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Seed Dormancy and Germination Characteristics of Endemic Elder Species (*Sambucus racemosa* subsp. *pendula*) and Common Elder Species (*S. williamsii*) in Korea

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Abstract

This study was conducted to determine the seed dormancy types of *Sambucus racemosa* subsp. *pendula* Nakai and *S. williamsii* Hance. Low temperature stratification (1, 2, 3, 4 months) and GA_3 treatment (1,000 mg/L) were performed on seeds to determine the type of seed dormancy. After the treatment, seeds were placed on a petri dish at 25°C under light conditions. The germination rate and mean germination time were investigated. Results showed that cold stratification was effective in breaking the dormant state of the seed in both species. In the low temperature stratification treatment, the seed germination performance was improved as the treatment period was prolonged. Gibberellin treatment was effective in breaking the dormant state of *S. racemosa* subsp. *pendula* without low temperature stratification. However, *S. williamsii* did not break the dormant state of the seed by gibberellin treatment without low temperature stratification treatment. In the gibberellin treatment, germination performance was improved according to the low temperature stratification treatment period. As a result of this study, the seeds of *S. williamsii* have both an intermediate complex and a deep complex morphophysiological dormancy (MPD). In comparison, it was found that the *S. racemosa* subsp. *pendula* had intermediate composite MPD.

Key Words: *Sambucus*, cold stratification, GA_3, germination performances

Introduction

In forests, the process of growing from seed to seedlings is a very important step because it determines the survival and settlement of populations in the future (Harper 1977). The germination of seeds and the occurrence of seedlings are species-specific, which varies depending on the living environment, habitat environment, and geographical distribution of each species (Nikolaeva 1999). Seeds need a certain period of time to germinate, which is greatly affected by environmental conditions. Therefore, seeds have various dormant characteristics, which is an important strategy to increase the probability of survival of the species (Baskin and Baskin 1998). Dormancy of seeds means a state in which seeds do not germinate even in a suitable environment, which is reported to result from adaptive mechanisms to match the environment suitable for the successful survival and growth of seedlings (Finch-Savage and Leubner-Metzger 2006). Seed dormancy is largely divided into the following five categories: (1) physiological dormany (PY), (2) mor-

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phological dormany (MD), (3) morphophysiological dormany (MPD), (4) physical dormany (PD), and (5) combinational dormany (PD+PY) (Baskin and Baskin 2004). Among them, morphophysiological dormant (MPD) can be classified into eight categories as the following three conditions are identified: (1) temperature for breaking seed dormancy and promote seed germination, (2) temperature during embryo development, and (3) whether gibberellin (GA_3) breaking seed dormancy (Baskin and Baskin, 1998, 2004). In other words, when immature embryos develop at relatively high temperatures, they are referred to as simple morphophysiological dormany (simple MPD), and when embryos develop at relatively low temperatures, they are referred to as complex MPD. It is again classified into three stages (nondeep, intermediate, deep) according to physiological dormant depth.

About 20 species of the genus *Sambucus* are distributed in temperatures and subtropical regions, and six taxa (*S. racemosa* L. subsp. *kamtschatica* (E.L. Wolf) hultén, *S. racemosa* subsp. *sieboldiana* (Miq.) Hara, *S. racemosa* subsp. *pendula* (Nakai) H.I.Lim and Chin S. Chang, *S. williamsii* Hance, *S. williamsii* var. *coreana* (Nakai) Nakai, *S. latipinna* Nakai) are known to grow naturally in Korea (Lee 1996; Im 2000; Lim 2009). Recently, *S. williamsii* var. *coreana*, *S. latipinna*, and *S. williamsii* were referred to the same taxon (*S. williamsii*) (Korean Plant Names Index 2022). The fruit of *Sambucus* has been used for food such as jam and juice, and has been widely used for medicinal purposes (Brinkman and Johnson 2008). Among them, *S. racemosa* var. *coreana* species which is distributed throughout the Korean Peninsula is used as a good medicine for bones. In particular, *S. racemosa* subsp. *pendula* is distributed only on Ulleungdo Island, and is recognized as Korean endemic species based on their unique morphological characteristics with smaller fruits and unique inflorescence trait compared to other *Sambucus* species (Lim et al. 2009).

