# Seed Dormancy Class and Germination Characteristics of *Prunus maackii* and *P. virginiana*

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KEYWORDS. Endocarp, germination rate, gibberellin, stratification, temperature regimes

ABSTRACT. Prunus species exhibit physiological dormancy (PD), requiring dormancybreaking treatments for successful germination in horticultural and ecological applications. Although stratification, gibberellic acid (GA<sub>3</sub>) application, and endocarp removal are commonly used, their effectiveness varies by species and dormancy depth. Despite the ornamental and ecological value of *Prunus maackii* and *P. virginiana*, their dormancy characteristics remain poorly understood. This study evaluated seed dormancy characteristics and effective germination treatments for both species. Seeds and stones were exposed to four alternating temperature regimes, seven stratification treatments, four GA<sub>3</sub> concentrations with or without endocarp removal, and combined GA<sub>3</sub> and stratification treatments. Water imbibition tests indicated that both species lack physical dormancy (PY), and fully developed embryos excluded morphological dormancy (MD). In P. maackii, the highest germination rate (65.6%) was achieved through a warm and cold stratification regime consisting of 30 days at 20 °C followed by 60 days at 4 °C. In P. virginiana, the highest germination rate (70.8%) was achieved with warm and cold stratification (30 days at 20 °C followed by 90 days at 4 °C) combined with 1000 mg·L<sup>-1</sup> GA<sub>3</sub>. Based on their responses to stratification and GA<sub>3</sub> treatment, P. virginiana was classified as having intermediate PD. In comparison, P. maackii exhibited characteristics of both nondeep and intermediate PD, indicating the presence of interspecific variation in dormancy types between the two species. These results provide species-specific protocols for improving the propagation efficiency of underutilized *Prunus* species.

Prunus (Rosaceae) species are economically and ecologically important as fruit and ornamental trees (Shi et al. 2013). Although grafting is widely used for propagation, seed propagation remains essential for rootstock production, breeding programs, and ecological restoration. However, grafting success is limited in some species, such as *P. pendula* var. ascendens, which has reported success rates

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as low as 24% (Park et al. 2000). Therefore, improving seed germination is critical for enhancing propagation efficiency in this genus.

Most *Prunus* species possess PD, requiring specific pretreatments such as stratification to break dormancy and promote germination (Baskin and Baskin 2014; Gendreau and Corbineau 2009). Although a combination of warm and cold stratification over 3 months is generally effective (Moreira et al. 2012), many species still exhibit inconsistent or low germination rates (Iliev et al. 2012). Stratification requirements can vary substantially among Prunus species and genotypes. For instance, P. spachiana required 12 weeks of cold stratification, whereas P. avium required 6 weeks of warm stratification followed by 5 months of cold stratification to effectively break dormancy (Iliev et al. 2012; Kim et al. 2024).

Germination is a critical stage in the propagation of ornamental plants and contributes significantly to the expansion of urban greening and landscaping (KFS 2021). However, extended delays between dormancy release and seedling emergence can compromise propagation efficiency. Many woody perennials, including *Prunus* species, require stratification periods of 3 to 4 months, posing logistical challenges in large-scale production.

To overcome PD, various approaches such as scarification and movealong stratification have been tested in woody perennials (Baskin and Baskin 2003b, 2004). These techniques have been applied successfully to several Prunus species, including P. mahaleb (Pipinis et al. 2012), P. azorica (Moreira et al. 2012), and P. spachiana (Kim et al. 2024). Plant hormones, particularly gibberellic acid (GA<sub>3</sub>), have also shown promise in stimulating germination in seeds exhibiting intermediate PD (Baskin and Baskin 2003a). Furthermore, combined treatments involving move-along, GA<sub>3</sub> application, and moist stratification have shown greater efficacy than single treatments (Kim et al. 2024; Moreira et al. 2012).

