

Seed Germination Response to Temperature, Cold Stratification Period, and Gibberellin Treatment in *Spiraea fritschiana*

Hyun Jin Kim¹, Ki Cheol Lee², Hyun Jin Kim², and Yoon Jin Kim^{1*}

¹Department of Horticulture, Biotechnology and Landscape Architecture, Seoul Women's University, Seoul 01797, Korea

²Useful Plant Resources Center, Korea National Arboretum of Korea Forest Service, Gyeonggi-province 12519, Korea

*Corresponding author: yj1082@swu.ac.kr

Abstract

To improve the germination of *Spiraea fritschiana* seeds for mass propagation, we evaluated the effect of a range of temperatures, cold stratification periods, and gibberellic acid (GA₃) treatments on three germination characteristics. Final germination percentage (FGP) increased as the temperature for seed germination increased, up to 30°C, while the mean germination time (MGT) and the mean number of days to 30% germination (T₃₀) decreased when seeds were germinated at 25-30°C. The optimum germination temperature of *S. fritschiana* seeds is approximately 30°C considering FGP, MGT, and T₃₀ together. FGP increased with the duration of cold stratification up to a period of 6 weeks, but declined after 8 weeks of cold stratification, as prolonged cold stratification can induce dormancy with a resultant decline in germination. Pretreatment with 6-8 weeks of cold stratification or soaking seeds in distilled water or 500 mg·L⁻¹ GA₃ for 24 h accelerated and increased the germination of *S. fritschiana* seeds, regardless of temperature. However, further study might be required to evaluate the effect of GA₃ concentrations lower than 500 mg·L⁻¹ on the promotion of germination in *S. fritschiana* seeds.

Additional key words: fritsch spirea, optimum germination temperature, ornamental plant, propagation, shrub

Introduction

Spiraea L. belongs to the Rosaceae family and comprises 50-80 species distributed throughout northern temperate regions (Potter et al., 2007). These ornamental plants produce colorful flowers and are highly ecologically adaptable, making them ideal for horticulture in many countries. For example, the North American native species *S. tomentosa* and *S. alba* are used primarily for landscaping and habitat restoration (Mickelbart et al., 2012). *S. fritschiana* (fritsch spirea), valued for landscaping, is native to the mountains and valleys of South Korea. This species has potential as a hedge shrub and a cut flower with its short height and abundant small flowers (compound corymb) (Kim and Sun, 1996), however little research has been conducted to examine the cultivation of *S. fritschiana*.

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The success of mass propagation in woody plants is highly dependent on the propagation method chosen. Propagation by cuttings of woody plants can often be more complex and more restricted by harvest time than parallel applications in herbaceous plants. When plants are required for a rapid coverage of slopes or habitat restoration, seed propagation can be much less labor intensive than cutting propagation, however each *Spiraea* species has different temperature range requirements for optimum seed germination. In the eight cultivars of Japanese spirea (*S. japonica*) over 94% of seeds germinated in field conditions, whereas germination of *S. chinensis* did not occur when grown in night/day temperatures of 15/25, 20/30 or 25/35°C (10/14 h), nor under field conditions (Kim et al., 2002; Wilson and Hoch, 2009).

Seeds of Rosaceae species often require cold stratification to break seed dormancy and increase germination, and their growth can be aberrant and slow without it (Baskin and Baskin, 1998). In *S. prunifolia* for. *simpliciflora*, stratification at 3°C for 7 d increased the seed germination (Cho et al., 2012). During cold stratification, the balance of growth factors in seeds changes considerably. The abundance of growth promoters such as gibberellic acid (GA) is elevated in seeds subjected to low temperatures of 4–5°C (Yamauchi et al., 2004), and cold stratification also increases tissue sensitivity to GA (Derks and Karsen, 1993).