The *Sambucus* species distributed in Korean Peninsula bloom with the leaves from April to May, and the fruits mature from June to July (Lee 1980; Lim 2009). Remove the fruit and store the seeds after drying, and dried seeds of *Sambucus* are considered as orthodox, which can be store for more than 10 years (Hidayati et al. 2000). The species of the genus *Sambucus* are reported to be difficult to germinate seeds by dormant embryos and hard seed coats (Bolli 1994; Hidayati et al. 2000). In addition, it is known that there are various breaking seed dormancy methods for each species, such as high temperature stratification treatment, low temperature stratification treatment, seed coat treatment, and hormone treatment (Brinkman and Johnson 2008). In addition, it has been reported that different dormant types appear due to ecological differences in native habitat distributed even in the same genus *Sambucus* species (Hidayati et al. 2000). Therefore, the purpose of this study was to investigate the seed dormancy types of *S. williamsii* distributed throughout the Korean Peninsula and *S. racemosa* subsp. *pendula* distributed only in Ulleungdo Island, a endemic species of Korea.

Materials and Methods

Seed collection

The seeds of *S. williamsii* and *S. racemosa* subsp. *pendula* species used in this study were collected in July 2012 from individuals with good growth and health in Mt. Gyejoksan (Daejeon city) and in June 2012 Ulleungdo Island, respectively. The collected seeds were randomly selected for selected seeds after drying in the shade for two weeks, and the length, width, thickness, and seed index (length/width) of the seeds were investigated. To determine the seed dormancy types of two *Sambucus* species, dried seeds were selected and used in experiments from June 2012.

Low temperature stratification and gibberellin treatment

To investigate the effect of low temperature stratification treatment on seed germination, seeds were treated in filter paper (Anchor heavy weight seed germination paper, 76 lb., Anchor Paper Co. St. Paul, MN) and stored in a low temperature storage (4°C). Two stored species of *Sambucus* seeds were used in the experiment for a total of 4 months at 1-month intervals, from July to October 2012 in *S. williamsii* and from June to September 2012 in *S. racemose* subsp. *pendula*, respectively.

To investigate the effect of plant growth regulators on seed germination, gibberellin (GA_3, G-7645, Sigma-Aldrich Co., St. Louis, MO) were immersed at a concentration of

1,000 mg·L^{-1} for 24 hours for low temperature stratification seeds (0, 1, 2, 3, and 4 months), respectively.

The seed germination experiment was conducted on a seed germination chamber under conditions in which two sheets of Whatman No. 42 filter paper with a diameter of 9 cm were laid and sterile water was added to maintain the wet state. After four replications of 25 seeds each were placed in a complete random arrangement method, they were repeatedly irradiated at 25°C under light conditions (2,000 lux) for 14 hours. The germination investigation of seeds was considered to have germinated only with a root length of 2 mm or more for a total of 80 days. Using the number of germinated seeds, the germination percentage (GP) and the mean germination time (MGT) were calculated (Scott et al. 1984).

Statistical analyses

To compare the seed morphological characteristics between species, t-test was conducted at $p < 0.05$, and analysis of variance (ANOVA) was performed to compare the seed germination characteristics according to low temperature stratification and gibberellin treatment to analyze the difference between treatments. In addition, the germination percentage and the mean germination time were conducted by Duncan's multiple tests to indicate the difference between treatments at $p < 0.05$. All analyses were conducted using the agricolae package in R statistical software (4.0.5 for Windows).

Results and Discussion

The length, width, and thickness of the seed of *S. williamsii* were found to be significantly larger than that of the *S. racemosa* subsp. *pendula* seed, but the seed index was clearly higher for the *S. racemosa* subsp. *pendula* than that of the *S. williamsii* ($p < 0.05$) (Table 1).

