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Research Article

Evaluation of reproduction biology of *Prunus cerasoides*

Suluh NORMASIWI[1]*, Siti S. HAFIZHAH[2], Ida ADVIANY[3], Muhammad I. SURYA[1]

[1]*Research Center for Plant Conservation, Botanic Gardens and Forestry, National Research and Innovation Agency (BRIN), Cibinong 16911, West Java, Indonesia; snsuluhsiwi10@gmail.com (*corresponding author); muha108@brin.go.id*
[2]*Universitas Islam Nusantara, Agriculture Faculty, Agrotechnology Department, Program of Agronomy, 40286 Bandung, West Java, Indonesia; salmaahafizhah@gmail.com*
[3]*University of Islam Nusantara, Agriculture Faculty, Agrotechnology Department, 40286 Bandung, West Java, Indonesia; advianyi@gmail.com*

Abstract

Prunus cerasoides have a high value in phytochemistry and pharmacology. It was classified as the Least Concern globally based on the IUCN red list due to its widespread distribution in eastern Asia. This research aims to evaluate the reproductive biology of *P. cerasoides* through the study of pollen morphology, pollen viability, stigma receptivity, and pollination in the Cibodas Botanical Garden, located in the Cibodas subdistrict of West Java, Indonesia. The pollen morphology was observed using SEM. Moreover, the pollen viability test was followed by the staining method (aceto-orcein 2%, I_2KI 1%, TTC 1%) and *in vitro* pollen germination with thirteen treatments (aquadest [control]; 5-30% sucrose; and 5-30% sucrose + 5 ppm boric acid). Stigma receptivity was observed daily, from 7 days before anthesis until the anthesis stage. Furthermore, several types of pollination were evaluated, including open pollination, autogamy, geitonogamy, and allogamy. The results showed that the best staining method on *P. cerasoides* was aceto-orcein 2%, with pollen viability at 87.87%. The sucrose concentration of 25% at 72 hours of observation gave the highest pollen viability results, 52.48%. Stigma receptivity was optimal in the two days before anthesis until anthesis. The highest pollination efficiency was cross-pollination at 53.33%, with an average percentage of the total fruit set of 24.17%.

Keywords: anthesis; pollen; pollination; *Prunus cerasoides*; stigma; SEM

Introduction

Prunus cerasoides grows in temperate forests and is native to China, Bhutan, India, Sri Lanka, Nepal, Laos, Myanmar, Thailand, and Vietnam. Moreover, it grows at a wide range of altitudes from 700 m to 3,700 m asl (eFloras, 2012). Several studies on ethnomedicine, phytochemistry, and pharmacology have reported that *P. cerasoides* highly potential to be developed as a medicine (Joseph *et al.*, 2018). Due to its widespread distribution in eastern Asia, *P. cerasoides* is classified as the Least Concern globally. Although deforestation is a threat to this species, it has been chosen as an integral part of forest regeneration programs in northern Thailand. It is thus presumed to be present in sufficient numbers. Despite this, there is still a lack of information

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about the species' population size and trend, and more research into these areas would benefit the species. Ex situ collection should be expanded to ensure that the full range of genetic diversity found in situ is represented in genebanks (Rhodes *et al*., 2016). *P. cerasoides* is a decorative collection from the Himalayas that was planted in Cibodas Botanical Garden (CBG) in 1963. CBG is an ex-situ conservation area located on the slope of Mount Gede Pangrango at an elevation of 1425 m asl that successfully propagated and cultivated *P. cerasoides*.

The flowering phenology of *P. cerasoides* in CBG has been reported by Hatta *et al*. (2005), and it could be flowering twice a year. Furthermore, Normasiwi (2015) reported that from 2009 to 2012, the flowering period was twice a year, and a little fruiting usually followed the flowering period from July to September. However, it has never become a mature fruit. In addition, the data showed that between 2001 and 2020, the mature fruit only occurred once in 2015 with very rare or little intensity of mature fruit (Kurniawan *et al*., 2021).

