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Research Report

Non-deep Simple Morphophysiological Dormancy in Seeds of *Thalictrum rochebrunianum*, an Endemic Perennial Herb in the Korean Peninsula

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Abstract. The aims of this study were to determine the requirements for dormancy break and germination and to characterize the type of seed dormancy of *T. rochebrunianum*. Ripe seeds (achenes) were collected in late Sept. To determine the temperature requirements for embryo growth and germination in the field, the seeds were sown in field soil. Every 1 or 2 weeks, the seeds were exhumed, and the phenology of embryo growth, germination and seedling emergence were investigated. In the laboratory, effects of temperature and GA_3 treatment on embryo growth and germination were also investigated to characterize the type of seed dormancy. Seeds had underdeveloped embryos, which were about 20% of the size of those in fully matured seeds. In natural conditions, embryo growth and germination occurred in early and late March next year, respectively, and embryos in the seeds of this species had to grow to a critical length before germination was possible. Thus, the seeds had morphological dormancy (MD). When tested at the time of dispersal, few seeds germinated after 4 weeks of incubation at 25/15°C. Therefore, the seed dormancy type for *T. rochebrunianum* seemed to be morphophysiological. Embryos in the seeds grew rapidly at warm temperature conditions following cold stratification at low temperatures. After 4-12 weeks of cold stratification at 1°C or 5°C, the seeds germinated rapidly during an incubation at 25/15°C. Cold stratification requirements could be substituted by GA_3 treatment in seeds of *T. rochebrunianum.* After 1000 $mg \cdot L^{-1}$ GA_3 treatment, high percentages (≥ 80%) of the seeds germinated after 4 weeks of incubation at 15/6, 20/10, and 25/15°C. Consequently, seeds of *T. rochebrunianum* could be characterized as expressing non-deep simple morphophysiological dormancy (MPD). The cold stratification requirements for dormancy break enabled the seeds to produce seedlings at the beginning of the growing season in natural conditions.

***Additional key words*:** ecophysiology, seed dormancy, seedling emergence, underdeveloped embryos

Introduction

Dormancy is very common in seeds of native wild flowers of the Northern Hemisphere and it takes various periods of time from several weeks to more than a year to break the dormancy (Geneve, 2003; Heather et al., 2010). Although seed dormancy is an important plant trait from ecological perspective, it can impede the propagation and production of wild species that would otherwise have potential in horticultural markets.

Seeds of many species in Amaryllidaceae, Apiaceae, Berberidaceae, Caprifoliaceae, Hyacinthaceae, Liliaceae, Melanthiaceae, and Ranunculaceae have small, underdeveloped embryos at dispersal, meaning that they must grow within the seed before germination occurs (Baskin and Baskin, 1998; Mamut et al., 2014; Vandelook and van Assche, 2008). If seeds have underdeveloped embryos at dispersal and germinate in about 30 days or less, the seeds will have morphological dormancy (MD) and require no dormancy-breaking pretreatments for germination (Baskin and Baskin, 1998; Nikolaeva, 1977). On the other hands, if seeds with underdeveloped embryos need relatively long time from 30 days to several months to complete germination and seedling emergence, the seeds have both morphological and physiological dormancy which is known as morphophysiological dormancy (MPD) (Baskin and Baskin, 1998; Nikolaeva, 1977). To break MPD, warm (≥ 15°C) and/or cold (0-10°C) stratification is needed (Baskin and Baskin, 2004). Nine different types (levels) of MPD have been proposed, based on the temperature requirements for embryo growth, breaking of physiological dormancy (PD), and the ability of gibberellic acid to overcome dormancy (Baskin and Baskin, 2004, 2014).

The genus *Thalictrum* (Ranunculaceae) includes 120-200 species of herbaceous, perennial flowering plants mostly native to temperate regions (eFloras, 2008). Jeon et al. (2007) reported that 16 species of *Thalictrum* are distributed throughout the Korean Peninsula. Among them, *T. rochebrunianum* var. *grandisepalum* (H. Lev.) Nakai has been known as an endemic perennial species on the Korean Peninsula (Korea National Arboretum, 2014). It grows in mesic temperate deciduous forest in Korea except on Jeju Island, which has a mild oceanic climate and the narrowest annual temperature range throughout the year (Ahn and Lee, 1997). The *T. rochebrunianum* var. *grandisepalum* blooms bright purple flowers from July to August and grows well in both dry and moist soil (Ahn and Lee, 1997); thus, it has great potential as a garden and landscape plant. However, the exertions towards understanding the ecological requirements of this species, including seed dormancy and germination, have been limited.

Previous studies on germination of seeds from various taxa of *Thalictrum* suggest that these seeds are dormant at dispersal. Martin (1946) reported that *T. polygamum* seeds have small, underdeveloped embryos at maturity. Cold stratification of 18 weeks for *T. occidentale* (Kaye, 1997), 120 days for *T. fendleri* (Hoffman, 1985), and 6 months for *T. simplex* var. *brevipes* seeds (Washitani and Masuda, 1990) did not completely break the dormancy. Walck et al. (1999) reported that *T. mirabile* seeds had underdeveloped embryos and MPD at dispersal, and the seeds germinated to more than 80% after cold stratification (8 - 12 weeks) at 1°C. Low temperature exposure, acid treatment, and GA_3 have a positive effect on growth and dormancy breaking in different ornamental plants (Nadeem et al., 2013; Ramzan et al., 2014; Younis and Riaz, 2007). Cold stratification of *T. rochebrunianum* seeds for more than 4 weeks at 4°C and treatment of *T. coreanum* seeds with 400 $mg \cdot L^{-1}$ GA_3 for 24 h promoted seed germination to 26.7 and 36%, respectively (Lee et al., 2007). However, whether the embryos were underdeveloped and needed to grow inside the seed before they germinated was not specifically determined. Furthermore, the type of seed dormancy was not identified in those species.

