germination

For the cold stratification, GA_3 treatment, and temperature change experiments, we set up light (12 h/12 h light/dark) and dark (24 h dark) treatments under the same conditions to examine the effect of light conditions on seed germination. The light conditions inside the growth chamber were 12 h light/dark photoperiod with fluorescent lamps at 40 ± 10 μmol/m^2s PPFD. Dark treatments were performed by wrapping the Petri dishes with aluminum foil to completely block access to light. The germination percentage for each treatment was measured at weekly intervals for 30 weeks.

2.9. Phenology of Embryo Growth, Germination, and Seedling Emergence under Natural Environmental Conditions

To observe seasonal changes in seeds in their natural state, we dug the ground to a depth of 5 cm on the slope of a deciduous forest and planted plastic containers. The interior of the plastic containers was filled with topsoil; the mixing ratios of the topsoil were 64.3% coco peat, 15% peat moss, 2.5% zeolite, 10% perlite, 8% zeolite, 0.19% fertilizer, and 0.01% wetting agent. We then created treatment plots for seed germination, emergence, and embryo and endosperm change observation experiments and planted them on plastic containers. The experiment site was located in the nursery area of the National Baekdu Daegan Arboretum on Munsu-ro, Chunyang-myeon, Bonghwa-gun, Gyeongbuk, and the experiment period was 30 weeks (from 19 December 2019 to 9 July 2020).

2.9.1. Embryo Growth

About 400 seeds were wrapped in polyethylene netting with coarse sand and planted 3 cm deep into the topsoil of the plastic containers. Then, every 2–4 weeks, 10 seeds were removed, and changes in the embryo and endosperm were observed. The seeds were surface cleaned with distilled water, cut with a razor blade to visualize the embryo, and photographed under a digital microscope. The E:S ratio, which is the length ratio of the embryo to the seed, was measured from the cross-section of the photographed seed.

2.9.2. Germination

Four sets of 25 seeds each were wrapped in a polyethylene net with coarse sand. Seeds were planted 3 cm deep in the topsoil of the plastic container, and the germination was monitored at weekly intervals. Subsequently, the germinated seeds were removed, the net was resealed, and the seeds were replanted in their original positions.

2.9.3. Seedling Emergence

Twenty-five seeds were planted 2–3 cm deep in plastic pots with a diameter of 7 cm filled with topsoil. Four pots were prepared and planted on a plastic container filled with

topsoil. The heights of the soil in the pot, on the plastic container, and outside the plastic container were adjusted to be the same. Emergence was observed at weekly intervals.

2.10. Statistical Analyses

The results of each experiment were analyzed by one-way ANOVA using SPSS Program (SPSS version 21, SPSS Inc., Chicago, IL, USA), and the statistical significance of the mean differences among treatments was compared by Duncan's Multiple Range Test ($p \leq 0.05$).

3. Results

3.1. Investigation of Morpho-Anatomical Characteristics of Seeds

Seeds were photographed using a scanning electron microscope and a digital microscope to investigate the morpho-anatomical characteristics of *B. amurensis* var. *latifolia* (Figure 1). The color of the seed coat was reddish-brown (RED-PURPLE GROUP 59-A; RHS color chart) based on mature seeds. The results of the SEM showed that the seed surface was wavy (Figure 1a,b). Upon cutting the seeds and observing their morpho-anatomical characteristics using a digital microscope, we found that the embryo size was approximately 60–70% of the seed length at the point of seed abscission (Figure 1d).

