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Seed and Germination Characteristics of *Allium koreanum* H.J. Choi & B.U. Oh for Effective Propagation

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Abstract. Native plants are unique genetic resources that have the potential to be used as ornamental, medical, and food resources. *Allium koreanum* H.J. Choi & B.U. Oh is one of the native plants distributed in the coastal rocky land of Korea, which has high conservation value due to climate change and reduction of its habitat. This study was conducted to investigate the effects of temperature and hydrogen peroxide on the germination of *A. koreanum*. The seeds were prepared as untreated (control) or those treated with 1% hydrogen peroxide for 90 minutes (H_2O_2), and the treatments were placed in plant growth chambers set at 15, 20, and 25°C. Regardless of the H_2O_2 treatment, the germination percentage at 15°C, which was 42%, was more than two times higher than that at 20°C and 25°C, which were 18% and 0%, respectively, 23 days after sowing. The number of days to attain 50% of the final germination percentage (T_{50}) was the shortest at 20°C, but the mean daily germination (MDG) was the highest at 15°C. Therefore, 1% of H_2O_2 treatment did not have a significant effect on the germination percentage of *A. koreanum*, and a temperature of 15°C was considered to be optimum to increase the germination percentage of *A. koreanum*. The results of this study can be used as basic research data for the germination of *A. koreanum*.

Additional key words: conservation, mean daily germination, native plant, plant growth chamber, rocky land

Introduction

Allium is a genus of plants, including garlic, onion, and leek, and is commonly cultivated for its pungent flavor and medicinal properties (Fenwick et al., 1985; Czech et al., 2022). *Allium* comprises more than 600 different species throughout North America, Europe, North Africa, and Asia (Fenwick et al., 1985; Choi et al., 2004a). As various species are distributed over a wide area, many species are native to specific areas, such as *A. ampeloprasum* (Mediterranean region) and *A. hirtifolium* (Iran) (Taran et al., 2006; Sharifi-Rad et al., 2016). Six species of native plants of the genus *Allium*, including *A. koreanum*, *A. taquetii*, and *A. deltoide-fistulosum*, have been reported in Korea (Choi et al., 2004a). As native plants of the genus *Allium* in other regions have been reported to have pharmacological value, Korean native plants may also have high potential utilization value (Fenwick et al., 1985; Taran et al., 2006; Sharifi-Rad et al., 2016).

A. koreanum was first reported as a native Korean plant in 2004 (Choi et al., 2004b). The hermaphroditic, perennial herb has paired ovoid bulbs and three to eight linear leaves (Choi and Oh, 2011). It blooms from August to November, and 70 to 200 reddish-purple-colored flowers form an umbel at the end of the flower stalk (Korea Forest Service, 2008). *A. koreanum* is distributed on the rocky land of Korea. It was first reported in Mountain Mai, Korea, but the most frequently found location is coastal rocky land (Choi et al.,

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2004b; National Biodiversity Center, 2018; Naturing, 2019). Recently, it has been reported as a genetic resource that can be eaten and used as an ornamental plant; however, the species has only been reported more recently in academia, and no effective propagation and cultivation methods have been reported (NIBR, 2023). Nevertheless, it is necessary to conserve *A. koreanum* to preserve the biodiversity of Korea and ensure the right to use biological and genetic resources according to the Nagoya Protocol (Park and Jung, 2017).

Seeds are one of the most basic reproductive organs of plants, and in many countries, species are maintained by preserving them in seed banks (Lee and Lee, 2014; Na et al., 2014; Rajametov et al., 2014). Seeds must be stored for a long time, but it is also important to germinate the seeds that have been stored (Na et al., 2014; Rajametov et al., 2014). For the seeds to germinate, appropriate moisture, temperature, oxygen, and light environments must be provided (Gardner et al., 2020). After the seeds absorb water, catabolism and anabolism by enzymes occur; at this time, an appropriate temperature must be provided to maximize the activity of enzymes and achieve the highest germination percentage in the shortest period (Gardner et al., 2020). Since the optimum temperature for seed germination is different for each species, it is important to determine the optimum germination temperature for seed propagation of native species (Thompson et al., 1977).

