Scientia Horticulturae 243 (2019) 451–456

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Practical methods for rapid seed germination from seed coat-imposed dormancy of *Prunus yedoensis*

Du Hyun Kim

Department of Life Resources Industry, Dong-A University, Busan 49315, Republic of Korea

ARTICLE INFO

Keywords:
Gibberellic acid
Seed-coat dormancy
Yoshino cherry
Germination inhibitor

ABSTRACT

The seeds of *Prunus yedoensis* have slow and poor germination when intact (with endocarp). Cold and/or warm stratification, washing, and scarification with liquid nitrogen and sulfuric acid, in combination with or without gibberellic acid (GA_3) application, were investigated as facilitators for rapid and high germination. No germination was observed without GA_3 in any treatment for intact seeds. GA_3 treatment for one day significantly shortens the mean germination time (MGT) and improves seed germination percentage. Immersion in liquid nitrogen followed by GA_3 treatment, GA_3 application to intact seeds and seed coat removed seeds significantly enhanced the germination and MGT to 63 ± 7.6% (8.6 weeks), 71 ± 5.0% (4.7 weeks), and 93 ± 5.1% (0.8 weeks), compared to untreated seeds or stratified seeds (0%) chilled for six months. Depending on the GA_3 application method, results could be different in intact seeds. GA_3 application with vigorous shaking accomplished higher germination and lower MGT (71 ± 5.0%, 4.7 weeks) than gentle shaking incubation (33 ± 6.0%, 10.4 weeks). The germination inhibitor(s) were located in the seed coat because seed coat extract inhibited germination in decoated seeds. Therefore, coat removal alleviates the inability to promote germination and it has the same beneficial effect as does the application of GA_3. According to the results, it suggest that the effective application of GA_3 on intact seeds or seed coat removal could overcome seed dormancy in *P. yedoensis*.

1. Introduction

Prunus yedoensis Matsumura, commonly known as *P.* × *yedoensis* or the Yoshino cherry, is one of the most popular ornamental *Prunus* species (*Prunus* subgenus *Cerasus*) in the world, and is widely planted in parks and roadsides for its beautiful spring flowers (Bailey and Bailey, 1976; Cho et al., 2014).

Reproductive capability of *P. yedoensis* has not been reported, but seeds purchased from a commercial source (F.W. Schumacher Co., Inc., Sandwich, MS, USA) failed to germinate after natural warm and cold stratification (Roh et al., 2007). Because of difficulties in seed collection, low seed germination, and lack of seedling cultivation techniques, *P. yedoensis* is typically propagated asexually using grafting, cuttings, or tissue culture (Cheong and Kim, 2001; Kim and Kim, 2007). However, asexual propagation has a risk of temporary extermination by disease due to the lack of genetic diversity. Therefore, especially in the case of *P. yedoensis*, which is sensitive to disease and low temperatures, propagation by seeds might be preferable to maintain genetic variability.

Prunus seeds have embryo dormancy and require a period of after ripening in the presence of moisture to break dormancy. Because of their stony endocarps, *Prunus* seeds are often thought to have physical dormancy. The endocarp may offer some resistance to germination, but it is permeable to water, and *Prunus* is not truly hard-seeded (Heit, 1967). Various methods have been used to break seed dormancy in *Prunus* species. Both mechanical and chemical methods have been used in attempts to crack, or soften the endocarp, by applying mechanical scarification, boiling water, sulfuric acid, citric acid, or hydrogen peroxide (Grisez et al., 2008; Tewari et al., 2011).

Furthermore the application of hormones during seed stratification or after mechanical and chemical treatments has been used to promote dormancy breaking. It was reported that addition of gibberellins has also been shown to increase germination percentage in *Prunus* species (García-Gusano et al., 2004; Imani et al., 2011; Martínez-Gómez and Dicenta, 2001). Gibberellin (GA_3) treatments can replace a portion of the stratification period in several *Prunus* spp., but only after endocarp removal (Grisez et al., 2008). Likewise, GA_3 addition promotes the germination of non-stratified *P. campanulata* seeds after endocarp removal (Chen et al., 2007) and significantly increases the germination percentages of *P. avium* and *P. serotine* seeds (Çetinbaş and Koyuncu, 2006; Phartyal et al., 2009).

However, only a few studies have been carried out on seed propagation of *P. yedoensis*, which is considered the most common and widespread flowering cherry species. Practical applications of increasing percentage and speed of germination would have positive

E-mail address: dhkimhort@dau.ac.kr.

https://doi.org/10.1016/j.scienta.2018.08.039
Received 29 June 2018; Received in revised form 22 August 2018; Accepted 24 August 2018

economic effects for nurseries by increasing seedling production within a short period. Additionally, it would be a valuable technical strategy for seedbanks concerned with conservation of genetic diversity, the regeneration of preserved germplasm, and for seedbank viability checks for species in deep dormancy.

The aim of this study was to determine treatments that are able to break the dormancy of *P. yedoensis* seeds and increase germination percentage and speed, improving the efficiency of its propagation. To do so, the effects on seed germination percentage of several pretreatments, including stratification regime, endocarp and seed coat removal, and gibbellic acid treatment methods (via gentle or vigorous shaking) evaluated.