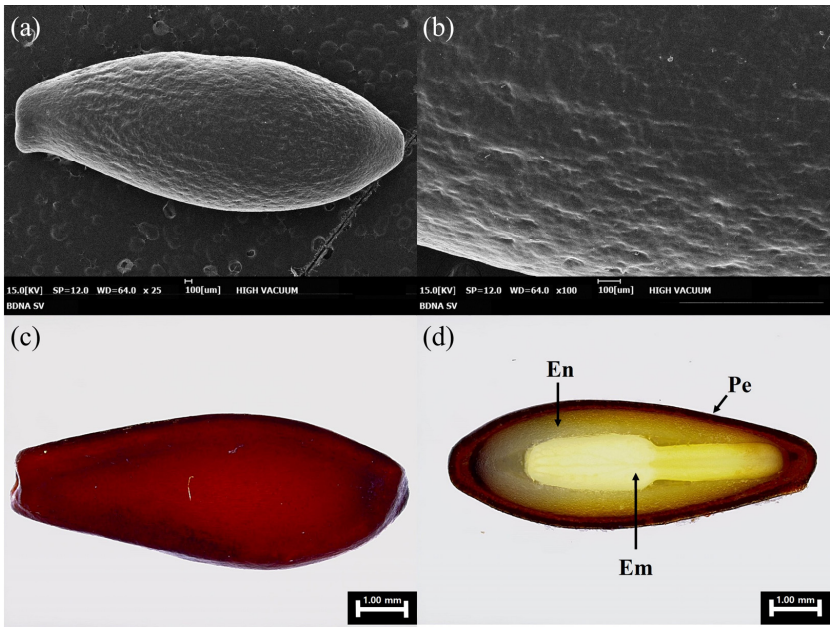

Figure 1. Seed external (**a**–**c**) and internal morphology (**d**) of *B. amurensis* var. *latifolia* seeds. Scale bars are 100 μm (**a**,**b**) and 1.00 mm (**c**,**d**). Em, embryo; En, endosperm; Pe, pericarp.

The measurements of the embryo and seed length showed that the embryo was 5.02 ± 0.07 mm on average, and the seed was 7.67 ± 0.07 mm on average. The E:S ratio (embryo: seed ratio), which is the size ratio of the embryo to the seed, was 0.65 ± 0.003 on average. The color of the embryo in the cross-section of the seed was light yellow (GREEN-YELLOW GROUP 1-D; RHS color chart), and the color of the endosperm was white (GREYED-WHITE GROUP 1-D; RHS color chart) (Figure 1d).

3.2. Water Imbibition Test

A water absorption experiment was conducted to test the imbibition of *B. amurensis* var. *latifolia*. The mass of seeds increased by 26.84 ± 1.18% and 45.81 ± 1.87% compared to their initial dry weight after 24 and 48 h, respectively (Figure 2).

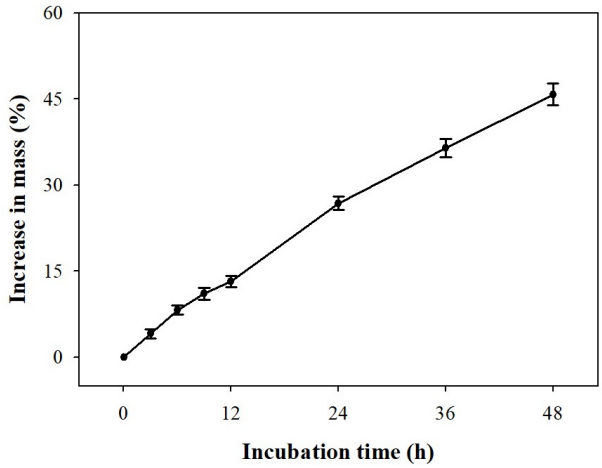

Figure 2. Water absorption by intact seeds of *Berberis amurensis* var. *latifolia*, indicated by a rise in mass, was observed. The seeds were incubated under ambient conditions (22–25 °C) on filter paper dampened with distilled water for 48 h. Vertical error bars denote standard error (n = 3).

3.3. Effect of GA_3 Treatment on Seed Germination

The results of culturing for 30 weeks at 20 °C under light/dark conditions after treatment with different concentrations of GA_3 are as follows. The final germination percentages under light conditions were 6.0 ± 2.58, 1.0 ± 1.00, 1.0 ± 1.00, 1.0 ± 1.00, and 1.0 ± 1.00% for GA_3 concentrations of 0, 10, 100, 500, and 1000 mg/L, respectively. And the final germination percentage under dark conditions were 5.0 ± 1.63, 0, 1.0 ± 1.00, 7.0 ± 3.42, and 3.0 ± 1.00%, respectively. The final germination percentage was <7% in all treatments, and no trend was observed according to the GA_3 treatment concentration (Figure 3).

