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OPTIMIZING GERMINATION AND DETERMINING PHYSIOLOGICAL MATURITY OF *RUBUS FRAXINIFOLIUS* SEEDS FOR STANDARDIZED SEED TESTING PROTOCOLS

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Abstract - *Rubus fraxinifolius*, a species of *Rubus* found in Indonesia, is an edible plant with high value and significant potential for domestication as a fruit crop. The requirements for seed germination are integral to the domestication process and necessitate improvement and standardization for *R. fraxinifolius* to produce high-quality seeds for breeding programs. Seed quality reaches maximum physiological maturity and is characterized by seed germination, vigor, and moisture content. This study aimed to enhance germination tests and determine the quality of *R. fraxinifolius* seeds. Two experiments were conducted, comprising seed germination testing and determination of seed physiological maturity. *R. fraxinifolius* seed germination demonstrated optimal results when seeds were treated with 1000 ppm KNO_3 using pleated paper, yielding a germination percentage of 82.5%, vigor index of 27.5%, and speed of germination of 1.94%/day. Physiological maturity was attained 39-40 days after anthesis, from fully ripe fruits with a red color that readily detached from the pedicle, exhibiting a maximum seed dry weight of 0.194 g, germination percentage of 95.8%, and vigor index of 71.6%. These germination test protocols were subsequently employed to evaluate the quality of *R. fraxinifolius* seeds in the breeding program and overcome seeds dormancy, potentially resulting in improved germination outcomes.

Keywords: final count, first count, germination protocols, pleated paper, top of paper, wild-type raspberry.

Introduction

Commercial Rubus fruits, such as raspberries (*Rubus idaeus*), blackberries (*Rubus* sp.), and dwarf raspberries (*Rubus pubescens*), hold significant value in several countries, including the United States, Russia, China, and Japan. However, there is limited information regarding the cultivation and utilization of Rubus species in Indonesia. Despite their presence in various regions such as West Java, Kalimantan, and the mountainous forests of Sulawesi, the cultivation and commercialization of these fruits are not yet widespread (Normasiwi et al., 2021). In Cianjur, West Java, *R. fraxinifolius* is native species known locally as "arben", has not only gained popularity but also become a part of the local community's identity (Surya et al., 2018). This local recognition and acceptance of *R. fraxinifolius* present a promising opportunity for its domestication and commercialization as a commercial fruit crop (Normasiwi et al., 2021).

The reproductive biology of the species to be domesticated is crucial for comprehending the mode of pollination, the necessity for pollinators, fruit development and maturation patterns, and the requirements for seed germination (Fredes et al., 2016). Although cultivars of raspberries are propagated clonally, significant traits for improvement through breeding programs are present in wild germplasm, which is accessed from stored seeds of wild-collected plants (Wada & Reed, 2011b). One of the challenges in *Rubus* breeding is the exceptionally slow and irregular germination of seeds and the limited number of seedlings obtained, as protocols for

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germination of wild species are not readily available (Wada & Reed, 2011b; Wada et al., 2011; Żurawicz et al., 2017).
Physiological maturity is a crucial element for the production of high-quality seeds, which is characterized by the highest seed dry weight because the accumulation of storage substances reaches its peak and vigor is at its highest, and the seed moisture content begins to decrease (Ilyas, 2012). During histodifferentiation and cell division, fresh weight and seed moisture content increase and then decrease in parallel with the increase in dry weight. Seed drying occurs during the ripening stage (Bewley & Black, 1994). The seeds should be harvested at physiological maturity, as their viability and vigor will decrease if left in the field after this stage (Suharsi et al., 2015). Fruit maturity is one of the methods that can be used to determine the physiological maturity of seeds (Aminah & Siregar, 2019). This involves assessing the fruit color, smell, hardness of the fruit or seed skin, fruit or seed loss, and fruit rupture (Rusmin & Darwati, 2018), which are all benchmarks associated with physiological seed quality in the seed germination test.
Rubus seed germination is constrained by physical, physiological, or both factors (Wada & Reed, 2011a). The raspberry seed germination problem was not only the scarification and stratification method, but also the varied response of seeds of different species to seed pre-treatment before sowing (Clark et al., 2007). According to Żurawicz et al. (2017), the best germinating raspberry seeds were treated with sulfuric acid for 30 min. Wada & Reed (2011b) recommended that *Rubus* seeds would be scarified with H_2SO_4 and stratified by treatment with 34 ppm GA_3+KNO_3. However, during seed treatment with acid chemical material, the seed coat can be damaged, as can the seed embryo (Żurawicz et al., 2017). However, since there were no protocols for germination and dormancy breaking of *R. fraxinifolius*, this study used modification of germination protocols for other *Rubus* species by Wada & Reed (2011b) and ISTA (2018).
The main goals of this study were to establish criteria for seedlings, as well as to identify the optimal germination method and seed treatment for faster germination. These findings will be valuable in setting seed testing protocols because no such information is currently available for *R. fraxinifolius* seeds in the ISTA reference. These germination test protocols were then used to evaluate the physiological maturity of *R. fraxinifolius* seeds to produce high-quality seeds.