The aim of the current study was to enhance seed propagation efficiency by determining the type of dormancy in seeds of *T. rochebrunianum* and, we investigated: i) the phenology of embryo growth, germination, and seedling emergence in nature; and ii) the effects of temperature and GA_3 on embryo growth and germination.

Materials and Methods

Seed Collection

Seeds (achenes) of *T. rochebrunianum* var. *grandisepalum* (H. Lev.) Nakai were collected from plants growing in the Hantaek Botanical Garden (37°09′N, 127°40′E), Yongin, Korea between 12 and 24 Sept. 2011. Seeds were allowed to dry under laboratory conditions (20-25°C) for 1-2 weeks, packed in sealed plastic bags, and stored at 5°C.

Water Imbibition Test

This experiment was conducted to determine whether the seed coat blocks water uptake and subsequent physical dormancy (PY). Three replications of 20 seeds each were placed on two sheets of a filter paper (Whatman No. 2, GE Healthcare, Buckinghamshire, UK) in 90 mm × 15 mm petri dishes, moistened with distilled water, and kept in the laboratory (20-25°C). Initial seed weight of air-dry seeds and water-imbibed seed masses were measured after 12, 24, and 48 h of incubation. Water uptake percentages were calculated as fresh mass increase by the seeds based on the formula:

$\%Ws = [(Wh - Wi)/Wi] \times 100$

where, Ws = increase in seed mass, Wh = mass of seeds after a given interval of imbibition, and Wi = initial seed mass.

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

Seeds were buried at a depth of 3 cm in field soil (almost loamy sand) in the experimental garden at Seoul National University in Seoul (28 Sept. 2011) and phenology of embryo growth, germination, and seedling emergence were investigated from 28 Sept. 2011 to 19 Apr. 2012. The soil temperature at 3 cm of depth was recorded every 30 min with a thermo-data logger (Watch Dog Model 450, Spectrum Technologies, Plainfield, IL, USA), and weekly maximum and minimum temperatures were calculated.

***Embryo growth*:** About 400 seeds were placed in fine-mesh polyester bags filled with potting soil (Plant World, Nongwoo Bio, Suwon, Korea) and buried in trays filled with the same potting soil. The potting soil is composed of peatmoss (25 - 25%), cocopeat (40 - 50%), perlite (10 - 14%), vermiculite (8 - 10%), and zeolite (8 - 13%). Trays were placed at ground level in the experimental garden. Every 1 or 2 weeks, a bag was exhumed and 20 seeds were selected randomly for embryo growth measurement. 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 ratio of embryo length to seed length (E:S ratio) was calculated to correct for a positive correlation between seed length and embryo length. The term critical length meant the embryo length just before radicle protrusion from the seeds in this study. After measuring the length of seeds and

embryos using the dissecting microscope, the sections of the seeds were viewed at 60 to 120× magnification and photographed with a MiView USB digital microscope (MV 1302U, CosView Technologies, Shenzhen, China). Images were used to measure the area of both the embryos and the endosperm from the seeds to quantify the embryo development during the field experiment.

***Germination*:** Three replicates of 30 seeds were sown in 10-cm plastic pots filled with the potting soil (Plant World) and placed in trays filled with the same potting soil. Trays were placed at ground level in the experimental garden. Seeds with an emerged radicle were counted and removed every one or two weeks. A seed was considered to be 'germinated' when the radicle protrusion reached at least 1 mm long. Intact seeds that had not germinated were buried back to the field.

***Seedling Emergence*:** Timing of seedling emergence was monitored by sowing three replicates of 30 seeds at a depth of 3 cm in plastic pots filled with the potting soil and placed in the trays described above. The trays were placed in a shady site where *Syringa oblata* var. *dilatata* (Nakai) Rehder was planted in the experimental garden. Therefore, the direct sun-light was prevented from reaching the soil surface. Emerged seedlings were counted and removed every 1 or 2 weeks during the field experiment. The pots were covered with a net to prevent disturbance by birds.

Temperature Effects on Dormancy Break under Controlled Environmental Conditions

For the laboratory experiment, seeds were treated with 500 mg·L^{-1} Benomyl (Dongbu HiTek, Bucheon, Korea) for 3-4 h for the fungal 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 distilled water. All dishes were wrapped with a parafilm to restrict water loss during incubation. At all temperature regimes, a 12-h light/dark photoperiod was provided by cool white fluorescent lamps that produce a photon flux density of approximately 30-40 μmol·m^{-2}·s^{-1} at the position of the petri dishes in the incubators (DS-13MCLP, Dasol Scientific, Hwaseong, Korea). Radicle emergence was monitored to calculate percent germination.

