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Research Article

Seed dormancy mechanism and dormancy-breaking methods in wild raspberry (*Rubus fraxinifolius* Poir.)

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Abstract

Raspberries are subtropical plants that contain high levels of vitamin C, antibacterial and anti-inflammatory. They can potentially be developed as horticultural and medicinal plants. Dormancy is a challenge in the cultivation of raspberries (*Rubus fraxinifolius* Poir.). This study was conducted as two separate experiments. The first experiment aimed to identify the dormancy mechanism of *R. fraxinifolius* seed. In a two-factor factorial design, the first factor was seed storage, as unstored and three-month-stored, and the second factor was chemical-immersed treatment consisting of control, H_2SO_4, acetone, GA_3, KNO_3, H_2SO_4-GA_3, acetone-GA_3, H_2SO_4-KNO_3, acetone-KNO_3. The second experiment was aimed at determining dormancy-breaking methods for *R. fraxinifolius* seeds. In main plots were filter paper and cocopeat germination substrates. The subplots included control, immersed with distilled water, H_2SO_4, ultrafine bubble water, and temperature treatment at −80 °C, 50 °C, and 70 °C. The germination of unstored and three-month-stored seeds increased after H_2SO_4 treatment (36 to 82% and 82 to 94%, respectively). Seed germination increased after three months of storage. There was an increase in cytokinin hormone levels along with germination enhancement. The seeds went into physical dormancy because their seed coat was hard, and they went into physiological dormancy because of low cytokinin concentration. Stratification at 50 °C increased germination (78.5 to 93.0%), reduced dormancy intensity (15 to 6.5%), and increased the percentage of the speed of germination (1.99 to 3.12 %NS.day^{-1}) on filter paper substrate.

Keywords: after-ripening; cytokinin; hard-seed; scarification; seeds; seed-coat

Introduction

Raspberries are consumed as fresh fruits, whereas their leaves are used as traditional medicine (Carvalho *et al.*, 2013). Raspberry stems have anti-tyrosine and antioxidant properties (Desmiaty *et al.*, 2020). The benefits of raspberries as fruit plants and their derivative products promote their development in Indonesia.

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The availability of raspberries in Indonesia is scarce, which is caused by a lack of information and plant cultivation (Surya *et al*., 2018).

Raspberries cultivation is needed to increase its quantity and quality as well as the diversity of its germplasm. *R. fraxinifolius* Poir., *R. rosifolius* Sm, *R. chrysophyllus* Reinw. ex Miq., *R. lineatus* Reinw. ex Blume are wild raspberries species that are collected at the Cibodas Botanical Gardens as germplasm, plant breeding, and domestication materials in Indonesia (Surya *et al*., 2018). Raspberries seeds have low germination. Low and non-uniform germination of raspberries seeds is caused by dormancy (Choi *et al*., 2016). Wada and Reed (2011) explained that dominant dormancy in raspberries is caused by the hard seed coat. Low seed germination in *R. coreanus* is caused by the seed coat being impermeable to water and gas and the embryo being dormant (Rehman *et al*., 2011). The proanthocyanidin compounds found in the seed coat of several species of raspberry seeds result in obstructed gas exchange and a more extended embryo dormant period (Choi *et al*., 2016). The dormancy-breaking method helps breeders shorten the plant assembly time, but each raspberry species responds differently to the given dormancy-breaking method.

The different responses of each raspberry species to the dormancy-breaking method are challenging for breeders (Zurawicz *et al*., 2017). The dormancy-breaking method by Chemical-immersed treatment with H_2SO_4 (95%) for 30 minutes and followed by cold stratification for three months *Rubus idaeus* L seeds resulted in higher germination than without scarification and stratification. However, the germination percentage was <40% (Contreras *et al*., 2016). Dormancy-breaking with H_2SO_4 (98%) for 30 minutes broke dormancy in *R. hoffmeisterianus* Kunth & C. D. Bouche but not in *R. coreanus* Miq. and *R. occidentalis* F. pallidus (Wada and Reed, 2011). The same method used by Choi *et al*. (2016) was effective on *R. corchorifolius* L. fil. Fauri species but not others.

