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Dormancy breaking and germination requirements of seeds of *Thalictrum uchiyamae* (Ranunculaceae) with underdeveloped embryos

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ABSTRACT

Thalictrum uchiyamae Nakai is an herbaceous perennial plant that has potential for greater utilization for landscaping and medicinal purposes. In the present study, we investigated some of the factors that affect seed dormancy and germination in this species. To determine the temperature requirements for embryo growth and germination in field conditions, the seeds were buried in field soil. Every 1 or 2 weeks, the seeds were exhumed, and embryo growth, germination, and seedling emergence were investigated. In the laboratory, the effects of temperature and GA_3 on dormancy break and germination were investigated in order to characterize the seed dormancy. The seeds had underdeveloped embryos that were physiologically dormant at maturity in late September. In natural conditions, embryo growth and germination occurred in March of the following year, and seedlings emerged in early April. The physiological dormancy of the embryos was broken by cold stratification at 5 °C for 8 weeks, but warm temperatures (25/15 °C) were required to promote embryo elongation even after the physiological dormancy was broken. GA_3 treatment could substitute for the cold stratification requirements and broke seed dormancy of *T. uchiyamae*, 87.3% of which germinated after 8 weeks of incubation at 25/15 °C at 100 mg $\cdot L^{-1}$ GA_3 soaking treatment. These results suggest that the seeds of *T. uchiyamae* exist in a state of non-deep simple morphophysiological dormancy. The temperature requirements for the dormancy break of the seeds enabled the seedlings to emerge in the very beginning of the growing season the following year. These results represent practical knowledge for propagation of these plants from seed.

1. Introduction

The genus *Thalictrum* in the family Ranunculaceae consists of 120–200 species of herbaceous perennial plants distributed primarily in the temperate regions of the world (eFloras, 2008). There are approximately 16 species of *Thalictrum* distributed primarily in the mesic deciduous forests of the Korean Peninsula (Ahn and Lee, 1997; Jeon et al., 2007). Among them, *Thalictrum uchiyamae* Nakai is endemic to Korea (Korea National Arboretum, 2014). *T. uchiyamae* blooms light purple flowers from June to July, and its seeds (achenes) mature to purple brown in the fall (from September to October). *T. uchiyamae* is used as an ornamental plant for gardening and landscape purposes. In addition, the genus *Thalictrum* has great medicinal potential because its members contain high levels of thaliblastine and acutiaporberine, which have anticancer properties (Chen et al., 1993; Chen et al., 2002).

The seeds of several Ranunculaceae species, such as *Delphinium tricorne* (Baskin and Baskin, 1994), *Hepatica nobilis* (Nomizu et al., 2004), *Helleborus niger* (Niimi et al., 2006), and *Aconitum lycoctonum* (Vandelook et al., 2009), have small and underdeveloped embryos at dispersal from their parent plants, meaning that their embryos have to grow to a critical size within the seed before germination can occur (Baskin and Baskin, 1998; Mamut et al., 2014; Vandelook et al., 2008; Vandelook and Van Assche, 2008). If seeds with underdeveloped embryos germinate within 30 days, the seeds are considered to have morphological dormancy (MD) and require no dormancy-breaking pretreatments for germination. If the underdeveloped embryos are dormant at seed dispersal, then the seeds are considered to have morphophysiological dormancy (MPD) (Baskin and Baskin, 1998; Nikolaeva, 1977). MPD can be broken by warm (≥15 °C) or cold (0–10 °C) stratification or a sequence of warm followed by cold temperature (Baskin and Baskin, 1998).

There are only a few reports on seed dormancy or the germination process from sowing in *Thalictrum* species. A previous study showed that no *T. occidentale* seeds germinated after moist cold stratification for