Effect of Temperature Sequences on Embryo Growth

Twenty seeds were sown in each of the 90 mm × 15 mm petri dish that was filled with potting soil (dark condition), moistened with distilled water, and kept in each of the specified temperature sequences. Each temperature sequence treatment included 6 petri dishes. Seeds were incubated either at a constant 5°C or at an alternating of 25/15°C (day/night) for 17 weeks. Another group of seeds were subjected to the following two temperature sequences: (i) beginning with warm temperature (25/15°C) for 9 weeks, and then kept under cold temperature conditions (5°C) for 11 weeks; (ii) beginning with cold temperature (5°C) for 9 weeks, and then kept under warm temperature conditions (25/15°C) for 5 weeks. Every 1 or 2 weeks, 20 seeds from each petri dish were cut into thin sections using a razor blade and the length of embryos and seeds were measured as described above.

Effect of Temperature Sequences on Germination: move-along test

A move-along experiment (Baskin and Baskin, 2003) adapted to seasonal temperatures in Korea was used to determine whether seeds required warm, cold, or warm followed by cold stratification. Each of the three replicates of 30 seeds was incubated at the four control temperatures (5, 15/6, 20/10, or 25/15°C) for 24 weeks, while other seeds were incubated at two temperature sequences: (i) beginning with warm temperature condition (25/15°C) for 10 weeks, slightly cooler temperatures (20/10°C) for 4 weeks, further reduced temperature conditions (15/6°C) for 4 weeks, and cold conditions (5°C) for 8 weeks, before slowly returning the seeds back to 15/6°C and 20/10°C; and (ii) beginning with cold temperature 5°C for 10 weeks and slowly warming the temperature to 15/6°C for 4 weeks, then 20/10°C for 4 weeks, 25/15°C for 6 weeks, and finally returning the seeds back to 20/10°C and 15/6°C.

Effect of Cold Stratification on Dormancy Break and Germination

Seeds were stratified at 5°C or 1°C for 0, 4, 8, or 12 weeks before being incubated at 25/15°C for 12 weeks. Since seeds of the *Thalictrum* species are dispersed in the late summer and early autumn, the seeds could experience several weeks of warm temperatures (≥ 15°C) before receiving cold stratification in the natural winter. Thus, seeds were subjected to 25/15°C temperatures (warm stratification) for 6 weeks and then cold stratified at 5°C or 1°C for another 12 weeks. After each of the cold stratifications, seeds were incubated at 25/15°C for 12 weeks.

Effect of Exhumed Date on Dormancy Break and Germination

Seeds that were buried on the 28 Sept. 2011 in the experimental garden were exhumed on the 31 Oct. and 20 Dec. 2011 and 21 Jan. 2012. Exhumed seeds were incubated at 25/15°C for 2 weeks and then the germination percentage

was calculated.

Effect of GA_3 on Dormancy Break and Germination

Seeds were soaked in solutions at concentrations of 0 (with distilled water, control), 10, 100, or 1000 $mg \cdot L^{-1}$ 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 at 4, 8, and 12 weeks of incubation.

Statistical Analyses

All laboratory experiments were conducted with a completely randomized design with three replications. Final percentages of germination in each experiment were analyzed statistically using the general linear model (GLM) procedure of the SAS program (SAS Institute, Cary, NC, USA). Means were compared using Tukey's honestly significant difference (HSD) test at the 5% level to compare treatment differences within the germination data.

Results

Water Imbibition Test

Fresh mass of seeds of *T. rochebrunianum* increased by more than 175% of their initial mass after 48 h of imbibition at 25/15°C (Fig. 1).

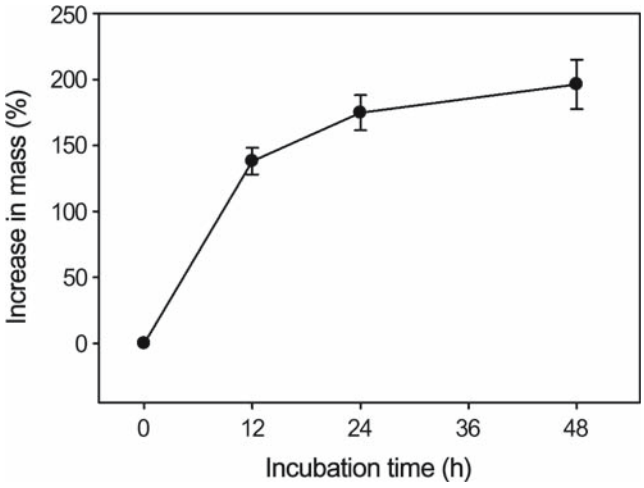

Fig. 1. Water uptake by intact seeds of *Thalictrum rochebrunianum* as represented by an increase in mass. Seeds were incubated at room temperature (22-25°C) on filter paper moistened with distilled water for 48 h. Vertical error bars represent SE.