Dormancy-breaking methods are needed to overcome various types of dormancies. Dormancy-breaking for seeds of tropical and subtropical plants was treated with dry-heat stratification at 50±2 °C (ISTA, 2014). Treatment with dry-heat scarification at 70 °C for 2-3 hours was reported to break dormancy and increase 22-24% germination of *Luffa cylindrica* (L.) M.Roem. (Chaodumrikul *et al*., 2016). The dormancy breaking method with *Ultra Fine Bubbles* (UFB) water is used in agriculture to accelerate the growth of seeds with physical and physiological dormancy (Maia *et al*., 2020). The mechanisms and dormancy-breaking methods of *R. fraxinifolius* have not been discovered. *R. fraxinifolius* is a cultivated species in Indonesia that contains higher sugar and vitamin C than other wild raspberries species (Surya *et al*., 2018). This research aimed to study the dormancy mechanism and obtain an effective dormancy-breaking method for *R. fraxinifolius* seeds.

Materials and Methods

Seed collection and preparation

The seeds were obtained from a collection of plants in Cibodas Botanical Garden. The seeds were harvested in September 2022 and January 2023. Freshly matured fruits detached from the receptacle are extracted manually using a filter and running water (Choi *et al*., 2016; Fuentes *et al*., 2019). The seeds were air-dried for five days (Wada and Reed, 2011). The initial moisture content of the seeds was measured using the low-constant-temperature method (103±2 °C) for 17±1 hours (ISTA, 2014). Seeds were germinated in IPB 73-2A/B germination equipment at 20±3 °C and 60-70% RH.

Dormancy mechanism

The experiment was aimed to identify the dormancy-mechanism of *R. fraxinifolius* seed. Its two-factor factorial experiment was arranged in a randomized completely block design (RCBD). The first factor is storage treatment that are unstored and three-months stored. The content of gibberellins, cytokinins (CKs), and ABA hormones in unstored and three-months seeds lots (control) was measured using High-Performance Liquid Chromatography (HPLC) with three replications. The second factor is the chemical-immersed treatment, which consisted of no treatment, Chemical-immersed with H_2SO_4, acetone, GA_3, KNO_3, H_2SO_4-GA_3, acetone-GA_3, H_2SO_4-KNO_3, and acetone-KNO_3. Thus, 18 treatment combinations with four replications resulted in 72 units, where each experimental unit used 50 seeds.

Seed were immersed by chemical solutions include H_2SO_4, acetone, GA_3, KNO_3 and their combination. Chemical solution treatment using H_2SO_4 (50%) for 5 minutes (Eyob, 2009; Choi *et al*., 2016). Acetone (25%) for 15 minutes (Khan *et al*., 2015). GA_3 (100 $mg.L^{-1}$), 32 $mg.L^{-1}$ KNO_3 for 24 hours (Saffari *et al*., 2021; Thapliyal *et al*., 2021; Wada and Reed, 2011). Chemical combination treatment of H_2SO_4-GA_3, Acetone-GA_3, H_2SO_4-KNO_3, and acetone-KNO_3 was carried out by immersed the seeds using H_2SO_4 (50%) for 5 minutes or acetone (25%) for 15 minutes. Afterward, the seeds were rinsed with distilled water and immersed with 100 $mg.L^{-1}$ GA_3 or KNO_3 32 $mg.L^{-1}$ for 24 hours. Treated seeds are then placed on two sheets of filter paper moistened with distilled water in a Petri dish. Germination observations were done daily for 65 days after planting (DAP) to observe germination percentage and dormancy-intensity.

Germination percentage (%)

Germination observation was carried out on normal seedlings (ISTA, 2014). Germination Percentage (GP)= [(ΣNS I + ΣNS II) / total seed sown] x 100%. NS I is normal seedlings on the first count, while NS II is on the final count. The first and final count were calculated at 36 and 54 DAP, respectively.

Dormancy-intensity (%)

Dormancy intensity is the percentage of fresh seeds that do not germinate at the end of the observations of the experiment. The percentage of dormancy intensity is calculated by a formula referring to Sari et al. (2021). Dormancy intensity (DI) = [(Fresh seed that does not germinate/total seed sown) x 100%]

Determination of dormancy-breaking method

The experiment was aimed to determine the dormancy-breaking methods to *R. fraxinifolius* seed. Its split-plot design and arranged according to the RCBD. The main plots were germination substrate, include filter paper and cocopeat. The subplots were dormancy-breaking method which consisted of control, immersing with distilled water, Ultra Fine Bubbles (UFB) water dissolved oxygen (20 $mg.L^{-1}$), H_2SO_4 (50%), temperature treatment at −80 °C, dry-heat stratification at 50 °C and scarification at 70 °C. There were 14 treatment combinations with four replications to obtain 56 experimental units, where each experimental unit used 50 seeds.