GA functions to overcome the mechanical restriction conferred by tissues that surround the embryo, such as the aleurone and the testa, and increases the growth potential of the embryo (Ogawa et al., 2003). Exogenous GAs including GA₃, GA₄, and GA₇ increase the success of germination in seeds of Rosaceae species (De Mello et al., 2009); for example, around 62% of *Prunus serotina* seeds soaked in 500 ppm GA₃ germinated, whereas germination was only half as successful in seeds soaked in distilled water after 120 d of cold stratification (Phartyal et al., 2009). The inhibitory effect of a GA biosynthesis inhibitor on germination was partially reversed by treatment with GA₄, and an increase in GA₄ content in embryos led to germination (Chen et al., 2007).

To find efficient ways to increase and enhance the germination of *S. fritschiana* for mass propagation, revegetation, or habitat restoration, we investigated the optimal germination temperature, duration of cold stratification, and GA treatment, with the aim of improving the germination of *S. fritschiana* seeds.

Materials and Methods

Seed Material

S. fritschiana seeds were harvested in Geumwonsan Ecological Arboretum, Guchang, Korea (latitude 35°43'19.9"N, longitude 127°47'00.6"E) in October 2013. The seeds were air-dried at room temperature (22–25°C) for about one month then stored in sealed plastic jars at 5°C. Seeds were sorted by hand to ensure size uniformity. The mean seed weight was 0.013 g (n = five replicates, 300 seeds per replicate). The mean seed size was 1.44 × 0.33 mm (n = 50).

Temperature Treatment

Seeds were germinated at constant temperatures of 5, 10, 15, 20, 25, 30, or 35°C in a multi-room incubator (HB-302S-2, Hanbaek Scientific Co., Bucheon, South Korea) and illuminated by fluorescent lamps (FCL32SD/30, Kumho Electric, INC., Seoul, South Korea) with a 12 h photoperiod (20 μmol·m⁻²·s⁻¹). All seeds were incubated in 9 cm Petri dishes containing two layers of filter paper (Whatman No. 2) moistened with distilled water that was warmed to each experimental

temperature to eliminate water temperature effects. A seed was considered to be germinated when the radicle protruded through the seed coat (> 1 mm). Germinated seeds were recorded and then removed throughout the 30 d experiment. Each experimental treatment used 50 seeds, with three replications per treatment.

Cold Stratification Period

Seeds were sufficiently moistened on 9 cm Petri dishes containing two layers of filter paper (Whatman No. 2) and incubated at 5°C for 0, 6, 8, 10, 12, or 14 weeks in the dark. After cold stratification, the seeds were germinated at constant temperatures of 25 and 30°C. Seed germination tests were conducted using the method described above.

GA₃ Treatment

Seeds were soaked in 0 (distilled water), 500, or 1000 mg·L⁻¹ GA₃ at a constant temperature of 25°C for 24 h. After soaking, the seeds were rinsed several times with tap water. All seeds were germinated at constant temperatures of 25 or 30°C. Seed germination tests were conducted using the method described above.

Data Collection and Analysis

The germination characteristics were examined the final germination percentage (FGP), mean germination time (MGT), and the mean number of days for 30% germination (T₃₀). MGT was calculated as $MGT = \frac{\sum (t_i \times n_i)}{\sum n_i}$, where t_i is the time (d) from the beginning of the test and n_i is the number of germinated seeds at the recorded time (Ellis and Roberts, 1981). The entire germination test was conducted in a completely randomized block design. Data were subjected to ANOVA and Duncan's multiple range test. Statistical analysis was performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). A p value < 0.05 was considered to indicate a statistically significant result. Regression and graph module analyses were performed using Sigma Plot software version 10.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Effects of Temperature on Seed Germination