The two *Sambucus* species were not seed germinated at all when sown without low temperature wet treatment (Fig. 1).

Table 1. Morphological characteristics of seeds in *S. williamsii* and *S. racemosa* subsp. *pendula*

Species	Length (mm)	Width (mm)	Thickness (mm)	Seed index
S. williamsii	2.7±0.1	1.6±0.1	0.9±0.1	1.6±0.1
S. racemosa subsp. *pendula*	2.5±0.1	1.4±0.1	0.8±0.1	1.7±0.1
Pr > t	***	***	***	**

The t-test was used to compare the results of two species, ** and *** indicates significant difference at $p \leq 0.01$ and $p \leq 0.001$.

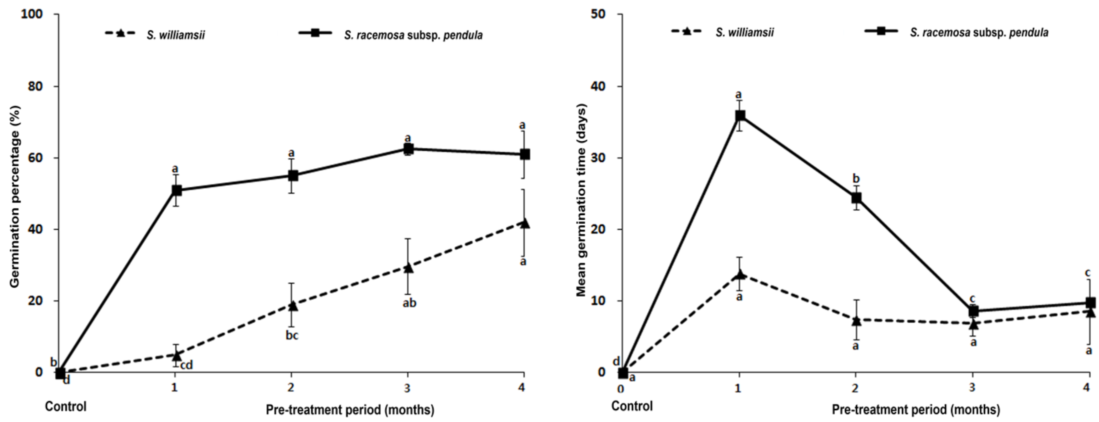

Fig. 1. Change of germination performances in different cold stratification treatment at 4°C of *S. williamsii* and *S. racemosa* subsp. *pendula*. The same letters are not significantly different at the 5% probability level by the Duncan's multiple range tests.

As a result of ANOVA on the germination characteristics of two *Sambucus* species seeds by low temperature wet treatment period, distinct differences were recognized only in germination rate, and *S. racemosa* subsp. *pendula* seeds showed distinct differences in both germination rate and average number of germination days (Table 2). In addition, as a result of ANOVA according to the treatment of gibberellin, there was a clear difference in the germination rate and the average number of germination days for *S. williamsii* seeds, and only the average germination days for *S. racemosa* subsp. *pendula* (Table 3).

The two *Sambucus* species were not seed germinated at all when sown without low temperature wet treatment. However, in both species, seeds began to germinate from one month of low temperature stratification, and the germination percentage tended to increase as the period increased (Fig. 1). The *S. williamsii* showed the highest seed germination percentage at 42.0% when treated for 4 months, while the *S. racemosa* subsp. *pendula* showed the highest seed germination percentage at 62.5% when treated for 3 months. However, the *S. racemosa* subsp. *pendula* showed a high seed germination percentage of 51.0% within one month of treatment, and there was no significant difference according to the treatment period ($p < 0.05$). The mean germination time was the shortest at 6.8 days and 8.7 days, respectively, for *S. williamsii* and *S. racemosa* subsp. *pendula* after low temperature wet treatment for 3 months. The germination percentage of *S. williamsii* seeds differed significantly depending on the period of low temperature stratification, but the mean germination times did not differ significantly. However, although there was no significant difference in germination percentages according to the low temperature stratification period for *S. racemosa* subsp. *pendula* seeds, the mean germination time showing a distinct difference depending on the treatment period.

