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Non-deep simple morphophysiological dormancy in seeds of heavenly bamboo (*Nandina domestica* Thunb.)

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ABSTRACT

Heavenly bamboo (*Nandina domestica*) is a popular ornamental shrub used as an indoor plant and for landscaping. However, seed propagation has been limited due to its very slow germination (9 months under natural conditions) caused by an extended seed dormancy period. We used various temperature treatments, including simulations of seasonal temperature changes, to investigate the temperature requirements for dormancy breaking and the germination of heavenly bamboo seeds at the embryo and whole-seed levels. Heavenly bamboo seeds were found to have no physical dormancy because the seeds imbibed water readily. However, its mature seeds contained underdeveloped embryos (E:S ratio <0.2) that had morphological dormancy. The seeds did not germinate within a month of incubation at fluctuating temperatures of 25/15, 20/10, and 15/6, or at 5 °C alone, which indicated that they had morphophysiological dormancy as well as morphological dormancy. A simulation study of seasonal temperatures (move-along test) showed that warm stratification (25/15 °C) was needed to stimulate embryo growth and germinate the seeds, but cold stratification (5 °C) was not required. Warm stratification at a constant 20 °C hastened seed germination more than a fluctuating temperature at 25/15 °C, and it led to a germination time that was shorter by 4 months than that under natural conditions. Application of gibberellic acid (GA_3) at 100 and 1000 mg L^{-1} could substitute for warm stratification and broke dormancy in seeds incubated at 15/6 °C. These results suggested that heavenly bamboo seeds had non-deep simple morphophysiological dormancy, which could be broken by warm stratification at 25/15 or 20 °C.

1. Introduction

Nandina domestica Thunb., also known as heavenly bamboo or nandina, is a broadleaf evergreen, upright, flat-topped shrub native to Northeast Asia (Jull and Blazich, 2008). The young leaves are bright pink in spring before turning green in summer, but old leaves blush to red or purple with the onset of low temperatures. The fruit is a bright red 5–10 mm diameter berry with that ripens in late fall and often persists through the winter. Heavenly bamboo was introduced to the United States as an ornamental plant in 1804 (Coats, 1992) and has been cultivated by 14.9% of the responding nurseries and total statewide sales were estimated at $3.3 million in 2003 (Wilson et al., 2014). Heavenly bamboo is one of the most popular landscape plants in the southeastern United States because of its ornamental characteristics, tolerance to pruning, resistance to disease, and ability to grow in full sun to shade and in moist to dry soils in USDA hardiness zones 6–10 (Dirr, 1998; Wilson et al., 2014). Over 40 different cultivars, including interior foliage and dwarf plants, are commercially available in the United States. Heavenly bamboo has also been reported to have indoor air remediation potential, and was the most effective in the removal of volatile organic compounds such as formaldehyde among the 20 Korean native plants that have been tested (Kim et al., 2010).

Although vegetative propagation methods, such as growing cuttings, are generally used to propagate heavenly bamboo, rooting tends to be slow, and when stems experience a winter, they become more difficult to root (Jull and Blazich, 2008). Protocols for micropropagation have been developed for heavenly bamboo (Ozudogru et al., 2013), but it requires appropriate facilities and skills, and is not cost-effective. Although seed propagation has mass production advantages, the germination of heavenly bamboo seeds has

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been known to be very slow, and optimum conditions for their seed germination are not well understood.

Delayed germination is generally caused, in many species, by seed dormancy, an ecological adaptation that allows seasonal timing so that germination occurs under favorable conditions; this increases the probability of seedling survival (Baskin and Baskin, 2014; Fenner and Thompson, 2005). An ecophysiological approach is needed to identify the best method to break seed dormancy and promote the germination of seeds. Most types of seed dormancy can be classified into five categories that are based on seed ecophysiological traits (Baskin and Baskin, 2014). Physical dormancy (PY) is caused by water-impermeable seed coats or fruit coats. Seeds with morphological dormancy (MD) have an embryo that is undifferentiated or underdeveloped at harvest and requires time for further development before germination. Physiological dormancy (PD) is characterized by a low embryo ability to rupture their seed coverings. Morphophysiological dormancy (MPD) is a combination of MD and PD, and combined dormancy is affected by PY and PD. The seed propagation recommendations for heavenly bamboo are that seeds are sown in a moist soil, and that they require two years to germinate if the seeds are sown in the autumn (Dirr, 1998). Previous studies have reported that heavenly bamboo seeds should be subjected to a period of moist, cold conditions (cold stratification) at 4 °C, and then sown in late spring or summer to obtain uniform and rapid germination (Dehgan, 1984; Hartmann et al., 2011). Previous research investigated the effect of various chemical compounds, such as vitamin B_1, hydrogen peroxide, or potassium permanganate; increasing oxygen pressure during germination; or varying the time of planting in order to replace the cold stratification requirement for heavenly bamboo seeds. However, all these attempts have been unsuccessful (Afanasiev, 1943). Dirr and Heuser (1987) recommended warm conditions followed by cold stratification for several months, whereas Hartmann et al. (2011) reported that cold stratification was not necessary for seed germination. Dirr and Heuser (1987) indicated that the cause of the delayed seed germination was probably related to a rudimentary embryo and the slow rate of embryo growth, but the factors needed to hasten embryo growth have not yet been identified.