Many factors influence fruiting success, including those related to reproductive effort and the effects of floral display on pollinators, which can be seen in fruit production (Stephenson, 1981; Susko and Lovett-Doust, 1999). On the other hand, rainfall, relative humidity, and temperature may also impact the flowering and fruiting period of *P. cerasoides* (Kurniawan *et al*., 2021). Environmental factors such as low temperatures and rain negatively correlate with pollinator activity and result in lower fruit sets (Roversi and Ughini, 1996). The growing conditions were influenced by the climate, where the vegetative growth of the sweet cherry tree or *P. avium* was very vigorous. However, the flower organs performed poorly, with more malformed flowers and low fruit settings (Li *et al*., 2010).

Prunus has several factors that intervene in the progamic phase, ranging from pollination to fertilization. Pollen viability, pollen transfer and germination into the stigma, pollen-pistil incompatibility reaction, synchrony between pollen tube arrival to the ovule and embryo sac maturation, fertilization, and successful early embryo development are all examples of these (Guerra and Rodrigo, 2015). Although there are several histological studies of pollen and stigma in *P. cerasoides*, evaluation of pollen viability and its relationship to stigma acceptance is lacking. Therefore, this study aimed to evaluate the reproductive biology of *P. cerasoides* through pollen morphology, pollen viability, stigma receptivity, and pollination experiment.

Materials and Methods

Material

The plant used in this study was *P. cerasoides*, a Cibodas Botanical Gardens (CBG) collection, Cianjur, West Java, Indonesia. *P. cerasoides* at CBG was planted around 2002 by natives from the Himalayas. CBG is located in the Cibodas biosphere reserve at an altitude of 1300-1450 masl, with an average daily temperature of 16-22 °C and a relative humidity of 86-90%.

Pollen morphology

The observation of pollen morphology was conducted using a scanning electron microscope (SEM) in Zoology Characterization Laboratories. The sample was cleaned by immersing it in cacodylate buffer for 2 hours and agitating it in an ultrasonic cleaner. The specimens were prefixed in 2.5% glutaraldehyde and fixed in 2% tannic acid, then the samples were dehydrated in 70% alcohol and dried using a vacuum drier. Furthermore, the specimens were dried using an Ion coater and observed with an electron microscope JSM-IT200 *InTouchScope*™ with 500 to 3000× magnification. We observed pollen grains samples in each replicate to determine pollen ornamentation and pollen characteristics.

Pollen viability

Observation the pollen viability using two methods namely the staining method and *in vitro* germination. The staining method used 2% aceto-orcein, 1% iodine potassium iodide (I_2KI), and 1% 2,3,5-Triphenyl tetrazolium chloride (TTC) solution to observe pollen viability. Pollen grains in the preparations were observed directly using a light microscope. Viable pollen was recorded when the pollen already showed a visible colour change in the treatment. Meanwhile, pollen that is not stained or looks faded is marked as non-viable. In non-viable pollen grains, the form is irregular, so the pollen wall looks wrinkled, and the pollen colour looks faded (Sari *et al*., 2010). Pollen viability was calculated by dividing the number of stained pollen grains by the total number of pollen grains observed.

To pollen *in vitro* germination, the pollen grain was grown in thirteen different media, namely aquadest (M1), sucrose 5% (M2), sucrose 10% (M3), sucrose 15% (M4), sucrose 20% (M5), sucrose 25% (M6), sucrose 30% (M7), sucrose 5% + boric acid 5 ppm (M8), sucrose 10% + boric acid 5 ppm (M9), sucrose 15% + boric acid 5 ppm (M10), sucrose 20% + boric acid 5 ppm (M11), sucrose 25% + boric acid 5 ppm (M12) and sucrose 30% + boric acid 5 ppm (M13). Moreover, after 24 hours, germination and pollen viability were assessed. The pollen germination and viability parameters were measured using an Optilab microscope at 100× magnification and a micrometer. Pollen germination was calculated by dividing the number of germinated pollen grains by the total number of pollen grains observed.

