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Effect of Seed Priming on the Germination Properties of *Allium hookeri*

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ABSTRACT

Effects of seed priming on the germination properties of *A. hookeri*, an important subtropical vegetable in Korea, were tested in Petri dishes and soil conditions. In the Petri dish, the effects of two priming solution s, calcium chloride ($CaCl_2$) and polyethylene glycol (PEG), and five priming times (6, 12, 18, 24, and 48 h) were evaluated. The results indicated that both $CaCl_2$ and PEG priming enhanced the germination of *A. hookeri*, whereas PEG priming resulted in a higher germination percentage, energy, rate, and time to reach 50% germination, compared to the control and the $CaCl_2$ priming. We further investigated whether seed priming had the same effect on *A. hookeri* germination in the soil. For this experiment, we selected two priming conditions that showed the best result for each group in a Petri dish by observing germination properties and initial growth characteristics. We found that both priming solutions positively affected the germination and early-stage growth of *A. hookeri*, although $CaCl_2$ priming had a more potent effect. These results suggested that seed priming can be effectively used for improving the germination properties of *A. hookeri*, even as its effect can differ depending on the environment of seed germination and growth.

Introduction

As of 2020, the number of foreigners living in Korea has exceeded 2.5 million, and approximately 25% of them have come from Southeast Asian countries such as Vietnam, Thailand, and the Philippines. This has become an important factor in promoting the cultivation of subtropical crops in Korea. The planting area of subtropical crops in Korea was only 99 ha in 2012, but this has increased sharply to 991 ha by 2020. In addition, 62.3% of the farmland in Korea is expected to belong to the subtropical climate zone by 2080 if greenhouse gases are emitted with the current trends (Jeong et al., 2020). Considering that one of the crucial factors for the stable growth of plants is climatic conditions (Arisz et al., 2018), the importance of cultivating subtropical crops in Korea is predicted to increase over time; thus, extensive research to utilize them more efficiently is required.

Allium hookeri (*A. hookeri*), one of the most widely cultivated subtropical vegetable crops in Korea, has a unique taste and demonstrates potent antioxidant activity (Rho et al., 2020; Shin and Jeong, 2021). Seeds of *A. hookeri* are obtained from wild individuals and are usually supplied to farmers in Korea. Seeds have a diverse distribution in size and maturity, and it is difficult to secure the collection of standardized seeds during the process of seed gathering. They often exhibit low and uneven germination patterns. Poor seed germination of *A. hookeri* results in a decrease in productivity and quality and is an important factor that deteriorates the profitability of *A. hookeri* farms. To solve this problem, it is necessary to identify the factors

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leading to improved germination properties of *A. hookeri.*

Seed priming, which can induce the physiological and biochemical changes required for successful seed germination, has been utilized as an important method for improving the germination properties of various commercial crops (Adnan et al., 2020; Zulfiqar, 2021). However, the effect of seed priming varies depending on the crop, priming solution, and time (Kim et al., 2021; Sadeghizadeh and Zarea, 2022; Tuiwong et al., 2022). Therefore, it is important to evaluate their effects when applying them for cultivation purposes. However, there have been no studies on this occasion in *A. hookeri*. This study aimed to examine whether seed priming can be used to improve the germination properties of *A. hookeri* by comprehensively investigating the effects of different priming solutions and treatment times on the germination properties *A. hookeri*.