Many seeds of plants in temperate regions have complicated types of dormancy and ecophysiological studies have revealed that many have MPD, including peony (Hao et al., 2014), ash (Steinbauer, 1937), yew (Chien et al., 1998), and *Ilex* species (Ives, 1923; Tezuka et al., 2013). Currently, nine different types of MPD are recognized, and are based on the degree and complexity of dormancy (Baskin and Baskin, 2014). A number of species with MPD have been categorized into these nine types. However, limited information exists on the dormancy mechanisms or germination requirements for heavenly bamboo species. Therefore, the objective of this study was to identify factors, particularly temperature, that affect seed dormancy and the germination of heavenly bamboo, and to investigate the potential for mass production of the species using seed propagation.

2. Materials and methods

2.1. Seed collection and storage

Ripe fruits of heavenly bamboo were collected from plants growing in an experimental garden located at Seoul National University, Suwon, Gyeonggi-do, South Korea, on 16 December, 2013. The seeds were separated from the fleshy pulp after the fruits had been soaked in water for one day and macerated. The seeds were manually cleaned and allowed to dry at room temperatures (20–25 °C) for one week. Then they were stored in sealed plastic bags at 5 °C until needed for the experiments.

2.2. Water uptake

Water uptake was investigated to determine whether seeds have physical dormancy or not. Three replicates of 30 seeds were placed on filter paper moistened with distilled water in 9-cm Petri dishes and kept at room temperature. Seed mass was determined to the nearest 0.1 mg at various times during incubation, which lasted 60 h. These were at 0, 1, 3, 6, 9, 24, 48, and 60 h. Percentage water uptake (%W_s) was calculated as $\%W_s = [(W_i - W_n)/W_n] \times 100$, where W_s = increase in mass of seeds, W_i = mass of seeds after a given interval of imbibition, and W_n = dry mass of seeds (Rhie et al., 2015).

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

The embryo growth of heavenly bamboo seeds under natural conditions was measured by putting 100 seeds in a fine-mesh polyester bag that was then placed in an experimental garden at the campus of Seoul National University, Seoul, Korea. The bag was buried under the soil to about 3 cm depth and covered with dead broad leaves. Every four weeks from 3 December, 2013 to 14 August, 2014, 10 seeds were removed from the bag and cut in half under a dissecting microscope using a razor blade. Then the embryo length was measured with an ocular micrometer, which allowed the ratio of the embryo length to seed length (E:S ratio) to be calculated. Germination and seedling emergence was monitored by filling three fine-mesh polyester bags with 30 seeds each and burying the bags in the experimental garden. Each week, the bags were exhumed and the numbers of germinated seeds were counted. Seedling emergence times were determined by sowing 20 seeds at 1 cm depth in plastic pots filled with potting soil. Four replicate pots were buried at soil level in a shady site in the garden. The emerged seedlings were counted each week. The soil temperature at a depth of 3 cm was measured every 30 min with a thermo-data logger (Watch Dog Model 450; Spectrum Technologies, Inc., Plainfield, IL, USA), and weekly maximum and minimum temperatures were calculated.

2.4. Temperature requirement assessment by simulating the seasonal temperature (move-along experiment)

The purpose of this experiment was to determine whether warm and/or cold stratification treatments promoted germination. For the move-along experiment, we assumed that incubating at constant 5 °C simulated winter stratifying temperatures, 25/15 °C (12 h day temperature alternated with 12 h night temperature) simulated summer, 20/10 °C represented early autumn and late spring, and 15/6 °C was late autumn and early spring (Baskin and Baskin, 2003). The seeds were subjected to the following two temperature sequences: (i) beginning with the summer season (warm stratification), 25/15 °C (12 weeks) → 20/10 °C (4 weeks) → 15/6 °C (4 weeks) → 5 °C (12 weeks); and (ii) beginning with the winter season (cold stratification), 5 °C (12 weeks) → 15/6 °C (4 weeks) → 20/10 °C (4 weeks) → 25/15 °C (12 weeks) → 20/10 °C (4 weeks) → 15/6 °C (4 weeks) → 5 °C (12 weeks). Then all the seeds were moved to a 15/6, 20/10, and 25/15 °C sequence, which was continued if they had not germinated. The E:S ratio and germination were measured as previously described. The experiments were conducted in light- and temperature-controlled incubators using a 12 h daily photoperiod (PPF of 30–40 μmol m^{-2} s^{-1}, with light provided by cool white fluorescent lamps). In the alternating regime, the high temperature was applied for 12 h in the light each day, and the low temperature for 12 h in the dark.