Stigma receptive

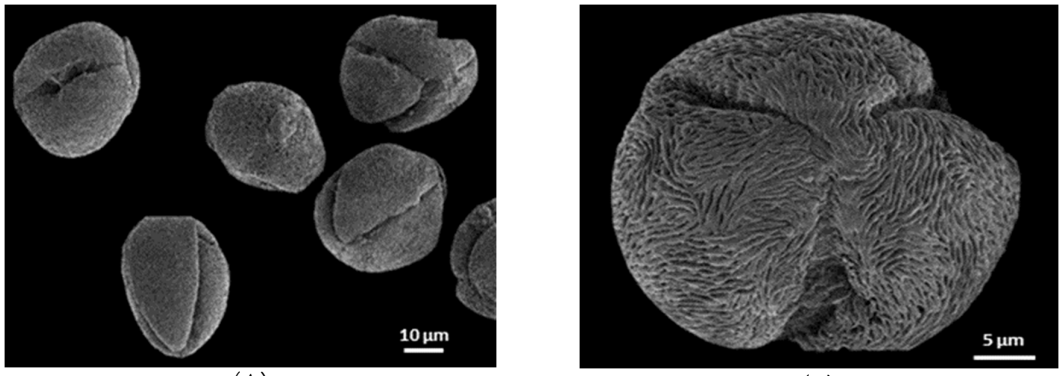
Before anthesis, the flowers were taken at seven stages (Day/D-7, D-6, D-5, D-4, D-3, D-2, D-1, A/Anthesis). Stigma receptivity was investigated in a laboratory using a hydrogen peroxide test under a microscope (Dafni and Maues, 1998). Flowers' stigmas were placed on a cavity slide, and 3% hydrogen peroxide was applied. The bubbling from stigma was thought to be a sign of stigma receptivity. Furthermore, the observations were captured using a microscope with 100× magnification.

Pollination

P. cerasoides from the Sakura Garden in the Cibodas Botanical Garden were used in the study. Pollination of *P. cerasoides* was noted when the treated flowers dropped their petals and formed young fruit. Four pollination techniques were used to evaluate the pollination experiment: geitonogamy, cross-pollination, open pollination, and self-pollination. Regular observations were made by counting the successfully formed fruit from pollination until harvesting.

Results

SEM observations showed that *P. cerasoides* pollen has a monad type unit (single), prolate spheroidal pollen, and three apertures (tricolporate), with the pollen structure belonging to the striate type (Figure 1). The polar axis (P) length is 30.94 μm with an equatorial diameter (E) of 34.29 μm, so the P/E index ratio is 1.10.



(A) (B)

Figure 1. *Prunus cerasoides* pollen on SEM. (A) EV (X^{1000}); (B) PV (X^{3000})

The viability of *P. cerasoides* pollen based on the staining method was observed directly in each treatment using 2% aceto-orcein solution, 1% I_2KI, and 1% TTC. Viable pollen was recorded when the pollen already showed a visible color change in the treatment. The results of pollen viability observations based on the staining method are presented in Table 1. The results showed that the 2% aceto-orcein treatment significantly differed from the 1% TTC treatment. While the 1% I_2KI treatment was not entirely different from the 2% aceto-orcein and 1% TTC treatment. The pollen viability test using 2% aceto-orcein was significantly higher, giving a pollen viability value of 87.87% compared to a 1% TTC solution of 78.62%.

Pollen grains in the preparations were observed directly using a light microscope. Pollen that looks densely coloured and has a regular shape is counted as viable pollen (Figure 2). Meanwhile, pollen that is not stained or looks faded is marked as non-viable. In non-viable pollen grains, the form is irregular, so the pollen wall looks wrinkled, and the pollen colour looks faded (Sari *et al*., 2010).