The close relationships between temperature and FGP, MGT, and T₃₀ are illustrated in Fig. 1, with R^2 ranging from 0.60 to 0.88 ($p < 0.01$ or 0.001). Seeds of *S. fritschiana* did not germinate at 5 and 10°C, and MGT and T₃₀ were not calculated at 5, 10, and 15°C (Fig. 1). FGP increased as the temperature for seed germination increased up to 30°C (Fig. 1A). The lowest MGT was obtained at 25-30°C (Fig. 1B). The fastest T₃₀ occurred when seeds were germinated at 30°C (Fig. 1C). The optimal temperature for seed germination is considered as the temperature yielding the highest germination percentage in the shortest time. These results indicate that the optimal temperature for germination of *S. fritschiana* seeds was about 30°C, although the maximum germination percentage was below 80%. High germination temperatures may be preferred because *S. fritschiana* is adapted to occupy forest edges and rocky areas with greater exposure to sunlight and higher temperatures (Korea NIBR., 2014; Matlack, 1993).

Some species have a specific temperature range for seed germination because temperature acts to regulate germination under natural conditions and prevents germination under unfavorable thermal conditions for seedling growth. Seeds of a

few shrubs native to deciduous forests, including *Rosa rugosa* and *Rubus idaeus*, germinate well at 20-30°C (Baskin and Baskin, 1998).

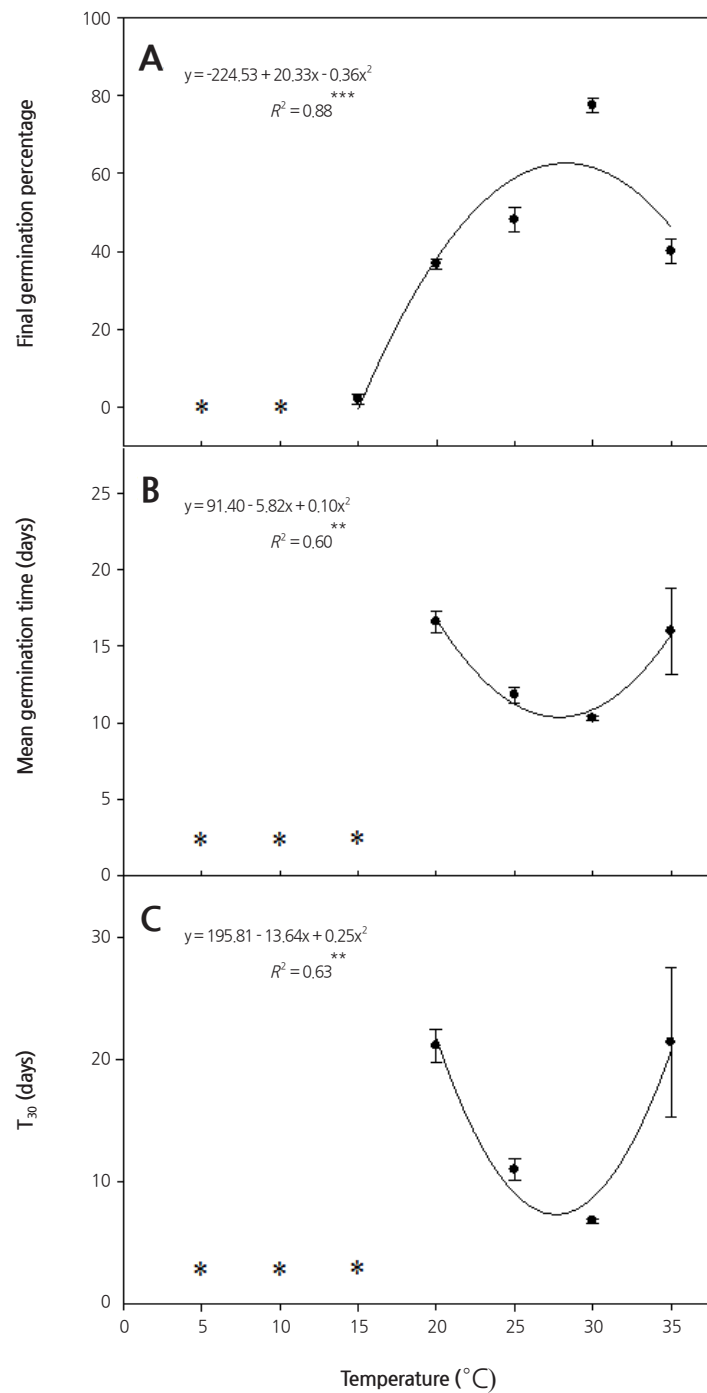


Fig. 1. Relationship between germination temperatures and final germination percentage (A), mean germination time (B), and the mean number of days for 30% germination (T_{30}) (C) of *S. fritschiana* seeds. Bars represent standard error of the mean. An asterisk (*) indicates that no information was available for this field.