Materials and Methods

The experiment was undertaken at the Cibodas Botanical Garden and Seed Health Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University between January and July 2023. The Cibodas Botanical Garden where *R. fraxinifolius* is cultivated is at an elevation of 1300 masl, with an average temperature of 24.43 ± 0.44 °C, an average humidity of 88.98 ± 1.69%, and a daily rainfall of 20.48 mm (NASA, 2023).

Germination method and treatment soaking seeds determination

The seeds were obtained from *R. fraxinifolius* fruits that were harvested at 40 days after anthesis (DAA) in January 2023. These fruits were considered morphologically ripe due to their full red color and ability to detach easily from the pedicle (Perkins-Veazie & Nonnecke, 1992; Fuentes et al., 2019). The study was designed as a two-factor factorial experiment with a completely randomized design. The first factor consisted of two germination test methods: top of paper (TP) and pleated paper (PP). The second factor was a seed-soaking treatment, consisted of control, distilled water, 30 ppm KNO_3, 1000 ppm KNO_3, 5 ppm GA_3, 100 ppm GA_3, and a mixture of 30 ppm KNO_3 and 5 ppm GA_3. Chemical KNO_3 and GA_3 used from Merck KGaA, Germany. There were 14 treatment combinations in total, and each was repeated four times with 50 seeds per replication.
The seed extraction involved carefully separating the seeds from the fruit and rinsing them with clean tap water to eliminate any residual pulp. Subsequently, the seeds were desiccated at room temperature for five days to prevent mould growth and ensure optimal condition for storage or planting (Wada & Reed, 2011b). The seeds were subsequently immersed in a seed-soaking treatment solution for 24 h at an incubation temperature of 20 ± 2°C before sowing. The top of paper (TP) method utilized three sheets of filter paper with a piece of tissue as the base, while the pleated paper (PP) method employed a folded filter paper with each fold measuring 2 cm in width. The filter paper that utilized was composed of cellulose cotton material with a thickness of 200 ± 10 µm and an ash content of 0.15%. Distilled water was meticulously applied to moisten the media. The germination process was conducted in a plastic container with dimensions of 8 cm × 8 cm × 5 cm, using a room germinator at a temperature of 20 ± 2°C.
The daily counts of normal seedlings were recorded and formed into a curve to determine the initial and final germination counts. The frequency of normal seedlings increased progressively until they reached their peak, which served as the first count for the evaluation of germination. The germination percentage was determined based on the rapidity of the peaks. The final count was established when the graph of seedling accumulation exhibited a slope,

indicating that few or no seeds germinated. The inclusion of a small number of normal seedlings per day results in a sloping and stationary graph (Sadjad, 1994). The interaction of factors facilitating the germination of *R. fraxinifolius* was considered for the initial and final count determination with the *R. fraxinifolius* seed germination test, consisting of:

a. Germination percentage (GP) was carried out on normal seedlings

$$GP\ (\%) = \frac{\sum \text{Normal Seedling in first count} + \text{Normal Seedling in final count}}{\text{number of seeds tested}} \times 100\%$$

b. Speed of germination (GS) was calculated based on the sum of normal seedlings every day during the testing period.

$$GS\ (\%/\text{day}) = \sum_{i=1}^{n} \frac{\%\ \text{Normal seedlings at day i}}{n}$$

c. The vigor index (VI) was calculated based on the number of normal seedlings that appeared in the first count.

$$VI\ (\%) = \frac{\text{Normal Seedling in first count}}{\text{number of seeds tested}} \times 100\%$$