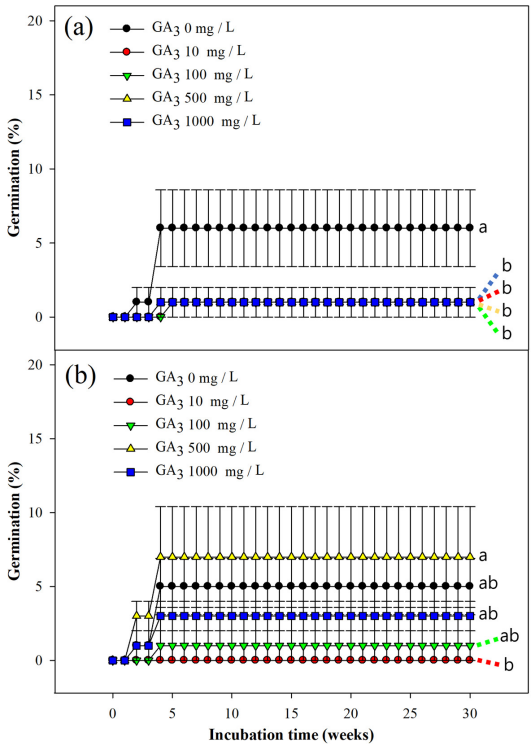

Figure 3. Germination of *B. amurensis* var. *latifolia* seeds as affected by GA_3 treatment. Seeds were soaked in a GA_3 solution for 24 h and then incubated for 30 weeks at 20 °C: (**a**) seeds were subjected to a light/dark cycle of 12 h each; (**b**) seeds were kept in constant darkness for 24 h. Vertical error bars indicate standard error (n = 4). Different lowercase letters indicate significant differences in final germination percentages at $p \leq 0.05$ (Duncan's multiple range test).

3.4. Effect of Cold Stratification Experiment on Seed Germination

The final germination percentage of the 12-week cold stratification treatment was the highest, regardless of light conditions (Figure 4). Moreover, the 12-week low-temperature stratification treatment showed most germination during the 5 °C stratification process. When comparing the light or dark conditions, the final germination percentage under light conditions was 49.0 ± 6.40%, and the final germination percentage under dark conditions was 63.0 ± 3.42%, showing a significant difference.