Table 1. Percentage of pollen viability based on the staining method

Treatments	Percentage of pollen viability (%)
Aceto-orcein 2%	87.87 ± 4.70 [a]
I_2KI 1%	81.80 ± 4.75 [ab]
TTC 1%	78.62 ± 3.92 [b]

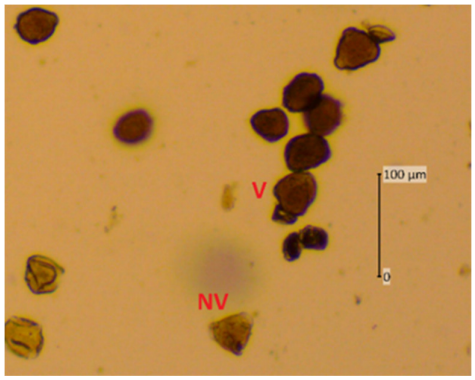
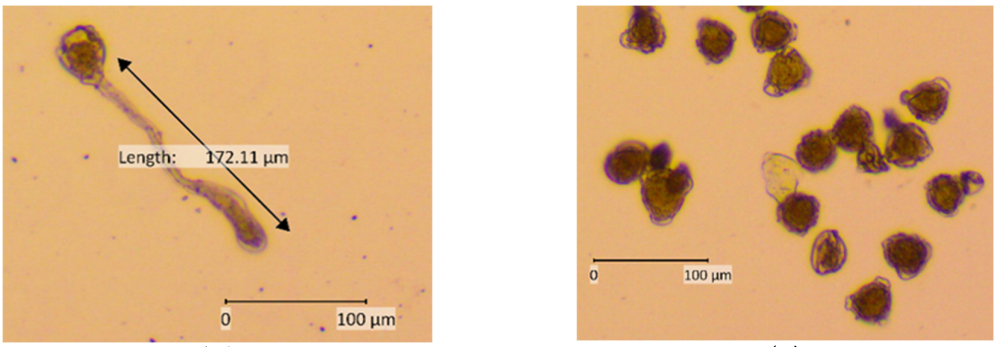


Figure 2. Pollen viability of *P. cerasoides*
V: viable; NV: non-viable

Pollen is categorized as germinating when the length of the pollen tube has reached at least the same diameter as the pollen grain (Shivanna and Rangaswamy, 2012). A not-viable pollen is characterized by pollen that does not form a pollen tube or could also form a pollen tube; however, its size does not exceed the pollen

diameter (Figure 3). Germinated pollen was recorded when the pollen tube length had reached at least the same as the diameter of the pollen. Pollen germination for 24, 48, and 72 hours increased in each treatment medium tested. Table 2 showed that *in vitro* pollen germination in 25% and 30% sucrose medium was significantly and consistently highest at 24, 36, and 72 hours of observation, with the results percentage of pollen germination was 52.48% (on 25% sucrose treatment medium; 72 hours), followed by 47.78% (on 30% sucrose medium; 72 hours). Conversely, the lowest germination during 72 hours was consistent in the control treatment (aquadest). On the other hand, 5% sucrose, 10% sucrose, 10% sucrose + 5 ppm boric acid, 25% sucrose + 5 ppm boric acid, and 30% sucrose + 5 ppm boric acid, showed a high percentage of pollen germination in the first 24 hours; however, percentage of pollen germination slowed down in the next 48 and 72 hours.