The seeds that had been stored for a month were used in this experiment. The dormancy-breaking methods include immersed and temperature treatment. The seeds were immersed in distilled water, UFB water for 24 hours (Maia *et al*., 2020), and H_2SO_4 (50%) for 5 minutes. The temperature treatment at −80 °C was carried out using a refrigerator for 2 hours. Dry-heat stratification treatment at 50 °C for 48 hours (ISTA, 2014) and scarification at 70 °C for 2 hours using an oven (Chaodumrikul *et al*., 2016). Afterward, the seeds were planted on two sheets of filter paper moistened with distilled water or cocopeat that was sterilized using an autoclave at 120 °C for 30±2 minutes. The seeds were plated on cocopeat substrate with a thickness of 2 cm in a germination box sized 11 cm x 8 cm x 6 cm. Seed germination was observed daily throughout 54 DAP. Observations were made on the germination percentage, dormancy intensity and speed of germination.

Speed of germination

Speed of germination (GS) was done by counting normal seedlings every day (24 hours) from the day after planted until the last day of observation. It is calculated by the formula Sadjad (1994) and Fridayanti *et al*. (2023). $GS = \sum_0^t (\%NS.\, day^{-1})$, whereas NS is a normal seedling and t is last day of observation.

Statistical analysis

The data were analysed by analysis of variance (ANOVA) using the SAS 9.4 software. If it showed a significant effect, the Duncan Multiple Range Test (DMRT) compared mean separation at $P < 0.05$.

Results and Discussion

The factors of first and second experiments affected germination variable, significantly (Table 1). The seed-storage and chemical-immersed treatment significantly affected GP and DI as a single factor and as an interaction between the two. The germination substrate and dormancy-breaking methods significantly affected GP, DI and GS as a single factor and as an interaction between the two.

Table 1. ANOVA results of germination percentage, dormancy-intensity, speed of germination on dormancy-mechanism and dormancy-breaking experiments

Variable	SV	Df	SS	MS	F-value	p-value
Dormancy Mechanism						
GP	Storage treatment (S)	8	31749.444	3968.680	429.883	0.000
	Chemical Treatment (C)	1	10416.055	10416.050	1128.255	0.000
	S x C	8	9047.445	1130.930	122.501	0.000
	Error	51	470.833	9.232		
	Total	68	51683.777			
	CV (%)			5.90		
DI	Storage treatment (S)	8	1876.340	234.542	56.326	0.000
	Chemical Treatment (C)	1	1615.013	1615.013	387.851	0.000
	S x C	8	2011.173	251.396	60.374	0.000
	Error	51	212.388	4.164		
	Total	68	5714.914			
	CV (%)			9.20		
Dormancy-Breaking Method						
GP	Germination Substrat (G)	1	25543.142	25543.142	9067.498	0.000
	Dormancy-breaking (D)	6	6440.714	1073.452	381.062	0.000
	G x D	6	2337.857	396.309	140.685	0.000
	Error	36	101.428	2.817		
	Total	49	34423.141			
	CV (%)			2.77		
DI	Germination Substrate (G)	1	19240.071	19240.071	7554.013	0.000
	Dormancy-breaking (D)	6	4377.428	729.571	286.443	0.000
	G x D	6	2671.428	445.238	174.809	0.000
	Error	36	91.714	2.547		
	Total	49	26380.641			
	CV (%)			5.23		
GS	Germination Substrate (G)	1	26.139	26.139	13069.500	0.000
	Dormancy-breaking (D)	6	11.809	1.968	984.000	0.000
	G x D	6	1.355	0.225	112.500	0.001

	Error	36	0.086	0.002		
	Total	49	39.389	28.334		
	CV (%)	3.06				

P-value < 0.05 denote significant difference on single or interaction between two factor treatments; SV = Source of variation; Df = Degree of freedom, GP = Germination percentage; DI = Dormancy-intensity; GS = Speed of germination; CV% = Coefficient of variation

Dormancy mechanism

The mean value of seed germination percentage affected by chemical-immersed treatment is presented in Table 2.