P. maackii, native to East Asia including Korea, is widely planted in landscape architecture because of its ornamental value and adaptability (Pooler et al. 2012). In contrast, P. virginiana, distributed across North America, is appreciated for its early spring flowering and ecological functions such as supporting wildlife and stabilizing habitats (Pliszko and Jermakowicz 2022). Despite their distinct geographic origins and applications, both species hold horticultural potential as rootstocks or ornamental trees. Improving seed germination in these species not only enhances propagation efficiency but also contributes to their broader utilization in cultivation and breeding programs (Souza et al. 2017). Previous studies have addressed aspects such as breeding strategies in P. maackii (Lenivtseva et al. 2017) and postharvest physiology in P. virginiana (Green and Low 2013). However, little attention has been paid to seed dormancy mechanisms and germination physiology in either species. Notably, no comparative studies have investigated how differences in their ecological backgrounds and evolutionary histories may influence their dormancybreaking requirements. This study addresses that gap by examining the physiological responses of P. maackii and P. virginiana seeds under stratification and hormonal treatments, thereby

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providing new insights into interspecific variation in seed dormancy within the genus *Prunus*.

This study aimed to identify effective dormancy-breaking protocols for *P. maackii* and *P. virginiana* by examining the roles of thermal regimes, stratification treatments, hormonal stimulation, and mechanical seedcoat modification. Specifically, we evaluate the individual and combined effects of 1) day/night temperature regimes, 2) moist warm and cold stratification, 3) GA<sub>3</sub> application and endocarp removal, and 4) integrated stratification and GA<sub>3</sub> treatments on germination success.

### **Materials and methods**

PLANT MATERIAL. Seeds of Prunus maackii and P. virginiana were provided by Sejong National Arboretum on 19 Aug 2022 and stored at 3 to 4 °C in a refrigerator at Seoul Women's University until used in each experiment. In this study, "seeds" refers to true seeds without the endocarp, whereas "stones" refers to seeds with the endocarp intact. Some stones were hand-opened using stainless steel nippers to remove the endocarp entirely and were classified as seeds. The remaining stones or seeds were used as controls. The seeds of P. maackii and P. virginiana measured  $\sim$ 4 to 5 mm and 5 to 6 mm in length, respectively (Fig. 1). A tetrazolium test was conducted on 27 Dec 2022 to assess seed viability by staining embryos with 1% tetrazolium solution for 18 h after halving the stones. Before each experiment, all seeds and stones were disinfected using a 5000 mg·L<sup>-1</sup> Benoram solution (Samkong Korea, Seoul, Korea).

WATER IMBIBITION. To determine whether physical dormancy (PY) was present, water uptake was measured for stones and seeds of P. maackii and P. virginiana under laboratory conditions (20 to 25 °C) from 10 to 12 Nov 2022. The experiment was conducted using three replicates of 30 stones or seeds. For each replication, the mean value of 30 seeds was used in the statistical analysis. Each sample was placed in a  $100 \times 50$ -mm culture dish containing two 9-cm filter papers (Cat. No. 1002 090; Whatman Int'l Ltd., Leeds, England) moistened with ~15 mL of distilled water. Seed moisture content was

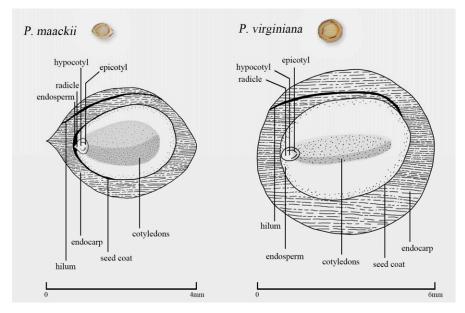


Fig. 1. Longitudinal sections through the endocarp of *Prunus maackii* and *Prunus virginiana*. A small amount of endosperm adheres to the hypocotyl and radicle. Stone refers to the seed with the endocarp intact, whereas seed represents the seed without the endocarp.

determined following ISTA (1996). Weights were recorded at 0, 2, 4, 8, 16, 24, and 48 h after surface moisture was removed. The water absorption rate ( $W_s$ , %) was calculated using the following formula:

$$W_s(\%) = [(W_i - W_d)/W_d] \times 100,$$

where  $W_s$  = relative weight ratio of seeds increased through water absorption,  $W_i$  = the weight of the seeds at each time point after water supply, and  $W_d$  = the weight of seeds before water absorption.