Studies on the use of peroxide as a germination inducer are increasing in number. Hydrogen peroxide (H_2O_2) is used as an antimicrobial agent for germination because of its oxidative reactivity (Ogawa and Iwabuchi, 2001). In addition, reactive oxygen species generated by H_2O_2 act as signal messengers in the cell membranes of seeds (Bailly et al., 2008). Barba-Espín et al. (2010) proposed an interaction between the redox state and phytohormones coordinated by H_2O_2 in the induction of proteins associated with plant signaling and development during pea seed germination. Wojtyla et al. (2016) reported that H_2O_2 promotes germination by affecting the interaction of plant growth hormones, such as gibberellic acid (GA), indole-3-acetic acid (IAA), and abscisic acid (ABA), in seeds. However, the effect of H_2O_2 as a germination inducer depends on the species, type of dormancy, and germination environment (Lariguet et al., 2013). Therefore, it is necessary to study the seed germination inducing effect of H_2O_2, which has a seed disinfection effect and is cheaper than other plant growth regulators.

Therefore, this study was conducted to investigate the effect of temperature and H_2O_2 on the germination of *A. koreanum* using a basic propagation method.

Materials and Methods

1. Investigation of Basic Seed Characteristics

A. koreanum seeds were collected from the coastal rocky area of Busan, Korea, from July 16 to August 20, 2022. For the investigation of morphological characteristics of *A. koreanum* seeds, ten seeds were randomly selected for each of the three replicates. The seed length, width, color, E:S ratio (embryo:seed length ratio) were measured using a stereoscopic microscope (SMZ 745T, Nikon Co., Ltd., Tokyo, Japan) with ToupView software package (version 4.11, ToupTek Photonics Co., Ltd., Hangzhou, China). The 100 seed weight was determined by measuring the weight of 100 seeds using an electronic balance (EW220-3NM, Kem&Sohn GmbH., Balingen, Germany), and the mean value of the three replicates was applied. The seed moisture absorption was investigated as follows: 0.1 g of *A. koreanum* seeds was placed in 15 mL microtubes and immersed in 5 mL of distilled water, then stored at room temperature. Every 24 hours, the seeds were taken out, and the moisture on the seed coat was removed using wiper (Smart-science wiper, SciLab, Seoul, Korea) before measuring the weight. Then, the seeds were re-immersed in fresh distilled water and observed for 7 days (Han et al., 2022).

2. Effects of Temperature and Hydrogen Peroxide on Germination of *A. koreanum*

After drying and selection, the seeds were immersed in distilled water (control), and the hydrogen peroxide treatment seeds were immersed in 1% hydrogen peroxide (Sungkwang hydrogen peroxide, Firson Co., Ltd., Cheonan, Korea) for 90 min (Nongsaro, 2008; Imani et al., 2011). After immersion, 10 seeds were sown on a layer of wet filter paper (90 mm diameter, CHMLAB Co., Ltd., Barcelona, Spain) in a petri dish (90 mm diameter, Daihan Scientific Co., Ltd., Wonju, Korea). Five petri dishes were placed in a plant growth chamber (C1200H3, FC Poibe Co., Ltd., Seoul, Korea) set

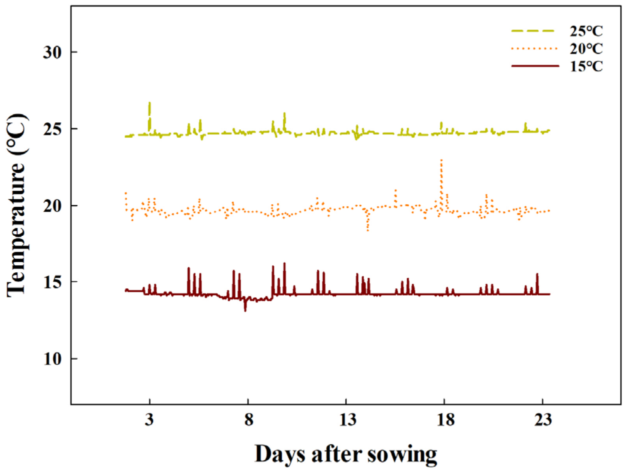

Fig. 1. Temperature inside the plant growth chamber for 23 days after sowing.

at 15, 20, and 25°C, and the actual temperatures were 15±0.8, 20±0.8, and 25±0.5°C (mean±SD), respectively (Fig. 1). Most *Allium* species native to Korea tend to germinate well in dark conditions (Kim et al., 2021), therefore experiment was conducted in the dark for 24 h. The moisture content of the filter paper was maintained by supplying 10 mL of distilled water once daily. The experiment was conducted for 23 days, from September 19, 2022, to October 11, 2022.

