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Germination Characteristics and Seed Dormancy of *Iris dichotoma* Pall., an Endangered Species Native to Korea

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ABSTRACT

Iris dichotoma Pall. found on Daechung Island in Korea has been designated as an endangered species. To aid in conservation efforts of this species, this study investigated its germination characteristics and seed dormancy type. Four sets of seeds were incubated at different temperatures (4/1°C, 15/6°C, 20/10°C, and 25/15°C). One set of seeds was cold stratified (4 weeks at 4/1°C). The final germination rate and mean germination time showed that the optimal germination temperature was 25/15°C. Final germination rates were ~70%, showing no significant difference among temperature treatments. However, mean germination time were significantly different among all temperature treatments except for 4/1°C. Mean germination time for seeds with temperature treatments of 15/6°C, 20/10°C, and 25/15°C were 3.2, 2.1, and 1.5 weeks, respectively. At 25/15°C, the mean germination time was half of that at 15/6°C. Seeds of *I. dichotoma* had fully developed embryos at the time of dispersal. No additional growth of the embryo was observed. Cold stratification did not affect the final germination rate or the mean germination time. This study shows that seeds of *I. dichotoma* have no physiological or morphological dormancy, unlike other members of the Iris genus known to have seed dormancy that needs a relatively high incubation temperature (≥25/15°C) for mass propagation to occur. These results will be useful for understanding ecophysiological mechanisms related to the species' habitat. They are also useful for mass propagation of *I. dichotoma* for the purpose of conserving this endangered species.

Keywords: Ecophysiology, Final germination rate, Mass propagation, Mean germination time, Seed morphology

Introduction

External factors (such as habitat destruction, exploitation, and pollution) and internal factors (such as ecophysiological adaption, reproduction, and growth rates) are associated with decreased biodiversity (Kang *et al*., 2010). According to International Union for Conservation of Nature (IUCN), 5,727 plant species and 3,325 plant species were listed as endangered and critically endangered, respectively, in 2020. In Korea, a total of 88 species were listed as endangered species in 2020, with 11 species listed as endangered I and 77 listed as II (National Institute of Biological Resources (NIBR), 2021). There are four endangered *Iris* species native to Korea. Among these, *Iris dichotoma* Pall. is an endangered species distributed on the Daechung Island and islands off the west coast of Korea. Presently, as this species and its habitat are restricted, ex situ conservation is important to conserve *I. dichotoma*. Research studies on its propagation and habitat restoration are warranted due to insufficient previous studies.

Propagation using seed germination provides a large number of plant individuals at once. However, since each species has different germination characteristics, further research is needed. This is especially true for seeds that re-

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quire a specific environment during dormancy, e.g., cold temperature or hormone treatment. Such seeds will not germinate until particular conditions are met, even if they are in a favorable environment (Finch-savage & Leubner-Metzger, 2006). Seed dormancy types are classified into five main categories: 1) physical dormancy, 2) physiological dormancy, 3) morphological dormancy, 4) morphophysiological dormancy, and 5) combinational dormancy (physical dormancy + physiological dormancy). These five categories of seed dormancy are subdivided based on the depth and pattern of dormancy (Baskin & Baskin, 1998; Finch-savage & Leubner-Metzger, 2006). Seed dormancy plays a decisive role in controlling germination timing and adaption of seeds to natural environments (Geneve, 2003). However, seeds of species in the same genus can exhibit various types of dormancy and germination characteristics (Hidayati *et al*., 2000; Park *et al*., 2019). In a research study conducted by Park *et al*. (2019), four *Lonicera* species native to Korea showed different germination characteristics and dormancy types under four temperature treatments, although they were the same species native to different regions. Seeds of eight *Veronica* species native to Korea also exhibited interspecific germination responses that varied under identical temperature treatments (Song *et al*., 2018). Thus, classification of seed dormancy and investigation of germination characteristics are needed to provide insight into how each species adjusts to different natural habitats (Park *et al*., 2019).