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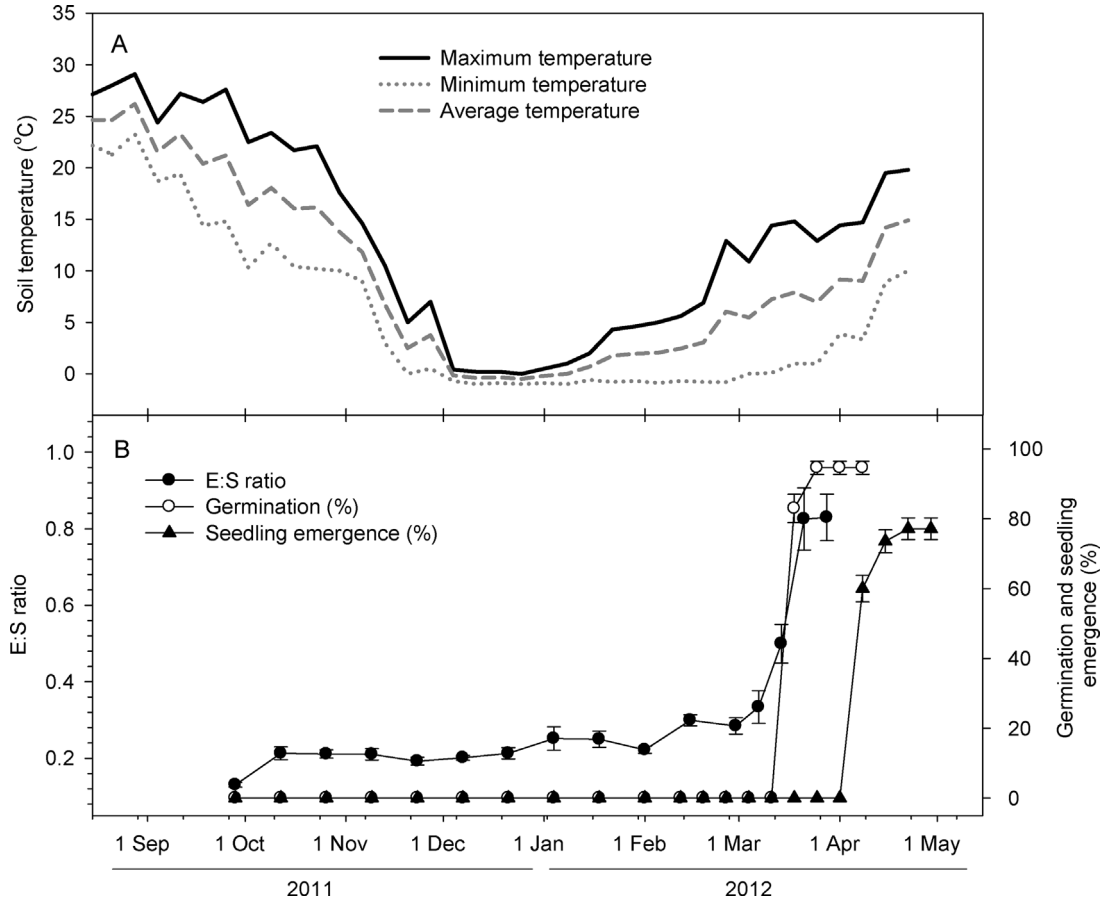

Fig. 1. Mean weekly maximum, minimum, and average soil temperatures (A), and phenology of embryo growth, germination, and seedling emergence (B) of *Thalictrum uchiyamae* seeds buried at a depth of 3 cm in 2011–2012. The E:S ratio is the ratio of embryo length to seed length. Vertical bars represent SE (n = 20 for embryo growth, n = 3 for germination and seedling emergence).

18 weeks at 5 °C (Kaye, 1997). The germination percentage of *T. fendleri* was previously found to be 28% after moist cold stratification for 120 days at 4 °C (Hoffman, 1985). The germination percentages in *T. simplex* var. *brevipes* seeds reached 42% following cold stratification for 6 months at 4 °C (Washitani and Masuda, 1990). Walck et al. (1999) reported that *T. mirabile* seeds had underdeveloped embryos and MPD at seed dispersal, and the seeds germinated to 95% following 12 weeks of cold stratification at 1 °C, whereas germination was observed in only 25% of those cold stratified for 12 weeks at 5 °C. The results of our previous study showed that the seeds of *T. uchiyamae* had underdeveloped embryos and experienced deep dormancy, demonstrating 3.3% germination after 4 weeks of incubation at 25/15 °C (data not shown).

The germination of *T. uchiyamae* seeds has only been partially studied, and detailed information is lacking on the growth of underdeveloped embryos, type of seed dormancy, practical dormancy-breaking techniques to promote germination for commercial plant propagation, and optimum conditions for germination. Therefore, the objective of the present study was to characterize seed dormancy in *T. uchiyamae*. We investigated the phenology of embryo growth, germination, and seedling emergence in the natural environment. We also investigated the effects of warm and/or cold stratification on embryo growth and germination in a laboratory experiment. In addition, we analyzed dormancy break of the seeds after gibberellic acid treatment, which has been used to break seed dormancy in many wild plants.

2. Materials and methods

2.1. Seed collection

Seeds (achenes) of *T. uchiyamae* were collected from plants growing in the Hantaek Botanical Garden (37°09′ N, 127°40′ E, Yongin, Korea) between 24th and 30th of September 2011. The seeds were air-dried under laboratory conditions (20–25 °C) for 1–2 weeks, packed in sealed plastic bags, and stored at 5 °C until needed for the experiments.

2.2. Phenology of embryo growth, germination, and seedling emergence

The seeds were buried at a depth of 3 cm in field soil (almost loamy sand) in an experimental garden at Seoul National University (37°46′ N, 126°95′ E) in Seoul, Korea, on September 28, 2011. The phenology of dormancy break was monitored from September 28, 2011 to April 29, 2012. The buried seeds were placed in a shady site closed to a plot of *Syringa oblata* var. *dilatata* (Nakai) Rehder. Therefore, the direct sunlight did not reach the soil surface. The soil temperature at a depth of 3 cm was recorded every 30 min with a thermo-data logger (Watch Dog Model 450, Spectrum Technologies, Plainfield, IL, USA), and weekly maximum, minimum, and average temperatures were calculated.