Phenology of Embryo Growth, Germination, and Seedling Emergence

The initial E:S ratio of fresh matured seeds of *T. rochebrunianum* was 0.21 (Fig. 2B). The length of embryos was relatively small as compared to the length of the seeds. The embryos grew very slowly from autumn to mid-winter

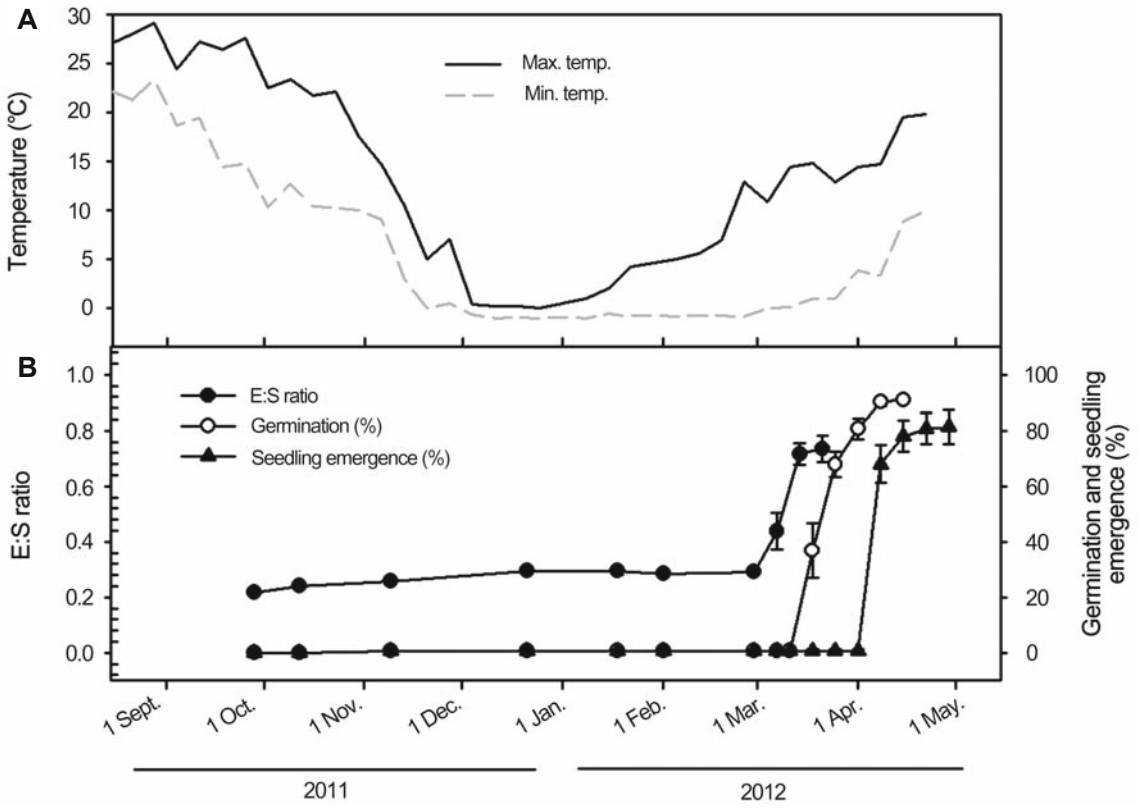

Fig. 2. Mean weekly maximum and minimum soil temperatures (A) and phenology of embryo growth, germination, and seedling emergence (B) of *Thalictrum rochebrunianum* seeds buried at a depth of 3 cm in 2011. Vertical bars represent SE.

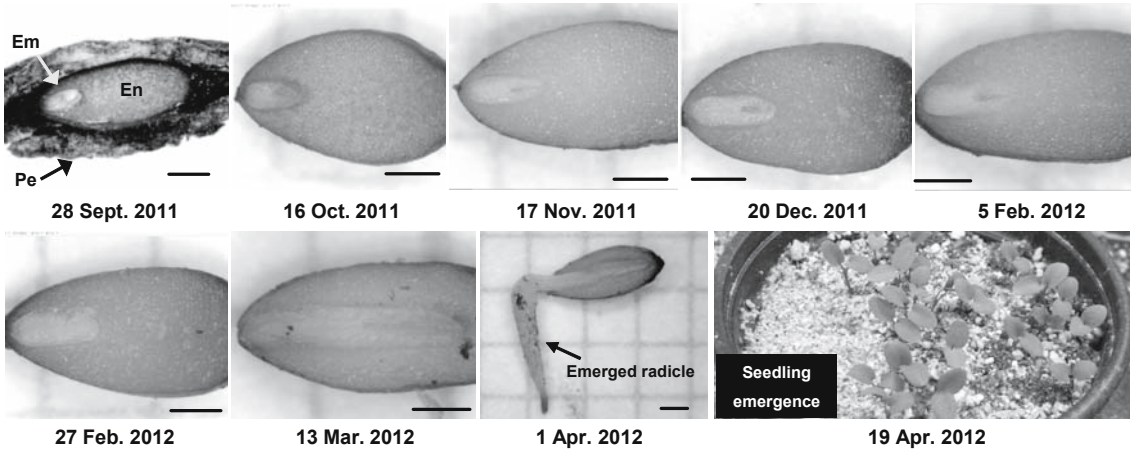

Fig. 3. Embryo growth, radicle emergence, and seedling emergence in *Thalictrum rochebrunianum* seeds kept outdoors in Seoul, Korea in 2011. Scale bars are 0.5 mm. Em, embryo; En, endosperm; Pe, pericarp.