EXPERIMENTAL DESIGN AND TREATMENTS. The experiments were designed to examine the effects of 1) four alternating temperature regimes, conducted from 5 Jan to 18 Aug 2023; 2) seven moist stratification regimes including a control, initiated on 2 Sep 2022, with sowing dates staggered from 2 Oct to 31 Dec 2022; 3) four concentrations of GA<sub>3</sub> with or without endocarp removal, starting on 26 Sep 2022; and 4) combined stratification and GA<sub>3</sub> treatments, starting on 28 Jun 2023, with sowing dates staggered from 2 Jun to 27 Oct 2023. A plant growth chamber (HV-302S-2; Hanbaek Science, Seoul, Korea) was used for both the alternating temperature regimes experiment and the warm and cold stratification treatments. A 12-h photoperiod was provided using six fluorescent lamps

with a photosynthetic photon flux density of 19 to 22  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>. Germination was defined as radicle emergence of at least 1 mm.

To assess the effect of temperature, stones were incubated under four alternating day/night temperature regimes (10/5, 15/10, 20/ 15, and 25/20°C), following the method of Moreira et al. (2012). Each treatment consisted of 90 stones (three replicates of 30) placed in Petri dishes  $(100 \times 50 \text{-mm})$  with two moistened 9-cm filter papers. To prevent fungal contamination, stones were periodically washed with tap water, and the dishes and filter papers were replaced weekly using new papers moistened with 1% (wt/vol) Benomyl solution. Germination was recorded weekly for 32 weeks.

To evaluate the effects of stratification, stones were subjected to six stratification treatments and a control. Treatments included moist stratification at 4°C for 30, 60, or 90 d, and warm stratification at 20°C for 30 d followed by cold stratification at 4°C for 30, 60, or 90 d. For each treatment, 90 stones (three replicates of 30) were placed in mesh bags and buried in 12 × 10.5-cm plastic pots filled with a 1:1 mixture of perlite and peat. After stratification, the stones were sown in 72-cell trays containing horticultural soil (Hanareum Soil No. 2;

Asia Seed, Seoul, Korea). The commercial soil is composed of 51% coco peat, 10% peatmoss, 13% vermiculite, 15% perlite, 10% zeolite, 0.1% humic acid, and 0.4% fertilizer. The trays were then placed in an Information and Communication Technology (ICT)-based smart greenhouse at Seoul Women's University, where germination was monitored every 2 d for 30 weeks.

To test the effect of GA<sub>3</sub> concentration and endocarp removal, both stones and seeds were soaked for 24 h in either distilled water (GA<sub>3</sub> 0 mg·L<sup>-1</sup>) or GA<sub>3</sub> solutions at 250, 500, or 1000 mg·L<sup>-1</sup>, following the method of Moreira et al. (2012). Each treatment consisted of 48 samples (three replicates of 16), immersed in 2-mL tubes. After treatment, all samples were sown in 16-cell trays containing horticultural soil and placed in the ICT smart greenhouse. Germination was recorded every 2 d for 30 weeks.

Throughout both the stratification experiment and the experiment on GA<sub>3</sub> concentration and endocarp removal (Sep 2022 to Apr 2023), environmental conditions in the greenhouse were maintained at an average temperature of  $15.7\,^{\circ}\text{C}$ , relative humidity of 65.6%, and daytime light intensity of  $100.7\,\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

The combined effect of stratification and GA<sub>3</sub> was also evaluated using a factorial design. Seeds were subjected to the same stratification regimes described previously and then soaked in GA<sub>3</sub> solutions (0, 500, or  $1000 \text{ mg} \cdot \text{L}^{-1}$ ) for 24 h. Each treatment consisted of 48 seeds (three replicates of 16) stratified in mesh bags buried in  $12 \times 10.5$ -cm plastic pots filled with a 1:1 mixture of perlite and peat. After GA<sub>3</sub> treatment, seeds were sown in 72-cell trays filled with horticultural soil and maintained in the ICT smart greenhouse. Germination was measured every 2 d for 50 d. During the experimental period (Jun to Dec 2023), the average daytime and nighttime temperatures and relative humidity in the greenhouse were 21.5 °C and 72.5%, respectively. The average light intensity during the daytime was  $108.9 \, \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ .

STATISTICAL ANALYSIS. Each experiment was arranged in a randomized complete block design with three replicates. Water absorption for *P. maackii* and *P. virginiana* stones and seeds was analyzed using a nonlinear regression

model to describe water uptake dynamics over time. The fitting equation was based on the exponential rise to a maximum model:

$$f(x) = y_0 + a(1 - e^{-bx}),$$

where f(x) is the increase in mass (%), x is incubation time (hours),  $y_0$  is the initial water uptake at x = 0, a is the asymptotic maximum water absorption, and b is the rate constant of uptake.

Model fitting was conducted using nonlinear least squares regression in SAS (PROC NLIN; SAS Institute Inc., Cary, NC, USA) to estimate parameters. Germination percentages were analyzed using analysis of variance (ANOVA), followed by Tukey's honestly significant difference test at P < 0.05. For factorial experiments involving GA<sub>3</sub> concentration (G) and stratification treatments (S), two-way ANOVA was conducted to assess the main effects of each factor and their interaction (G × S). All statistical analyses were conducted separately for P. maackii and P. virginiana using SAS software (version 9.4). Graphs were generated using SigmaPlot (version 10.0; Systat Software Inc., Chicago, IL, USA).

#### Results

WATER IMBIBITION. The stones and seeds of Prunus maackii and P. virginiana absorbed water and showed a typical pattern of rapid initial water uptake as seed mass increased (Fig. 2). At room temperature, all Prunus samples showed water absorption rates of more than 20% relative to their initial weight. Stones of P. maackii absorbed  $25.7\% \pm 2.0\%$  water after 4 h of immersion (Fig. 2A) and seeds absorbed  $37.4\% \pm 1.1\%$  within the same period. Stones of P. virginiana absorbed  $20.8\% \pm 0.4\%$  after 12 h of immersion (Fig. 2B). Seeds of P. virginiana absorbed  $24.2\% \pm 0.9\%$  within 2 h and reached  $44.1\% \pm 0.8\%$  by 12 h.

SEED GERMINATION. No *P. maackii* stones germinated within 32 weeks at any of the four alternating temperature regimes of 10/5, 15/10, 20/15, and 25/15 °C (Fig. 3A). For *P. virginiana*, germination under temperature treatments was minimal, with rates below 6% across the 10/5, 15/10, and 20/15 °C treatments (Fig. 3B). No germination was observed at 25/20 °C.

No significant difference was found in all treatments.

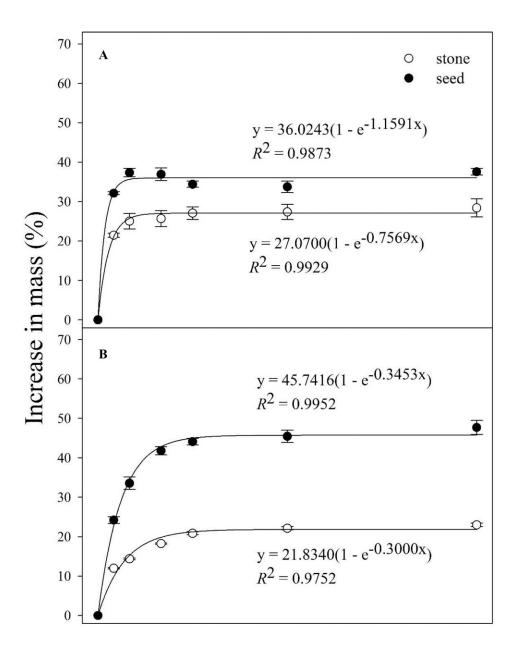
In stratification treatments, the highest germination in P. maackii was  $65.6\% \pm 5.9\%$  in  $20\,^{\circ}\text{C}$  30 d +  $4\,^{\circ}\text{C}$  60 d treatment (Fig. 3C). With cold stratification alone, P. maackii showed germination rates ranging from  $16.7\% \pm 8.4\%$  to  $32.2\% \pm 11.6\%$ , whereas the control without stratification resulted in only  $5.6\% \pm 2.9\%$  germination. The highest germination rate of P. virginiana was  $24.4\% \pm 7.3\%$  in  $4\,^{\circ}\text{C}$  90 d treatment (Fig. 3D). The germination rates of P. virginiana in other treatments were less than 5%.