A fine-mesh polyester bag filled with a total of 400 seeds and potting soil (Plant World, Nongwoo Bio, Suwon, Korea) was buried in a tray filled with the same potting soil. In the fine-mesh polyester bag, each of the 20 seeds was sown in white sand, covered with a net, and mixed with potting soil in order to easily distinguish the seeds from the soil. The potting soil was composed of peat moss (25%), cocoa peat (40–50%), perlite (10–14%), vermiculite (8–10%), and zeolite

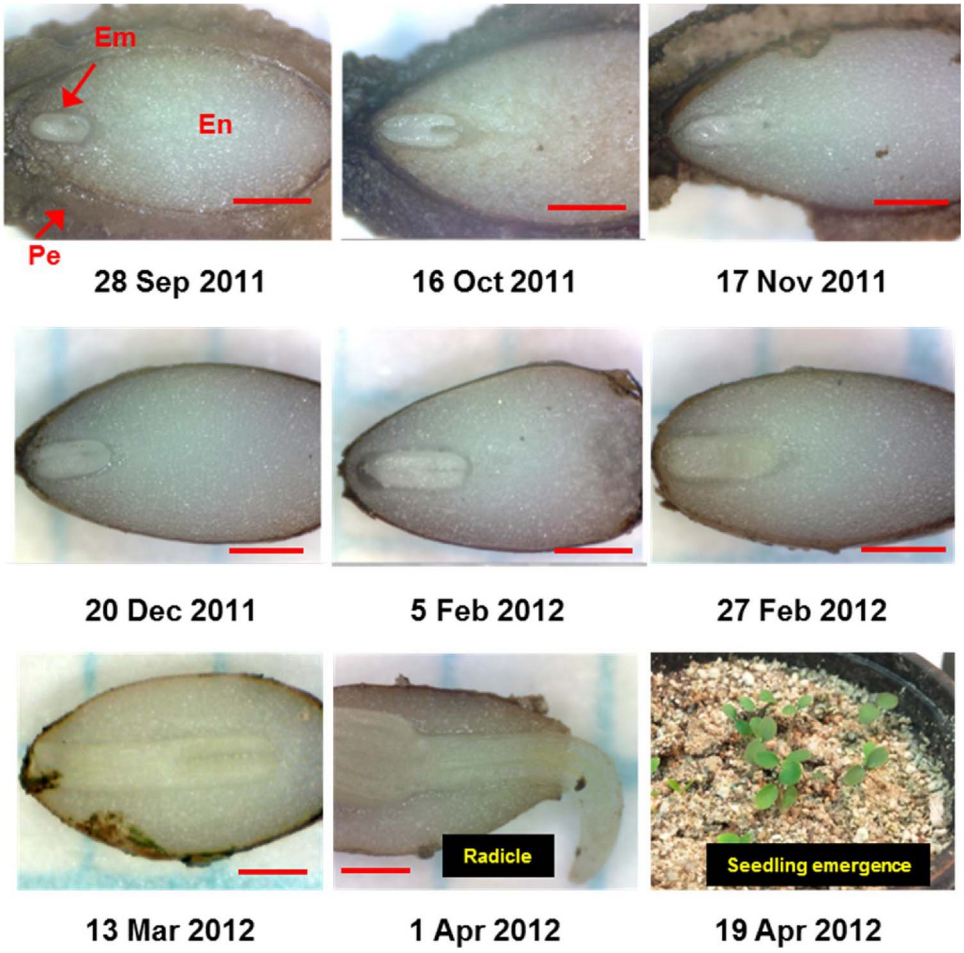

Fig. 2. Embryo growth, radicle emergence, and seedling emergence in *Thalictrum uchiyamae* seeds kept outdoors in Seoul, Korea, in 2011 – 2012. Scale bars are 0.5 mm. Em, embryo; En, endosperm; Pe, pericarp.

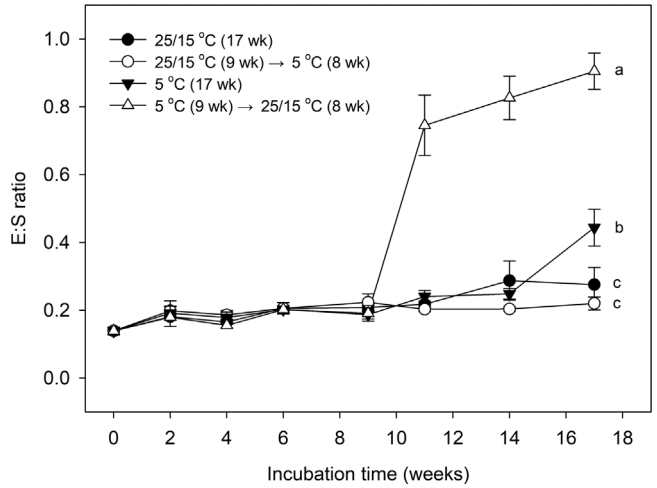

Fig. 3. Embryo growth of *Thalictrum uchiyamae* seeds as affected by temperature treatments. Vertical bars represent SE (n = 3). Final E:S ratio among the temperature sequences followed by different letters indicate significant differences (HSD test, $p < 0.05$).