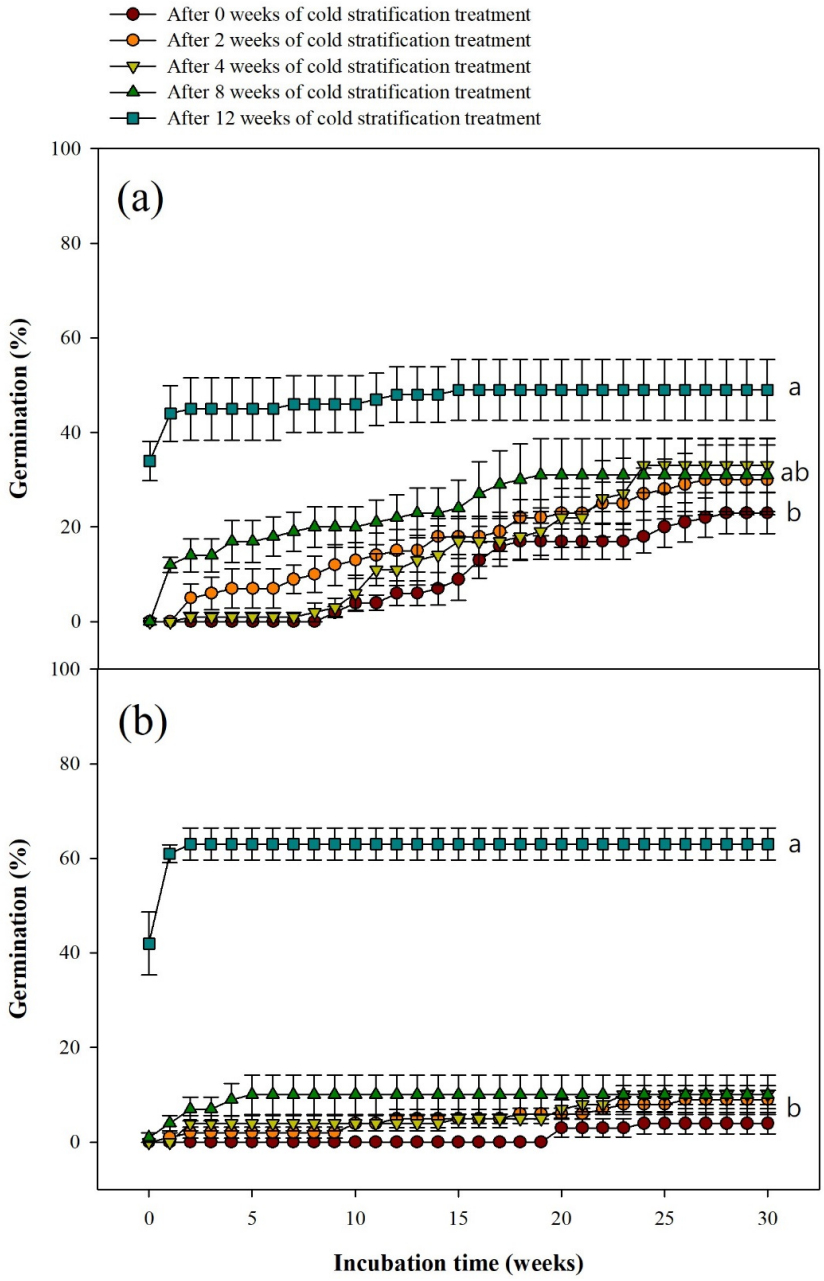

Figure 4. Germination of *B. amurensis* var. *latifolia* seeds subjected to cold stratification (0, 2, 4, 8, or 12 weeks) treatment: (**a**) seeds were subjected to a light/dark cycle of 12 h each; (**b**) seeds were kept in constant darkness for 24 h. Vertical error bars indicate standard error (n = 4). Different lowercase letters indicate significant differences in final germination percentages at $p \leq 0.05$ (Duncan's multiple range test).

3.5. Seed Germination Based on Temperature Conditions: A Move-along Experiment

The results of the move-along experiment were 44.0 ± 4.61% in the T1 (5→15→20→25 °C) treatment and 17.0 ± 4.12% in the T2 (25→20→15→5 °C) treatment, which was a significant difference (Figure 5).

