Application of Gibberellic Acid (GA_3) and Coconut Water with Stratification on Morphological, Anatomical, and Germination of Cherry Seed (*Prunus jamasakura*)

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Abstract. *Prunus jamasakura* Siebold ex Koidz. (hill cherry) is included in the family of Rosaceae and used for ornamental plants. The dormant cherry seed has become an obstacle since it takes a prolonged time to germinate. Seed germination can be induced by the application of gibberellic acid (GA_3) and coconut water (CW) at low-temperature. This research aimed to determine the effect of GA_3 and CW combination followed by stratification in temperature 5 °C on the morphological and anatomical characters of cherry seed germination and to determine which combination is the most effective to trigger cherry seed germination. Completely randomized design (CRD) was performed on various concentrations; control GA_3 0 ppm + CW 0% (K_0), GA_3 750 ppm + CW 50% (K_1), GA_3 750 ppm + CW 75% (K_2), and GA_3 750 ppm + CW 100% (K_3). Cherry seeds were soaked in those combinations for 72 h. Then they were through 8 weeks of stratification. Germination was conducted within 30 days. The viability test showed that 100% of seeds are viable. The morphology showed an increase in seed length and endocarp cracks. Seed mass increased significantly ($p < 0,05$). The highest imbibition rate was 80,1% found in the K_1 group. No seeds germinated (germination percentage 0%) in all treatments. Based on the anatomy of the seeds showed an increase in cell size. This treatment was not effective in increasing the germination of cherry seeds, which were characterized by seeds that did not germinated.

Keywords: Germination · Gibberellic Acid and Coconut Water · Morphology and Anatomy · Prunus jamasakura · Stratification

1 Introduction

Prunus jamasakura Siebold ex Koidz. known as yama-z(s)akura "hill cherry" is a classic cherry in Japan that grows at 1,300–2,000 m above sea level. Sakura is very attractive during flowering because after blooming the flowers will fall out simultaneously [1, 2]. *Prunus* seeds have seed coat and embryo dormancy that inhibit germination. Seed dormancy can be broken through several ways such as low-temperature treatment [3]. The length of time at which dormant seeds are viable and able to germinate varies from

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a few days to decades. It is a problem for seed producers, so needed knowledge about seed dormancy and how to break it [4].

The low-temperature stratification treatment 5 °C was able to increase the germination of sakura seeds because it could activate the embryo. In the study of cherry seeds germination, stratification increased germination results with warm temperatures above 10 °C followed by low temperatures 1–5 °C to create a suitable temperature for germination. Stratification can improve hormone balance through temperature fluctuations that affect the activity of genes in hormone synthesis for metabolism [1].

In [5], to stimulate the germination of *P. jamasakura* seeds, gibberellic acid (GA_3) was applied in 0, 250, and 750 ppm concentrations for 24 h. Stratification was carried out for two months at low-temperature 5 °C and then tested for germination in 30 days. A significant increase in seed imbibition rate was found in GA_3 250 and 750 ppm. The highest percentage of seed germination (60%) was obtained at 750 ppm.

The addition of organic material (both from fruit and vegetable) along with plant growth regulators can help the plants to grow. Organic materials are used because they are cheaper than using chemicals [6]. The combination affects germination rate, germination, plumule length, and seed weight [7]. Coconut water is widely used, the liquid endosperm contains minerals such as amino acids, sugars, vitamins, and plant regulators in the form of auxins, cytokinins, and gibberellins so they can stimulate germination and growth in cell division [8].

Soaking the seeds in water is effective in promoting germination by reducing the hardness of the seed coat and diluting the inhibitory substances. The treatment of soaking soybean seeds in coconut water with concentrations of 25%, 50%, and 75% gave the highest germination percentage at 25%. Presumably, at this concentration, the composition of the hormone content is suitable for germination [9]. The results of the study of soaking rice seeds in coconut water with various concentrations obtained the best concentration of 55% for germination. Soaking coconut water on soursop (*Annona muricata* L.) seeds for 24 h gave varying results. Coconut water concentration of 75% obtained the best results on height, number of leaves and plant dry weight, while the highest germination percentage was at 50% [10, 11].

Recent research on cherry seed is still limited, especially to increase the percentage of germination, as well as the morphological and anatomical characters of cherry seeds. In this study, the effect of the combination GA_3 and coconut water also stratification (5 °C) on these characters in stimulating germination was examined.

2 Materials and Method

2.1 Study Area

The research was conducted from September 2019 until December 2020 at the Laboratory of Plant Physiology, Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta.