(8–13%). The trays were placed at ground level in the experimental garden. Every 1 or 2 weeks, the bag was exhumed, and a net filled with 20 seeds was selected at random for embryo growth measurements. The embryo length was measured from September 28, 2011 to March 28, 2012. The seeds were cut into thin sections using a razor blade, and the lengths of seeds and embryos were measured under a dissecting microscope fitted with an ocular micrometer (KSZ-1B, Samwon Scientific, Seoul, Korea). The average ratio of embryo length to seed length (E:S ratio) was determined. Germinated seeds were assigned an E:S ratio of 1. The term critical length referred to the embryo size just before radicle protrusion from the seeds. After measuring the lengths of seeds and embryos using the dissecting microscope, sections of the seeds were viewed at 60 × to 120 × magnification and photographed using a MiView USB digital microscope (MV 1302U, CosView Technologies, Shenzhen, China).

Three replicates of 30 seeds were sown in white sand, covered with a net, placed in 10-cm plastic pots filled with the potting soil, and then placed in trays filled with the same potting soil. The trays were placed at ground level in the experimental garden. The seeds with an emerged radicle were counted and removed every one or two weeks from September 28 to April 15, 2012. A seed was considered to be 'germinated' when the radicle reached at least 1 mm in length. Intact seeds that had not germinated were reburied in the field.

The timing of seedling emergence was monitored by sowing three replicates of 30 seeds at a depth of 3 cm in each of the plastic pots filled with the potting soil and then placed in the trays as described above. The pots were covered with a net in order to avoid damage by birds and insects. The emerged seedlings were counted and removed every 1 or 2 weeks during the field experiment.

2.3. Laboratory experiments

For the laboratory experiments, seeds were soaked in a solution of 500 mg·L^{-1} benomyl (Dongbu HiTek, Bucheon, Korea) for 3–4 h for bacterial control. Unless otherwise stated, each of the three replicates of 30 seeds was used. The seeds were placed on two sheets of filter paper (Whatman No. 2) in 90 × 15 mm petri dishes and moistened with

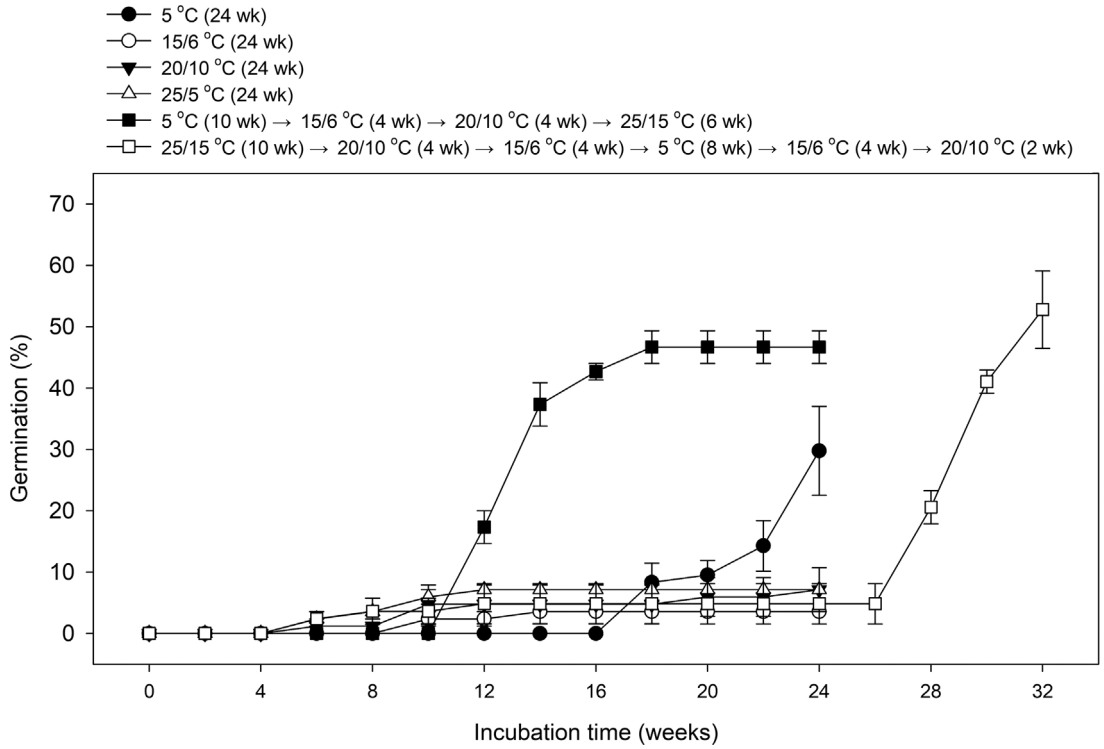

Fig. 4. Germination in seeds of *Thalictrum uchiyamae* incubated under a constant temperature or a temperature sequence beginning at 25/15 °C or at 5 °C. Vertical bars represent SE (n = 3).