Materials and Methods

The present study was conducted at the plant breeding laboratory of Gangneung-Wonju National University in Korea. Seeds of *A. hookeri* were produced by the World Seed Company in Korea and were used for this study. The effect of priming solutions and time on the germination properties of *A. hookeri* was assessed using Petri dishes at first. In this experiment, 1.0% calcium chloride ($CaCl_2$) and 13.5% polyethylene glycol (PEG) 6000 were selected as priming solutions. The priming treatment time was set at 12, 24, or 48 h. Seeds that were not subjected to any treatment were used as controls to determine the effects of the priming treatment. Thirty primed and control seeds were placed in Petri dishes with four replications, and germination was induced in an environment at a temperature of 20±1℃, a humidity of 60±10%, the photosynthetic photon flux density of 120 μmol m^{-2} $second^{-1}$, and a photoperiod of 16 h. The number of germinated seeds was determined as germinated individuals whose rootlets were elongated by 1 mm or more and surveyed every day from the day after the seeds were incubated to the 10th day. Based on this, germination percentage, energy, and the time to reach 50% germination were calculated according to the formula described by Kim et al. (2022).

Since there are environmental differences between Petri dishes and soils in terms of moisture, oxygen, and light environment required for seed germination, the positive priming effect observed in the Petri dish would not appear in the soil. Thus, to confirm the usefulness of priming treatments in *A. hookeri*, we further tested whether the two priming conditions that showed the best results in each priming solution group from the Petri dish experiment showed equally good results in the soil. To accomplish this task, germination properties and initial growth characteristics were investigated. For this experiment, *A. hookeri* seeds with or without priming treatment were sown in three replications each at a depth of 0.5 cm in a 32-hole plug tray filled with horticultural soil (Best; Shinsung Mineral, Seongnam, Korea). The germination of *A. hookeri* in soil was investigated daily from the 1st to the 20th day after sowing seeds, and the germination properties were assessed using the same indicators and calculation methods as in the Petri dish experiment. The comparison of initial growth characteristics between control or seed-primed *A. hookeri* seedlings was performed by measuring fresh and dry weight and the number of leaves in seven randomly selected seedlings grown for 40 days. The fresh weights of above- and below-ground parts were measured using an electronic balance (WBA-220; Witeg Labortechnik GmbH, Wertheim, Germany), and their dry weights were measured after drying them in an incubator (HK-BI025; Hankuk S&I, Hwaseong, Korea) at 70 °C for 48 h (Jang et al., 2020). The lengths of the whole plant and root were measured using a ruler. The experiment was conducted in a randomized block design. For the comparison of data, the analysis of variance (ANOVA) was performed using SPSS (Version 24; IBM, New York, USA) and then the Duncan test at $P \leq 0.05$ was used to compare significant means.

Results

Effects of priming solution and time on germination properties of *A. hookeri* are summarized in Table 1. Germination patterns of *A. hookeri* in petri-dish were slightly different depending on experimental conditions. Generally, germination started on the 1st day of the experiment and was completed on the 7th day. The germination percentage in the control was 47.0%, whereas the percentage in the PEG and $CaCl_2$ priming group was 50.0~78.0%. The longer the priming time in the PEG priming group, the higher the germination percentage (Fig. 1).

As a result, the highest germination percentage (78.0%) was observed with 48-hour PEG priming among all conditions. The germination percentage was higher in the $CaCl_2$ priming group compared to the control. However, the germination percentage gradually increased with 18-hour priming, and then decreased with an increase in priming time, unlike the PEG priming

group. In addition, the overall germination percentage in the $CaCl_2$ priming group was lower than that observed in the PEG priming group. The T_{50} value of *A. hookeri* with PEG priming was shorter than that of the control by at least 0.4 days to a maximum of 0.8 days. In particular, the lowest T_{50} was observed with 48-hour PEG priming, which led to the highest germination percentage. In $CaCl_2$ priming, only 12 and 18-hour priming showed significantly lower levels of T50 compared to the control. It was observed that the germination energy and rate were commonly improved in the groups with statistically significantly higher values of germination percentage.