In previous studies, there have been reports that low temperature treatment breaks down the dormancy of seeds (Kim et al. 1987), and low temperature wet treatment of seeds breaks down the dormancy by controlling the balance of germination inhibitors and germination-promoting substances present in the seed coat and embryo of seeds. In this study, it was also found that low temperature wet treatment breaks down the dormancy of seeds of two *Sambucus* species (*S. williamsii* and *S. racemosa* subsp. *pendula*). The seeds of *Weigela subessilis* L.H. Bailey included in the same Caprifoliaceae are known to germinate as a result of low temperature wet treatment rather than non-treatment, similar to the results of this study (Lee et al. 2003).

Gibberellin (GA) is effective in promoting dormant breaking of seeds, has different germination effects depending on plant species, and is effective in promoting germination of seeds with morphological and physiological dormant types (Hidayati et al. 2000). In addition, GA is generally known to break down seed dormancy by replacing low temperature treatment (Yoon et al. 1999). As a result of GA_3 treatment without low temperature wet treatment in *S. williamsii*, the seeds not germinated (Fig. 2). However, the germination rate of *S. williamsii* seeds increased when low temperature wet treatment and GA_3 treatment were performed together. The seeds of *S. racemosa* subsp. *pendula* showed a high germination rate of 67.5% by GA_3 alone treatment even without low temperature wet treatment, and showed a higher germination rate when low temperature wet treatment and GA_3 treatment were combined (Fig. 3). In particular, as the period of low temperature wet treat-

Table 2. ANOVA analysis of variance for two germination behaviors according to storage periods of cold stratification treatment in *S. williamsii* and *S. racemosa* subsp. *pendula*

Species	Mean square	
	Germination percentage (%)	Mean germination time (days)
S. williamsii	1,201.5***	97.8$^{n.s.}$
S. racemosa subsp. *pendula*	2,719.2***	816.9***

***$p \leq 0.001$, n.s., non-si6gnificance.

Table 3. ANOVA analysis of variance for two germination behaviors according to storage periods of cold stratification treatment with GA3 treatment in *S. williamsii* and *S. racemosa* subsp. pendula

Species	Mean square	
	Germination percentage (%)	Mean germination time (days)
S. williamsii	1,162.0***	232.3***
S. racemosa subsp. *pendula*	291.2***	214.8***

***$p \leq 0.001$, n.s., non-significance.

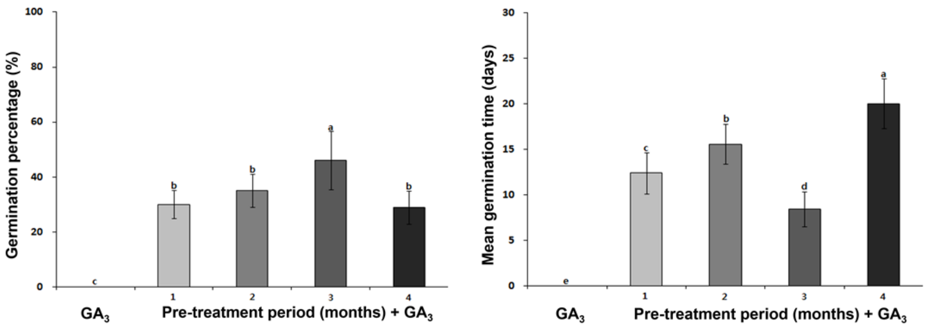

Fig. 2. Change of germination performances in different cold stratification at 4°C with 1,000 mg·L^{-1} GA_3 treatment of *S. williamsii*. The same letters are not significantly different at the 5% probability level by the Duncan's multiple range tests.