2.2 Procedures

Prunus jamasakura seeds were selected in the range of 0.5 ± 1 cm. Seed viability test was carried out using 1% tetrazolium solution. The combination of GA_3 750 ppm was

added to coconut water with a concentration of 50% (K_1), 75% (K_2), and 100% (K_3). K_0 used distilled water as a comparison.

The stratification of cherry seeds was first soaked in NaOCl for 5 min to inhibit fungal growth. The seeds were rinsed in aqua dest 3 times. Seeds were soaked in each combination concentration of GA_3 and coconut water for 72 h in a dark glass bottle at room temperature 28–30 °C. The seeds were put in a closed container with sand media and then stored at 5 °C for 60 days. Humidity is maintained by adding aqua dest during the stratification period [5].

Seed germination test was carried out for 30 days with 10 repetitions placed at 20 °C for 16 h in the dark and then in the light at 25 °C for 8 h. This is related to the photoperiod and ensures high humidity levels. Humidity was maintained every day. The presence or absence of radicles that appeared with a length of 0.2 cm was observed as a parameter [5, 12].

Seed imbibition rate was done by weighing the seeds as their initial weight and then soaking them in a combination of GA_3 and coconut water for 24 h. Seeds were re-weighed after soaking. Seeds were weighed at the same intervals to see the water content during germination.

Seed morphology observations were conducted through a stereo microscope including the presence or absence of cracks in the endocarp, seed length, and seed weight every two weeks during stratification. Seed anatomy observations were made using the paraffin method by two seeds from each treatment at the beginning and end of stratification. Measured parameters include seed germination percentage, seed germination rate, and biomass also the morphology of cherry seedlings.

2.3 Statistical Analysis

Quantitative data including germination percentage, changes in seed weight during stratification, and seed imbibition rate for 4 treatments were analyzed in SPSS by Analysis of Variance (ANOVA) with 10 repetitions. If there is a significant difference in the group, the Duncan's Multiple Range Test (DMRT) test can be carried out with a 95% confidence level. Seed viability test, seed imbibition rate, seed morphology, and anatomy were analyzed descriptively.

3 Results and Discussion

3.1 Tetrazolium Test

Seed viability testing using 1% tetrazolium solution (2,3,5-triphenyl tetrazolium chloride/bromide) provided information on the physiological quality of seeds in a short time before germination even though the seeds were in a dormant state. The results of the viability test of cherry seeds (*Prunus jamasakura* Siebold ex Koidz.) showed viable 100%, indicated by the change in color of the seeds to red in Fig. 1 is absorbed into the seeds and then forms a red precipitate of triphenyl formazan due to bonds with hydrogen released by the dehydrogenase enzyme in the reduction process in living cells. These deposits indicate live seeds.

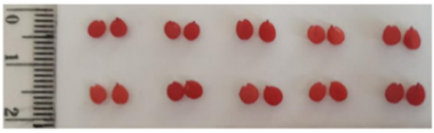

Fig. 1. Seed color changes after treatment in *Prunus jamasakura.*

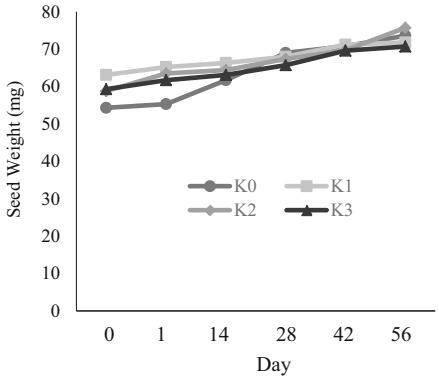

Fig. 2. Changes in weight of *Prunus jamasakura* during stratification.

3.2 Seed Morphology

Observation of cherry seeds stratification in K_0 (control) showed an increase in seed length 0.02 cm. Cracks along the endocarp begin at the 6th week then more open by the 8th week. The radicle has not been able to emerge through the micropyle even though it is supported by a slightly open endocarp until the stratification period is complete. K_1 showed an increase in seed length up to 0.03 cm related to water uptake. K_2 and K_3 showed an increase of 0.02 cm in seed length.

Seed weight was measured at the beginning, after 24 h, and every 2 weeks during the stratification period. In the K_0, K_1, K_2, and K_3 treatments, the weight of the seeds increased until the 8th week of stratification. The graph is shown in Fig. 2. The changes that occur are related to the water absorbed by the seeds which are needed for germination. A significant increase was seen in the K_0 treatment at 24 h towards the 2nd week of stratification. The graph of seed weight increased after 24 h due to the presence of cracks in the seed coat.