In previous studies, seeds of species in the Iris genus showed physiological dormancy. Such seed dormancy is a typical characteristic of plant species native to the Northern Hemisphere (Baskin & Baskin, 1998). Seeds with physiological dormancy can germinate when gibberellic acid concentration in seeds increases due to particular conditions, such as a cold or a warm stratification treatment. Seed dormancy of *I. suaveolens* Boiss can be broken via stratification treatments, especially with 2 weeks of cold followed by 6 weeks of warmth (Hajyzadeh *et al*., 2019). NaOH scarification and stratification can effectively break seed dormancy and improve seed germination of *I. lactea* Pall (Sun *et al*., 2006). Although previous research has examined germination rates of *I. dichotoma* and *I. setosa* Pall according to seed storage method (Lee *et al*., 2015), studies about seed dormancy type of irises native to Korean seeds are insufficient.

Thus, the objective of the present study was to investigate germination characteristics under various temperatures (4/1°C, 15/6°C, 20/10°C, and 25/15°C) and cold stratification treatment (4/1°C at 4 weeks). Seed morphology was observed and seed dormancy was classified for *I. dichotoma*. Results of this study can improve our understanding of seed ecophysiological mechanisms in a natural habitat. They could be used for mass propagation of *I. dichotoma*.

Materials and Methods

Plant materials

This study was carried out at the Endangered Resources Center in Yeong -yang, Korea. Fruits of *I. dichotoma* were collected in 2020 from plants growing at the center's greenhouse. These seeds were dehisced, and dried at an ambient room temperature (~25°C) for 2 weeks, and then stored at 4°C until further analysis.

Temperature treatments

To investigate seed germination characteristic and type of dormancy of *I. dichotoma*, three replicates of 50 seeds were placed in 10-cm-diameter Petri dishes on top of two sheets of filter papers moistened with distilled water. All Petri dishes were sealed with Parafilm to prevent water loss during the experiment. Temperature- and light-controlled multiroom chambers were used in this study. These chambers were set at 4/1°C, 15/6°C, 20/10°C, and 25/15°C, respectively. Cool white florescent lamps were set to an alternating 12-h/12-h light/dark photoperiod.

Germination investigation was carried out once per week. Germinated seeds were removed from Petri dishes. Distilled water was frequently supplied to Petri dishes to prevent water loss. Rotten seeds were excluded from the calculation of germination rate. Measured traits included final germination rate (FGR) and mean germination time (MGT). They were calculated with the following equations:

$$FGR = (N/S) \times 100$$
$$MGT = \sum(t_i \times n)/N$$

where N was the total number of germinated seed, S was the total number of seed sown, t_i was the number of days from sowing, and n was the number of seeds newly germinated at time t.

Seed morphology

To investigate morphological characteristics, seed morphology characterization was carried out on June 8, 2021. Iris seeds were incubated at 25/15°C. These seeds were halved using a surgical blade (stainless blade; Feather Safety Razor Co., LTD). Lengths of the embryo of each seed at dispersal and just before germination were measured using a USB microscope (AD7013MZT Dino-Lite; AnMo Electronics Co., Taiwan). The embryo–seed ratio (E:S ratio) was then calculated and compared.

Cold stratification

Cold stratification treatment was conducted to investigate its effect on seed germination. Seeds were placed in 10-cm-diameter Petri dishes on two filter papers moistened with distilled water. These seeds were stored in a chamber set to 4/1°C with a 12-h alternating light and dark cycle for 4 weeks. They were moved to the chamber at 25/15°C after 4 weeks of cold stratification. Germinated seeds were counted every week and removed from Petri dishes. Distilled water was frequently supplied to Petri dishes to prevent water loss. Rotten seeds were excluded from the calculation of germination rate.

Statistical analysis

Statistical analysis software version 9.4 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses of the data. Differences between mean FGRs of seeds under four temperature treatments were assessed using Tukey's honestly significant difference tests. Differences between E:S ratios of seeds at dispersal and just before germination were assessed using paired t-tests. Results with p values < 0.05 were considered statistically significant.

