

Effects of cold stratification and duration on seed germination of Korean ginseng (*Panax ginseng* C.A. Meyer)

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Abstract

This study investigated the effects of cold stratification temperature and duration on seed germination of Korean ginseng (*Panax ginseng* C.A. Meyer). Seeds were subjected to four temperatures (5–20 °C) and three durations (1–3 months) in a factorial design. Radicle emergence and seedling establishment were significantly affected by stratification temperature, duration, and their interaction ($P < 0.05$). The highest germination (78.00%) and seedling establishment (74.67%) were achieved at 5 °C for 3 months. Increasing stratification duration improved germination, whereas higher temperatures (≥ 15 °C) reduced performance. These results indicate that prolonged low-temperature stratification effectively promotes dormancy release. Stratification at 5 °C for 3 months is recommended to optimize seed propagation efficiency in *P. ginseng*.

Keywords: *Panax ginseng*; Seed dormancy; Cold stratification; Germination; Seedling establishment

1. Introduction

Korean ginseng (*Panax ginseng* C.A. Meyer) is one of the most important medicinal plants worldwide and has been widely used in traditional Asian medicine for centuries. Its pharmacological properties are mainly attributed to ginsenosides, a group of triterpenoid saponins with antioxidant, anti-inflammatory, neuroprotective, and immunomodulatory activities (Attele et al., 1999; Christensen, 2009). Owing to its high medicinal and economic value, ginseng has become an important crop in countries such as Korea, China, and Japan.

Seed propagation is commonly used in ginseng cultivation to maintain genetic diversity and plant vigor. However, seeds of *P. ginseng* exhibit deep morphophysiological dormancy, requiring prolonged stratification before germination can occur (Bewley et al., 2013). Under natural conditions, dormancy release may take more than one year, resulting in low and uneven germination.

Dormancy release is primarily regulated by the balance between abscisic acid (ABA) and gibberellins (GA), where ABA maintains dormancy and GA promotes germination through embryo growth and activation of hydrolytic enzymes (Finch-Savage and Leubner-Metzger, 2006). Cold stratification is widely recognized as an effective method to overcome dormancy in temperate species. Previous studies have shown that low-temperature stratification significantly enhances germination of *P. ginseng*, although its effectiveness depends on both temperature and duration (Li et al., 2000; Stoltz and Snyder, 1985; Chang et al., 2020)

Despite these findings, optimal combinations of stratification temperature and duration for efficient dormancy release under controlled conditions remain insufficiently defined. Therefore, this study aimed to evaluate the effects of

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stratification temperature and duration on germination and seedling establishment of *P. ginseng* and to identify optimal conditions for improving seed propagation efficiency.

2. Materials and Methods

2.1. Plant materials

Uniform seeds of Korean ginseng (*Panax ginseng* C.A. Meyer) were selected based on size and weight. Seeds with a 1000-seed weight ≥ 40.08 g were used. Damaged or undersized seeds were discarded to ensure uniformity.

2.2. Experimental design

The experiment was arranged in a two-factor factorial design under a completely randomized design (CRD), with stratification temperature (5, 10, 15, and 20 °C) as factor A and stratification duration (1, 2, and 3 months) as factor B. A total of 12 treatment combinations (4×3) were established. Each treatment was replicated three times, with 50 seeds per replicate.

Seeds were placed in moist sterile peatmoss and incubated at the designated temperatures for the specified durations. Moisture content was maintained throughout the stratification period to prevent desiccation.

2.3. Germination conditions

After stratification, seeds were transferred to germination conditions at 18 ± 2 °C. Seeds were sown in trays containing sterile peatmoss and maintained under adequate moisture and aeration.

2.4. Measured parameters and statistical analysis

The numbers of germinated seeds and normal seedlings were recorded to calculate radicle emergence and seedling establishment (%). Radicle emergence was defined as the proportion of germinated seeds relative to the total number of seeds, while seedling establishment was defined as the proportion of normal seedlings relative to the total number of seeds.

All data were expressed as mean \pm SD ($n = 3$). Data were analyzed by two-way ANOVA to assess the effects of stratification temperature, duration, and their interaction. Mean comparisons were performed using Duncan's multiple range test at $P < 0.05$, with significant differences indicated by different letters. The coefficient of variation (CV %) was calculated to evaluate experimental precision. Statistical analyses were conducted using SAS software (version 9.0).