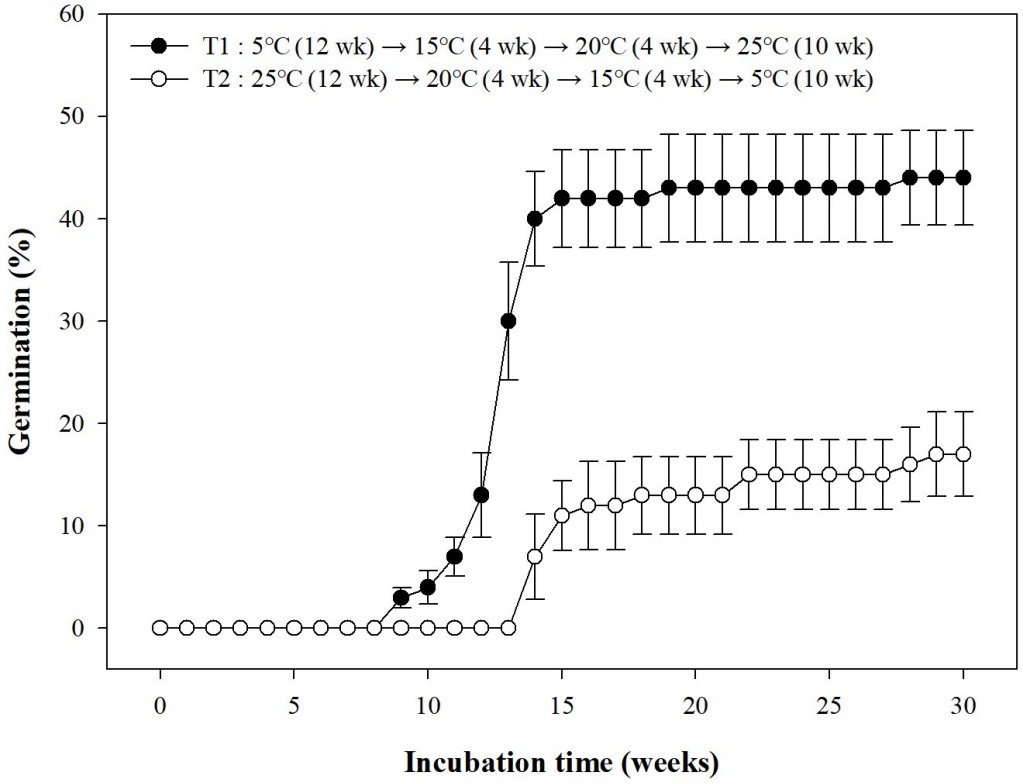

Figure 5. Seed germination of B. amurensis var. latifolia was observed under two different temperature sequences: starting at 5 °C (T1) or 25 °C (T2). Vertical error bars indicate standard error (n = 4).

Seeds of *B. amurensis* var. *latifolia* germinated from the 9th week in T1 treatment under the winter temperature condition (5 °C, 1–12 weeks) and reached 13.0 ± 0.07% germination by the 12th week. They germinated to 42.0 ± 4.76% under the early spring temperature condition (15 °C, 13–16 weeks) and recorded the final germination of 44.0 ± 4.61% at the 28th week after changing to the late spring temperature condition (20 °C, 17–20 weeks) and the summer temperature condition (25 °C, 21–30 weeks).

In the T2 treatment, seeds did not germinate at all under the summer temperature condition (25 °C, 1–12 weeks). They germinated to 12.0 ± 4.32% under the early autumn temperature condition (20 °C, 13–16 weeks) and recorded the final germination of 17.0 ± 4.12% at the 29th week after changing to the autumn temperature condition (15 °C, 17–20 weeks) and the winter temperature condition (5 °C, 21–30 weeks).

3.6. Phenology of Embryo Growth, Germination, and Seedling Emergence

Observation of the seed cross-section at 2–4 week intervals showed no increase in the E:S ratio (Figure 6). Germination started from the 14th week (26 March 2020) and was completed by the 20th week (7 May 2020), showing germination of 80.0 ± 5.16%. Seedling emergence was observed from the 18th week (23 April 2020) and was completed by the 26th week (18 June 2020), showing an emergence of 47.0 ± 3.42% (Figure 7).