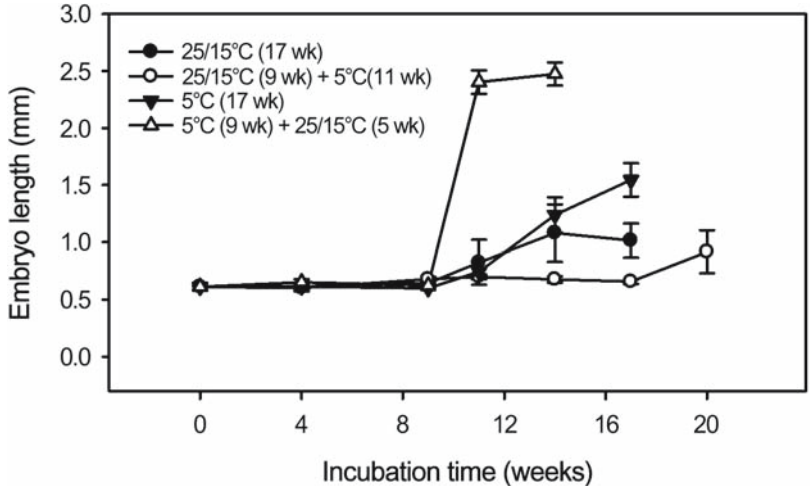

Fig. 4. Embryo length in seeds of *Thalictrum rochebrunianum* as affected by temperature treatments. Vertical bars represent SE.

in the seeds (Figs. 2B and 3). After a low-temperature period in winter, however, embryos grew rapidly starting in March and reached the critical E:S ratio of 0.71 required for germination on 13 Mar. 2012 (Figs. 2 and 3). Germination percentages started to increase between 11 and 24 Mar. 2012. On 15 Apr. 2012, 91.0% of the seeds had germinated. Most of the seedlings emerged between 1 and 15 Apr. 2012. 81.3% of the emerged seedlings were observed on 28 Apr. 2012; no additional seedlings emerged thereafter (Fig. 2B).

Effect of Temperature Sequences on Embryo Growth

Cold stratification was required for embryo growth in seeds of *T. rochebrunianum* (Fig. 4). At constant 5°C, embryos in some seeds started to grow at 6-8 weeks of incubation (data not shown). However, embryos in most of the seeds started to grow at 9-11 weeks of incubation at 5°C. The growth rate of the embryos was higher at 5°C than at 25/15°C. In the seeds incubated at 5°C, embryos eventually reached the critical length for germination (data not shown). After a cold stratification at 5°C for 9 weeks, embryos in seeds grew rapidly when transferred to warmer temperatures (25/15°C), and embryo length increased by more than 300%, indicating that physiological dormancy for embryo growth was broken during cold stratification.

Effect of Temperature Sequences on Germination

Cold stratification for several weeks was required for *T. rochebrunianum* seeds to break dormancy and start germination (Fig. 5). For seeds incubated in the temperature sequence beginning with 5°C, germination began after 10 weeks of incubation at 5°C and germination percentage increased rapidly to 92.9% at 15/6°C (Fig. 5). For seeds incubated in the temperature sequence beginning at 25/15°C, germination percentage was 24.7 ± 10.4% after 26 weeks of incubation, and then increased rapidly to 91.6 ± 2.6% by the following

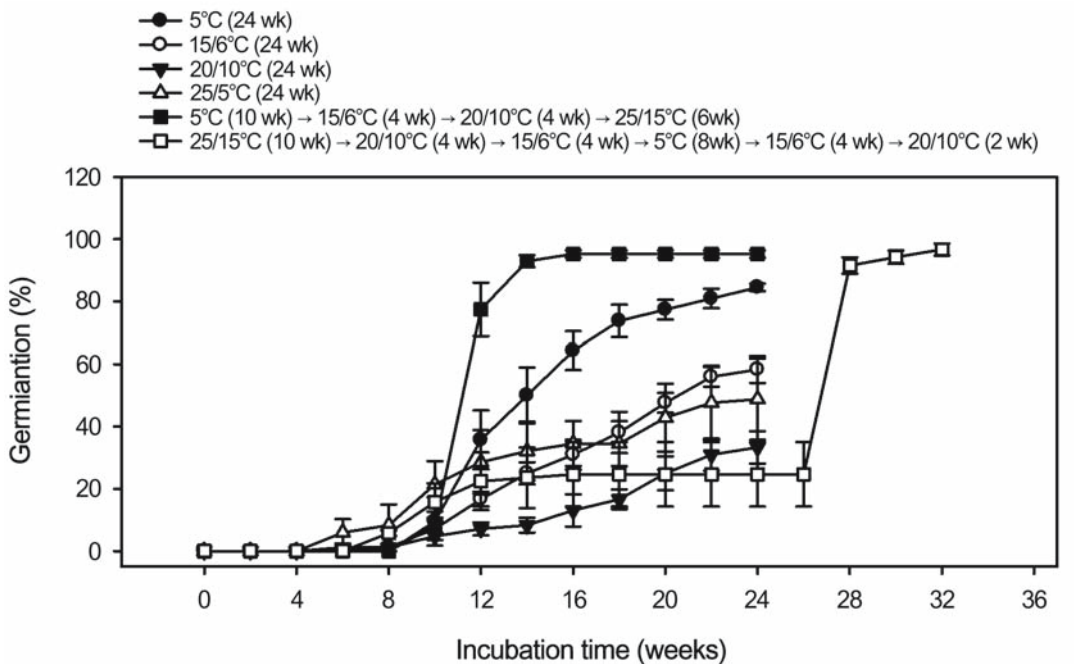

Fig. 5. Germination in seeds of *Thalictrum rochebrunianum* incubated under a constant temperature or a temperature sequence beginning at 25/15°C or at 5°C. Seeds collected in 2011 were used for this study. Vertical bars represent SE.