Results and Discussion

Seed morphology

The seed morphology experiment showed that seeds of *I. dichotoma* had fully linearly developed embryos at dispersal (Fig. 1). This embryo type is well-presented in gymnosperms, monocots, and dicots (Martin, 1946). E:S ratios of seeds were 0.70 ± 0.03 and 0.75 ± 0.02 at dispersal and just before germination, respectively (Fig. 2). There was no significant difference in the E:S ratio between dispersal and just before germination. In general, seeds with underdeveloped embryos have morphological dormancy or morphophysiological dormancy (Baskin & Baskin, 1998; 2004). Seeds with morphological dormancy and morphophysiological dormancy will disperse with underdeveloped embryos. Their embryos then show additional growth until just before germination within 30 days. Seeds of *I. dichotoma* have no morphological dormancy. Thus, they will germinate without additional growth of the embryo within 30 days.

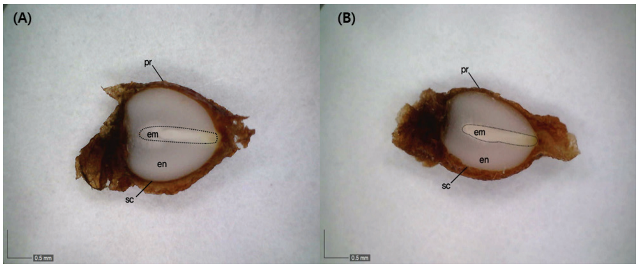

Fig. 1. Internal seed morphology of *Iris dichotoma* Pall. Fully developed embryos at seed dispersal (A) and at just before germination (B) are shown. pr, pericarp; sc, seed coat; en, endosperm; em, embryo. Scale bars are 0.5 mm.

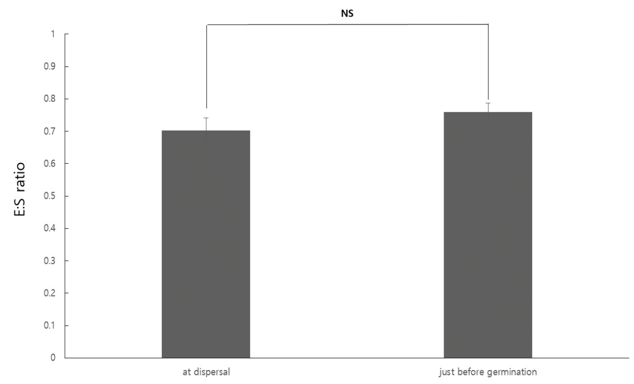

Fig. 2. Embryo:seed ratio (E:S ratio) in the seeds of *Iris dichotoma* Pall at seed dispersal and at just before germination. The seeds of *I. dichotoma* were incubated at 25/15°C. The vertical error bars represent SE (n = 10). Each E:S ratio at dispersal and just before germination was compared using paired t test.

Temperature treatments

At 15/6°C, seeds of *I. dichotoma* started to germinate at 3 weeks after sowing. At 5 weeks after sowing, the FGR of seeds was 81.3%. At 20/10°C, freshly matured seeds started to germinate at one week after sowing. At 5 weeks after sowing, the FGR of seeds was 75.3%. At 25/15°C, seeds started to germinate at one week after sowing. At 2 weeks after sowing, the FGR was 80.0% (Fig. 3). Its seeds did not germinate when they were incubated at 4/1°C. Final germination rates showed no significant differences among all temperature treatments except for 4/1°C. Seeds germinated within 2 weeks. According to Baskin and Baskin (2004), seeds with physiological dormancy will not germinate within ~30 days even in an optimal environment. To break the physiological dormancy, seeds need particular conditions, such as a cold or a warm stratification, or treatment with germination-improving chemicals such as GA_3, KNO_3, and so on. Several previous studies have reported that seeds of the *Iris* genus exhibit a typical physiological dormancy. They germinate when germination inhibitors are removed. They also need optimal conditions of light, temperature, and so on (Lashkarian *et al*., 2012; Lee *et al*., 2015). Seeds of *I. lactea* require NaOH scarification and cold stratification to break dormancy and experience greater rates of germination than the control (Sun *et al*., 2006). Combined alternate cold-warm stratification could effectively break the seed dormancy of *I. suaveolens* (Hajyzadeh *et al*., 2019). In this study, however, seeds of *I. dichotoma* germinated within 30 days after all temperature treatments except for the treatment at 4/1°C. These results indicate that the seeds of *I. dichotoma* have no physiological dormancy, unlike other species of the *Iris* genus.

