

The effect of alternating temperature treatment on seed germination of *Pulsatilla tongkangensis*

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ABSTRACT

The effect of alternating temperature on the germination of *Pulsatilla tongkangensis* seeds was investigated. Open pollinated seeds were wrapped in a moist paper towel for alternating temperature treatments. Seeds were sown in cells filled with Miracle-Gro Potting Mix and then topped with 0.5 cm of Miracle-Gro Seed Starting Potting Mix. The number of germinated seeds was counted daily until no further germination was observed for 3 to 4 days. The maximum germination rate (49%) was achieved in 18 days when seeds were treated at 22°C for 7 days followed by 5 °C for 7 days, and germinated at 20°C. The low germination rate could result from the inclusion of empty seeds. Mature *P. tongkangensis* seeds may not have a shallow physiological dormancy since germination was not improved with 14 days of 5°C.

Keywords: dormancy, full seeds, germination rate, Pasque flower, X-ray imaging.

INTRODUCTION

The genus *Pulsatilla* Mill. contains approximately 33 species, which grow in a wide area including Asia, North America, and Europe (Wang and Bartholomew, 2001; USDA, Agricultural Research Service, National Plant Germplasm System, 2024). *Pulsatilla tongkangensis* Y. N. Lee et T. C. Lee (<https://www.ipni.org/n/20005221-1>; accessed 22 August 2023) is growing limited regions in Korea, specifically near calcified rock cavity cliffs and in well-drained areas (Lee *et al.*, 2010; Lee and Lee, 2014; Oh *et al.*, 2014). This species is cultivated as ornamentals because of bell-shaped showy flowers, and white seed head. However, this species is considered a hybrid population in both natural habitats analyzed with molecular markers (Lee *et al.*, 2010).

The germination rate of *P. tongkangensis* seeds is highest (73.35%) when harvested 28 days after artificial fertilization (An *et al.*, 2010). Seeds of *P. koreana* germinate in 14 days at 25°C (Sang *et al.*, 1993, 1996). The germination rates (<38%) is lower than the 72% reported previously by Yuan *et al.* (2019) and might result from the inclusion of non-viable seeds (Yuan *et al.*, 2021).

The optimum temperatures for *P. koreana* seed germination are reported as 20°C (Yuan *et al.*, 2021), 25°C (Sang *et al.*, 1993), and 30°C (Yoo *et al.*, 2020). Additionally, germination rates were higher at 25/15°C and 30/20°C compared to 20/10°C and 35/25°C (Yoo *et al.*, 2020). However, the effects of alternating temperature treatments, such as high-low-high cycles, have not been studied in *P. tongkangensis*. The objective of this research was

therefore to investigate the effect of alternating temperature treatments on seed germination of *P. tongkangensis*.

MATERIALS AND METHODS

Open-pollinated seeds of *P. tongkangensis* seeds were collected from Jungsun, Kangwon-do Province, Korea, and were tested in this experiment. *Pulsatilla tongkangensis* seeds, received on 21 May 2023, after removing filaments were wrapped in a moist paper towel for alternating temperature treatments. The seeds (Frame A, Figure 1) were kept at 22°C or 5°C and then potted after 22°C treatment, as indicated by S (Table 1). Seeds were then sown into conical-shaped hexagon cells (3.8 cm top diameter × 2.5 cm bottom diameter × 6.5 cm depth, Ferry-Morse Home Gardening, Norton, MA, USA) (Frame B, Figure 1). The number of germinated seeds was counted daily until no further germination was observed for 3 to 4 days. Each treatment was replicated twice, with 50 seeds per replication. Data were analyzed separately for each species using Microsoft Excel (Microsoft Corporation, 2019). Means were compared using Tukey's honestly significant difference (HSD) test to determine significant differences.