Table 1. Effect of a cold (1°C or 5°C) stratification period on germination percentages in *Thalictrum rochebrunianum* seeds. Stratified seeds were incubated at 25/15°C. Seeds collected in 2011 were used for the stratification experiment.

Stratification weeks at			Germination (%)			
25/15°C →	5°C →	1°C	Incubation time after warm and/or cold stratification (week)			
			2	4	6	12
0	0	0	0.0 e[z]	1.1 c	12.2 b	42.2 b
0	4	0	89.8 abc	90.9 ab	93.2 a	96.6 a
0	8	0	79.2 cd	94.7 ab	94.7 a	96.0 a
0	12	0	95.4 ab	95.4 ab	95.4 a	96.6 a
0	0	4	79.8 bcd	84.6 b	88.1 a	90.5 a
0	0	8	67.7 d	94.2 ab	94.2 a	94.2 a
0	0	12	94.2 abc	96.5 ab	97.7 a	97.7 a
6	12	0	96.5 a	98.8 a	98.8 a	98.8 a
6	0	12	94.3 abc	98.9 a	98.9 a	98.9 a

[z]Mean separation within columns by Tukey's honestly significant difference test at $p < 0.05$.

incubation at 15/6°C. Although the percentage of germinated seeds increased when the temperature was constant at 5°C, both germination speed and final germination percentage were higher in temperature sequences that included 5°C period followed by 15/6°C incubation period than they were in the constant 5°C incubation.

Effect of Cold Stratification on Dormancy Break and Germination

Cold stratification pretreatment had a significant effect on dormancy break and germination (Table 1). Few seeds germinated without a cold stratification pretreatment. On the other hands, 84 - 96% of seeds germinated after 4 weeks of incubation at 25/15°C following 4 - 12 weeks of cold stratification at 1 or 5°C. When seeds were warm stratified for 6 weeks before they were transferred to 5°C or 1°C conditions for cold stratification, 94-96% of the seeds germinated after 2 weeks of incubation at 25/15°C.

Effect of Exhumed Date on Dormancy Break and Germination

Seeds of *T. rochebrunianum* were exhumed from the field

Table 2. Effect of exhumed dates on germination in seeds of *Thalictrum rochebrunianum*.

Exhumed date	Germination (%)
2011	
28 Sept. (at harvest)	0.0 c[z]
31 Oct.	16.1 b
20 Dec.	97.8 a
2012	
21 Jan.	91.7 a

[z]Germination percentages among the exhumed dates followed by different letters indicate significant differences at $p < 0.05$ (Tukey's honestly significant difference test).

and then incubated at 25/15°C (Table 2). When the seeds were exhumed on October 31, 2011, the germination percentage was 16.1% after 2 weeks of incubation. However, when the seeds were exhumed on 20 Dec. 2011 and 21 Jan. 2012, germination percentages were 97.8% and 91.7%, respectively, after 2 weeks of incubation.

Effect of GA_3 on Dormancy Break and Germination

In all temperature regimes, the final germination percentages of *T. rochebrunianum* seeds increased with increasing concentrations of GA_3 (Fig. 6). At 4 weeks after incubation, more than 80% of seeds treated with 1000 $mg \cdot L^{-1}$ GA_3 germinated under temperature conditions of 15/5°C, 20/10°C and 25/15°C; however, no seeds germinated at 5°C (Fig. 6A). After 12 weeks of incubation, more than 80% of seeds treated with 1000 $mg \cdot L^{-1}$ GA_3 germinated at 5°C (Fig. 6C).

Discussion

Seeds of *T. rochebrunianum* imbibed water readily, increasing in fresh mass by 175% after 24 h of incubation (Fig. 1). According to Baskin and Baskin (2003), if seed mass (fresh weight) does not increase, then the seed or fruit coat is impermeable to water. On the other hand, if seed mass increases ≥ 20% based on air-dried seed or fruit mass, it can be assumed that the seed coat is permeable to water. Thus, the seeds of *T. rochebrunianum* in this study expressed no physical dormancy (PY).

Mature seeds of *T. rochebrunianum* had small embryos at dispersal. Lee et al. (2014) reported that seeds of *T. rochebrunianum* had small, underdeveloped embryos when dispersed from mother plants in autumn. However, they did not mention any detailed embryo growth patterns in their study. In temperate regions, many herbaceous plant species have small, underdeveloped embryos in seeds at the time of dispersal (Baskin and Baskin, 1988). According to Baskin and Baskin (1998), rudimentary or small, linear embryos must elongate before germination occurs; thus, the seeds generally are referred to as having underdeveloped embryos. Previous studies showed that seeds of Ranunculaceae also had small, underdeveloped embryos (Baskin and Baskin, 1998; Lee et al., 2012; Martin, 1946). We found that embryos in seeds of *T. rochebrunianum* grew to critical length before radicles emerged in nature (Fig. 2), and thus, the embryos were found to be underdeveloped.