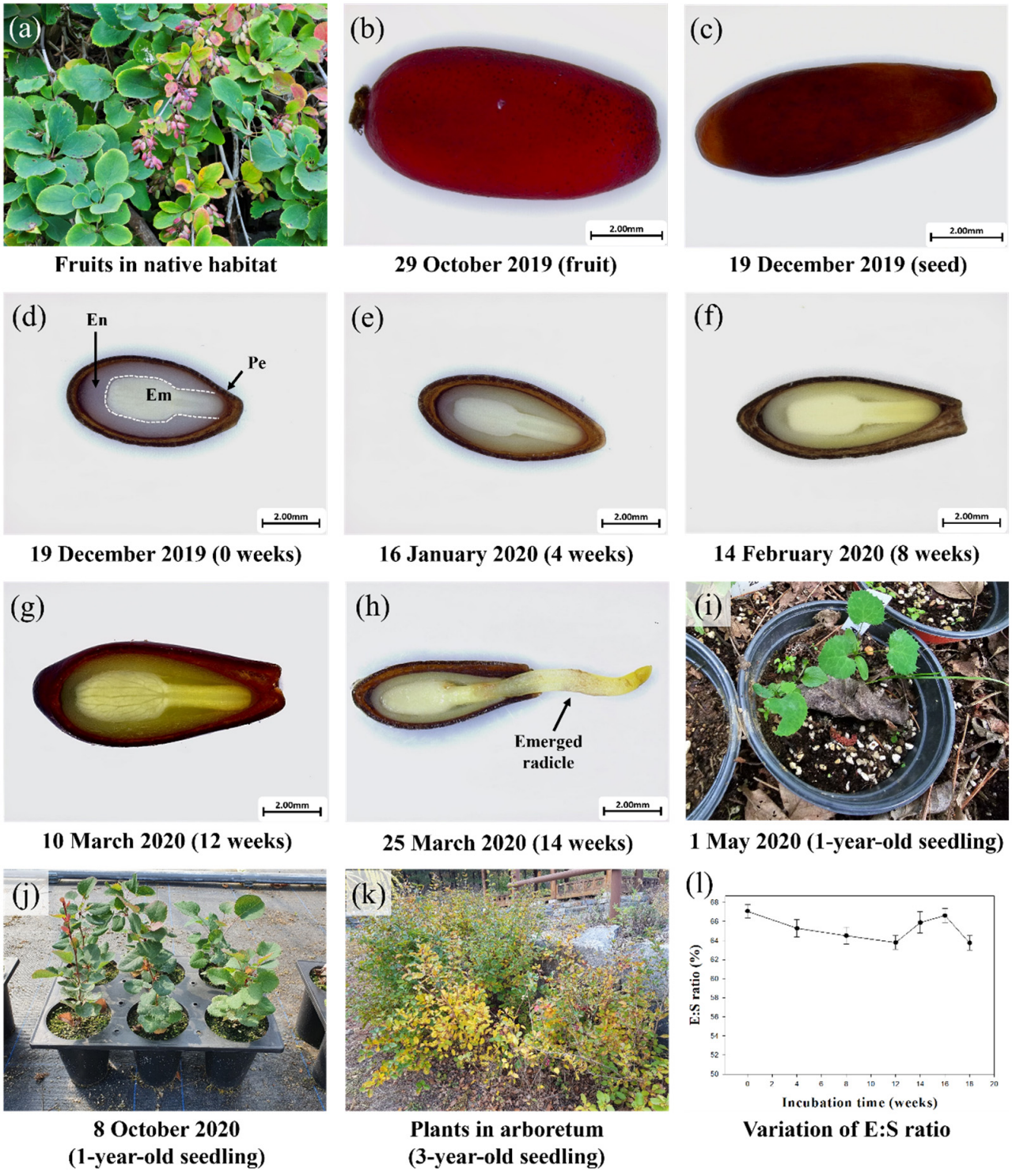

Figure 6. Embryo growth (E:S ratio) and radicle emergence of *B. amurensis* var. *latifolia* seeds kept outdoors in Bonghwa, Republic of Korea, in 2020: (**a**) the fruits of wild individuals of *B. amurensis* var. *latifolia*; (**b**) fruit; (**c**) seed; (**d**–**h**) changes in the embryo during seed germination; (**i**–**k**) the growth of plants of *B. amurensis* var. *latifolia*; (**l**) the variation in the embryo: seed ratio during seed germination. Scale bars are 2.0 mm. Em, embryo; En, endosperm; Pe, pericarp. Vertical bars represent standard error (n = 10).