3. Results and Discussion

The effects of stratification temperature and duration on radicle emergence and seedling establishment were highly significant (Table 1). The ANOVA further indicated a significant interaction between temperature and duration for both parameters.

Table 1 Analysis of variance for the effects of stratification temperature and duration on radicle emergence and seedling establishment of *Panax ginseng*

Variation factor	DF	Mean square (Radicle emergence, %)	Mean square (Seedling establishment, %)
Temperature (T)	3	3717.48**	3752.11**
Duration (D)	2	1749.78**	1655.11**
T \times D	6	97.93*	112.89*
Error	24	30.99	31.32
CV (%)		16.43	18.42

Note: * Significant at $P < 0.05$; ** Significant at $P < 0.01$; ns, not significant by ANOVA.

3.1. Effect of stratification temperature

Table 2 Effect of stratification temperature on radicle emergence and seedling establishment of *Panax ginseng*

Stratification temperature (°C)	Radicle emergence (%)	Seedling establishment (%)
5 °C	58.44 ± 3.06 a	55.56 ± 4.00 a
10 °C	37.33 ± 3.46 b	33.33 ± 3.46 b
15 °C	30.67 ± 2.31 c	26.67 ± 2.31 b
20 °C	9.11 ± 2.00 d	6.00 ± 1.15 c

Note: Values are mean ± SD (n = 9). Means within a column followed by different letters (a–d) are significantly different according to Duncan's multiple range test at P < 0.05.

Stratification temperature had a significant effect on both radicle emergence and seedling establishment of *Panax ginseng* seeds (Table 2). The highest radicle emergence (58.44%) and seedling establishment (55.56%) were recorded at 5 °C, followed by a marked decline at higher temperatures. At 10 °C, these values decreased to 37.33% and 33.33%, respectively, and further declined at 15 °C (30.67% and 26.67%). The lowest values were observed at 20 °C, with only 9.11% radicle emergence and 6.00% seedling establishment. Differences among temperature treatments were statistically significant (P < 0.05).

The superior performance at 5 °C confirms the essential role of low-temperature stratification in overcoming the morphophysiological dormancy of *P. ginseng* seeds. Cold conditions promote embryo development and stimulate metabolic processes associated with germination, including hydrolytic enzyme activation and reserve mobilization (Bewley et al., 2013). In addition, low temperatures contribute to hormonal regulation by increasing endogenous gibberellins and reducing abscisic acid, thereby promoting dormancy release (Finch-Savage and Leubner-Metzger, 2006; Shu et al., 2016).

In contrast, higher temperatures (≥15 °C) were less effective, as reflected by significantly reduced radicle emergence and seedling establishment. Under these conditions, embryo development may remain incomplete and dormancy-related inhibitory mechanisms may persist, resulting in poor germination performance. These findings are consistent with previous studies indicating that optimal stratification of ginseng seeds occurs at low temperatures (approximately 5–10 °C) (Bewley et al., 2013; Stoltz and Snyder, 1985; Chang et al., 2020).

Overall, the results demonstrate that stratification temperature is a key factor regulating dormancy release and germination in *P. ginseng*, with 5 °C identified as the most effective condition for maximizing both radicle emergence and seedling establishment.

3.2. Effect of stratification duration

Table 3 Effect of stratification duration on radicle emergence and seedling establishment of *Panax ginseng*

Stratification duration (months)	Radicle emergence (%)	Seedling establishment (%)
1 month	22.67 ± 3.06 c	19.50 ± 3.46 c
2 months	32.33 ± 3.06 b	28.83 ± 3.06 b
3 months	46.67 ± 16.65 a	42.83 ± 16.65 a

Note: Values are mean ± SD (n = 12). Means within a column followed by different letters (a, b, c) are significantly different according to Duncan's multiple range test at P < 0.05.

Stratification duration significantly affected radicle emergence and seedling establishment of *Panax ginseng* seeds (Table 3). Both parameters increased with time, with radicle emergence rising from 22.67% (1 month) to 46.67% (3 months) and seedling establishment from 19.50% to 42.83% (P < 0.05).