In this study, seeds of *T. rochebrunianum* did not germinate for several months in natural settings and not germinated in 25/15°C conditions for 4 weeks in laboratory experiments. If embryo growth and germination are completed at suitable temperatures within 30 days, without dormancy breaking treatments, seeds were regarded to have morphological dormancy (MD) (Baskin and Baskin, 1998; Nikolaeva, 1977). However, if seeds with underdeveloped embryos need a dormancy breaking pretreatment such as cold and/or warm stratification before they can germinate, they are expressing both morphological and physiological dormancy, which is known as morphophysiological dormancy (MPD). Lee et al. (2014) also reported that a few seeds of *T. rochebrunianum* germinated after incubation at 25/15°C for 4 weeks in their previous study, indicating that the seeds were morphophysiologically dormant. Therefore, we have classified seeds of *T. rochebrunianum* as expressing MPD.

Baskin and Baskin (1998, 2014) recognized nine types of MPD based on the temperature requirements for dormancy breaking, temperatures at the time of embryo growth, and responses to gibberellic acid. The nine types are non-deep simple, intermediate simple, deep simple, non-deep simple epicotyl, deep simple epicotyl, deep simple double, non-deep complex, intermediate complex, and deep complex MPD.

Based on temperature at the time of embryo elongation, seeds with MPD have been divided into two categories: simple and complex (Baskin and Baskin, 1998, 2004). Embryo growth occurs at relatively warm temperatures (≥ 15°C) in simple MPD; whereas, in complex MPD, embryo growth occurs at low temperatures (0-10°C) (Baskin and Baskin, 1998, 2004). Each type of MPD (simple or complex) can be subdivided into non-deep, intermediate, and deep types, depending on the physiological states of seeds (Baskin and Baskin, 1998, 2004; Nikolaeva, 1977).

In *T. rochebrunianum* seeds, there was little embryo growth while the temperatures were < 10°C in nature (Figs. 2 and 3). But when the temperature increased to about 10°C (or higher), the embryos grew rapidly in the natural conditions. Embryo growth occurred at both warm and cold temperatures in laboratory experiments in this study (Fig. 4). The growth rate of the embryo was higher at 5°C than at 25/15°C during 17 weeks of incubation. Although embryos in some seeds grew to a critical length for germination at

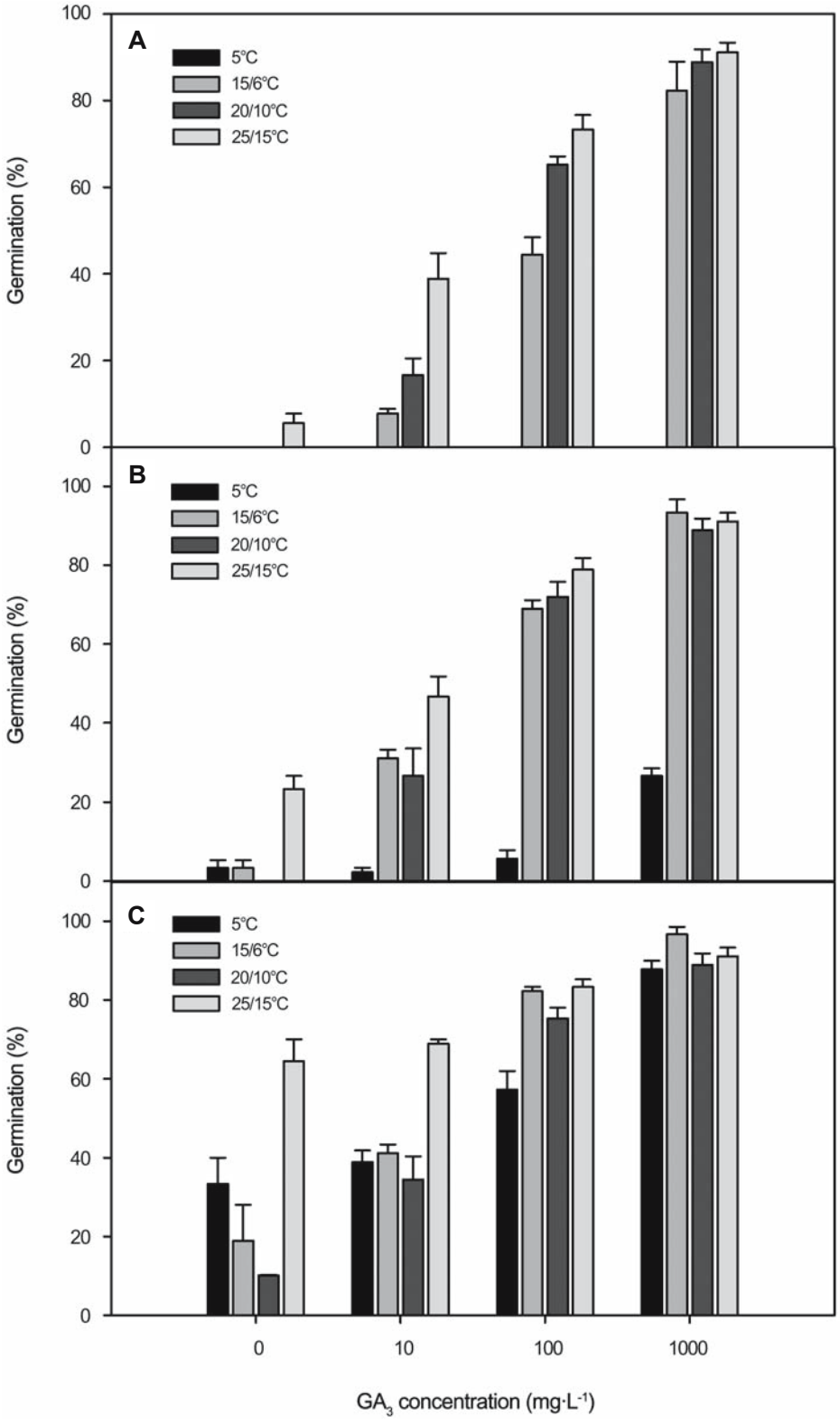

Fig. 6. Effect of GA_3 (0, 10, 100, or 1000 mg·L^{-1}) treatment on mean percentage germination in seeds from *Thalictrum rochebrunianum* after 4 (A), 8 (B), and 12 (C) weeks of incubation at different temperature regimes. Seeds collected in 2011 from *T. rochebrunianum* were used. Vertical bars represent SE.