Physiological maturity determination of *R. fraxinifolius* seeds

Randomized complete block design (RCBD) with a single factor, comprising six different fruit harvesting times, consisted of 35, 36, 37, 38, 39, and 40 DAA fruits. Each treatment was divided into four groups, with each replicate containing 50 seeds. The experiment commenced with the labeling of flowers that had not fully reached anthesis. The fruit color was assessed using the RHS color chart (RHS, 2015). The seed processing, germination method, and seed treatment were based on the optimal results obtained in the previous experiment. The collected seed was extracted and dried for five days at room temperature (Wada & Reed, 2011b). The seeds were immersed in 1000 ppm KNO_3 for 24 h at an incubation temperature of 20 ± 2°C before sowed. The germination was conducted in a plastic box with pleated paper method, and incubated in a room germinator at a temperature of 20 ± 2°C.

The viability of *R. fraxinifolius* seeds was assessed based on two variables: germination percentage (GP) and maximum growth potential (MGP). The physiological quality variables observed in *R. fraxinifolius* seed for physiological maturity determination were as follows:

a. Seed dry weight (g) was measured according to the procedures outlined in ISTA (2018) by weighing seeds in each replicate and drying them using the oven method at 80°C for 24 h. The seeds were then placed in a desiccator and weighed.

b. Moisture content (%) was determined using the direct method (oven) at 103 ± 2 °C for 17 ± 1 h. The seed sample used was ± 0.25 g. After being oven dried, the seeds were placed in a desiccator for 30 minutes and weighed (ISTA, 2018).

c. Maximum growth potential (MGP) was determined based on the percentage of seedlings at the end of observation (Sadjad, 1994):

$$MGP\ (\%) = \frac{\sum NS1 + NS2 + ANS1 + ANS2}{\text{number of seeds tested}} \times 100\%$$

NS1= normal seedlings in the first count; NS2= normal seedlings in the final count; ANS1= abnormal seedlings in the first count; ANS2= abnormal seedlings in the final count.

Data Analysis

Data were analyzed using ANOVA, and Duncan's multiple range test (DMRT) (α = 0,05) was used for further statistical analysis if there was a significant difference. tests were carried out using the Agricolae package (de Mendiburu, 2020) in R-Studio version 2022.02.3. Microsoft Excel 2019 and CurveExpert version 1.3 was also used for statistical analysis and to construct the curve.

Results

The structure of *R. fraxinifolius* seedlings are shown in Fig. 1.

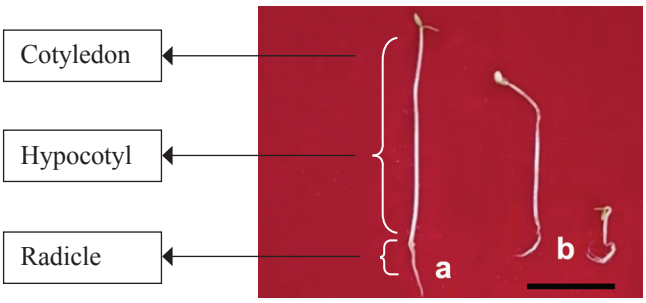

Figure 1. Structure of *R. fraxinifolius* seedlings (a) Normal seedling; (b) Abnormal seedling. Scale= 1 cm

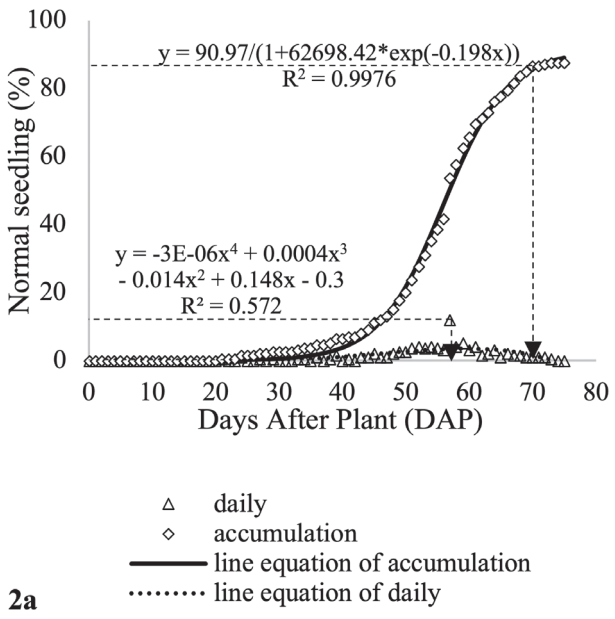

Figure 2a. Determination curve of *R. fraxinifolius* germination by soaking in 1000 ppm KNO_3 under TP method.