2.5. Temperature requirements for embryo growth

Five hundred seeds were incubated at 25/15, 20/10, 15/6, and 5 °C. Ten seeds were removed from the dishes at 4 week intervals and their E:S ratio was measured. The experiment was terminated 40 weeks after seed incubation.

2.6. Effects of incubation temperatures on germination

Three replicates of 30 seeds were incubated at 25/15, 20/10, 15/6, and 5 °C for 40 weeks and examined for germination at 1 week intervals. Mean germination time (MGT) and germination uniformity (GU) were calculated. MGT was calculated using the formula $\Sigma(T_x \cdot N_x)/N$ where T_x is the number of germinated seeds on each week, N_x is the number of weeks from the beginning of the test, and N is the total number of germinated seeds. GU was estimated according to the formula $\Sigma[(MGT - T_x)^2 \cdot N_x]/(N - 1)$.

2.7. Effects of gibberellic acid (GA_3) on germination under low temperature regimes

Seeds were soaked in solutions containing 0, 10, 100, or 1000 mg L^{-1} GA_3 for 24 h at room temperature. After imbibition, the seeds were washed with distilled water and placed on two sheets of filter paper moistened with distilled water, and then incubated at 15/6 °C for 12 weeks.

2.8. Effects of light conditions on germination

Three replicates of 30 seeds each were placed in the light or dark at 20 °C, and their germination percentages were determined after 20 weeks. Dishes containing seeds for the dark treatment were wrapped in two layers of aluminum foil and were not opened until the end of the incubation period.

2.9. Statistical analysis

The experimental design was a completely randomized design. The main effects of treatments including 1) incubation temperatures (four levels), 2) GA_3 concentration (four levels), and 3) light conditions (two levels) on the final germination percentage, MGT, and GU were tested by analysis of variance (ANOVA), followed by Tukey's honestly significant difference multiple comparison test using SAS (SAS Institute, Cary, NC, USA).

3. Results

3.1. Water uptake

Seed mass initially increased rapidly, which showed that water uptake had occurred (Fig. 1). Seed mass increased by 83.1 ± 5.9% (mean ± SE) after 9 h and stabilized after 60 h (96.3 ± 3.3%).

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

At harvest, the embryo occupied about 13% of the seed (Fig. 2A). The embryo grew slowly in the field for 7 months from 3 December, 2013 to 7 July, 2014, and the E:S ratio was below 0.17 (Fig. 3). However, between 7 July and 1 August the E:S ratio rapidly increased to 0.56 (more than 3-fold) and the seeds began to germinate on 1 August, 2014 (Figs. 2B–C and 3). Six weeks later, on 11 September 96.1% of the seeds had germinated, and no germination took place after this date. The first seedlings appeared on 21 August and most seedlings had emerged by 11 September (Figs. 2F and 3). The average maximum and minimum temperatures during the preceding week were 28.5 and 20.6 °C, respectively.

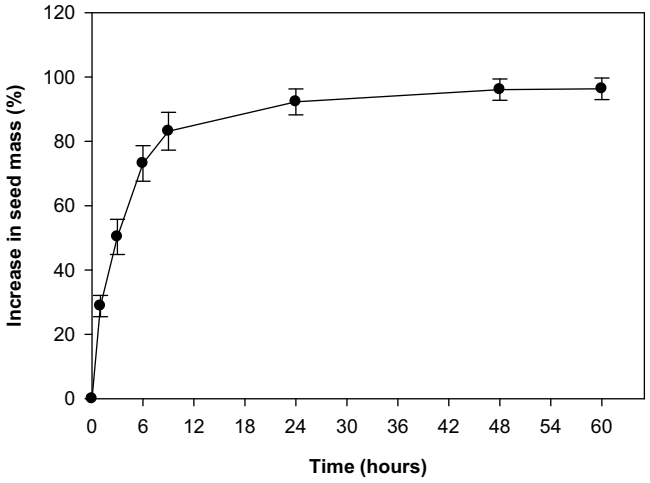

Fig. 1. Water uptake by *Nandina domestica* seeds incubated at room temperature (22–25 °C) on filter paper moistened with distilled water for 0–60 h. Vertical bars represent SEs.