3. Germination Characteristics

Seeds with radicles of 2 mm or more were considered germinated seeds. The number of germinated seeds in each petri dish was counted daily. Four parameters to evaluate germinability were calculated: germination percentage, mean germination time (MGT), mean daily germination (MDG), and days to attain 50% of the final germination percentage (T_{50}) (Farooq et al., 2005; Gairola et al., 2011; Nadimi et al., 2022).

$$Germination\ percentage = \frac{N_g}{N_t} \times 100 \quad (1)$$

where N_g and N_t represent the number of germinated seeds and total number of seeds, respectively.

$$MGT = \frac{\Sigma D \times n}{\Sigma n} \quad (2)$$

where D and n represent the number of days from the start of the germination experiment and the number of seeds germinated on day D, respectively.

$$MDG = \frac{N_{tg}}{D_t} \quad (3)$$

where N_{tg} and D_t represent the total number of germinated seeds and total number of days, respectively.

$$T_{50} = t_i + \frac{\left(\frac{N_{tg}}{2} - n_i\right)(t_j - t_i)}{n_j - n_i} \quad (4)$$

where n_i and n_j represent the cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N_{tg}/2 < n_j$, respectively.

4. Statistical Analyses

The experimental treatments were performed using a randomized completely randomized design. Ten seeds were used per replicate with 5 replications. Statistical analyses were performed using statistical analysis software (SAS 9.4, SAS Institute Inc., Cary, NC, USA). The experimental results were analyzed using analysis of variance and Tukey's range test. Differences were considered statistically significant at $p \leq 0.05$. The graph was plotted using SigmaPlot software package (SigmaPlot 14.5, Systat Software Inc., San Jose, CA, USA).

Results and Discussion

The length and width of *A. koreanum* seeds were 3.58±0.04 and 1.61±0.03 mm, respectively (Table 1). The length-width ratio was calculated as 2.23±0.05. The seed color was black (Fig. 2), and the E:S ratio was calculated as 0.85±0.03, and the 100-seed weight was measured as 0.21±0.005 g. Based on the observations from a cross-sectional picture of seed, it is determined that the type of *A. koreanum* seed is 'MA-seed miniature axile dwarf or micro' (Martin, 1946; Finch-Savage and Leubner-Metzger, 2006). When water absorption into the seed is prevented, it will delay germination; this phenomenon is referred to as physical dormancy

Table 1. Seed morphological characteristics of the *Allium koreanum* (n = 30).

Size			Color	E:S ratio[z]	100 seed weight (g)
Length (mm)	Width (mm)	Length-width ratio			
3.58±0.04	1.61±0.03	2.23±0.05	Black	0.85±0.03	0.21±0.005

[z]Embryo:seed length ratio.

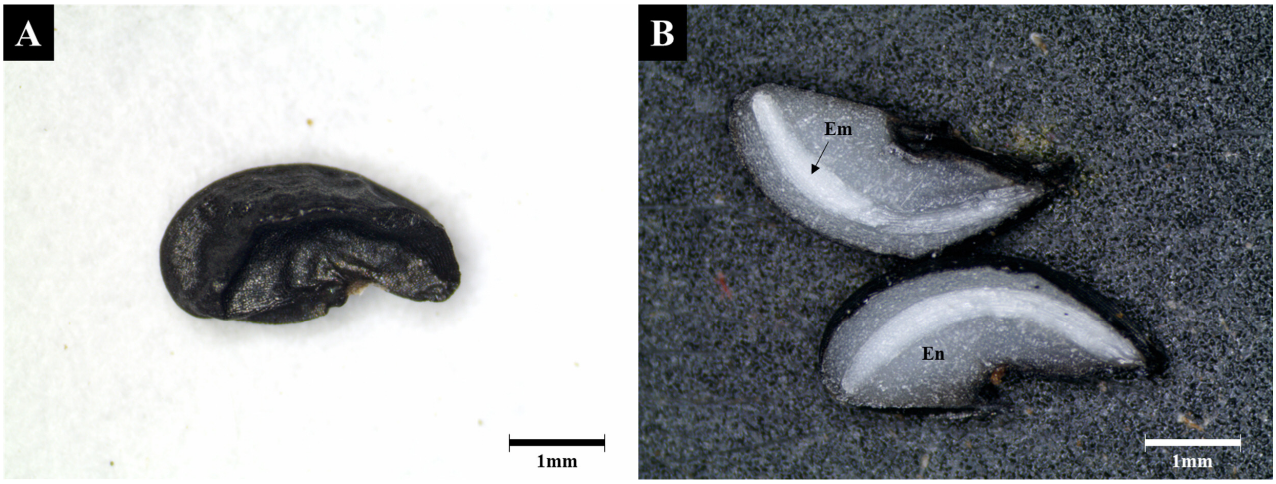

Fig. 2. Morphology (A) and cross section (B) of *Allium koreanum* seeds. Em: embryo; En: endosperm.