Table 1
Effect of warm and/or cold stratification periods on percentage germination of *Thalictrum uchiyamae* seeds. The seeds were stratified at 5 °C or 1 °C for 0, 4, 8, or 12 weeks and then incubated at 25/15 °C for 12 weeks. In the other two treatments, the seeds were cold-stratified at 5 °C or 1 °C for 12 weeks following 6 weeks of warm-stratification at 25/15 °C and then were incubated at 25/15 °C for 12 weeks.

Stratification weeks at			Incubation time after warm and/or cold stratification (weeks)			
25/15 °C	5 °C	1 °C	2	4	6	12
			% germination			
0	0	0	0.0 c[a]	0.0 e	4.4 d	11.1 e
0	4	0	8.4 c	27.0 d	35.5 c	36.7 de
0	8	0	66.4 a	74.4 a	77.1 ab	77.1 abc
0	12	0	56.3 a	70.7 ab	72.0 ab	73.1 abc
0	0	4	7.4 c	50.0 bc	56.7 bc	61.1 bcd
0	0	8	23.2 b	36.8 cd	40.5 c	41.6 d
0	0	12	12.1 bc	39.8 cd	51.9 bc	51.9 cd
6	12	0	55.5 a	76.0 a	77.7 ab	84.0 ab
6	0	12	68.4 a	87.5 a	88.6 a	88.6 a
Significance			***	***	***	***

NS,*,**,***:non-significant or significant at $p < 0.05$, 0.01 and 0.001, respectively.
[a] Mean separation within columns according to Tukey's honestly significant difference test at $p < 0.05$.

distilled water. All of the dishes were wrapped with a parafilm to prevent water loss during incubation. In all of the temperature regimes, a 12-h light/dark photoperiod was provided using cool white fluorescent lamps that produced a photon flux density of approximately 30–40 μmol·m^{-2}·s^{-1} at the positions of the petri dishes in the incubator (DS-13MCLP, Dasol Scientific, Hwaseong, Korea). Radicle emergence was monitored in order to calculate percentage of germination, and the germinated seeds were removed.

2.3.1. Effects of temperature sequences on embryo growth

Twenty seeds were sown in each of the 90 mm × 15 mm petri dishes that were filled with potting soil (dark conditions), moistened with distilled water, and incubated in the specified temperature sequence. The effects of four different temperature sequences on embryo growth were tested. Each of the temperature sequence treatments included 7 petri dishes. The temperatures of the seven petri dishes were maintained at 5 °C or 25/15 °C (day/night) for 17 weeks.

Another group of 7 petri dishes was treated with the following two temperature sequences: (i) a warm temperature (25/15 °C) for 9 weeks and then cold temperature conditions (5 °C) for 8 weeks; (ii) a cold temperature (5 °C) for 9 weeks and then warm temperature conditions (25/15 °C) for 8 weeks. Every 2 or 3 weeks, 20 randomly selected seeds from each of the petri dishes were cut into thin sections using a razor blade, and the E:S ratio was calculated as described above.

2.3.2. Effects of temperature sequences on dormancy break and germination

Each of the three replicates of 30 seeds was incubated at one of the four controlled temperatures (5, 15/6, 20/10, or 25/15 °C) for 24 weeks. Another group of three petri dishes was subjected to two temperature sequences: (i) warm temperature conditions (25/15 °C) for 10 weeks followed by slightly cooler temperatures (20/10 °C) for 4 weeks, then further reduced temperature conditions (15/6 °C) for 4 weeks, followed by cold conditions (5 °C) for 8 weeks, and then the seeds were slowly returned to 15/6 °C for 4 weeks and 20/10 °C for 2 weeks; or (ii) beginning with cold conditions (5 °C) for 10 weeks, followed by slowly warming the temperature to 15/6 °C for 4 weeks, then 20/10 °C for 4 weeks, and 25/15 °C for 6 weeks.