3.3. Temperature requirements determined by simulating seasonal temperatures

The E:S ratio increased from 0.13 to 0.49 during the 12 weeks of incubation at 25/15 °C (Fig. 4A) for the seeds incubated in the temperature sequence beginning with the simulation of the summer season (warm stratification at 25/15 °C). The seeds started to germinate after the temperature was changed to 20/10 °C, and germination reached 79.5% shortly after the seeds were exposed to 15/6 °C. Further incubation at 5, 15/6, and 20/10 °C for 20 weeks produced no additional germination. After the remaining seeds were transferred to the seventh stage in the sequence (25/15 °C), they began germinating again, and germination reached 93.4% in the next two stages of 20/10 and 15/6 °C. In the temperature sequence beginning with the simulation of the winter season (cold stratification at 5 °C), the embryos did not grow until they were moved to 25/15 °C (Fig. 4B). Embryo growth started after 8 weeks at 25/15 °C and the E:S ratio increased to 0.27. The seeds began to germinate at 25/15 °C and germination reached 86.3% after the seeds were transferred from the 25/15 °C stage to the 20/10 and 15/6 °C stages. Additional germination was observed during the tenth stage in the sequence (25/15 °C) and finally reached 99.2%.

3.4. Temperature requirements for embryo growth

Embryo growth reached to 0.61 and 0.30 when the seeds were kept at 25/15 and 20/10 °C, respectively (Fig. 5). However, the embryos did not grow at 15/6 °C and at a constant 5 °C during the 40-week incubation period.

3.5. Effects of incubation temperatures on germination

The germination of heavenly bamboo seeds reached 91.2, 93.4, and 28.6% when incubated at 20, 25/15, and 20/10 °C for 40 weeks, respectively (Table 1). However, the seeds did not germinate at 15/6 and 5 °C. Shorter seed MGTs and better GUs occurred at 20 °C than at 25/15 and 15/6 °C.

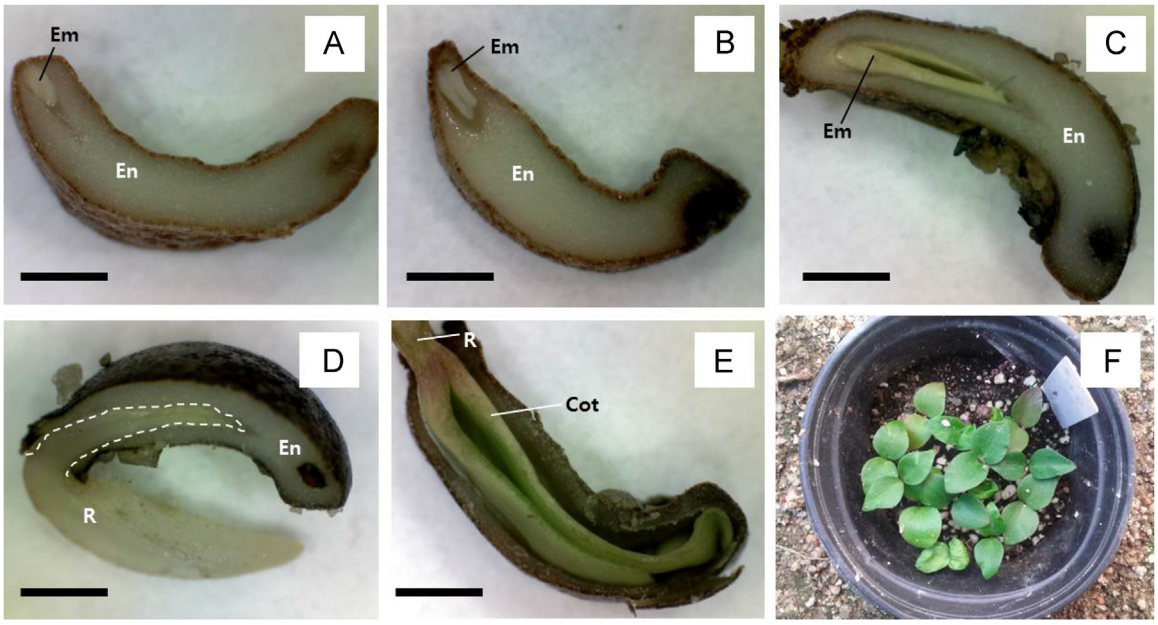

Fig. 2. Longitudinal section of a *Nandina domestica* seed showing the embryo (Em), endosperm (En), radicle (R), and cotyledon (Cot). Embryo growth in the seeds under natural conditions in Seoul, Korea, on 3 December, 2013 (A), on 7 July, 2014 (B), and on 1 August, 2014 (C); germinated seed on 14 August, 2014 (D) and 21 August, 2014 (E); and seedling emergence in September (F). Bars represent 2 mm.