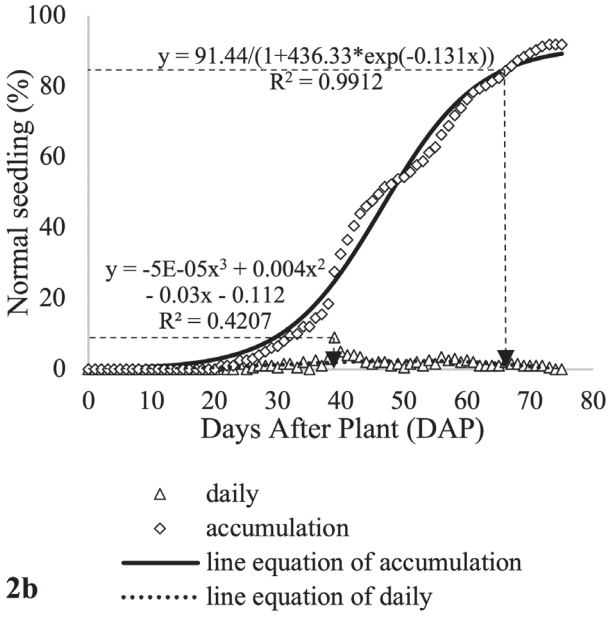

Figure 2b. Determination curve of *R. fraxinifolius* germination by soaking in 1000 ppm KNO_3 under PP method.

A healthy radicle with a minimum length of 0.5 cm, a straight hypocotyl of at least 2 cm, and a detached seed coat from the cotyledon (a) were indicative of normal seedling development. Abnormal seedlings of raspberry seeds were characterized by structures that were unable to exhibit normal growth symptoms, including short radicles, damaged, rotten, or coiled hypocotyls, and a seed coat still attached to the cotyledon (b).

Figure 2a and 2b illustrates the initial and final count determinations. The TP method, incorporating seed soaking treatment, achieved its peak at 57-59 days after planting (DAP). The application of 1000 ppm KNO_3 as a soaking treatment in the TP method resulted in the most rapid peak of normal seedling emergence at 57 DAP (Figure 2a).
The treatment involving 1000 ppm KNO_3 immersion in the PP method can serve as a benchmark for determining the

Table 1. Average seed quality variables of *R. fraxinifolius* based on germination method and seed soaking treatment.

Seed treatment	Germination method					
	PP	TP	PP	TP	PP	TP
	-----GP (%)-----		-----VI (%)-----		----GS (%/day)----	
Control	60.5^{cB}	71.0^{aA}	2.0^{cA}	0.5^{cB}	1.09^{cA}	1.23^{bA}
Distilled Water	69.0^{abcA}	75.5^{aA}	13.0^{bA}	1.5^{bcB}	1.45^{bA}	1.35^{abA}
30 ppm KNO_3	76.5^{abA}	71.0^{aA}	10.5^{bA}	2.5^{bB}	1.58^{bA}	1.30^{bA}
1000 ppm KNO_3	82.5^{aA}	77.5^{aA}	27.5^{aA}	6.5^{aB}	1.94^{aA}	1.51^{aB}
5 ppm GA_3	65.0^{bcA}	78.2^{aA}	20.0^{aA}	0.0^{cB}	1.46^{bA}	1.41^{abA}
100 ppm GA_3	73.0^{abcA}	68.5^{aA}	9.0^{bA}	1.0^{bcB}	1.49^{bA}	1.24^{bA}
5 ppm GA_3 + 30 ppm KNO_3	73.0^{abcA}	74.0^{aA}	25.5^{aA}	0.5^{cB}	1.74^{abA}	1.33^{abB}

Numbers followed by the same letter within a column or row indicate no significant difference based on DMRT at $\alpha = 5\%$. Capital letters in each row for the variables GP, VI, and GS represent the effect of the germination method (PP and TP), while lowercase letters in each column indicate the effect of seed treatment. PP=pleated paper; TP=top of paper; GP=germination percentage; VI=vigor index; GS=speed of germination.

initial and final counts. The increase in normal seedlings begins to decline, producing a sloping graph after 65 DAP, indicating that the final germination count can be established at 65 DAP (Fig. 2a). In contrast, the use of 1000 ppm KNO_3 in the PP method reached its peak at 39 DAP (Fig. 2b).