2.3.3. Effects of cold and/or warm stratification period on dormancy break and germination

The seeds were stratified at 5 °C or 1 °C for 0, 4, 8, or 12 weeks and then incubated at 25/15 °C for 12 weeks. *T. uchiyamae* seeds in the field are dispersed from late summer to early autumn, suggesting the need for a period of warm temperatures (≥15 °C) before cold stratification during winter to break dormancy in the natural environment. Therefore, the seeds were placed at 25/15 °C (warm stratification) for 6

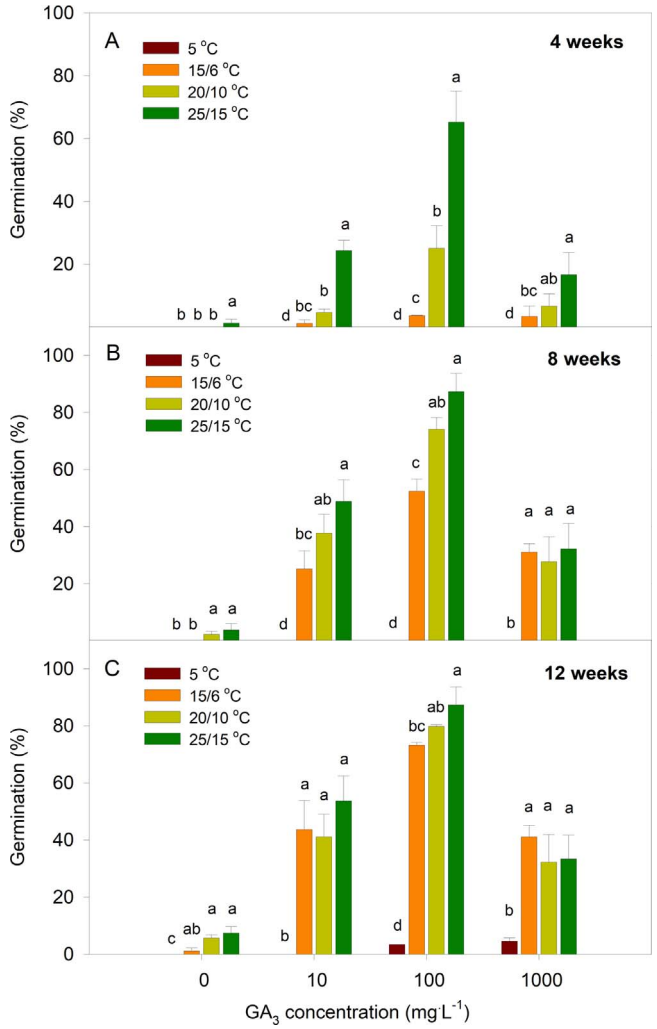

Fig. 5. Effect of GA_3 (0, 10, 100, or 1000 mg·L^{-1}) treatment on germination percentages of *Thalictrum uchiyamae* seeds after 4 (A), 8 (B), and 12 (C) weeks of incubation at different temperature regimes. Vertical bars represent SE (n = 3). Bars with the same letter within a temperature condition are not significantly different (HSD test, $p < 0.05$).

weeks and then stratified at 5 °C or 1 °C (cold stratification) for another 12 weeks. Following each of the stratification periods, the seeds were incubated at 25/15 °C for 12 weeks, and the germination percentages were measured at 2, 4, 6, and 12 weeks of incubation.

2.3.4. Effects of GA_3 treatment on dormancy break and germination under various incubation temperatures

The seeds were soaked in solutions at concentrations of 0 (with distilled water, control), 10, 100, or 1000 mg·L^{-1} GA_3 for 24 h at room temperature (20–25 °C) and then incubated at 5, 15/6, 20/10, or 25/15 °C. Germination percentages were calculated after 4, 8, and 12 weeks of incubation.

2.4. Statistical analysis

All of the laboratory experiments were conducted with a completely randomized design with three replicates. The effects of cold and/or warm stratification periods on germination percentages were analyzed statistically using the general linear model (GLM) procedure of the SAS program (SAS Institute, Cary, NC, USA). Mean values were compared using Tukey's honestly significant difference (HSD) test at the 5% level in order to compare the treatment differences.

3. Results

3.1. Phenology of embryo growth, germination, and seedling emergence

Ripe seeds of *T. uchiyamae* had an initial E:S ratio of 0.13. The embryos grew very little from September 28, 2011 to February 29, 2012 (Figs. 1 and 2). However, the embryos grew rapidly from March 7th to 21st 2012; during this time, the mean weekly maximum, average, and minimum soil temperatures were 14.3, 7.1, and −0.5 °C, respectively. In the seeds exhumed on March 21, 2012, the embryos had grown to the critical E:S ratio (0.83 ± 0.08) required for germination. For seeds buried in 2011, no germination was observed until March 11, 2012, when the mean weekly maximum, average, and minimum soil temperatures were 14.4, 7.5, and −0.1 °C, respectively (Fig. 1). The germination percentages started to increase between March 11th and March 18th, 2012. By March 25, 2012, 94.6% of the seeds had germinated. The first newly emerged seedlings of *T. uchiyamae* were observed on April 8, 2012 and the last on April 22, 2012; during this time, the mean weekly maximum, average, and minimum soil temperatures were 19.5, 13.9, and 8.5 °C, respectively. During this 2-week period, a total of 77.1% of the seeds produced seedlings (Fig. 2). Therefore, it took approximately 7 months to produce seedlings in the natural environment.