2. Materials and methods

2.1. Seed material

Fruits of *P. yedoensis* were collected at full maturity, when the fruit colour is nearly black, on 24 June 2016 in the NIFoS campus. Fruits were collected by spreading sheets under trees to catch the naturally falling fruit. Seed cleaning was performed using macerators with water to float off and screen out the pulp. After 3 h of drying in an aerated laboratory at room temperature, seed moisture content (SMC) was 27%. Seeds that were going to be used for scarification with liquid nitrogen (LN) were SMC controlled to 24% and 10% before LN immersion. For the other treatments, seeds at 8.5% SMC (fresh weight basis) were stored in a refrigerator in zipped plastic bags at 4 °C until treatment began. The viability of seeds was determined using the tetrazolium (TZ) test according to protocol for the *Prunus* genus as provided in AOSA (2010) and TZ tests showed mean viability of 89 ± 2.7%. Seed size were measured using digital callipers (Mitutoyo 500-196-20, Japan). Seed size and the mass of 100 seeds showed average width of 6.65 ± 0.09 mm, length of 7.46 ± 0.19 mm, and weigh of 12.5 ± 0.28 g · 100 seeds^{-1}.

2.2. Seed pretreatments

2.2.1. Warm or cold stratification

The substrate for seed stratification was < 1 mm thick with sieved river sand. Seeds mixed with the substrate at a ratio of 1:3 were placed in a plastic petri dish (90 mm) to protect seeds from excessive drying or moisture. Cold stratification of the seeds was conducted for three months or six months at 4 °C. Seeds for mixed stratification were treated at 18 °C for three months (warm stratification) followed by placed seed at 4 °C for three months (cold stratification). The condition of both the seeds and substrate was assessed and controlled every week during stratification. During stratification, moisture level of the sand was checked, and water was added if necessary.

2.2.2. Washing

Intact seeds were washed with tap water after three detergent washings, and then soaked in the running tap water for two days. Sonication washing was performed for 3 h/day for two days at 40 kHz frequency in an ultrasound bath (Branson Model8210, Branson Ultrasonic, USA). After washing treatments, GA_3 treated seeds were soaked in 50 mL of 1000 ppm GA_3 solution for 24 h at 18 °C under dark condition, using a shaking incubator with a gentle shaking speed (80 rpm).

2.2.3. Scarification with liquid nitrogen and sulfuric acid

Intact seeds that had 10% and 24% (fresh weight base) seed moisture content were immersed in liquid nitrogen (LN) at −196 °C for 10 min and rewarmed in a 37 °C water bath to overcome external hardness of the endocarp and seed coat. For sulfuric acid treatment, the seeds were immersed in 70% sulfuric acid solution for 10 min and rinsed thoroughly with tap water. After liquid nitrogen and sulfuric acid treatments, GA_3 treated seeds were soaked in 50 mL of 1000 ppm GA_3 solution for 24 h at 18 °C under dark condition, using a shaking incubator with a gentle shaking speed (80 rpm).

2.2.4. Imbibition in GA_3 solution

GA_3 was applied in one of two ways, via either a gentle or vigorous treatment, to increase treatment efficiency. Gentle treatment was as follows: intact seeds were soaked in darkness for 24 h in 50 mL of 1000 ppm GA_3 solution in an Erlenmeyer flask (60 mm diameter and 100 mL volume) at 18 °C, using a shaking incubator with a gentle shaking speed (80 rpm). This condition was applied to all above pretreated seeds for GA_3 treatment. Vigorous treatment was as follows: Intact seeds were soaked in darkness for 24 h in 50 mL of 1000 ppm GA_3 solution in a cylindrical culture vessel (90 mm diameter and 200 mL volume) at 18 °C, using a shaking incubator at a vigorous shaking speed (120 rpm)

2.2.5. Removal of endocarp or/and seed coat

Seeds were soaked in distilled water for 24 h at 20 °C in order to facilitate removal of the endocarp and seed coat. A vise used to exert pressing force on the seam to crack and remove the endocarp. Seed with the endocarp removed were soaked for 3–5 h in water, and then the seed coat and nucellus from the embryo were removed (AOSA, 2010). Seed coat extract was applied on seeds with removed seed coats, in order to clarify the inhibition effect of seed coats: 0.2 g of seed coat was homogenized with 10 mL of water and the resulting mixture was applied on the seeds for the seed germination test, instead of water. When seeds were placed for germination test in a petri dish, 1000 ppm GA_3 was applied instead of water to the seeds which removed endocarp and seed coats.

2.3. Germination test

The seeds after pretreatments were kept to germinate in the light/dark (16 h/8 h a day) at 20 °C in the sand (pretreatments no. 1-4) or two layers of filter papers (pretreatment no. 5) in 90 mm petri dish. Four replicates of 25 seeds were tested for germination. Germination counted when visible protrusion of radical (2 mm) occurred every week or day (ISTA, 2016), Germination percentage (GP) and mean germination time (MGT) were calculated as the average of the four replications. The MGT was calculated for each replication per treatment according to the following equation:

MGT = MGT = $n_i d_i/n$, where n is the total number of germinated seeds during the germination test, n_i is the number of germinated seeds on day d_i and i is the days during germination period (Falleri et al., 1997).

2.4. Statistical analysis

All data were statistically analysed using the analysis of variance (ANOVA). To examine the effects of seed treatments on germination, two-way ANOVA analysed the effects of GA_3, seed pretreatments and their interactions on germination. Means were compared using the Duncan multiple range test (DMRT) at the 5% level. Statistical analyses were done using SAS System for Windows, Version 8.01 (SAS Institute, USA).

3. Results

Untreated intact whole seeds did not germinate regardless of the length of the stratification period or temperature. Cold-stratified seeds that placed in moistened sand at 4 °C for either three or six months did not germinate. Additionally, seeds that were stratified for three months at 18 °C followed by three months at 4 °C showed no germination.

Seed germination percentage and MGT in *P. yedoensis* was significantly affected by GA_3 treatments ($p < 0.001$). However,