(A) (B)

Figure 3. *In vitro* pollen germination of *Prunus cerasoides* after 72 hours on 100x magnification (A) length of the pollen tube; (B) non germinate pollen

Table 2. The mean value of pollen germination and pollen tube of *Prunus cerasoides* on various media

Media	Percentage of pollen germination (%)			Length of the pollen tube (µm)		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
Aquadest	18.55 ± 1.78 ^{d}	20.01 ± 1.51 ^{f}	23.56 ± 1.00 ^{g}	47.66 ± 9.14 ^{d}	54.30 ± 7.46 ^{d}	70.64 ± 13.39 ^{d}
Sucrose 5%	32.59 ± 7.50 ^{ab}	39.60 ± 1.21 ^{ab}	42.09 ± 1.59 ^{cd}	71.33 ± 4.21 ^{ab}	82.95 ± 7.32 ^{cd}	93.35 ± 10.90 ^{cd}
Sucrose 10%	31.81 ± 2.99 ^{ab}	37.20 ± 3.95 ^{bc}	41.76 ± 0.31 ^{cd}	84.06 ± 7.18 ^{a}	131.29 ± 17.43 ^{a}	137.00 ± 9.34 ^{a}
Sucrose 15%	24.35 ± 5.10 ^{cd}	29.11 ± 4.16 ^{e}	38.16 ± 1.92 ^{def}	65.15 ± 7.16 ^{bc}	78.87 ± 15.75 ^{cd}	97.96 ± 13.95 ^{bc}
Sucrose 20%	25.11 ± 2.11 ^{cd}	34.55 ± 3.03 ^{bcde}	39.38 ± 4.28 ^{cdef}	49.11 ± 3.81 ^{cd}	73.21 ± 21.93 ^{cd}	113.15 ± 7.43 ^{abc}
Sucrose 25%	36.06 ± 0.68 ^{a}	44.30 ± 3.87 ^{a}	52.48 ± 3.72 ^{a}	75.49 ± 7.80 ^{ab}	80.41 ± 20.73 ^{cd}	124.08 ± 5.80 ^{ab}
Sucrose 30%	33.79 ± 1.40 ^{a}	40.57 ± 0.81 ^{ab}	47.78 ± 4.55 ^{ab}	80.46 ± 5.64 ^{ab}	113.59 ± 16.85 ^{ab}	119.60 ± 6.71 ^{abc}
Sucrose 5% + boric acid 5 ppm	26.83 ± 1.60 ^{bc}	31.34 ± 2.73 ^{cde}	37.91 ± 3.21 ^{def}	65.33 ± 17.14 ^{bc}	71.34 ± 16.26 ^{cd}	104.38 ± 15.79 ^{bc}
Sucrose 10% + boric acid 5 ppm	36.31 ± 7.31 ^{a}	40.24 ± 4.71 ^{ab}	42.56 ± 3.65 ^{cd}	79.44 ± 10.98 ^{ab}	114.57 ± 18.38 ^{ab}	134.98 ± 4.79 ^{a}
Sucrose 15% + boric acid 5 ppm	23.07 ± 0.75 ^{cd}	30.57 ± 1.83 ^{de}	36.26 ± 1.59 ^{ef}	52.10 ± 4.45 ^{cd}	88.17 ± 17.02 ^{bc}	102.98 ± 26.71 ^{bc}
Sucrose 20% + boric acid 5 ppm	24.65 ± 3.18 ^{cd}	30.94 ± 2,83 ^{de}	34.06 ± 3.12 ^{f}	51.03 ± 2.63 ^{cd}	59.13 ± 17.28 ^{cd}	90.54 ± 28.64 ^{cd}
Sucrose 25% + boric acid 5 ppm	34.02 ± 0.67 ^{a}	37.44 ± 3.79 ^{b}	44.45 ± 3.64 ^{bc}	49.28 ± 6.75 ^{cd}	57.56 ± 12.81 ^{cd}	98.93 ± 15.74 ^{bc}
Sucrose 30% + boric acid 5 ppm	31.96 ± 1.56 ^{ab}	35.68 ± 4.82 ^{bcd}	40.92 ± 0.74 ^{cde}	53.48 ± 16.12 ^{cd}	58.50 ± 18.96 ^{cd}	110.97 ± 12.62 ^{abc}