In *P. virginiana*, seeds exhibited higher germination rates than stones (Fig. 3F). Seed treatments with GA<sub>3</sub> 1000 mg·L<sup>-1</sup> showed the highest germination rate of 25.0%  $\pm$  6.3%. Among the stone treatments, the highest germination rate was observed in the GA<sub>3</sub> 500 mg·L<sup>-1</sup> treatment, reaching 14.6%  $\pm$  2.1%. *P. maackii* showed a germination rate of 14.6%  $\pm$  5.5% in seeds and 10.4%  $\pm$  2.1% in stones under GA<sub>3</sub> 0 mg·L<sup>-1</sup> treatment (Fig. 3E). All treatments with 250, 500, and 1000 mg·L<sup>-1</sup> resulted in germination rates below 10%.

ANOVA showed that the stratification period, GA<sub>3</sub> concentration, and their interaction significantly affected germination traits in P. virginiana (Table 1). Significant differences in germination percentages were observed under combined stratification and GA<sub>3</sub> treatments in P. virginiana (Fig. 4). In *P. virginiana*, the highest rate  $(70.8\% \pm 8.3\%)$  occurred under 20°C 30 d + 4 °C 90 d with GA<sub>3</sub> 1000 mg·L<sup>-1</sup> treatment (Fig. 4B). In all stratification regimes, GA<sub>3</sub> 500 and 1000 mg·L<sup>-1</sup> treatments resulted in higher germination rates than the  $GA_3 \ 0 \ mg \cdot L^{-1}$  treatment. The highest germination rate in P. maackii  $(33.4\% \pm 12.7\%)$  was observed under  $4 \,^{\circ}$ C 90 d with GA<sub>3</sub> 0 mg·L<sup>-1</sup> treatment (Fig. 4A).

#### Discussion

The germination percentage of *Prunus maackii* stones was enhanced by stratification, which proved to be the most effective method for breaking seed dormancy (Fig. 3C). In *P. virginiana*, germination significantly increased under combined treatments of moist stratification and gibberellic acid (GA<sub>3</sub>), resulting in high germination rates during



# Incubation time (h)

Fig. 2. Water uptake by stones and seeds of *Prunus maackii* (A) and *Prunus virginiana* (B) as represented by an increase in mass. Stones and seeds were incubated at room temperature (20 to  $25\,^{\circ}$ C) on filter paper with distilled water for 0 to 48 h. Error bars indicate means  $\pm$  standard error of three replicates.

the 50 d observation period (Fig. 4B). These findings are consistent with earlier studies on the *Prunus* genus (Esen et al. 2007; Phartyal et al. 2009). Although previous research reported that GA<sub>3</sub> soaking after endocarp removal was effective in breaking dormancy (Kim et al. 2024), the same treatment resulted in relatively low germination rates in both *Prunus* species in this study.

Water imbibition tests confirmed that the endocarp does not act as a

barrier to water uptake in *P. maackii* and *P. virginiana*. PY is typically caused by water-impermeable palisade or palisade-like cell layers in the seed or fruit coat (Baskin et al. 2000) and can be overcome by scarification (Mousavi et al. 2011). Seeds that absorb more than 20% of their initial weight in water are generally considered not to exhibit PY (Baskin and Baskin 2003b; Baskin et al. 2000). Kim et al. (2024) also reported that

P. spachiana f. ascendens lacks PY, as water was absorbed into the pith-like tissue inside the pericarp during safranin staining. According to the concept of trait stasis, physiological and ecological traits related to seed dormancy and germination have remained largely unchanged over geological time within certain lineages (Adams et al. 2005). Therefore, it is considered that P. maackii and P. virginiana do not have PY.