The increasing value of seed weight indicates the amount of water absorbed by *Prunus jamasakura* seeds during the stratification period. Changes in seed weight ranged from 8.7–19.3 mg. Within this range, in Fig. 3 the radicle did not appear until the end of the stratification period. The results of ANOVA ($F = 6.320$; $P = 0.005$) showed that the stratification, the combination of GA_3 and coconut water affected changes in cherry seed weight in Fig. 4, but the results of the combination of GA_3 and coconut water were not higher than the control. Based on these results, further DMRT tests were carried out showing that K_0 treatment had the highest amount of water absorption followed by K_2, K_3, and then K_1.

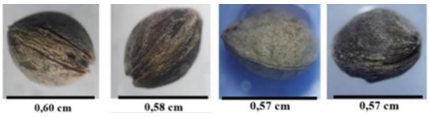

Fig. 3. The radicle did not appear until the end of the stratification period.

K_0	$0{,}0193^{c}$
K_1	$0{,}0087^{a}$
K_2	$0{,}0169^{bc}$
K_3	$0{,}0113^{ab}$

Fig. 4. The results of ANOVA for the stratification period.

K_0	$0{,}03154^{ab}$
K_1	$0{,}05744^{b}$
K_2	$0{,}01976^{a}$
K_3	$0{,}03874^{ab}$

Fig. 5. The results of ANOVA on the average change in seed weight at the end of germination.

Based on the morphological observation, the treatments affected changes in cherry seed where cracks are found in the endocarp, but the embryo has not emerged yet.

3.3 Imbibition Rate

Seed imbibition rate is the rate of water absorption in the seed so that it expands for seed germination. Seed imbibition was measured by initial and final weighing of *Prunus jamasakura* seeds after absorbing water every three days during the seed germination period. The initial average weight of cherry seeds ranges from 70.7–75.7 mg. The highest seed imbibition rate was obtained by K_1 at 80.1% followed by K_3, K_0, and K_2. The results of ANOVA on the average change in seed weight at the end of germination showed that the stratification in Fig. 5, the combination of GA_3 and coconut water did not have a significant effect ($F = 3.001$; $P = 0.062$), but the K_1 treatment had the highest average change than K_3, K_0, and K_2.

Seed weight for the control or the combination of GA_3 and coconut water informed an increase and decrease on the 1st to the 9th day as shown in Fig. 6. On the 12th day to the end of germination, there was an increase. This can indicate that the seeds are still in a dormant state. According to [13], seeds can absorb water up to twice their initial weight. Imbibition in dormant seeds usually occurs in a reversible way where the seeds can be dry again but no damage occurs.

Dormant seeds with hard seed coat conditions tend to have water absorption in the lag phase until they finally germinate. The endocarp may provide mechanical resistance to germination, but it is still permeable to water. Seeds with these conditions such as *Prunus serotina* did not germinate with low-temperature indicated the existence of various kinds of dormancy, both mechanical and physiological. The presence of inhibitors such as

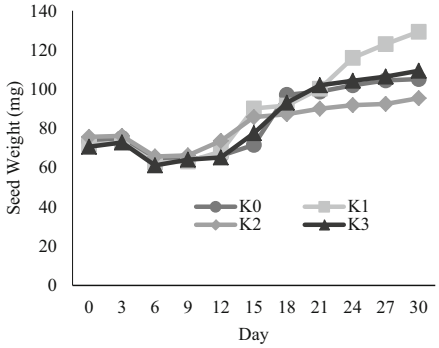

Fig. 6. Changes in weight of cherry seeds during 30 days of germination.

parasorbic, absisic, and isopropylmalic acid in seeds can also inhibit germination [14, 15].

In seed germination, rapid absorption of water occurs during phase I of imbibition. This absorption occurs in a balanced way, both dead and live seeds. The rate of increase depends on the media, the soil, and the location of the seeds in the soil. Phase II is based on the cell wall potential pressure in living cells. This phase is an active metabolism (enzyme synthesis) for the germination of both dormant and non-dormant seeds. Phase III occurs when the cells elongate so that the radicle appears with the crack of the seed coat. The duration of each phase depends on the quality of the seed such as the permeability of the seed coat and its size to environmental conditions such as temperature and humidity. Dormant seeds can normally reach phase II but do not show progress to phase III or without causing germination [16]. Based on the results, the treatments for cherry seeds reached phase I on the 3rd day of germination with an increase in seed weight, until the last day is still in the phase II where enzyme has been carried out optimally but the radicle has not emerged yet for phase III.