The length of the pollen tube data (Table 2) showed that the most extended consistent pollen tubes within 24, 48, and 72 hours of observation were 10% sucrose media with or without boric acid 5 ppm and 30% sucrose media. The pollen tube length at 10% sucrose and 10% sucrose + 5 ppm boric acid after 72 hours of observation were 137.29 µm and 134.98 µm, respectively. Meanwhile, in a 5% sucrose medium, pollen tube

growth in the first 24 hours was significantly high at 71.33 μm, and growth slowed down in the following 48 hours and 72 hours at 82.95 μm and 93.35 μm, respectively. Inversely proportional to 30% sucrose + 5 ppm boric acid medium, which showed slow pollen tube growth at 24-48 hours, then significantly high at 72 hours. On the other hand, the pollen tubes in the control medium (aquadest) could still grow lengthwise; however, it was deficient compared to other treatments.

The stigma receptivity was observed visually through a digital microscope from seven days before anthesis (D-7) to anthesis (A) (Figure 4). Anthesis in *P. cerasoides* flowers is characterized by fully blooming flowers, the surface of the stigma secretes mucus, and the mature pollen on the stamens will appear yellow. The results of observations of stigma receptivity in Sakura are presented in Table 3.

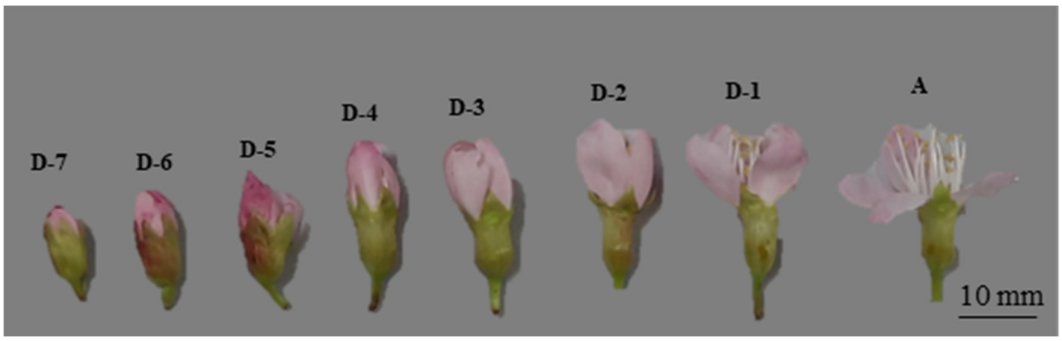


Figure 4. The flowering stage of *Prunus cerasoides* from flower bud to anthesis

Table 3. Indicator stigma receptive of *Prunus* sp. on 3% hydrogen peroxide

Flower stage	Indicator (O_2)	Notes
D - 7	+	less reactive
D - 6	+	less reactive
D - 5	+ +	less reactive
D - 4	+ +	less reactive
D - 3	+ + +	medium reactive
D - 2	+ + + +	high reactive
D - 1	+ + + +	high reactive
Anthesis	+ + + +	high reactive

Note: D-7 = 7 days before anthesis; D-6 = 6 days before anthesis; D-5 = 5 days before anthesis; D-4 = 4 days before anthesis; D-3 = 3 days before anthesis; D-2 = 2 days before anthesis; D-1 = 1 days before anthesis; A = Anthesis

The presence of mucus on the surface of the stigma indicates stigma receptivity *to P. cerasoides*. Based on the test results with hydrogen peroxide solution (H_2O_2) at the age of the flower two days before anthesis, the stigmas showed receptive results. It was indicated by much of the oxygen bubbles that emerged from the surface of the stigma in more significant numbers than the age of the flower seven days before anthesis to 4 days before anthesis (Figure 5). The optimal stage of *P. cerasoides* stigma receptivity begins at the flower two days before anthesis. *P. cerasoides* can be said to tend to self-pollinate (autogamy) because the stigma receptive period lasts quite a long time from the age of the flower, two days before anthesis until the anthesis takes place.