Germination method and pre-treatment soaking seeds determination

The germination method of *R. faxinifolius* did not have a substantial influence on the GP variable, but did have a significant effect on the VI and GS variables. Additionally, the seed soaking treatment factor had a singular impact on all three germination evaluation variables. Furthermore, the factors of germination method and seed soaking treatment demonstrated a significant interaction in their effect on VI and GS (as shown in Table 1).

The influence of seed soaking and germination methods on seed viability and vigor is significant. Seed viability can be evaluated by GP and MPG, while seed vigor by GS and VI. In the same seed treatment (control), PP method resulted in a GP of 60.5%, while the TP method had a GP of 71.0%. The interaction between the PP method and seed soaking treatment with 1000 ppm KNO_3 proved to be the most effective among the various combinations evaluated, as evidenced by the highest VI (27.5%) and GS (1.94%/day).

Physiological maturity determination of *R. fraxinifolius* seeds

The physiological maturity determination of *R. fraxinifolius* seeds was accomplished using an approach based on fruit maturity and harvest time. Fruits that were 25 DAA (Days After Anthesis) in age were still green and hard, and began to change color at 30 DAA (Table 2). There were visible changes occurred in both color and fruit hardness from 35 to 40 DAA, the fruit size increased until it detached from the torus. The physiological maturity of *R. fraxinifolius* seeds was determined at 35-40 DAA, with differences in fruit color being evident as shown in Table 2. Two physical attributes of seeds as benchmarks for assessing physiological maturity are seed moisture content and seed

Table 2. *R. fraxinifolius* fruit color at various harvest times.

Harvest time (DAA)	Fruit color		Color code (RHS)	Figure of fruit
25	Green Group	Strong Yellow Green	143 A	
30	Yellow-Green Group	Strong Yellow Green	144 A	
35	Yellow-Green Group	Strong Greenish Yellow	151 A	
36	Orange Group	Strong Orange	N 25 B	

Harvest time (DAA)	Fruit color		Color code (RHS)	Figure of fruit
37	Red Group	Vivid Red	44 A	
38	Red Group	Vivid Red	45 A	
39	Red Group	Vivid Reddish Orange	43 A	
40	Red Group	Vivid Reddish Orange	43 A	

DAA= Days After Anthesis; RHS = Royal Horticultural Society; Scale= 1 cm

dry weight. According to Table 3, the moisture content of *R. fraxinifolius* seeds exhibited a significant decline between 35 and 37 days after anthesis (DAA), followed by a gradual decrease that was not statistically significant until 40 DAA. This reduction in seed moisture content contrasted with the increase in seed dry weight (SDW) observed during seed maturation. The highest SDW value was recorded at 40 DAA, reaching 0.194 g, and was significantly influenced by other harvest times. This finding indicates the attainment of maximum SDW in seeds harvested at 40 DAA.

Rubus fraxinifolius seeds were found to be capable of germination at any time of fruit harvesting, but there were differences in GP value. Fruit harvested at 40 DAA produced seeds with the highest GP at 95.8%, but this did not have a significant effect on the GP at 39 DAA, which was 94.5%. The MGP value of seeds harvested at 38 DAA was not significantly different from the seeds harvested at 39 and 40 DAA, which was 93.8%. On the other hand, fruit harvested at 35 DAA had the lowest GP at 87.6%, and was significantly different compared to other fruit ages, in accordance with MGP value. The highest VI of *R. fraxinifolius* seeds was obtained at 40 DAA, but did not have a significantly difference compared to 39 DAA, about 71.6% (Table 4).

Discussion

The criteria for defining normal *R. fraxinifolius* seedlings were established by assessing the key structures of the radicle, hypocotyl, and cotyledons. The emergence of normal *R. fraxinifolius* seedlings occurred between 21 and 24 DAP. The first and final counts are essential for

Table 3. Moisture content and dry weight of *R. fraxinifolius* seeds based on different harvesting time

Harvest time (DAA)	Seed moisture content (%)	Seed dry weight (g)
35	35.9[a]	0.181[d]
36	34.5[b]	0.184[cd]
37	33.5[c]	0.185[bcd]
38	33.3[c]	0.188[bc]
39	33.3[c]	0.189[b]
40	32.8[c]	0.194[a]

Numbers followed by the same letter in the same column indicate no significant effect in the DMRT test α = 5%. DAA= Days After Anthesis.