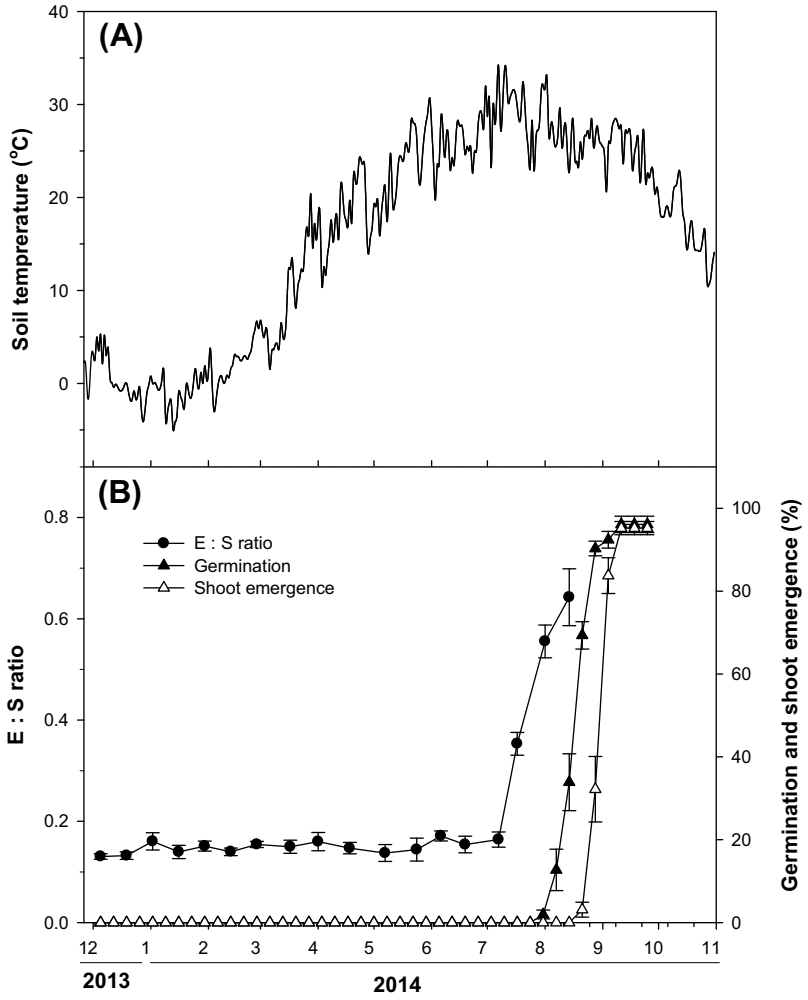

Fig. 3. Average daily soil temperatures during the experiment (A) and embryo growth (E:S ratio; the ratio of embryo length to seed length), mean percentage germination, and seedling emergence for *Nandina domestica* seeds sown at 3 cm depth (B). Vertical bars represent the SEs.