Moreover, the observations result of *P. cerasoides* in the third week after pollination was shown in Table 4. The highest pollination efficiency value of 53.33% was shown in cross-pollination (allogamy). Open pollination has a pollination efficiency value of 46.67% and geitonogamy of 43.33%. Meanwhile, the lowest was found in self-pollination (autogamy) at 36.67%.

Figure 5. Stigma receptive of *Prunus cerasoides* on the anthesis stage

Table 4. Percentage number of fruits artificial pollination on *P. cerasoides*

Type of pollination	Pollination (%)	Fruit set (%)
Open pollination	46.67 ± 13.94	23.33 ± 9.12
Self-pollination/ Autogamy	36.37 ± 13.94	16.67 ± 11.78
Geitonogamy	43.33 ± 14.91	26.67 ± 9.12
Allogamy	53.33 ± 21.73	30.00 ± 13.94

The fruit set in *P. cerasoides*, which showed the highest percentage of 30.0 %, was in cross-pollination (allogamy). On the other hand, the lowest fruit set percentage was in self-pollination (autogamy) at 16.67%. Overall, the average percentage of fruit set on *P. cerasoides* was 24.17%.

Discussion

Reproductive biology has a significant impact on production. Prunus biology-related characteristics, including flower bud density, number of flowers per bud, number of flowering nodes per branch, flower bud drop, flower quality, or fruit set, have an impact on production (Garcia-Montiel *et al*., 2010). According to Chang *et al*. (1987) reproductive bud and fruit set counts are the most important yield factors. Estimating bloom density and fruit set for any genotype is an important step in the breeding process. It is crucial to determine the phenological stages, viability, pollen grain germination capacity *in vitro*, and fertility of novel cultivars. Pollen performance includes pollen production, homogeneity of pollen morphology, pollen germination, growth of the pollen tube, and pollen competition (Davarynejad *et al*., 2008).

Saklani *et al*. (2018), in the study of pollen in Hamirpur Hills, India, reported that *P. cerasoides* pollen morphology with SEM 30.5 µm x 28.7 µm; triangular; colporate, tricolporate; striate; single. This statement has closed to the results observations of *P. cerasoides* in CBG.

In the 2% aceto-orcein treatment, almost all the pollen grains were stained. Frescura, et al. (2012) in their research on pollen viability on *Polygala paniculata* L., stated that a 2% aceto-orcein dye solution on pollen grains gave excessive viability results or appeared insignificant between viable and non-viable pollen because both show the same red colour. Therefore, determining viable pollen can only be distinguished by its intensity. In viable pollen, the colour will appear deep red with a larger size than in non-viable pollen with a faded red appearance.

I_2KI 1% solution in the pollen staining method can detect the presence of sugar or starch. Starch plays a role in supporting pollen, so it is assumed that the higher the starch content in pollen, the higher the pollen viability (Warid and Palupi, 2009). Pollen grains treated with I_2KI 1% in this study could be observed regarding the difference between viable and non-viable pollen grains. A brown colour appears to cover the entire pollen surface in viable pollen grains, while non-viable pollen appears colourless or transparent. The staining method

using 2% aceto-orcein solution and 1% I_2KI can be observed after 5-10 minutes, contrast, 1% TTC pollen solution can be observed after 30 - 2 hours. Observations of less than 30 minutes did not show significant colour changes, which hindered identifying viable and non-viable pollen. The colouring method using a 1% TTC solution requires more skill and appropriate magnification to be able to study an object in a coloured or colourless field of view (Sutopo, 2004).

The pollen viability test based on the staining method on cherry laurel (*Prunus laurocerasus* L.) showed that staining using TTC and I_2KI could determine cherry laurel pollen viability. TTC gave better results close to the pollen germination rate and showed the highest significant difference. Meanwhile, the I_2KI solution produces well-coloured pollen (Sulusoglu and Aysun, 2014). The low pollen viability of *P. cerasoides* can be influenced by several factors, one of which is because the germination media used are unsuitable for the species tested. Media concentration and composition in pollen germination tests can affect pollen viability in various plant species (Wang et al, 2004).