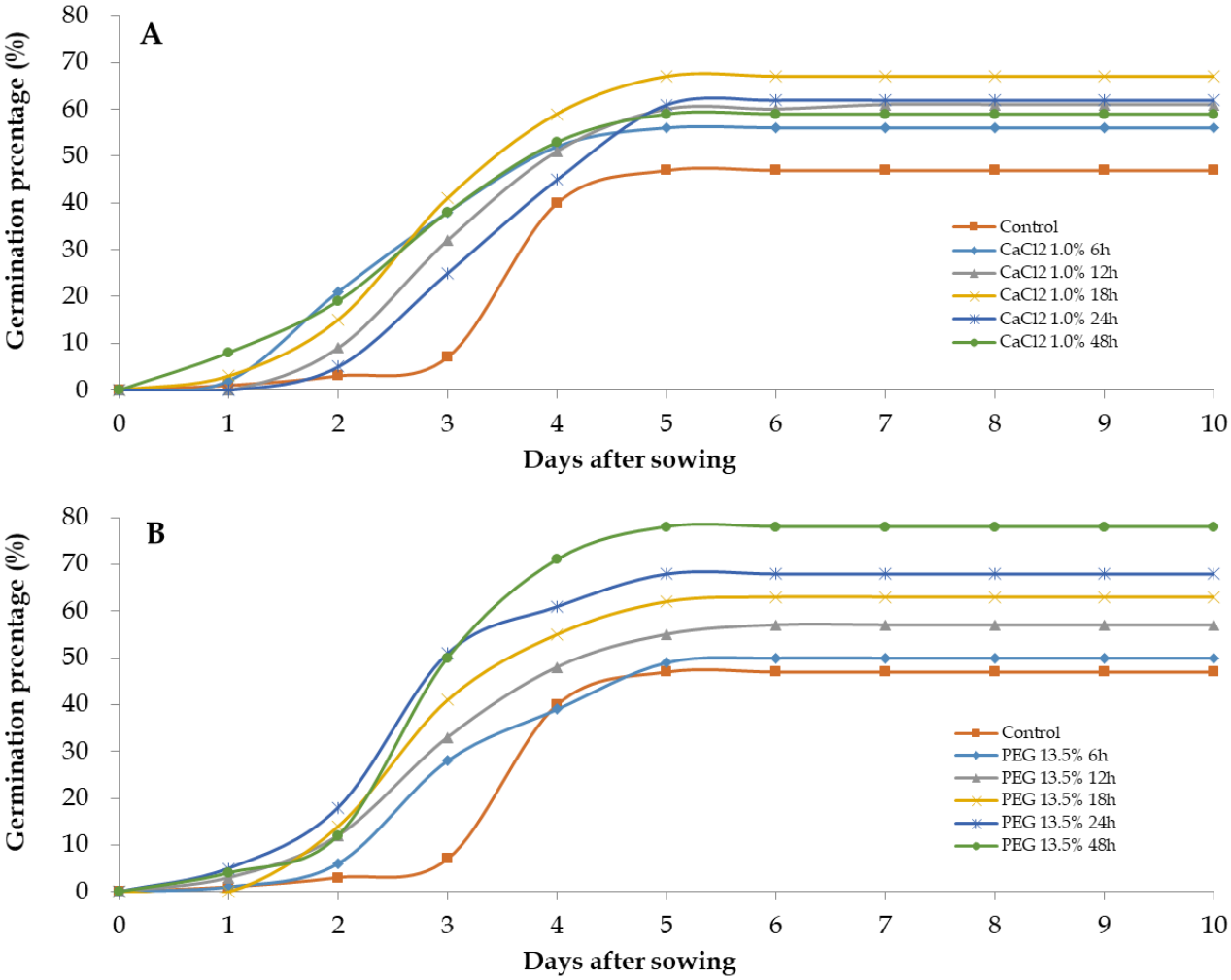

Fig 1. Effect of various priming conditions on germination pattern of *Allium hookeri* seedlings in petri-dish. (A) $CaCl_2$ treatment; (B) PEG treatment.