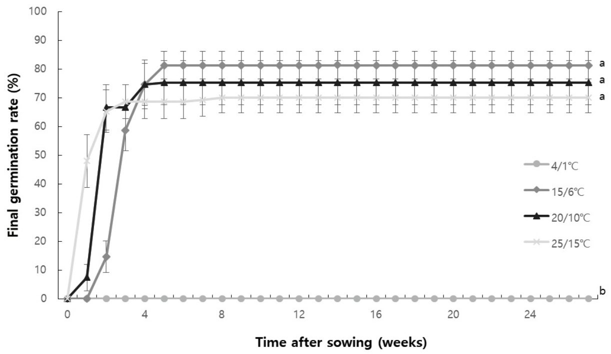

Fig. 3. Cumulative germination rate in seeds of *Iris dichotoma* Pall at four various temperatures (4/1°C, 15/6°C, 20/10°C and 25/15°C). The vertical error bars represent SE (n = 3). The different letters represent statistically significant differences, as determined by Tukey's HSD tests ($p < 0.05$).

It is theorized that in the evolution of seed dormancy, morphological dormancy and morphophysiological dormancy belong to the primitive dormancy class. Seeds with morphophysiological dormancy evolved as nondormant seeds with larger embryo size and lost physiological dormancy (Baskin & Baskin, 1998; FinchSavage & Leubner-Metzger, 2006; Nikolaeva, 2004). Therefore, nondormant seeds with fully developed embryo were hypothesized to have more evolved seed physiological characteristics. Thus, in this study, interspecific differences in seed dormancy type might have resulted from the evolution of seed dormancy in the *Iris* genus.

Seeds of *I. dichotoma* showed different MGTs under four temperature treatments (Fig . 4). MGTs of the seeds at 15/6°C, 20/10°C, and 25/15°C were 3.2, 2.1, and 1.5 weeks, respectively. There were significant differences in MGTs of these seeds under the four temperature treatments. As the temperature increased, the MGT decreased significantly. The MGT at 25/15°C was twice as fast as that at 15/6°C. MGT is not the only the parameter to predict the rate of germination. Plant growth post germination (e.g., root length, stem length, etc.) is also predictive (Demir *et al*., 2008; Matthews & Hosseini, 2006). In particular, MGT is highly correlated with mean emergence time (MET) and higher temperature (Demir *et al*., 2008; Demirkaya *et al*., 2020). In maize (Zea mays L.), there was a high correlation between MGT and MET. Root length was higher at lower MGT (Matthews & Hosseini, 2006). In a study on seeds of eight Veronica species native to Korea, MGT was found to be decreased with a higher temperature (Song *et al*., 2018). Thus, METs of seeds of *I. dichotoma* in laboratory and natural habitat can be predicted depending on MGT and the temperature of the environment. In natural habitats with relatively higher temperatures, seeds of *I. dichotoma* are expected to germinate faster than in habitats with relatively moderate temperatures. These results are helpful for mass propagation of *I. dichotoma* for the purpose of restoration.