Effects of Cold Stratification Period on Seed Germination

The germination of cold-stratified seeds was investigated at sub-optimal (25°C) and optimal (30°C) temperatures. FGP improved as the period of cold stratification increased up to a peak at 6 weeks at both germination temperatures (Fig. 2A). There were significant relationships between the cold stratification period and MGT and T_{30} (Fig. 2B, C; $p < 0.001$), as the cold stratification hastened germination of *S. fritschiana* seeds. These results suggest that seeds that fall in the autumn and overwinter under the snow will germinate rapidly at relatively high temperatures, which is a favorable condition for seedling development.

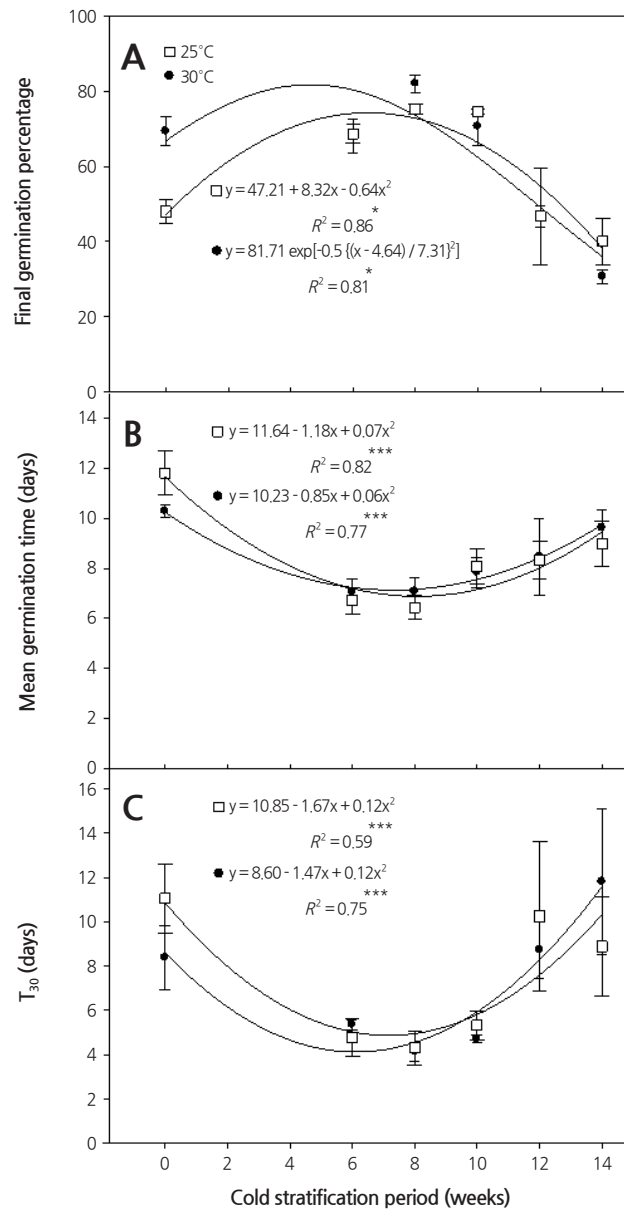


Fig. 2. Relationship between cold stratification period and final germination percentage (A), mean germination time (B), and the mean number of days for 30% germination (T_{30}) (C) of *S. fritschiana* seeds at 25 and 30°C. Bars represent standard error of the mean.