In the soil experiment, the germination percentage ranged from 34.4–47.8 %, which was lower than the value observed in the Petri dish experiment (Table 2). However, the germination percentages were 23% and 39% in the PEG and $CaCl_2$ priming groups, respectively, which were significantly higher than that in the control although there was no significant difference in germination energy, germination rate, and T50 among treatments.

Table 1. Effect of $CaCl_2$ and PEG priming on germination properties of *Allium hookeri* in petri-dish.

Method	Treatment	Germination percentage (%)[z]	Germination energy	Germination rate	T_{50}
Control	0 h	47.0 d	47.0 d	88.3 e	3.1 b
$CaCl_2$	6 h	56.0 bcd	52.0 cd	98.3 cde	3.6 a
	12 h	61.0 bc	60.0 bcd	114.0 bcde	3.1 b
	18 h	67.0 ab	67.0 ab	130.0 ab	2.8 bcd
	24 h	61.0 bc	61.0 bc	111.5 bcde	3.4 a
	48 h	59.0 bcd	59.0 bcd	118.0 bcd	2.7 cd
PEG	6 h	50.0 cd	49.0 cd	93.3 de	3.1 b
	12 h	57.0 bcd	55.0 bcd	109.0 bcde	3.0 bc
	18 h	63.0 bc	62.0 bc	121.8 bc	2.8 bcd
	24 h	68.0 ab	68.0 ab	135.8 ab	2.6 d
	48 h	78.0 a	78.0 a	151.3 a	2.8 bcd
ANOVA[y]					
Method (A)		NS	NS	NS	**
Treatment (B)		***	***	***	***
A × B		NS	NS	NS	***

z Within each column, the mean values followed by different letters are significantly different according to Duncan's multiple range test ($p \leq 0.05$).

y *, **, *** Significant at the 0.05, 0.01 and 0.001 probability level, respectively. NS = Not significant.

Table 2. Effect of $CaCl_2$ and PEG priming on germination properties of *Allium hookeri* in soil.

Method	Germination percentage (%)[z]	Germination energy	Germination rate	T_{50}
Control	34.4 b	32.2 a	140.0 a	7.1 a
$CaCl_2$ 18 h	47.8 a	35.6 a	181.3 a	7.9 a
PEG 48 h	42.2 ab	40.0 a	176.3 a	6.4 a

z Within each column, means followed by different letters are significantly different according to Duncan's multiple range test ($p \leq 0.05$).

The germination percentage differed between the two priming groups in the soil experiment, and $CaCl_2$ seemed to have a stronger effect on seed germination than PEG in the soil. The lengths of the aboveground part of seedlings obtained after non-priming and $CaCl_2$ and PEG priming were 16.2, 17.9, and 16.9 cm, respectively, but no significant difference was detected therein (Table 3). In contrast, root length increased with $CaCl_2$ and PEG priming by 34 % and 29 %, respectively, compared to the control. In addition, the number of leaves in seedlings was 4.1 and 4.0 with $CaCl_2$ and PEG priming, respectively, which were significantly higher than that of the control (3.3). Both fresh and dry weights of the above-ground and underground parts of seedlings were significantly higher in the priming groups than in the control. Regarding the initial growth

characteristics, we found that there was a slight difference between $CaCl_2$ and PEG priming, and $CaCl_2$ priming resulted in better seedling growth.

Table 3. Growth characteristics of *Allium hookeri* seedlings grown in soil for 4 weeks (n = 7).