The increase in radicle emergence with prolonged stratification can be attributed to the gradual release of morphophysiological dormancy in *P. ginseng* seeds. Extended cold stratification provides sufficient time for embryo growth and differentiation, enabling completion of morphological development prior to radicle emergence. Simultaneously, physiological dormancy is alleviated through hormonal regulation, characterized by increased endogenous gibberellins and reduced abscisic acid, which enhance metabolic activity and promote germination. In

addition, prolonged stratification stimulates hydrolytic enzyme activity and reserve mobilization, ensuring adequate nutrient supply for embryo elongation and seedling establishment (Bewley et al., 2013). These results are consistent with previous studies highlighting the critical role of stratification duration and temperature in dormancy release of *Panax* seeds (Stoltz and Snyder, 1985; Finch-Savage and Leubner-Metzger, 2006; Baskin and Baskin, 2014).

Overall, a stratification duration of 3 months is optimal for maximizing radicle emergence and seedling establishment.

3.3. * Interaction between temperature and duration

Table 4 Interaction between stratification temperature and duration on radicle emergence and seedling establishment of *Panax ginseng*

Temperature (°C)	Duration (months)	Radicle emergence (%)	Seedling establishment (%)
5	1	39.33 ± 3.06 d	36.00 ± 4.00 d
5	2	58.00 ± 2.00 b	56.00 ± 2.00 b
5	3	78.00 ± 2.00 a	74.67 ± 2.31 a
10	1	28.00 ± 3.46 f	24.00 ± 3.46 f
10	2	32.67 ± 3.06 e	28.67 ± 3.06 e
10	3	51.33 ± 16.65 c	47.33 ± 16.65 c
15	1	21.33 ± 2.31 g	17.33 ± 2.31 g
15	2	28.67 ± 4.16 f	24.67 ± 4.16 f
15	3	42.00 ± 10.58 d	38.00 ± 10.58 d
20	1	2.00 ± 2.00 i	0.67 ± 1.15 i
20	2	10.00 ± 4.00 h	6.00 ± 4.00 h
20	3	15.33 ± 4.16 h	11.33 ± 4.16 h

Note: Values are mean ± SD (n = 3). Means within a column followed by different letters are significantly different according to Duncan's multiple range test at P < 0.05.

The interaction between stratification temperature and duration significantly affected radicle emergence and seedling establishment of *Panax ginseng* seeds (Table 4). The highest radicle emergence (78.00%) and seedling establishment (74.67%) were obtained at 5 °C for 3 months, indicating that the combined effect of low temperature and prolonged stratification is optimal. At the same temperature, extending the duration from 1 to 3 months markedly improved both parameters, confirming the cumulative effect of stratification time.

At higher temperatures (10–15 °C), although radicle emergence increased with duration, the overall values remained significantly lower than those at 5 °C. In contrast, stratification at 20 °C resulted in extremely low radicle emergence (2.00–15.33%) and seedling establishment (0.67–11.33%), regardless of duration, suggesting that high temperatures are ineffective in promoting dormancy release.

These results indicate a strong interaction between temperature and duration in regulating dormancy release. Prolonged low-temperature stratification promotes embryo development and physiological activation, including enhanced hydrolytic enzyme activity, reserve mobilization, and favorable hormonal balance (increased gibberellins and reduced abscisic acid), which collectively facilitate germination (Bewley et al., 2013; Finch-Savage and Leubner-Metzger, 2006). Similar responses have been reported in *Panax* species, where optimal germination is achieved under prolonged cold stratification (Stoltz and Snyder, 1985).

Overall, a stratification regime of 5 °C for 3 months is identified as the most effective condition for maximizing both radicle emergence and seedling establishment in *P. ginseng*.

4. Conclusion

Stratification temperature and duration significantly influenced germination and seedling establishment of *Panax ginseng* seeds. Low-temperature stratification at 5 °C markedly enhanced germination compared to higher temperatures, while increasing duration from 1 to 3 months progressively improved both parameters. The optimal treatment was identified as 5 °C for 3 months, achieving the highest germination (78.00%) and seedling establishment (74.67%). In contrast, higher temperatures (≥ 15 °C) were ineffective regardless of duration. These findings demonstrate that successful dormancy release in *P. ginseng* requires both optimal temperature and sufficient stratification time.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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