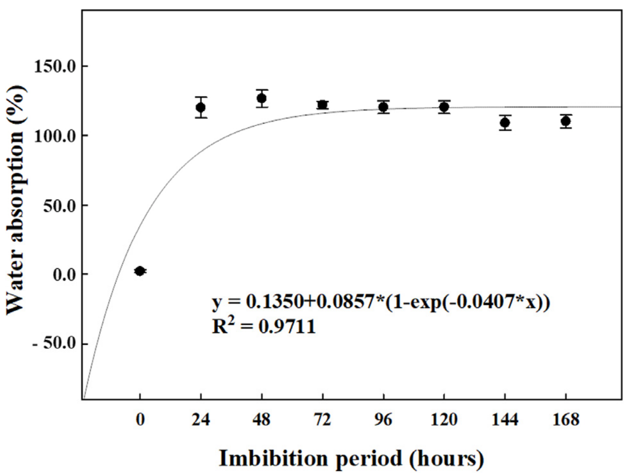

Fig. 3. Changes in water absorption of seeds on *Allium koreanum* soaked in water (n = 3).

(Baskin and Baskin, 2004; Finch-Savage and Leubner-Metzger, 2006). A seed is considered permeable if its weight increases by more than 20% after water uptake compared to its initial seed weight (Baskin and Baskin, 2004). *A. koreanum* is determined to have no physical dormancy, as it exhibited an increase in moisture absorption of over 105% within just 24 hours of seed soaking (Fig. 3).

The first germination was observed 6 d after sowing at 20°C (Fig. 4). Regardless of the H_2O_2 treatment, the 25°C treatment showed no germination. From 16 d after sowing, a significant difference in germination percentage was observed between the treatments. The germination percentage was affected by temperature but not by hydrogen peroxide treatment. At 15°C, the germination percentage tended to be higher than at 20°C. Some germination-inhibiting chemicals (typically ABA) inside the seeds become active at high temperatures (Yoshioka et al., 1998; Geneve, 2003). A wild plant of the same *Allium* genus (*A. tenuissimum* L., *A. truncatum, A. rothii*) showed a high germination percentage at temperatures in the range 15－20°C, and it was confirmed that the germination percentage decreased at temperatures above 20°C (Gutterman et al., 1995; Xiao et al., 2020). For plants that germinate during low-temperature periods, high temperatures induce secondary dormancy (Xiao et al., 2020). *A. koreanum* blooms from July to August and seeds germinate after winter (Choi et al., 2004b). The average temperatures of Korea in spring, summer, autumn, and winter are 13.4, 25.6, 15.5, and −0.9°C, respectively (KMA, 2023). Hydrogen peroxide did not have any significant effect on the germination percentage of *A. koreanum*. The treatment time and concentration were considered insufficient to affect seed germination. Therefore, it is considered that 15°C, which is approximately the average temperature in spring in Korea, is optimal for germination.

The MGT was not significantly different between 15 and 20°C with H_2O_2 treatment (Table 2). MDG was higher at 15°C than at 20°C. T_{50} was higher at 15°C with control. Unlike the 20°C, which has a gradual increase in germination, the 15°C has a burst of germination after 12 days (Fig. 4). And the final germination percentage was higher at 15 than at 20°C. Because of these unusual germination characteristics, it appears to have different germination characteristics than other *Allium* species, which generally have similar tendency in germination percentage, and T_{50} (Kim et al., 2021). *A. koreanum* is considered to be a seed, which germination is induced at low temperatures. However, Wojtyla et al. (2016) reported that, the H_2O_2 can decrease the germination time by improving the signaling function inside the seed but did not work in this experiment and further research is needed.