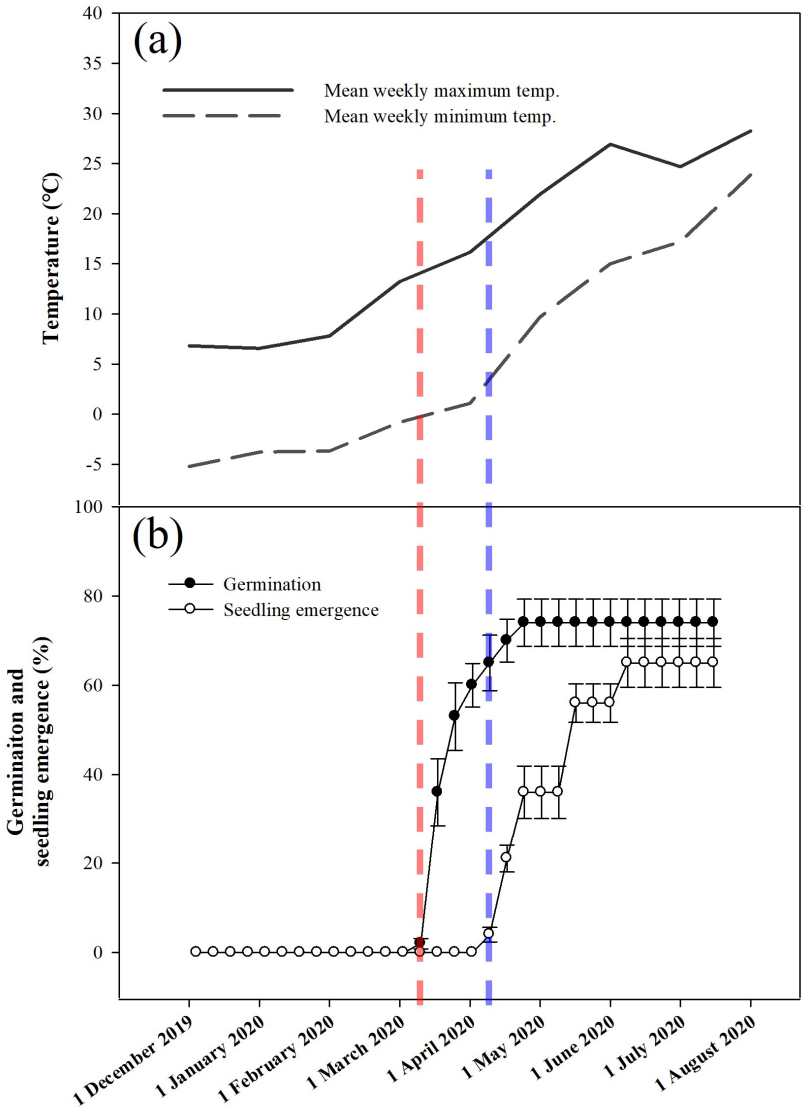

Figure 7. Changes in temperature and phenology of *B. amurensis* var. *latifolia* seeds buried 3 cm deep in 2019: (**a**) average weekly maximum and minimum temperatures; (**b**) germination and seedling emergence. The red line represents the start of germination, and the blue line indicates the start of seedling emergence. Vertical bars represent standard error (n = 10).

4. Discussion

Dried seeds of *B. amurensis* var. *latifolia* showed imbibition by increasing their weight by 26% after 24 h of soaking in water (Figure 2). When the imbibition test was conducted on dried seeds, it was judged that the seeds were imbibed if the mass increase was more than 20% within 24 h, the seeds were non-imbibed if the mass increase was less than 20%, and had physical dormancy (PY) [30]. Therefore, seeds of *B. amurensis* var. *latifolia* were considered imbibed seeds that did not exhibit physical dormancy (PY) or combined dormancy (PY + PD).

In seeds buried on 19 December 2019, and monitored for embryo growth until 25 March 2019, there was no increase in the E:S ratio until the seeds germinated, and no elongation of the embryo was observed (Figure 6). Typically, seeds containing immature embryos elongate within 30 days of cultivation under suitable conditions, a process referred to as morphological dormancy (MD) [18]. Therefore, seeds of *B. amurensis* var. *latifolia* do not have morphological dormancy (MD) and morphophysiological dormancy (MPD).