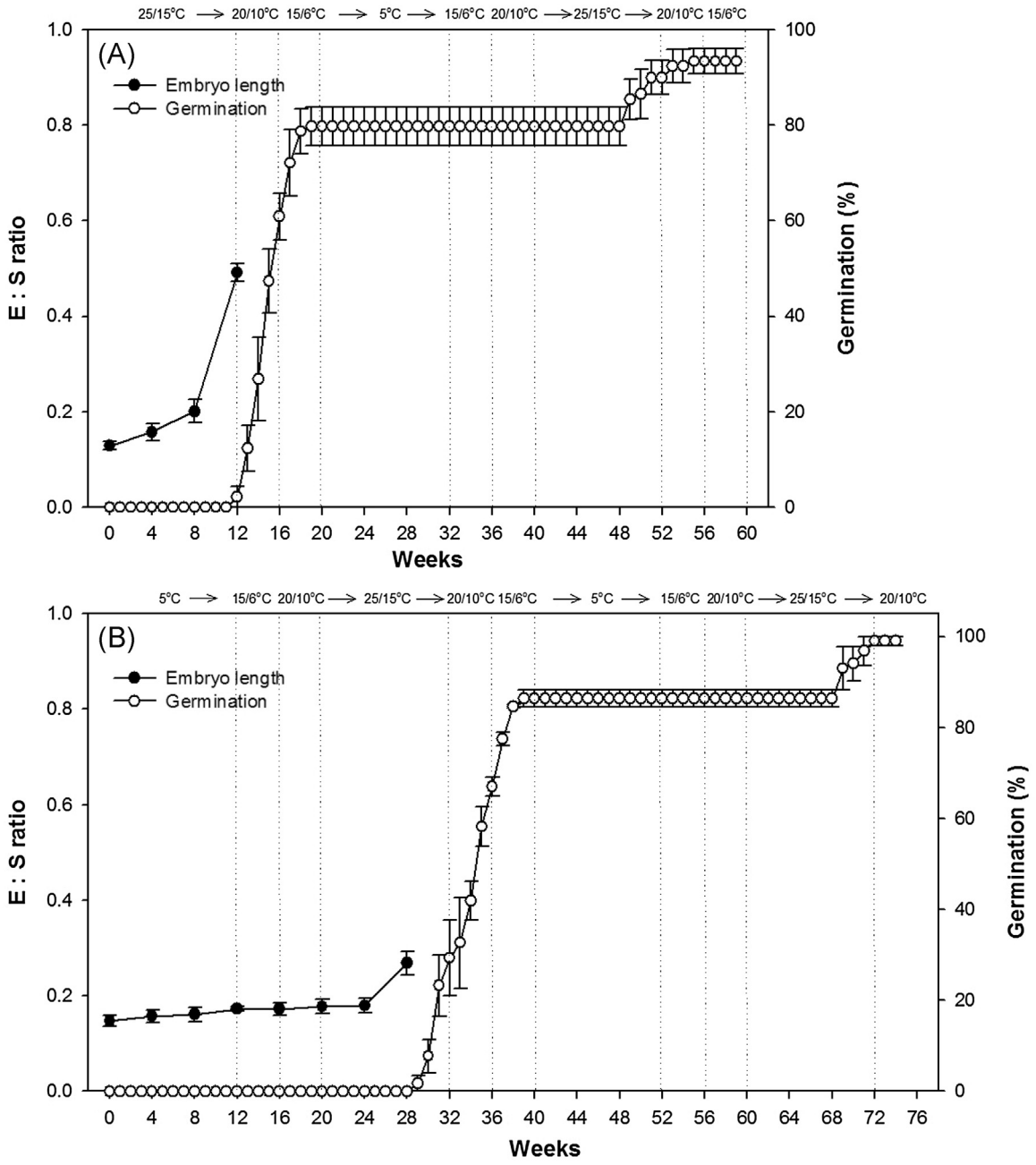

Fig. 4. Embryo growth (E:S ratio; the ratio of embryo length to seed length) and germination of seeds from *Nandina domestica* incubated under two temperature sequences: (A) beginning with summer season (warm stratification at 25/15 °C) or (B) beginning with winter season (cold stratification at 5 °C). This move-along procedure (Baskin and Baskin, 2003) was used to determine the effects of temperature changes on embryo growth and germination. Vertical error bars represent SEs.

3.6. Effects of GA_3 on germination under a low temperature regime

Although heavenly bamboo seeds did not germinate at 15/6 °C, the gibberellic acid (GA_3) treatment at 100 and 1000 mg L^{-1} had a positive effect on the germination percentage, MGT, and GU of the seeds incubated at under 15/6 °C (Table 1). No heavenly bamboo seeds germinated when soaked in distilled water or when they were treated with 10 mg L^{-1} of GA_3. However, seeds treated with GA_3 at 100 and 1000 mg L^{-1} produced germination percentages of 23.9 and 82.9%, respectively, and the MGT became shorter at the higher GA_3 concentration. However, there was no significant difference in GU between the 100 and 1000 mg L^{-1} treatments.

3.7. Effects of light conditions on germination

The germination rate for the seeds was 87.4 and 94.3% under the light and dark conditions, respectively, but there was no significant difference in the germination percentages between the two conditions (Table 1).

4. Discussion

According to Baskin and Baskin (2014), seed dormancy in plants can be categorized into physical dormancy (PY), morphological dormancy (MD), physiological dormancy (PD), morphophysiological dormancy (MPD), and combinational dormancy (PY + PD). Our results suggested that heavenly bamboo seeds showed no physical

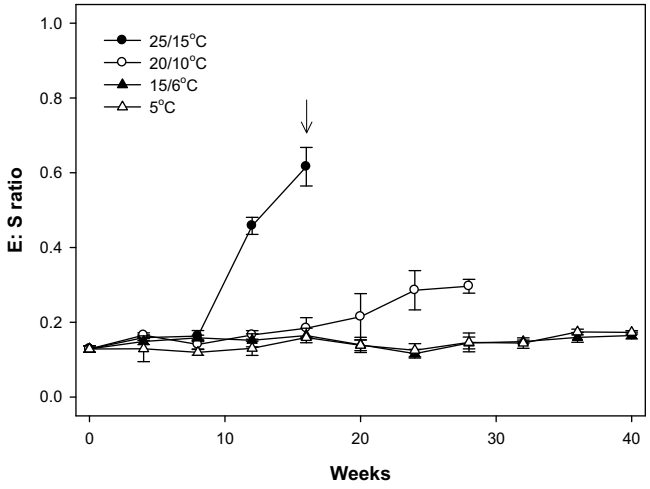

Fig. 5. Embryo growth (E:S ratio; the ratio of embryo length to seed length) of *Nandina domestica* seeds incubated in the light at 25/15, 20/10, 15/6, and 5 °C for 40 weeks. Arrow indicates when germination occurred. Vertical error bars represent SEs.

Table 1
Effects of incubation temperatures, GA_3, and light conditions on germination, mean germination time (MGT), and the germination uniformity (GU) of *Nandina domestica* seeds.