5°C within 8 weeks, those in most of the remaining seeds did not reach to the critical length for germination at 5°C (under dark conditions) within 17 weeks of incubation. After breaking physiological dormancy by cold stratification at

5°C, the rate of embryo growth increased rapidly at warm temperature conditions (25/15°C) and reached to the critical length for germination within 2 weeks of incubation. Germination percentages gradually increased in seeds kept at continuous 5°C with increasing time of incubation (Fig. 5). Although germination percentages at 12 and 16 weeks of incubation at 5°C were 35.7% and 64.3%, respectively, germination percentages in the seeds from *T. rochebrunianum* increased rapidly from 8.2% at 10 weeks to 92.9% at 14 weeks of incubation when the seeds were moved from 5°C to 25/15°C in this study. These results indicated that the seeds needed cold stratification to break physiological dormancy, after which embryo grew rapidly with the onset of warm temperature. Therefore, the seeds of *T. rochebrunianum* expressed a simple type of MPD.

In other species of Ranunculaceae, cold or outdoor winter stratification successfully overcame dormancy in seeds with MPD (Forbis and Diggle, 2001; Walck et al., 1999). Walck et al. (1999) reported that the germination percentage of *T. mirabile* increased to 95% after stratification for 12 weeks at 1°C. They also documented that embryos in seeds of *T. mirabile* grew at warm temperature following cold stratification at 1°C, whereas 5°C was not low enough to promote full loss of physiological dormancy (PD). Unlike the seeds *T. mirabile*, the embryos in the seeds of *T. rochebrunianum* grew during incubation at a cold temperature (5°C) and most of the seeds germinated at the low temperature. Interspecific variations in temperature requirements for embryo growth and germination were reported in *Lonicera fragrantissima* and *L. morrowii* (Hidayati et al., 2000) and *Sambucus* spp. (Hidayati et al., 2010).

There are three types of simple MPD (non-deep, intermediate, and deep) in seeds with underdeveloped embryos (Baskin and Baskin, 1998, 2004). Among the three types of MPD, seeds with non-deep or intermediate simple MPD can be overcame from dormancy by GA_3 treatment. In the two types of MPD, seeds with non-deep simple MPD require only cold or warm stratification for dormancy break. In seeds of *T. rochebrunianum*, dormancy was broken by cold stratification for more than 4 weeks at 1°C or 5°C (Table 1). A GA_3 treatment of 1000 $mg \cdot L^{-1}$ also successfully resulted in dormancy break in the seeds (Fig. 6), indicating that GA_3 was an adequate substitute for cold stratification requirements. It has been demonstrated that GA/ABA ratio, but not the absolute hormone contents, controls dormancy break and germination (Finch-Savage and Leubner-Metzger, 2006). From the results, we can assume that GA_3 treatment could increase the GA/ABA ratio, and thus dormancy was broken in the seeds with MPD. Therefore, it can be concluded that the seeds express non-deep simple MPD.

The germination percentage was high (≥ 90%) at an incubation temperature of 25/15°C when the seeds of *T. rochebrunianum* were buried outdoors in autumn and exhumed on 20 Dec. 2011 (Table 2). This indicated that cold stratification requirements for dormancy break in the seeds were fulfilled in late December in Korea. Thus, cold stratification, fulfilled naturally by the cold period in winter, is one of the factors delaying dormancy break and seedling emergence in nature.

We might explain the pattern of dormancy break using the temperature requirements of *T. rochebrunianum* seeds from our results. In the outdoors, seeds are dispersed from late summer to early autumn, but do not germinate immediately as the seeds have underdeveloped embryos at dispersal. Cold temperatures are needed to break physiological dormancy and promote embryo growth. Therefore, embryos did not grow in autumn immediately after dispersal, the embryos grew after a cold period in the winter. Since embryo growth was delayed until the beginning of the growing season next year, both germination and seedling emergence were also delayed until early spring. Many woodland herbs maintain the spring emergence strategy, but they have evolved different mechanisms that result in spring emergence (Baskin and Baskin, 1998; Mamut et al., 2014). This is a typical mechanism found in seeds of which seed dormancy is broken by cold stratification in winter and seedlings emerge shortly after radical emergence the following spring. Very often, seeds of these kinds of species can germinate at low temperatures, enabling seedlings to emerge as soon as temperatures begin to rise very early in the growing season. It is suggested that such a delay mechanism within the seed population can be an ecologically advantageous strategy for unpredictable environmental conditions (Alves-Da-Silva et al., 2011; Doussi and Thanos, 2002). Therefore, vulnerable seedlings of the species can avoid severe winter climate conditions by emerging in the spring.

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