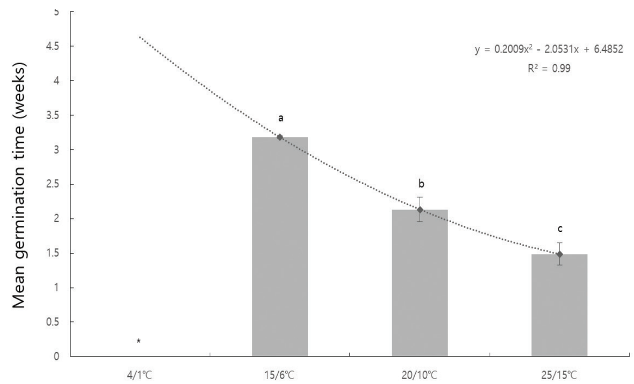

Fig. 4. Mean germination time of *Iris dichotoma* Pall under four temperature treatments. The vertical error bars represent SE (n = 3). The different letters represent statistically significant differences, as determined by Tukey's HSD tests ($p < 0.05$). An asterisk (*) indicates that no information was available for this field.

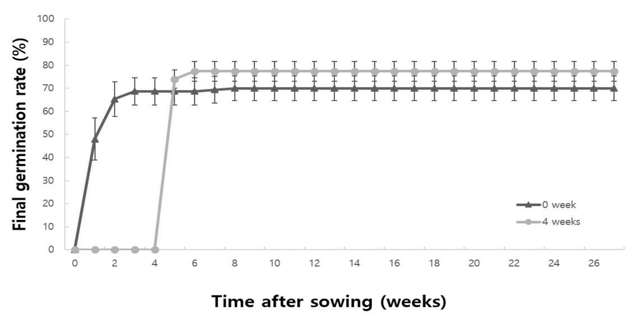

Fig. 5. Germination response of *Iris dichotoma* Pall according to cold stratification treatment (4 weeks at 4/1°C). The vertical error bars represent SE (n = 3). The different letters represent statistically significant differences, as determined by Tukey's HSD tests ($p < 0.05$).

Cold stratification

Cold-stratified seeds of *I. dichotoma* started to germinate at one week after the temperature was raised to 25/15°C. At 6 weeks after sowing, the FGR was 77.3%. There was no significant difference in the FGR be tween 0 week of cold stratification and 4 weeks of cold stratification (Fig . 5). Previous studies have shown that cold stratification can break physiological dormancy by removing germination inhibitors and improve germination improving hormone concentration (likely GA_3). However, in the present study, the temperature experiment showed that seeds of *I. dichotoma* had no physiological dormancy. Although cold stratification did not enhance the FGR, the highest FGR was maintained during cold stratification. The seed germination rate of *I. dichotoma* was the highest when seeds were stored at 2°C for 60 days among all treatments (Lee *et al*., 2015). Therefore, in natural habitats, seeds of *I. dichotoma* can disperse from October to November. These seeds can become cold stratified in the soil. As temperature increases in the following year, seeds can germinate without a decrease in germination rate.

Conclusion

Results of this study demonstrate that seeds of *I. dichotoma* have no seed dormancy, whereas seeds of other species in the Iris genus have physiological dormancy. This interspecific variation of seed dormancy in the same genus is considered to be due to the evolution of seed dormancy. *I. dichotoma* is considered to be a species that has differentiated or evolved more recently compared with other species of the Iris genus with seed dormancy. The FGR of *I. dichotoma* seeds was not affected by temperatures, although temperatures did affect MGT, which was the lowest at 25/15°C. In natural habitats, seeds of *I. dichotoma* can disperse with fully linearly developed embryos from October to November. These seeds then experience cold stratification in the soil. As temperature increases in the following year, seeds can germinate without a decrease in germination rate. Our results are helpful for understanding seed ecophysiological adaptions and mass propagation of *I. dichotoma*.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgments

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References

Baskin, C.C., and Baskin, J.M. (1998). *Seeds: ecology, biogeography, and, evolution of dormancy and germination*. San Diego, CA: Academic Press.