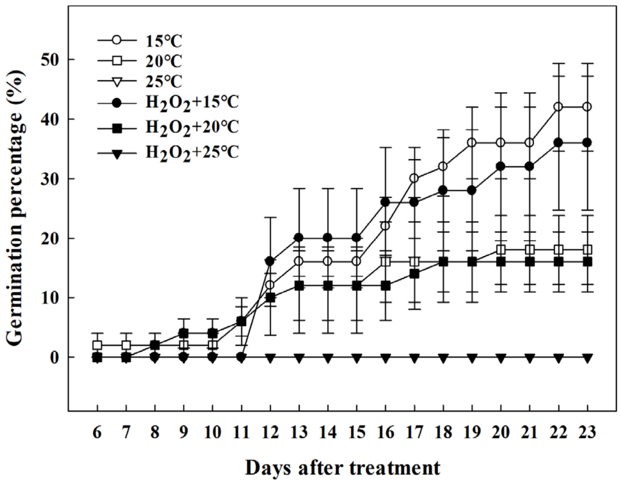

Fig. 4. Germination percentage of *Allium koreanum* affected by hydrogen peroxide (H_2O_2) and sowing temperature during 23 days after sowing. Vertical bars indicate standard errors of the means (n = 50).

Conclusion

In this study, we examined the optimum temperature environment and the effect of H_2O_2 on *A. koreanum* seed germination. The germination percentage was affected by temperature but not by H_2O_2 treatment. At 15°C, the germination percentage was higher than that at 20°C. There was no germination at 25°C. The MGT was not significantly different between 15 and 20°C. However, MDG and T_{50} tended to be higher at 15°C. Therefore, the appropriate temperature in this study environment for *A. koreanum* germination was considered to be 15°C. This finding can be helpful for basic propagation research on *A. koreanum*. The germination percentage of *A. koreanum* is low, at less than 50%, so further research is needed to increase the germination

Table 2. Germination characteristics of *Allium koreanum* affected by hydrogen peroxide (H_2O_2) and sowing temperature at 23 days after sowing (n = 50).

H_2O_2 treatment (A)	Temperature (°C) (B)	MGT[z]	MDG[y]	T_{50}[x]
None	15	14.7 a[w]	0.18 a	13.4 a
	20	14.6 a	0.08 b	9.5 b
H_2O_2	15	16.4 a	0.16 a	11.0 ab
	20	14.3 a	0.09 b	8.8 b
Significance	A	NS	NS	*
	B	NS	***	***
	A × B	NS	NS	NS

[z]Mean germination time.
[y]Mean daily germination.
[x]Days to attain 50% of the final germination percentage.
[w]Mean separation within columns by Tukey's range test at $p \leq 0.05$.
[NS,*,***]Nonsignificant or significant at $p \leq 0.05$ or 0.001, respectively.

percentage. For instance, plant growth regulators or cold stratification may be considered.

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효과적인 번식을 위한 돌부추의 종자 및 발아 특성

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적 요. 자생식물은 관상용, 약용, 식량자원으로 활용될 수 있는 잠재력을 지닌 고유 유전자원이다. 돌부추(*Allium koreanum* H.J. Choi & B.U. Oh)는 우리나라 해안 암반지대에 분포하는 자생식물 중 하나로, 기후변화와 서식지 감소로 인해 보전 가치가 높은 식물이다. 이번 연구는 온도와 과산화수소가 돌부추의 발아에 미치는 영향을 조사하기 위해 수행되었다. 종자를 무처리(대조군) 또는 1% 과산화수소(H_2O_2)로 90분간 처리한 종자를 준비해 15, 20, 25°C로 설정된 식물 생장 챔버에 배치하였다. 그 결과, 파종 23일 후 15°C에서 42%인 발아율이 20°C와 25°C에서 각각 18%와 0%인 발아율보다 2배 이상 높았으며, H_2O_2 처리 여부와 관계없이 15°C에서 발아율이 42%로 가장 높았다. H_2O_2 처리와 관계없이 최종 발아율 50%(T_{50})에 도달하는 일수는 20°C에서 가장 짧았지만, 일평균 발아율(MDG)은 15°C에서 가장 높았다. 따라서 1%의 H_2O_2 처리는 돌부추의 발아율에 큰 영향을 미치지 않았으며, 15°C의 온도가 돌부추의 발아율을 높이는 데 최적인 것으로 판단된다. 본 연구 결과는 돌부추의 발아를 위한 기초 연구 자료로 활용될 수 있을 것으로 기대된다.

추가 주제어 : 보존, 평균 일일 발아, 자생 식물, 식물 생장 챔버, 암석지