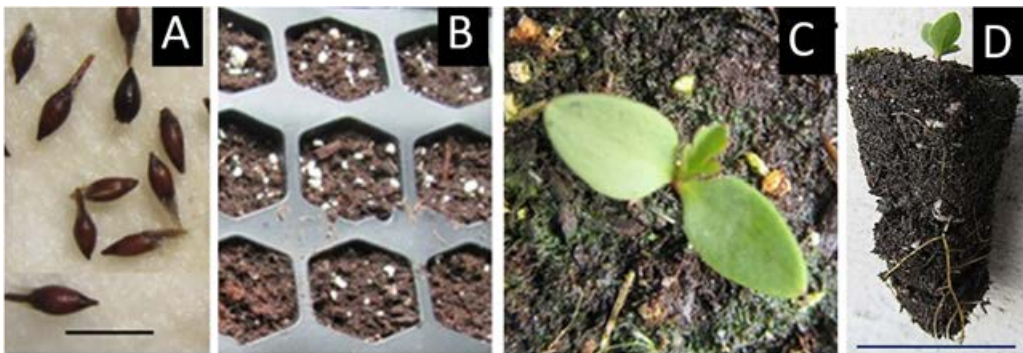


Figure 1. Preparation and germination of *Pulsatilla tongkangensis* seeds: Filaments were removed from seeds (frame A) and sown into conical-shaped hexagon cells (B), and germinated seeds were counted when cotyledon emerged from the sowing medium. Seeds that developed the first true leaves (C) and developed roots (D) were transplanted when seedlings formed 3 – 4 true leaves for subsequent experiments

RESULTS AND DISCUSSIONS

Pulsatilla tongkangensis seeds treated with moist treatment at 22°C for 28 days (Treatment 1; T1) exhibited an initial germination rate of 36.2% in 14 days, and plateaued in 21 days after sowing, which was significantly longer compared to the other treatments (Table 1). The highest final germination rate of 49.2% was observed when seeds were initially treated at 22°C for 7 days, followed by 5°C for 7 days, and 22°C (Treatment 2; T2). In accordance with other studies, the germination rate in this result was lower than the *in vitro* germination rate of 65% (An *et al.*, 2010) and the 72% germination rate in *P. koreana* (Yuan *et al.*, 2021). However, it is higher than the 38% germination rate observed after a 2-week at 5°C treatment reported by Yuan *et al.* (2019).

The relatively low germination rate observed in this study could be partially attributed to the germination temperature of 22°C, which is lower than the optimal 25°C reported in *P. tongkangensis* (An *et al.*, 2010). A higher germination rate of 55.3% was also reported in *P. koreana* at a 30°C temperature (Sang *et al.*, 1993; Yoo *et al.*, 2020). Additionally, Wang *et al.* (2013) reported that the optimal temperature range for *P. turczanovii* is between 25

and 30°C. The presence of empty seeds might have also contributed to the lower germination rates.

Table 1. *Pulsatilla tongkangensis* seeds received moist storage treatment at 22°C and 5°C at 7-day intervals

Treatment (T)	No. of days of temperature treatment (22 or 5°C) and sowing (S ^a)				No. of days to germination ^b		From A to B	Final germination rates (%)
	0-7	8-14	15-21	22-28	First seed (A)	Reaching plateau ^c (B)		
T1	22 (S)	22	22	22	14	21	7	36.2
T2	22	5	22 (S)	22	8	18	10	49.2
T3	22	5	5	22 (S)	10	19	9	30.1
T4	5	22 (S)	22	22	7	19	12	26.2
T5	5	5	22 (S)	22	6	13	7	27.0
HSD 1%^d					3.4	3.9	2.8	5.72

^a Seeds were sown (S) at the conclusion of the indicated 22°C treatment.

^b The number of days to germination was counted from the sowing dates and seeds germinated were recorded daily.

^c Recording of germinated seeds was terminated after reaching the plateau when there were no more seeds germinated for 3 to 4 days after reaching the plateau.

^d Tukey's honestly significant difference (HSD) test ($P \leq 0.01$).

To improve the germination rate of *P. tongkangensis* to over 50%, alternating temperature treatments of 30/20°C, as reported in *P. koreana* (Yoo *et al.*, 2020), applying aaaaaaaa temperature of 30–35°C during the first 7 days followed by 5°C for 7 days could be beneficial (An *et al.*, 2010). Since germination was not improved with 14 days of 5°C treatment before or after 14 days at 22°C, mature *P. tongkangensis* seeds may not have a shallow physiological dormancy (Baskin and Baskin, 2004).

CONCLUSIONS

The maximum germination rate of *P. tongkangensis* (49.2%) was achieved within 18 days by treating seeds first at 22°C for 7 days, followed by 5°C for 7 days. The relatively low germination rate observed in this study could be attributed to the mixture of both full and empty seeds and the suboptimal germination temperature of 22°C. Mature *P. tongkangensis* seeds may not have a shallow physiological dormancy since germination was not improved with 14 days of 5°C

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