Table 2. Seed germination percentage after chemical-immersed treatment

Chemical-immersed treatment	Seed	
	Unstored	Three-months stored
Control	36.0 ± 1.6 ef	82.0 ± 3.6 b
H_2SO_4	82.0 ± 4.4 b	94.0 ± 1.9 a
Aceton	74.0 ± 2.3 c	86.0 ± 1.6 b
GA_3	25.0 ± 3.8 i	84.5 ± 1.9 b
KNO_3	13.5 ± 1.9 j	28.0 ± 1.6 ghi
H_2SO_4-GA_3	31.5 ± 6.6 g	32.5 ± 1.0 fg
Aceton-GA_3	26.5 ± 1.9 ih	81.5 ± 1.0 b
H_2SO_4-KNO_3	37.0 ± 5.0 e	30.5 ± 1.0 gh
Aceton-KNO_3	30.0 ± 2.8 gh	52.0 ± 1.6 d

Mean values in percent (%) ± standard deviation; numbers followed by the same letter show no significant difference based on the DMRT test at P < 0.05

Chemicals-immersed with H_2SO_4 and acetone increased the seed germination of unstored and three-month stored seeds (Table 2). The treatment with H_2SO_4 (50%) for 5 minutes effectively increased the germination percentage of the unstored and three-month stored seed from 36 to 82% and 82 to 94%, respectively. It showed that the hard seed coat is an external factor (exogenous dormancy) causing dormancy in *R. fraxinifolius* seeds. The H_2SO_4 treatment showed low permeability of *R. fraxinifolius* seed coat to gas or water. Chemical-immersed with H_2SO_4 (50%) for 5 minutes as scarification treatment can scrape and soften the seed coat of *R. fraxinifolius*, leading to an increase in germination. H_2SO_4 is a strong acid that oxidises most organic compounds and damages cells (Saeid and Chojnacka, 2014). Yuniarti and Djaman (2015) reported that H_2SO_4 could increase imbibition rate by releasing hydrophilic colloids, removing the waxy layer on the seed coat and making it permeable to gas and water. H_2SO_4 treatment will damage the cell lining of the macrosclereid lumens resulting in water imbibition and the release of simple sugars for protein synthesis, which promotes germination (Taghizadeh and Sajadi, 2023).

Acetone increased germination of unstored and three-month stored seeds by up to 82% and 94%, respectively (Table 2). The enhancement is due to the dissolved inhibitor in acetone resulting in a dormancy-broken seed. Acetone is a solvent for plant growth inhibitors (Todorovic *et al*., 2005; Sánchez-Coronado *et al*., 2015). Terpenoids, phenols and aldehydes are inhibitory compounds in seeds that can lower pH, prevent chemical reactions needed for germination, interfere with germination promoter activity and inhibit cell division and elongation (Marcos-Filho, 2016). Therefore, dormancy in *R. fraxinifolius* can be suspected due to the biochemical compounds in the seeds.

GA_3 100 mg.L^{-1} for 24 hours did not increase the germination of unstored and three-month stored seeds (Table 2). Unstored and three-month stored seed resulted in a germination rate of 25% and 84% after GA_3-immersed, respectively. Applying the correct concentration of GA_3 can trigger growth and have a positive effect. If it is too high, it can give the opposite effect. Nimir et al. (2017) reported that GA_3 can reduce some

ions. The high affinity of the GA receptor, Gibberellin-Insensitive Dwarf (GID1) on GA_3, inhibits germination (Ge and Steber, 2018). The research results by Zhu *et al.* (2019) reported that immersing GA_3 for 24 hours reduced the accumulation of *Sorghum bicolour* [L.] Moench seed germination. GA_3 cannot increase germination and replaces the cold/warm stratification method to break seed dormancy with intermediate physiological dormancy (PD), which takes 1-6 months (Baskin and Baskin 2014; Tang *et al.*, 2019).

After immersed with KNO_3 32 $mg.L^{-1}$ for 24 hours, the germination percentage was lower than each control of unstored and three-month stored seeds, 13.5% and 28%, respectively. Abadi and Kaboli (2020) reported that immersing *Silybum marianum* (L.) Gaertn seeds with 1% KNO_3 for 48 hours resulted in a lower vigour index compared to 24 hours of immersing. The effect of low KNO_3 concentrations on germination is unknown. However, Cavusoglu *et al.* (2017) reported that high KNO_3 applications increased chromosomal aberrations and slowed mitotic activity in shallots (*Allium cepa*), reduced accumulation of lipids and carbohydrates, disrupted K+ metabolites, as well as increase and uptake of N in cells (Chen *et al.*, 2019).