Table 4. Viability of *R. fraxinifolius* seeds based on different harvesting time

Harvest time (DAA)	Germination percentage (%)	Maximum growth potential (%)	Vigor index (%)	Speed of germination (%/day)
35	87.6[d]	89.1[d]	42.1[d]	2.20[e]
36	91.0[c]	92.2[c]	49.4[d]	2.35[d]
37	92.2[bc]	92.9[bc]	59.5[c]	2.47[c]
38	93.0[bc]	93.8[abc]	63.1[bc]	2.52[bc]
39	94.5[ab]	95.2[ab]	67.9[ab]	2.62[ab]
40	95.8[a]	96.0[a]	71.6[a]	2.67[a]

Numbers followed by the same letter in the same column indicate no significant effect in the DMRT test α = 5%. DAA= Days After Anthesis.

evaluating seed germination. The observation of the first count serves as a test of seed vigor, enabling the assessment of the germination rate.

Germination method and pre-treatment soaking seeds determination

The pleated paper germination method is a widely utilized technique for germinating small seeds. It has been observed that when using the top of paper method for the germination of *Silphium perfoliatum* L., fungal infection occurs. However, the pleated paper method, due to its folded structure, serves as a barrier that prevents the spread of fungi from one seed to another (Bareke, 2018). The paper medium used in this method should possess exceptional moisture absorption and retention capabilities, as well as display capillary rise potential, maintain a neutral pH, and be free from any phytotoxic effects (Santos et al., 2022).

The germination phase is significantly influenced by hormonal regulation, particularly abscisic acid (ABA) and gibberellin acid (GA_3). ABA inhibits germination, while gibberellin supports it (De Wit et al., 2016). ABA accumulates in the embryo during seed maturation and plays a crucial role in primary dormancy, while GA_3 induces embryo growth and increases nutrient availability in the seed (Gansberger et al., 2017; Bareke, 2018). They promote growth by increasing cell wall plasticity, followed by the hydrolysis of starch into sugars that reduce cell potential pressure, allowing water to enter the cell for germination (Gupta & Chakrabarty, 2013; Agurahe et al., 2019). Potassium nitrate (KNO_3) is a nitrogen compound that promotes germination and breaks seed dormancy in various plants. The impact of KNO_3 is

contingent upon the concentration utilized and the specific type of plant being tested (Bareke, 2018).

The germination percentage below 80% suggests a state seed dormancy. *Rubus* seeds exhibit both physical and physiological dormancy, as reported by Wada & Reed (2011b). The seed coat of *Rubus* and other Rosaceae species is impermeable to both water and gas. Hydrogen cyanide (HCN) is formed when water uptake reaches inhibitory levels and remains until the seed coat and endosperm pellicle are either damaged or partially removed, as indicated by Wada et al. (2011b). The permeability of the seed coat to water is influenced by the seed coat's phenolic content and its degree of oxidation, as stated by Marbach & Mayer (1974). It is crucial to administer a dormancy-breaking treatment to *R. fraxinifolius* seeds in order to eliminate any compounds that inhibit germination and improve the seed coat's permeability.

The folds in the PP method prevent light from reaching smaller seeds, thereby inhibiting germination. Gansberger et al. (2017) found that the germination percentage of the PP method was similar to that of the TP method under minimal light conditions. Light is a regulator of plant growth and development, from the germination to the aging phase (Warpeha & Montgomery, 2016; Deepika et al., 2020). Hormones can replace the role of light by stimulating germination enzymes (Kołodziejek, 2017).

The vigor index signifies the proportion of seeds that germinate at first count and the PP method typically yields the highest VI. The TP method exhibits a low VI due to its extended peak of normal germination. This disparity is linked to the GS, reflecting that certain treatments in the PP method are more expeditious than TP method. The folds on PP method may possess better water-storing capabilities, maintaining the humidity of the germination environment, and sustaining germination over an extended period. In the study of Gundala et al. (2023), the small sized Sambiloto (*Andrographis paniculata*) seeds germinated twice as fast in the PP method, as they are enveloped by moist paper, resulting in a slower evaporation of water. ISTA (2018) also recommends pleated paper for small seeds, like *R. fraxinifolius* seed. The hard seed coat of *R. fraxinifolius* necessitates high humidity for germination. According to Baskin & Baskin (2004), light and substrate moisture serve as two essential dormancy-breaking factors that facilitate seed germination.