Abscisic acid (ABA) is related to the regulation of dormancy in seeds. Its concentration was reduced during the stratification. ABA concentrations tend to be high in the endocarp and seed coat of *Prunus campanulata*. Low ABA concentrations were obtained after stratification but the seeds failed to germinate because the concentration of GA is still lower so it does not stimulate germination. GA will increase with the presence of water imbibition in the seeds. The ABA content in the endocarp after 12 weeks of stratification was as high as fresh seeds but decreased in the seed coat and embryo. This may be due to reduced ABA catabolism. Fluridone and paclobutrazol can inhibit ABA biosynthesis and increase GA to induce seed germination [17].

3.4 Seed Germination

The germination percentage for all treatments was 0% because until the 30th day of germination there were no germinating *Prunus jamasakura* seeds. Parameters of seed germination rate, biomass, and morphology of cherry seedlings could not be determined. This result is supported by the radicle that has not emerged yet. The research was

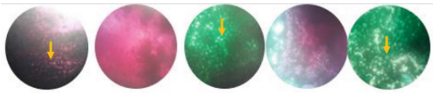

Fig. 7. Cell size of cherry seeds (beginning; last day of K_0, K_1, K_2, and K_3).

continued by opening the endocarp to see the condition of the seeds. Some seeds rotted for each treatment.

The success of seed germination can be affected by factors such as soil condition, temperature, aeration, light, salinity, and the presence of pathogenic organisms [4]. In this study, high soil moisture must be available to initiate physiological and biochemical processes in the seeds of sakura (*Prunus jamasakura*) which is a winter plant so that the embryo grows with watering. Temperature can affect the percentage and rate of seed germination, germination in this study used a temperature of 20 °C for 16 h in the dark and then the light temperature of 25 °C for 8 h. This is related to the photoperiod and ensures high humidity levels. Stagnant water that is not well absorbed during germination after daily watering can limit the oxygen availability to seeds. Fungi are found on the seed surface at the end of the germination period.

Research on seeds of *Malus sylvestris*, *Prunus avium*, and *Prunus padus* that have been stored for 2–3 years showed that the concentration of ascorbic acid decreased which affected their germination through their viability. The molecular response of different species of orthodox seeds with different storage can also affect their germination [18]. Three inhibitors that affect the germination of *Sorbus aucuparia* seeds are parasorbic acid, abscisic acid, and isopropylmalic acid. Scarification of *Sorbus alnifolia* seeds had a significant effect of 8% and the release of half cotyledons of 20% in seed germination. The lower temperature, the higher the germination rate [15]. The combination of GA_3 and young coconut water was not significant >0.05 on the germination of local chili (*Capsicum frutescens* L.). It is suspected that the content of GA_3 and coconut water can reduce its effect to promote germination due to the presence of phenolic compounds so that the germination observation parameters simultaneously run separately and do not affect each other [19].

3.5 Seed Anatomy

Cells in cherry seeds (*Prunus jamasakura* Siebold Ex. Koidz.) with stratification also the combination of GA_3 and coconut water have been differentiated to form procambium and protoderm. The increased length of the radicle could not be observed because the cutting was not perfect in all treatments. The difference in cell size for the combination of GA_3 and coconut water appears in Fig. 7 to be larger.

According to [4], after imbibition occurs, GA activates the amylase enzyme system which breaks down complex molecules into simpler ones for cell growth and division. Based on these results, the combination can increase the potential for embryos to germinate.

The combination treatment of GA_3 750 ppm and coconut water (50%, 75%, 100%) also stratification (5 °C) on the morphology of cherry seeds (*Prunus jamasakura*) showed changes in length up to 0.03 cm and cracks were found in the endocarp with significant

changes in seed weight ($p < 0.05$). No seeds germinated (germination percentage 0%) in all treatments. The highest seed imbibition rate was 80.1% found in the K_1 group. Seed anatomy showed an increase in cell size. This treatment was not effective in increasing the germination of cherry seeds, which were characterized by seeds that did not germinate.

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Authors' Contributions. IFA collecting data, data analysis, research methodology, manuscript writing, and editing. SO research methodology and review the manuscript as well SUR, and SUG as a supervisor. All authors read and approved the final manuscript.

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