The success of pollen germination in sucrose media depends on the humidity where when pollen is taken, the condition of the pollen grains has been previously exposed. In this viability test experiment through germination, pollen must be transferred directly from the anther to the practical solution as soon as possible to maintain the pollen conditions (Dafni, 1992). Moreover, sucrose in a medium need to be carefully calculated to optimally fulfil the need for germination. Even so, the treatment concentration may differ if used for pollen types from different plants. It is because the characteristics of a plant may respond differently to each treatment or environmental condition it receives, even though the plant is in the same environmental conditions. Even for each pollen grain taken at the same flower, it can still respond differently, including when it is germinated simultaneously and in the same media (Budiwati, 2014).

During 24, 48, and 72 hours of observation, a pollen tube continued to grow and elongate to form a pollen reed to be included in the category of viable pollen. Nevertheless, some emerge from the pollen surface and do not continue to grow beyond their diameter, so they are considered inviable pollen. In pollen, some form more than one tube (polysiphonus), and some only form one tube (monosiphonus). Pollen tubes that are still possible to grow are marked by a clear zone at the tip of the pollen, some of which break or stop growing. The clear zone at the tip contains vesicles containing wall precursors (Budiwati, 2014).

Receptive stigmas will secrete mucus containing sugar, protein, and other organic substances, making it a suitable medium for germinating pollen (Darjanto and Satifah, 1990). The esterase enzyme influences stigma receptivity in the stigma that appears on the bloomed flower. At the beginning of the anthesis, the activity of the esterase enzyme is only visible in a small part of the stigma surface (not evenly distributed). The esterase enzyme is visible in full bloom on the entire stigma surface. This esterase enzyme activity indicates that the stigma has begun to be receptive or ready to receive pollen (Hasanuddin, 2009).

Based on observations, not every pollination is followed by fertilization, and not a few young fruits experience loss before the fruit is ripe. Fertilization is suspected to occur only in the generative cell nucleus 2, which forms the endosperm. In contrast, the generative cell nucleus 1 fails to fertilize the ovum and cannot form seeds, so the young fruit cannot continue development and eventually dies. In addition, environmental factors, i.e., wind and humidity, also affect fruit loss.

Many factors can influence the success rate of pollination and fertilization. Apart from being influenced by compatibility between male and female gametes (compatibility), environmental conditions also influenced pollination. Each type of plant has an optimal temperature for germination and pollen growth ranging from 25 °C. Failure in pollination can be affected by low contact between pollen and stigma, inhibiting interaction with the ovule. Furthermore, the failure of the fertilization process in plants can be caused by sterile pollen and ovules or incompatibility between pollen and ovules (Kartikawati, 2008). In addition, fertilization failure is also caused by miscarriage and ovule damage due to low pollen quality, pollination failure, and competition for food reserves.

Conclusions

The staining method with aceto-orcein 2% showed the highest of *P. cerasoides* pollen viability with a percentage of 87.87% compared to I_2KI% and TTC 1%. In pollen germination *in vitro*, the highest viability value was shown in a 25% sucrose medium with 52.48%. Furthermore, optimal stigma receptivity is demonstrated from stage D-2 to anthesis. Pollination and fruit set with the highest percentage was found in cross-pollination (allogamy) of 53.33%, and the average percentage of total fruit set was 24.17%

Authors' Contributions

Conceptualization: SN and MIS; Data curation: SSH; Formal analysis: SSH; Funding acquisition: SN; Investigation: SSH; Methodology: SN and SSH; Project administration: SN and SSH; Resources: SSH; Software: SSH; Supervision; SN, IA, and MIS; Validation: MIS; Visualization: SSH; Writing - original draft: SSH; Writing - review and editing: SN, SSH, IA, and MIS. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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