3.2. Effects of temperature sequences on embryo growth

None of the seeds that were incubated at 25/15 °C or 5 °C reached the critical E:S ratio (0.83 ± 0.08) after 17 weeks of incubation (Fig. 3). After this incubation period, the E:S ratios of the seeds incubated at 25/15 °C or 5 °C were 0.28 ± 0.05 and 0.44 ± 0.05, respectively. Although the embryos in a few of the seeds incubated continuously at 5 °C reached the critical E:S ratio (0.8 ± 0.08) after 17 weeks, the embryos in most of the seeds did not grow to the critical E:S ratio. A rapid increase in embryo length was observed upon transfer from 5 °C to 25/15 °C (Fig. 3). When the seeds were moved to 25/15 °C following cold stratification at 5 °C for 9 weeks, the E:S ratio increased from 0.19 ± 0.02 to 0.75 ± 0.09 within 2 weeks of incubation.

3.3. Effects of temperature sequences on dormancy break and germination

The seeds incubated at 5 °C began to germinate after 18 weeks, and the final germination percentage was 29.8% after 24 weeks of incubation (Fig. 4). Only 3.6, 7.1, or 7.1% of the seeds germinated at 15/6, 20/10, or 25/15 °C, respectively, after 24 weeks of incubation. In the temperature sequence that began at 5 °C, none of the seeds maintained continuously at 5 °C germinated; however, the germination percentage increased to 46.7% when they were subjected to gradually increasing temperature for 14 weeks. In the temperature sequence that began at 25/15 °C, the germination percentage was 4.9% for 24 weeks. However, the germination percentage increased from 4.9 to 52.8% when the seeds were heated from 5 to 20/10 °C during incubation.

3.4. Effects of cold and/or warm stratification periods on dormancy break and germination

Warm and/or cold stratification pretreatments had a significant effect on dormancy break and germination (Table 1). Only 11.1% of the seeds germinated after 12 weeks of incubation without a cold stratification pretreatment. On the other hand, 77.1 and 73.1% of the seeds germinated after 12 weeks of incubation at 25/15 °C following 8 or 12 weeks of cold stratification at 5 °C, respectively. After 4, 8, or 12 weeks of cold stratification at 1 °C, seed germination percentage was 61.1, 41.6, or 51.9% after 12 weeks of incubation at 25/15 °C. When the seeds were warm stratified at 25/15 °C for 6 weeks before they were transferred to 5 or 1 °C conditions for cold stratification, 84.0 and 88.6% of the seeds germinated after 12 weeks of incubation at 25/

15 °C.

3.5. Effects of GA_3 on dormancy break and germination under various incubation temperatures

In all of the temperature regimes, the final germination percentages of the *T. uchiyamae* seeds increased with increasing concentration of GA_3 from 0 to 100 mg·L^{-1}, whereas the percentage was low in the 1000 mg·L^{-1} GA_3 treatment (Fig. 5). At 4 weeks after incubation, 65% of the seeds treated with 100 mg·L^{-1} GA_3 germinated at 25/15 °C (Fig. 5A). After 12 weeks of incubation, more than 73% of the seeds treated with 100 mg·L^{-1} GA_3 germinated at 15/6, 20/10, and 25/15 °C of incubation, whereas only a few of the seeds (less than 5%) germinated at any of the GA_3 concentrations during incubation at 5 °C (Fig. 5C).

4. Discussion

The fresh seeds of *T. uchiyamae* had an E:S ratio of 0.13, which increased to 0.82 prior to radicle emergence (Fig. 1). Therefore, the seeds of *T. uchiyamae* had underdeveloped embryos. Baskin and Baskin (1998) documented that seeds with an underdeveloped embryo did not germinate within 30 days in cases when an additional physiological mechanism delaying radicle emergence was present. Since the seeds did not germinate for several months in natural environments and did not germinate in 25/15 °C conditions for 4 weeks in laboratory experiments (Figs. 1 and 4), we concluded that the seeds of *T. uchiyamae* had MPD.

Nine levels (types) of MPD have been proposed based on temperature requirements for embryo growth, warm and/or cold stratification for germination, and responses of seeds to gibberellic acid (GA_3) pretreatments (Baskin and Baskin, 2004, 2014). The nine levels of MPD can be sub-divided into two categories: simple and complex (Baskin and Baskin, 2004; Walck et al., 1999). In the simple levels of MPD, embryos grow at warm temperatures (≥15 °C), and some or all of the physiological dormancy (PD) is broken prior to embryo elongation. On the other hand, low temperatures (0–10 °C) are required for embryo elongation in the complex levels of MPD (Baskin and Baskin 2004, 2014). Although embryo growth was more highly promoted under incubation at continuous 5 °C than at continuous 25/15 °C for 17 weeks, the embryos did not reach the critical length required for germination in the laboratory conditions (Fig. 3). On the other hand, when the fresh seeds of *T. uchiyamae* were moved from 5 °C to 25/15 °C, the embryos grew rapidly at 25/15 °C. Therefore, we concluded that the seeds of *T. uchiyamae* had a simple type of MPD.