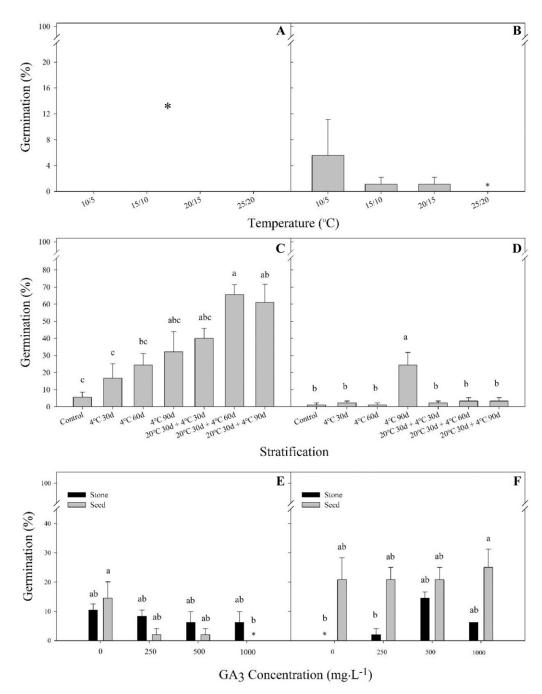


Fig. 3. Germination percentages of *Prunus maackii* (A, C, and E) and *Prunus virginiana* (B, D, and F). Effects of day/night temperatures (10/5, 15/10, 20/15, and 25/20 °C) on stone (A and B). Effects of moist stratification on stones with seven treatments: no stratification; 30, 60, or 90 d at 4 °C; or 30 d at 20 °C followed by 30, 60, or 90 d at 4 °C (C and D). Effects of GA<sub>3</sub> at four concentrations (0, 250, 500, and  $1000 \text{ mg} \cdot \text{L}^{-1}$ ) on both stones and seeds (E and F). Error bars represent means  $\pm$  standard error of three replicates. Different letters indicate statistically significant differences as determined by the results of Tukey's honestly significant difference test at P < 0.05.

In the temperature regimes experiment, the germination rate was less than 10% in all species, indicating that a single application of alternating day/night temperature was not effective in breaking dormancy in either species (Fig. 3A and 3B). MD is defined as dormancy caused by underdeveloped embryos that

require time (typically within 30 d) to grow before germination (Baskin and Baskin 2003b). Because both species had fully developed embryos (Fig. 1) and germination did not occur even after extended periods, MD can be ruled out, supporting the conclusion that both species exhibit PD.

Moist warm and cold stratification was effective in promoting germination of *P. maackii* (Fig. 3C). This finding aligns with previous reports that moist stratification is effective in breaking PD (Geneve 2003). Seeds with stony endocarps need a warm stratification period followed by cold stratification to germinate (Densmore

Table 1. Probability values of the two-way analysis of variance of *Prunus maackii* and *Prunus virginiana* germination treatments and treatment interactions.

Effects	P. maackii	P. virginiana
GA <sub>3</sub> concentration (G)	NS	***
Stratification (S)	**	***
$G \times S$	NS	*

<sup>\*</sup>P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 levels, and NS denotes not significant.

and Zasada 1977; Young and Young 1992). Crocker and Barton (1957) found that species like *Arctostaphylos*, *Cornus*, *Cotoneaster*, *Crataegus*, *Halesia*, *Rhodotypos*, and *Symphoricarpos* had impermeable endocarps and dormant embryos. They proposed that soil microorganisms help degrade the endocarp during warm, moist summer conditions (Baskin and Baskin 2014). Cold stratification during winter breaks embryo dormancy, allowing germination

in spring. However, Kim (2019) reported that *P. yedoensis* seeds did not germinate after cold stratification at 4 °C for 3 or 6 months. Germination was also not observed when seeds were stratified at 18 °C for 3 months followed by 4 °C for another 3 months. Stratification requirements can vary even among seeds within the same genus. Interspecific variation in seed germination requirements was also reported in the genus *Corylopsis*. *C. coreana* seeds germinated

(about 26% of partial germination) without cold stratification, whereas *C. sinensis* var. *calvescens* failed to germinate to more than ~30% following 3 months of cold stratification at 5 °C (Roh et al. 2008). Stratification requirements can vary even among seeds within the same genus, particularly because of differences in dormancy traits among specific species.