Woody species generally require extensive cold stratification treatments, which can be up to 180 d although 60-90 d is usually satisfactory (Bewley and Black, 1994). *S. betulifolia* seeds germinate at 0-2°C when kept under cold stratification conditions for more than 120 d (McLean, 1967), however in the present study, FGP of *S. fritschiana* seeds declined after 8 weeks of cold stratification. In some *S. fritschiana* seeds, dormancy had been induced by the prolonged cold stratification, preventing germination during winter (Milberg and Andersson, 1998).

Effects of GA₃ Treatment on Seed Germination

Commercial seeds exhibit a high germination percentage, but their germination tests are conducted at optimal temperatures (Demir et al., 2011). Germination varies depending on the environmental conditions, which are not always ideal for seed germination in the field, however seeds for commercial use are required to achieve over 90% germination regardless of optimal temperature. To increase the germination percentage of *S. fritschiana*, exogenous GA₃ was applied to the seeds.

The application of GA₃ often stimulates the germination of seeds belonging to Rosaceae, such as *P. serotina* (Phartyal et al., 2009). In the present study, the germination of *S. fritschiana* seeds increased from 48 to 82% by soaking them in distilled water at 25°C (Fig. 1A and Table 1), however soaking the seeds in GA₃ solution did not improve FGP more than distilled water alone. The MGT of seeds treated with either 500 or 1000 mg·L⁻¹ GA₃ was 5.5 and 5.9 d respectively, which was lower than the 8.9 d MGT of seeds soaked in distilled water at 25°C (Table 1). The T₃₀ of seeds treated with 500 mg·L⁻¹ GA₃ at 25°C was approximately 1.6 times faster than the 6.38 d taken by seeds soaked in distilled water. The similar results were obtained when seeds treated with 0, 500, and 1000 mg·L⁻¹ GA₃ germinated at 30°C.

Table 1. Final germination percentage (FGP), mean germination time (MGT), and the mean number of days for 30% germination (T₃₀) of *S. fritschiana* seeds at 25 and 30 °C treated with 0, 500, or 1000 mg·L⁻¹ GA₃ at 25 °C for 24 h.

GA ₃ concentration (mg·L ⁻¹)	Germination temperature					
	25°C			30°C		
	FGP	MGT (days)	T ₃₀ (days)	FGP	MGT (days)	T ₃₀ (days)
0	82.0 a ^z	8.9 a	6.38 a	78.7 a	8.5 a	6.16 a
500	91.3 a	5.5 b	3.96 b	83.3 a	5.5 b	4.13 b
1000	90.0 a	5.9 b	3.91 b	85.3 a	5.5 b	3.90 b

^zAverage values in a column with a different letter are significantly different ($p < 0.05$) using Duncan's multiple range test.

Enhancing the germination and uniformity of seeds is required for mass production; however, wild-type seeds can have large variations in germination percentage, which results in non-uniform germinability (Qu et al., 2005). Variations in seed germination may have important ecological consequences, because a plant that produces seeds with different germination requirements has a higher chance of leaving descendants than one that does not (Fenner, 1991). In the present study, both cold stratification and GA₃ treatment hastened the MGT of *S. fritschiana*. Exogenous application of GA₃ has previously been substituted for cold stratification to germinate seeds in a short period of time (Kitchen and Meyer, 1991).

In conclusion, the optimal germination temperature is approximately 30°C for *S. fritschiana* seeds. Pretreatment with 6-8 weeks of cold stratification or by soaking seeds in distilled water or 500 mg·L⁻¹ GA₃ for 24 h accelerated and increased the germination of *S. fritschiana* seeds regardless of temperature. Further research might be useful to evaluate the effect of low concentrations of GA₃ solution, such as 10, 20, 50, or 100 mg·L⁻¹ on the promotion of germination in *S. fritschiana* seeds.

Prolonged cold stratification can induce dormancy and result in a decline in germination. These results provide useful information for predicting germination characteristics of *Spiraea* species.

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