Treatments	Final germination (%)	MGT[z] (wk)	GU[y]
Incubation temperatures			
20 °C	91.2a[x]	11.3b	5.9b
25/15 °C	93.4a	18.2a	12.3b
20/10 °C	28.6b	18.8a	139.7a
15/6 °C	0c	–	–
5 °C	0c	–	–
Temperature regimen significance	***	*	*
GA_3 concentration (incubated at 15/6 °C)			
0 mg L^{-1}	0b	–	–
10 mg L^{-1}	0b	–	–
100 mg L^{-1}	23.9b	18.6a	41.1a
1000 mg L^{-1}	82.9a	14.1b	9.9a
GA_3 concentration significance	***	**	NS
Light conditions (incubated at 20 °C)			
Light	87.4a	–[w]	–
Dark	94.3a	–	–
Light regimen significance	NS		

NS, *, **, *** Non significant or significantly different at $P < 0.05$, 0.01, and 0.001, respectively.

[z] Mean germination time, $MGT = \Sigma(T_x \cdot N_x)/N$ where T_x is the number of germinated seeds observed each week, N_x is the number of weeks from the beginning of the test, and N is the total number of germinated seeds.

[y] Germination uniformity, $GU = \Sigma[(MGT - T_x)^2 \cdot N_x]/(N - 1)$.

[x] Means followed by different letters are significantly different in each category ($P < 0.05$, one-way analysis of variance with Tukey's honestly significant difference test).

[w] Data were not available because in the light condition experiment germination in the dark was checked once after 20 weeks of incubation.

dormancy because the seeds imbibed water readily and, increased their mass by 83.1% within 9 h after imbibition had started (Fig. 1). However, mature seeds of the species contained small embryos that required further growth prior to germination (Figs. 2 and 3), which suggests that they have underdeveloped embryos. This means they are categorized as having either MD or MPD (Baskin and Baskin, 2014). If the seeds germinated within 30 d under favorable conditions, then heavenly bamboo seeds would have MD only. However, the seeds required several weeks (>10 weeks) to germinate at temperature of 25/15 or 20 °C, which indicated that they have MPD.

Under natural conditions, the embryo growth and seed germination of heavenly bamboo occurs in the following autumn after the seeds have been subjected to winter temperatures followed by summer temperatures (Fig. 3). However, these data did not indicate whether cold stratification and/or warm stratification were required to promote embryo growth and germination. The move-along experiments showed that the warm-to-cold temperature sequence initiated embryo growth and that the seeds began to germinate during incubation at 25/15 °C (Fig. 4A). In the sequence starting with 5 °C, the embryos did not grow during the 5, 15/6, and 20/10 °C temperature periods, but only began to grow after the seeds were exposed to 25/15 °C. Although the seeds of this species are dispersed during the winter season and undergo the sequence winter → spring → summer → autumn for about 8 months before germination, only warm stratification (summer temperatures) was required to begin germination, and seedlings could be obtained in about 3–4 months under controlled temperature conditions. The seeds from *Cardiocrinum cordatum* (Liliaceae) grown under natural conditions require about 18–19 months from dispersal to germination, but *C. cordatum* seeds germinated in about 9 months after a warm-to-cold temperature sequence, indicating that this sequence could shorten the germination time by 10 months (Kondo et al., 2006).

Baskin and Baskin (2014) categorized nine basic types of MPD. These nine types of MPD are divided into two categories: simple and complex, on the basis of the temperature at the time of embryo growth. In the six simple levels for MPD, embryo growth occurs at warm (usually 15 °C or above) temperatures, whereas in the three complex levels, embryo growth occurs during cold stratification (0–10 °C). The heavenly bamboo seeds required relatively high temperature (25/15 °C) for embryo growth, which means, they could be categorized as simple MPD seeds. Simple MPD includes six types of seed dormancy: non-deep simple MPD, intermediate simple MPD, deep simple MPD, non-deep simple epicotyl MPD, deep simple epicotyl MPD, and deep simple double MPD. Non-deep simple MPD is the only level of MPD in which physiological dormancy can be broken and embryos will grow after a relatively short period of warm stratification. In contrast, both warm and cold stratification is needed for the other simple MPD levels (Baskin and Baskin, 2014). Since heavenly bamboo seeds did not require cold stratification to stimulate embryo growth and germination, and only required warm stratification (Figs. 4 and 5), the seeds can be categorized as non-deep simple MPD.

It has been reported that seeds from the Berberidaceae family generally have underdeveloped embryos, and further development of the embryo is stimulated under specific temperature conditions (Barton, 1944). Other species of Berberidaceae have been reported to have seed dormancy, such as deep simple MPD [*Jeffersonia diphylla* and *J. dubia* (Baskin and Baskin, 1989; Rhie et al., 2015)] and deep simple double MPD [*Caulophyllum thalictroides* (Barton, 1944)], which require both warm and cold stratification for germination. The family Berberidaceae contains 19 genera, including *Nandina*. Heavenly bamboo is the only member of the *Nandina* genus and our results suggested that seeds of this species have non-deep simple MPD. This appears to be the first report of a Berberidaceae member that displays non-deep simple MPD, which means that it only requires a few weeks of warm stratification to overcome dormancy.