There are three levels of simple MPD, namely, non-deep, intermediate, and deep. An important consideration when trying to classify the level of simple MPD is the warm and/or cold stratification requirements for dormancy break and the response of seeds to gibberellic acid pretreatment (Baskin and Baskin 2004, 2014; Walck et al., 1999). In non-deep simple MPD, only warm or cold stratification is required for dormancy break and germination, and GA_3 pretreatment can overcome the dormancy. On the other hand, seeds require warm followed by cold temperature sequences in both intermediate and deep simple MPDs (Baskin and Baskin, 2004; Rhie et al., 2015; Vandelook et al., 2009; Vandelook and Van Assche, 2009). GA_3 pretreatment is known to promote germination of seeds with intermediate simple MPD but not those with deep simple MPD (Baskin and Baskin, 2004, 2014). In the seeds of *T. uchiyamae*, germination was promoted at warm temperatures following cold stratification at 5 °C (Fig. 4 and Table 1). In addition, the germination of *T. uchiyamae* seeds was promoted by GA_3 treatment (Fig. 5). After a 100 mg·L^{-1} GA_3 application, 65.2 or 87.3% of the seeds germinated at 25/15 °C after 4 or 8 weeks of incubation, respectively. Therefore, we concluded that the seeds of *T. uchiyamae* had non-deep simple MPD. In other species of *Thalictrum*, cold stratification (5 or 1 °C) successfully overcame seed dormancy (Lee et al., 2015; Walck et al., 1999). In *T. mirabile*, seed germination reached 94% after 2 weeks of incubation at 25/15 °C following 12 weeks of cold stratification at 1 °C (Walck et al., 1999). In *T. rochebrunianum*, 89% of seeds germinated after 2 weeks of incubation at 25/15 °C following 4 weeks of cold stratification at 5 °C (Lee et al., 2015).

T. mirabile is native to eastern North America, whereas both *T. rochebrunianum* and *T. uchiyamae* are native to eastern Asia. However, it appears that these three eastern North America–eastern Asia disjuncts exhibit the same dormancy type (non-deep simple MPD). Ricklefs and Latham (1992) reported that trait stasis related to ecological distribution had occurred because congeners between eastern North America-eastern Asia of herbaceous perennials had a significant correlation in area of geographic range. Baskin and Baskin (2014) reported that the type of seed dormancy and germination requirements are important traits that influence the ecological and geographic distributions of plants.

Based on the experiments of Walck et al. (1999), the seeds of *T. mirabile* incubated at 25/15 °C following 12 weeks of cold stratification at 1 °C experienced 94% germination, whereas those incubated at 25/15 °C following 12 weeks of cold stratification at 5 °C showed a germination percentage of only 25%. In our study, however, a cold stratification temperature of 5 °C was more effective than one at 1 °C for dormancy break and germination of *T. uchiyamae* seeds. Interspecific variation in germination requirements has been reported in *Corylopsis* species (Rho et al., 2008), *Crocus* species (Carta et al., 2014), and *Stellaria* species (Vandelook et al., 2008).

Germination percentage was higher in warm (6 weeks at 25/15 °C) followed by cold (12 weeks at 1 or 5 °C) stratification sequences than with only cold stratification for 12 weeks at 1 or 5 °C (Table 1). Although 77.1 or 73.1% of the seeds germinated after 12 weeks of incubation at 25/15 °C following 8 or 12 weeks of cold stratification at 5 °C, respectively, a higher germination percentage occurred when the seeds were warm-stratified for 6 weeks at 25/15 °C before being cold-stratified at 1 or 5 °C. In nature, the seeds of *T. uchiyamae* are exposed to a period of relatively high temperatures in late summer and early autumn, followed by a period of low temperatures in winter. These results indicate that, although most of the seeds in *T. uchiyamae* require only cold stratification to break dormancy, a period of relatively high temperatures in late summer and early autumn might play a role in promoting dormancy break and germination.

5. Conclusions

The seeds of *T. uchiyamae* have underdeveloped embryos upon dispersal from their parent plants, and they exhibit non-deep simple MPD. The seed dormancy was broken by 8 weeks of cold stratification at 5 °C. GA_3 treatment substituted for cold stratification requirements: 87.3% of the seeds germinated after 8 weeks of incubation at 25/15 °C after a GA_3 soaking treatment at 100 mg·L^{-1}. The information obtained in this study could be used for commercial propagation of this ornamental plant.

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