Determining the optimal combination of treatment and germination method is a challenging task when relying on the GP value, as the resulting value lacks significant practical implications. Based on the three germination variables examined, it is concluded that the combination of 1000 ppm KNO_3 soaking treatment with the PP method is the most effective treatment for *R. fraxinifolius* germination percentage. The application of KNO_3 is known to involve hydrogen receptors such as nitrate, which play a role in the reoxidation process of NADPH, a coenzyme that facilitates the chemical reactions and respiration required for seed germination (Bareke, 2018).

Physiological maturity determination of *R. fraxinifolius* seeds

Wet or fleshy fruit, such as those of *R. fraxinifolius,* does not experience a sharp decline in moisture content like dry fruit (Marcos-Filho, 2016). *R. fraxinifolius* seeds belong to the orthodox seed group, characterized by a rapid increase in seed moisture content during the embryogenesis phase, where cell division and food reserves accumulation occur (Bonner, 1996). The decrease in moisture content is due to the release of water from cells as food reserves accumulate (Bradford, 1994). The increase in seed dry weight (SDW) of *R. fraxinifolius* with longer harvest time was observed. The end of seed development is reached when seeds physiological mature (Marcos-Filho, 2016).

The maximum growth potential of seeds refers to their ability to germinate both normally and abnormally. The higher the level of fruit ripeness, the greater the MGP value. The MGP value of seeds harvested at 38 DAA was not significantly different from the seeds harvested at 39 and 40 DAA. This suggests that seeds at 38 DAA had high viability, whereas at 35 DAA, the MGP value was low and significantly different from other harvest times. According to Kartika & Ilyas (1994), Jogo nut seeds do not germinate well at the beginning of the seed development stage due to insufficient food reserves to support germination. Copeland & McDonald (2001) state that seeds at physiological maturity have sufficient food reserves to support germination.

The vigor index, which represents the percentage of normal seedlings in the initial count, serves as an indicator of the swiftness with which seeds germinate (Sadjad et al., 1999). This research revealed that the highest VI of *R. fraxinifolius* seeds was obtained at 40 DAA, but did not demonstrate a significant difference compared to 39 DAA. Based on these results, it can be inferred that maximum vigor has been achieved 39 DAA. Additionally, the speed of germination (GS) variable is another measure of seed vigor. The highest GS value was observed in 40 DAA seeds, but not significantly different compared to 39 DAA seeds, about 2.67%/day. Seeds with high vigor are likely to grow faster than those with low vigor (Rori et al., 2018).

The values of GP, MGP, VI, and GS have reached their peak at 39 DAA during the germination of seeds, while seed dry weight only attained its maximum at 40 DAA. Therefore, the time of physiological maturity of seeds can be estimated to be around 39 and 40 DAA. It is recommended that seed harvesting be carried out shortly after the seeds

reach physiological maturity and the moisture content has decreased. The optimal time for harvesting *R. fraxinifolius* seeds is at 40 DAA, when the physiological quality variables and dry weight have attained their maximum, and the moisture content has begun to decline. After 40 DAA, *R. fraxinifolius* fruit will fall by itself from the pedicle, so it cannot be detected which the fruit has recently or a long time fallen. Although determining physiological maturity by the level of fruit ripeness is a straightforward and simple method, it is subject to the influence of environmental factors that can affect the period of physiological maturity. High humidity and rainfall can cause flower and fruit development to be delayed, resulting in a shift in the physiological maturity of seeds (Normasiwi et al., 2021; Wlodarczyk et al., 2023). Another approach that can be employed to obtain measurable and objective results in determining physiological maturity is to quantify fruit color or calculate the accumulation of heat units in the fruit of *R. fraxinifolius* or other *Rubus* species.

Conclusions

Germination of *R. fraxinifolius* seeds can be initiated through treatment involving immersion in 1000 ppm KNO_3 for 24 hours, followed by germination using the pleated paper method on filter paper media. Incubation was conducted at a temperature of 25 ± 2°C, with the first count performed at 39 days after plant (DAP) and the final count at 65 DAP. Seeds harvested at 39-40 days after anthesis (DAA) exhibited optimal quality, including maximum dry weight, viability, and vigor. These seeds were physiologically mature, characterized by fully ripened fruits displaying complete red coloration and facile detachment from the pedicle. These findings can serve as preliminary information for developing methods to enhance germination and overcome dormancy in *R. fraxinifolius* seeds, potentially resulting in improved germination outcomes**.**

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