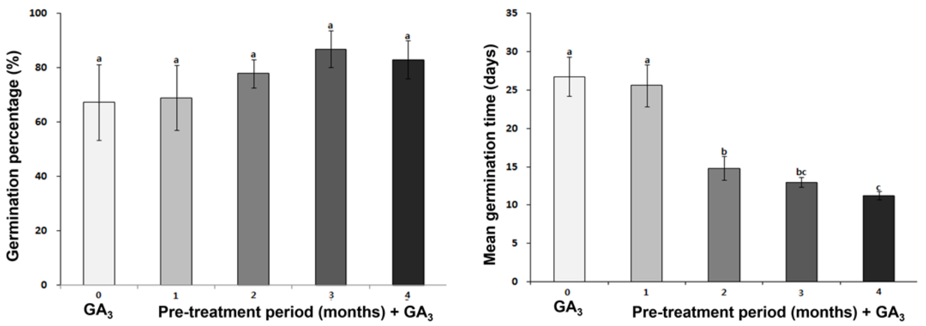

Fig. 3. Change of germination performances in different cold stratification at 4°C with 1,000 mg·L^{-1} GA_3 treatment of *S. racemosa* subsp. *pendula*. The same letters are not significantly different at the 5% probability level by the Duncan's multiple range tests.

ment increased, the average number of germination days decreased significantly when GA_3 treatment was added. Therefore, it was found that GA_3 does not replace low temperature wetting treatment to break down the dormant seeds in *S. williamsii*, but replaced in *S. racemosa* subsp. *pendula*.

According to the eight criteria for dormant types proposed by Baskin and Baskin (1998), complex MPD in which embryo development occurs at low temperatures is divided according to the degree of physiological dormancy. If the temperature conditions required to break down seed dormant are low temperature wetting, they are divided into intermediate complex MPD and deep complex MPD. Among them, it is divided into intermediate complex MPD when GA break down seed dormancy, and deep complex MPD when GA has no effect on dormant seed breaking (Baskin and Baskin 1998). In both *Sambucus* species, seed dormancy was break down by low temperature wetting treatment, and among them, *S. racemosa* subsp. *pendula* seeds were break down by GA_3 alone treatment. Therefore, the seed dormancy type of *S. williamsii* was found to be intermediate complex MPD and deep complex MPD based on the results that low temperature wetting treatment was effective in breaking down seed dormancy and GA_3 was not effective. However, *S. racemosa* subsp. *pendula* can be classified as a seed dormancy type of intermediate complex MPD because they effectively break through low temperature wetting and GA_3. In this study, it was found that the dormant physiology of seeds was different depending on the species even within the same genus of *Sambucus*, and different dormant seed types have been reported in previous studies on *S. canadensis* L. and *S. pubens* Michx (Hidayati et al. 2000). These results are thought to be due to geographical, ecological, and evolutionary differences in the dormant physiology of seeds depending on the species even within the same genus (Baskin and Baskin 1998, 2004; Finch-Savage and Leubner-Metzger 2006).

Conclusion

S. racemosa subsp. *pendula* is distributed only on Ulleungdo Island, and is recognized as Korean endemic species. The *S. racemosa* subsp. *pendula* has a unique flower morphological characteristic, which is distinguished from the other *Sambucus* species. It is necessary to investigate the dormant characteristics of the seeds as basic data for the conservation of the Korean endemic *Sambucus* species. In this study, we compared and analyzed the seed dormant type of the endemic species, *S. racemosa* subsp. *pendula*, and the inland species, the *S. williamsii*. With these results, seeds of *S. williamsii* have both intermediate complex and deep complex morphophysiological dormancy (MPD). In comparison, seeds of the *S. racemosa* subsp. *pendula* have intermediate complex MPD. Therefore, GA_3 and low temperature wetting treatment were found to be effective for seed germination. It is expected that our study will provide insights for the conservation and propagation of not only Korean endemic species, *S. racemosa* subsp. *pendula* but also *S. williamsii*.

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