Method	Length of the aboveground (cm)[z]	No. of leaves	Root length (cm)	Fresh weight (g/plant)		Dry weight (g/plant)	
				Shoot	Root	Shoot	Root
Control	16.2 a	3.3 b	10.1 a	0.1231 c	0.0852 b	0.0134 c	0.0181 b
$CaCl_2$ 18 h	17.9 a	4.1 a	13.5 a	0.3636 a	0.3810 a	0.0405 a	0.0667 a
PEG 48 h	16.9 a	4.0 a	13.0 a	0.2345 b	0.2613 a	0.0281 b	0.0542 a

z Within each column, means followed by different letters are significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Discussion

PEG and $CaCl_2$ are osmoprimed solutions with low water potentials that can control seed water uptake (Chen et al., 2021). With the application of these solutions, water enters the seed slowly, which allows gradual seed imbibition and generates a series of pre-germination metabolic activities in the early phases of germination by adjusting water uptake (Thakur et al., 2019). PEG has a high molecular weight. It does not penetrate the seed tissue and has a direct negative influence on the embryo (Lei et al., 2021). Moreover, it can be applied to seeds at an appropriate concentration and time; thus, PEG can assist seed respiration, which is important for seed viability and synchronization of germination (Valdovinos et al., 2021). Thus, PEG is widely used for seed priming in diverse crop species (Lei et al., 2021; Kim et al., 2022). In this study, we found that the germination of *A. hookeri* was also improved by PEG priming. It was presumed that PEG positively affected the germination of *A. hookeri*, for reasons similar to those reported by other researchers. $CaCl_2$ priming is an osmopriming method that uses a salt solution. Salt solutions can regulate the water potential and nutritional status of seeds (Girolamo and Barbanti, 2012). The positive effect of $CaCl_2$ priming observed in this study has also been reported for important crops, including rice (Anwar et al., 2021) and wheat (Mim et al., 2021). Carbohydrate metabolism is promoted when the activity of protease and α-amylase increases in seeds, thereby improving the germination properties of seeds (Khan et al., 2021). Calcium is strongly linked to carbohydrate metabolism, and it has been reported that $CaCl_2$ can increase a-amylase and protease activities (Jafar et al., 2012). Referring to other studies, the reason behind the improved germination properties of *A. hookeri* could be the induction of α-amylase and protease activities by $CaCl_2$ priming. From the Petri dish experiment, we observed that the effect of $CaCl_2$ priming could be weakened after a specific priming time had elapsed, whereas the effect of PEG priming was enhanced with a longer priming time. These results indicate that priming time is also important for seed germination in *A. hookeri* and are in agreement with the results of previous studies.

Another important result observed in this study was that the highest germination percentage was observed in the PEG priming group in the Petri dish, whereas $CaCl_2$ priming had a better germination percentage in the soil. This might be due to different interactions between the conditions of the germination environment and the activation of priming materials under soil and Petri dishes. Calcium is a central element in the cell wall and enhances the binding force of the middle layer within the cell (Park et al., 2022). It can protect the structure of the cell and plays an important role in water and nutrient uptake from the soil during germination and seedling development (Siddiqui et al., 2012). In this regard, it has been reported that $CaCl_2$ priming promotes germination percentage and root growth of seedlings by allowing seedlings to use water and nutrient-available soil (Rehman et al., 2021). In this experiment, $CaCl_2$ primed seedlings demonstrated significantly better root development and better growth characteristics of whole parts in terms of biomass than the control and PEG primed seedlings. Thus, it was assumed that $CaCl_2$ was more efficient in improving the

capacity of water absorption and nutrient supply to the seeds and seedlings in the soil, contributing to better germination and growth characteristics. From our experiment, we found that the effect of priming can differ depending on germination and priming conditions, which supports previous research that several factors should be considered to determine the optimal priming conditions for the practical application of seed priming technology. In addition, our results demonstrated that specific PEG and $CaCl_2$ priming conditions can not only improve germination properties but also lead to early growth in *A. hookeri.* Hence, the results of this study could be used as important information to solve the problem of poor germination properties in *A. hookeri.*

Conclusion

The results of this study indicated that the seed germination properties of *A. hookeri* were substantially influenced by the priming conditions, while seed priming significantly improved the germination success of *A. hookeri* in several priming conditions, thereby providing important insights for better and more efficient usages.

Conflict of interest

The authors indicate no conflict of interest in the present work.

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