Most seeds of the genus *Berberis* have physiological dormancy, and that is divided into three types: non-deep, intermediate, and deep PD; non-deep PD can be broken by GA_3

treatment [31], such as *B. vulagaris* seeds dormancy being broken at outdoor temperature conditions [32], and *B. buxifolia* seeds dormancy being broken at 20 °C (or 20/10 °C) temperature conditions and relatively dark environments [33,34]. *B. aristata* seed dormancy is broken at 20 °C temperature conditions [35].

Therefore, seeds of *B. amurensis* var. *latifolia* have physiological dormancy (PD). However, as a result of the GA_3 treatment experiment, all treatments showed a germination percentage of less than 7%, and no effect depending on the GA_3 concentration was observed; therefore, it was judged that they had a deeper level of physiological dormancy than non-deep PD. Seeds of *B. amurensis* var. *latifolia* increased their germination as the period of cold stratification increased, and they recorded the highest germination of 63.0 ± 3.42% at 12 weeks of cold stratification treatment (Figure 4). In earlier research, *B. dicrophylla* and *B. kansuensis* were reported to require 80 days of cold stratification to break seed dormancy [20,36]. Also, the dormancy of *B. dubia* and *B. vernae* seeds was reported to be broken after 168 days of cold wet treatment under 20/15 °C temperature conditions [18], and Deb et al. [27] reported that cold treatment and light effect could break the dormancy of *B. manipurana* seeds. Seeds of *B. amurensis* var. *latifolia* seeds examined in this study also showed that more than 12 weeks of cold stratification was required to break dormancy, similar to previous studies.

According to Baskin and Baskin [24], among the levels of physiological dormancy, intermediate PD can be broken by 2–3 months of cold treatment. Considering that more than 12 weeks (3 months) of cold treatment were effective in the cold treatment experiment and that the germination increased sharply between 3 and 4 months of cold treatment in the phenology experiment, the kind of dormancy in seeds of *B. amurensis* var. *latifolia* seeds is intermediate PD. The research findings were consistent with the results of previous studies conducted on *B. koreana* Palib [25].

However, in the cold treatment experiment, the final germination percentages were 49.0 ± 6.40% in the light condition (light 12 h/dark 12 h) and 63.0 ± 3.42% in the dark condition (dark 24 h), which was a significant difference. Some plants require completely dark conditions for germination; in such cases, they are classified as dark-germinated seeds [18]. For *B. amurensis* var. *latifolia* seeds, the germination percentages increased even under light conditions, so it was not a completely dark germination seed, but it was considered to be a seed that prefers dark conditions because the germination percentage was significantly higher under dark conditions.

In seeds with physiological dormancy (PD), the dormancy can be broken by cold stratification [33]. The results of the germination percentage change experiment based on the move-along experiment showed that seeds of *B. amurensis* var. *latifolia* showed an increase in germination percentage up to 44.0 ± 4.61% in T1 treatment and up to 17.0 ± 4.12% in T2 treatment (Figure 5). Therefore, we conclude that *B. amurensis* var. *latifolia* seeds were effective at breaking dormancy under the cold stratification treatment.

5. Conclusions

To break the seed dormancy of *B. amurensis* var. *latifolia*, cold stratification for more than 12 weeks was required. Also, GA_3 was not effective in breaking dormancy. In this study, only the GA_3 hormone was used to investigate the hormonal effects of GA. In follow-up studies, we suggest using other hormonal treatments such as GA_{4+7}.

After 12 weeks of cold stratification, the most effective way to germinate seeds was to incubate them at 20 °C in the dark for over 2 weeks. Therefore, the seeds of *B. amurensis* var. *latifolia* exhibited an intermediate PD among the seed dormancy types. These results can be used as important basic data for mass propagation of *B. amurensis* var. *latifolia*.

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