Previous studies have reported that gibberellic acid treatment overcame seed dormancy in many species with non-deep simple MPD (Baskin and Baskin, 1990; Lee et al., 2015; Walck et al., 2000). Our results suggested that heavenly bamboo seed embryos did not grow as well as expected because the seeds did not germinate under the low temperature regime of 15/6 °C (Fig. 5 and Table 1). However, GA_3 application promoted heavenly bamboo seed germination and 100 and 1000 mg L^{-1} GA_3 increased germination percentages to

23.9 and 82.9%, respectively (Table 1). These results indicated that GA_3 substituted for warm stratification and was able to promote embryo growth and germination in heavenly bamboo. This was consistent with previous studies that also showed that GA treatment breaks the dormancy of seeds with non-deep simple MPD (Baskin and Baskin, 2014).

In both temperature sequence move-along experiments, the seed germination percentage increased by about 80% after a period of warm stratification (25/15 °C) but stalled at 5 °C and under any following temperature sequences (Fig. 4A and B). The germination rate was increased again by about 100% after a second warm stratification treatment. Although seed dormancy in many species can be broken by warm and/or cold stratification, the seeds often experience secondary dormancy when environmental conditions are unfavorable for germination (Bewley and Black, 1994). Baskin and Baskin (2014) reported that unfavorable temperatures, prolonged light or darkness, water stress, and that anoxia can induce secondary dormancy in weed species. Furthermore, Banovetz and Scheiner (1994) reported that incubating the nondormant seeds of *Coreopsis lanceolata* L. at 5 °C induced secondary dormancy. We assumed that secondary dormancy of heavenly bamboo seeds had been induced at the temperature sequence beginning with 5 °C because the seeds did not germinate at 15/6 and 20/10 °C immediately after the 5 °C stage (Fig. 4A and B). The seeds began to germinate again after they were subjected to warm stratification for 8 or more weeks, which indicated that secondary dormancy was broken by exposure to the second warm stratification period.

Fluctuating temperature (25/15 °C) delayed the MGT of heavenly bamboo seeds to about 7 weeks compared seeds incubated at a constant temperature (20 °C) although the average temperature was the same between 25/15 °C and 20 °C treatments (Table 1). This indicated that a constant 20 °C was a more effective warm stratification temperature than the 25/15 °C fluctuating temperature during seed germination. However, the opposite results were observed for *Cynara cardunculus*, where incubating seeds at a 25/15 °C fluctuating temperature promoted seed germination compared to incubation at 20 °C (Huarte and Benech-Arnold, 2010). It has been known that temperature fluctuation is a requirement for seed germination in many species (Probert, 1992), and its ecological significance is related to the strategies seeds use to detect seed burial depth in soil (Thompson and Grime, 1983). The diurnal temperature difference is larger in near-surface soil than below vegetative cover or under water (Balisky and Burton, 1993). Previous studies on the mechanism behind dormancy breaking have suggested that fluctuating temperatures broke seed dormancy mainly by promoting GA_3 biosynthesis and by reducing ABA biosynthesis and sensitivity (Ali-Rachedi et al., 2004; Huarte and Benech-Arnold, 2010). The effective temperature range for warm stratification varies among species, but each species has a minimum temperature for breaking dormancy and incubating below this temperature is ineffective (Baskin and Baskin, 2014). Our results suggested that 25 °C and 20 °C were ideal temperatures to use during the warm stratification of heavenly bamboo, but 15 °C was too low to break dormancy. Furthermore, a constant temperature of 20 °C is more effective at breaking the seed dormancy of heavenly bamboo than fluctuating 25/15 °C conditions.

The germination rates of heavenly bamboo were not significantly different under light and dark conditions at 20 °C (Table 1), although seed dormancy breaking and germination can be affected by various light factors, such as light intensity, light quality, and photoperiod (Baskin and Baskin, 2014). Our results indicated that warm stratification could hasten heavenly bamboo seed germination, but further study on different light factors, such as light quality and photoperiods, may lead to enhanced germination of heavenly bamboo seeds.

5. Conclusion

Heavenly bamboo seeds could be classified into the non-deep simple MPD category, which means their dormancy can be broken by warm stratification at 25/15 or 20 °C. Although seeds dispersal to germination requires about 9 months under natural conditions, it should be possible to germinate up to 90–100% of the seeds and shorten the germination time by 4 months if a warm stratification treatment is used or GA_3 at higher concentration than 100 mg L^{-1} is applied. The results presented here will enable horticulturalists and seed ecologists to reduce the time required to obtain heavenly bamboo seedlings.

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