Baskin, J.M., and Baskin, C.C. (2004). A classification system for seed dormancy. *Seed Science Research*, 14, 166. doi:10.1079/SSR2003150

Demir, I., Ermis, S., Mavi, K., and Matthews, S. (2008). Mean germination time of pepper seed lots (Capsicum annuum L.) predicts size and uniformity of seedlings in germination tests and transplant modules. *Seed Science and Technology*, 36(1), 21-30. doi:10.15258/sst.2008.36.1.02

Demirkaya, M., Şekerci A.D., Teke, E., and Gülşen, O. (2020). Effects of osmotic conditioning treatments at different temperatures on mean germination time and rate of heliotro pium greuteri. *Journal of Biological & Environmental Sciences*, 14(40), 1-3.

Finch-Savage, W.E., and Leubner-metzger, G. (2006). Seed dormancy and the control of germination. *New Phytologist*, 172, 501-523. doi:10.1111/j.1469-8737.2006.01787.x

Geneve, R.L. (2003). Impact of temperature on seed dormancy. *HortScience*, 38(3), 336-341. doi:10.21273/HORTSCI.38.3.336

Hajyzadeh, M., Yildirim, M.U., Mokhtarzadeh, S., Sarihan, E.O., and Khawar, K.M. (2019). Breaking of seed dormancy in iris suaveolens boiss. Et reuter under in vitro conditions. *Acta Scientiarum Polonorum Hortorum Cultus*, 18(4), 15-24. doi:10.24326/asphc.2019.4.2

Hidayati, S.N., Baskin, J.M., and Baskin, C.C. (2000). Dormancy-breaking and germination requirements of seeds of four Lonicera species (Caprifoliaceae) with underdeveloped spatulate embryos. *Seed Science Research*, 10(4), 459-469. doi:10.1017/S0960258500000507

Kang, H.S., Shin, S.Y., and Whang, H.J. (2010). Are the conservation areas sufficient to conserve endangered plant species in Korea?. *Journal of Ecology and Environment*, 33(4), 377-389. doi:10.5141/JEFB.2010.33.4.377

Lashkarian, G., Kalatehjari, S., and Khosroshahli, M. (2012). Seed dormancy breaking evaluation of two subspecies seashore Iris, Iranian Native, through *in vitro culture. Journal of Agriculture and Food Technology*, 2(3), 56-60.

Lee, S.G., Kim, H.Y., Lee, K.C., and Ku, J.J. (2015). Effects of seed storage methods and shading on seed germination and seedling growth of endangered species, Iris dichotoma and Iris setosa. *Journal of Korean Society of Forest Science*, 104(1), 60-66. doi:14578/jkfs.2015.104.1.60

Martin, A.C. (1946). The comparative internal morphology of seeds. *The American midland naturalist*, 36, 513-660. doi:10.2307/2421457

Matthews, S., and Hosseini, M.K. (2006). Mean germination time as an indicator of emergence performance in soil of seed lots of maize (Zea mays). *Seed Science and Technology*, 34(2), 339-347. doi:10.15258/sst.2006.34.2.09.

National Institute of Biological Resources (NIBR). (2021). *National list of species of Korea*. Retrieved June 17, 2021 from http://species.nibr.go.kr.

Nikolaeva, M.G. (2004). On criteria to use in studies of seed evolution. *Seed Science Research*, 14(4), 315-320. doi:10.1079/SSR2004185

Park, H.B., Ko, C.H., Lee, S.Y., Kim, S.Y., Yang, J.C., and Lee, K.H. (2019). Ecophysiology of seed dormancy and germination in four Lonicera (Caprifoliaceae) species native to Korea. *Journal of Ecology and Environment*, 43(1), 1-9. doi:10.1186/s41610-019-0121-8

Song, S.J., Shin, U.S., Oh, H.J., Kim, S.Y., and Lee, S.Y. (2018). Seed germination responses and interspecific variations to different incubation temperatures in eight Veronica species native to Korea. *Horticultural Science and Technology*, 37(1), 20-31. doi:10.12972/KJHST.20190003

Sun, Y.C., Zhang, Y.J., Wang, K., and Qiu, X.J. (2006). NaOH scarification and stratification improve germination of Iris lactea var. chinensis seed. *HortScience*, 41(3), 773-774. doi:10.21273/HORTSCI.41.3.773