Germination responses to GA<sub>3</sub> and endocarp removal differed between species. *P. virginiana* seeds with the endocarp removed exhibited higher germination rates than stones, regardless of GA<sub>3</sub> concentration. This suggests that the presence of abscisic acid (ABA) within the endocarp inhibited germination, and that the removal of this ABA-rich tissue was effective in enhancing germination. According to a previous

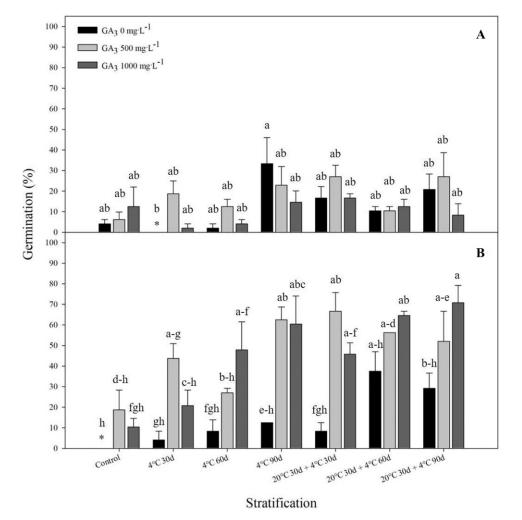


Fig. 4. Germination percentages of *Prunus maackii* (A) and *Prunus virginiana* (B) seeds as affected by different combination treatments of moist stratification and gibberellic acid (GA<sub>3</sub>). Stratification (seven levels): no stratification; 30 to 90 d at  $4^{\circ}$ C; 30 d at  $20^{\circ}$ C followed by 30 to 90 d at  $4^{\circ}$ C. GA<sub>3</sub> concentrations (three levels): 0, 500, and 1000 mg·L<sup>-1</sup>, respectively. Error bars represent means  $\pm$  standard error of three replicates. Different letters indicate statistically significant differences as determined by the results of Tukey's honestly significant difference test at P < 0.05.

Table 2. Summary of germination traits seed dormancy and in *Prunus maackii* and *Prunus virginiana*, including water imbibition, embryo development, temperature regimen, warm and cold stratification, GA<sub>3</sub> treatment and endocarp removal, and combined GA<sub>3</sub> and stratification treatments.

Trait	P. maackii	P. virginiana
Water imbibition	>20%, no PY <sup>i</sup>	>20%, no PY
Embryo development	Fully developed, no MDii	Fully developed, no MD
Temperature regimen effect	No germination	<6%
Warm and cold stratification effect	$20^{\circ}\text{C}$ $30\text{ d} + 4^{\circ}\text{C}$ $60\text{ d} \rightarrow 65.6\%$	$4^{\circ}\mathrm{C}$ 90 d $ ightarrow$ 24.4%
GA <sub>3</sub> and endocarp removal effect	≤15% across all GA <sub>3</sub> concentrations; endocarp removal not effective	Seed GA <sub>3</sub> 1000 mg·L <sup>-1</sup> $\rightarrow$ 25.0%
GA <sub>3</sub> and stratification effect	$4 ^{\circ}\text{C}$ 90 d + $GA_3$ 0 mg·L <sup>-1</sup> $\rightarrow$ 33.4%	$20 ^{\circ}\text{C}$ 30 d + $4 ^{\circ}\text{C}$ 90 d + $\text{GA}_3$ 1000 mg·L <sup>-1</sup> $\rightarrow$ 70.8%
Dormancy type	Nondeep to intermediate PD <sup>iii</sup>	Intermediate PD

<sup>&</sup>lt;sup>i</sup> Physical dormancy.

study, GA<sub>3</sub> treatment did not promote germination in P. spachiana unless the pericarp was removed, after which a 100% germination rate was achieved within 2 weeks (Kim et al. 2024). Similarly, in P. campanulata, removing both the endocarp and seedcoat before stratification significantly improved germination, as ABA concentrations were much higher in these layers compared with the embryo (Chen et al. 2007). In the present study, removing the endocarp from P. virginiana seeds led to higher germination rates than seeds with the stone intact across all GA<sub>3</sub> concentrations (Fig. 4). These results support that physical removal of ABA-rich outer tissues, rather than GA<sub>3</sub> application alone, was a critical factor in overcoming dormancy. Gibberellins (GAs) such as GA<sub>3</sub> and GA<sub>4+7</sub> are commonly used to break seed dormancy (Szabó et al. 2012). In P. mahaleb 'Korponay', GA3 and GA4+7 treatments yielded final germination rates of 81.6% and 100%, respectively (Szabó et al. 2012). However, P. maackii and P. virginiana seeds treated with GA<sub>3</sub> exhibited germination rates of less than 30% (Fig. 3E and 3F), suggesting species-specific GA effectiveness. As germination also varies with GA<sub>3</sub> concentration (Zeinalabedini et al. 2009), further studies are needed to determine optimal treatment levels.

Combined treatment of GA<sub>3</sub> and stratification resulted in higher germination rates in *P. virginiana* compared with single treatments. This could be attributed to the thick endocarp of *P. virginiana* seeds, which is presumed to contain a higher concentration of ABA, a dormancy-inducing compound. Therefore, the combined

application of GA<sub>3</sub> and stratification to seeds without the endocarp resulted in significantly higher germination rates than either treatment alone, highlighting the importance of removing dormancy-related physical and chemical barriers. Diaz and Martin (1972) found higher concentrations of ABA in the seedcoat than in the embryo of Lovell peach seeds, suggesting that both physical and chemical barriers from the outer layers contribute to dormancy. ABA and GAs are known to regulate seed dormancy and germination; ABA maintains dormancy during stratification, whereas GAs initiate germination (Bewley 1997; Chen et al. 2005; Koornneef et al. 2002). Combined treatment of GA<sub>3</sub> and stratification also increased seed germination in other *Prunus* species (Phartyal et al. 2009; Pipinis et al. 2012). Baskin and Baskin (2003b) summarized the characteristics of each dormancy level. Nondeep PD is characterized by normal seedling formation after embryo excision, positive responsiveness to GA treatment, and warm and/or cold stratification requirements. After-ripening and scarification may further enhance germination in nondeep dormant seeds. Intermediate PD exhibits normal seedling formation upon embryo excision, variable responsiveness to GA treatment, and a requirement for 2 to 3 months of cold stratification to break dormancy. In species with intermediate PD, dormancy break is promoted by sequential warm and cold stratification, which mimics natural seasonal conditions and reduces the required cold period (Baskin and Baskin 2003a).

Based on the results, P. virginiana is considered to exhibit intermediate PD, as germination improved after cold stratification and was further enhanced by warm and cold stratification, with increased responsiveness to GA<sub>3</sub> following stratification (Table 2). In contrast, P. maackii appears to show dormancy characteristics consistent with both nondeep and intermediate PD. A relatively short period of cold stratification increased germination, suggesting nondeep PD in part of the seed population and additional improvement following warm and cold stratification supports the presence of intermediate PD in another portion of the population. The results offer practical guidance for improving germination protocols in Prunus species. For P. maackii, implementing a cold stratification regimen is sufficient for breaking dormancy in a portion of seeds. For P. virginiana, however, dormancy-breaking requires a combination of physical (endocarp removal), environmental (stratification), and hormonal (GA<sub>3</sub>) treatments. These findings are applicable to rootstock production and conservation efforts, where reliable and efficient seed propagation is essential. Future studies should explore the use of alternative gibberellins such as  $GA_{4+7}$ , as well as the precise hormonal composition of seedcoats in both species to further clarify dormancy mechanisms.

## **Conclusions**

This study demonstrated that both *Prunus maackii* and *P. virginiana* exhibit PD but differ significantly in dormancy depth and germination responses. Water imbibition and embryo inspection confirmed the absence of PY and MD, indicating that dormancy-breaking efforts

ii Morphological dormancy.

iii Physiological dormancy.

should focus on overcoming physiological barriers. Stratification alone was effective in promoting germination in P. maackii, whereas P. virginiana required combined stratification and GA<sub>3</sub> treatment to achieve optimal germination, highlighting interspecific variation in dormancy mechanisms. Based on germination responses, P. virginiana fulfilled the criteria for intermediate PD-characterized by low responsiveness to GA<sub>3</sub> without stratification, a requirement for ≥60 d of cold stratification, and a pronounced increase in germination when combined with GA<sub>3</sub>. In contrast, *P. maackii* exhibited population-level heterogeneity, with some seeds responding to short cold stratification and others requiring sequential warm and cold stratification. P. maackii may show different depth of PD between nondeep and intermediate levels. Together, these findings underscore the importance of developing species- and genotype-specific dormancy-breaking protocols. They provide a scientific basis for enhancing propagation efficiency and formulating optimized seedling production and conservation strategies for Prunus species.

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