The germination percentage without chemicals-immersed treatment increased after three-months of storage from 36% to 82% (Table 2). Its germination was higher than unstored seed, except for chemicals-immersed by H_2SO_4-KNO_3. On the other hand, H_2SO_4 and acetone were effective for three-month stored seed resulted in germination rates ≥ 85% and higher compared unstored seed. These results predict that the seeds have an after-ripening period. Seeds with an after-ripening period will break dormancy and have high germination after passing a dry storage process for a certain period (Marcos-Filho, 2016). The dormancy of *R. fraxinifolius* is a physically hard seed coat and physiologically indicated by changes in the composition of endogenous seed hormones after three-months stored (Figure 1).

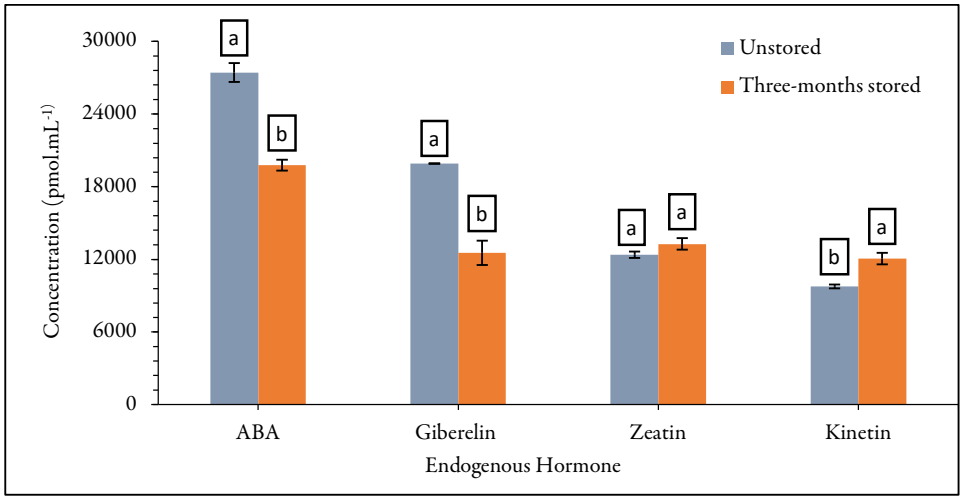

Figure 1. The endogenous hormones of *R. fraxinifolius* seed after three-months stored
Bars shows mean value ± SD. The same lowercase above bars indicates no significant difference before and after three-months stored based on the DMRT test at $P < 0.05$

56 $pmol.mL^{-1}$ and 19917.60 to 12544.49 $pmol.mL^{-1}$, respectively (Figure 1). Its decrease was accompanied by an increase in the hormone CKs in the form of kinetin from 9762.24 to 12067.43 $pmol.mL^{-1}$. The ABA:GA_3 ratio after storage increased from 1.37 to 1.58. Wang *et al.* (2022) explained that a low ABA:GA_3 ratio will stimulate germination. The increase in CKs in *R. fraxinifolius* seeds is predicted to trigger germination. Kinetin is one of the CKs hormones. CKs and GA_3 work antagonistically with ABA in plant germination. Haq *et al.* (2023) reported that the levels of ABA, GA_3 and CKs in seeds fluctuated during storage, an increase in CKs and ABA:GA_3 after storage resulted in high germination of CU-1051 cucumber seeds. The

equilibrium of CKs and GA_3 can suppress the effect of ABA as an inhibitor in germination (Marcos-Filho, 2016). CKs and GA_3 work synergistically in stimulating anaphase in the mitotic process by activating cyclin-dependent kinases (Tank *et al*., 2014). CKs interfere Abscisic Acid Insensitive 5 (ABI5) transcription by inducing ABI5 protein degradation via the 26S proteasomal pathway (Guan *et al*., 2014; Shu *et al*., 2016). Kinetin in seeds will reduce the role of ABA as a germination inhibitor (Kelly and Lacroix 2019). Kinetin increases protein and chlorophyll synthesizes in plants (Kaya *et al*., 2018). Araujo et al. (2019) reported that kinetin accelerated radicle emergence in *Medicago truncatula* Gaertn increasing the cotyledon size of soybean seeds and apricot seedlings (Kurdi and Alzebari, 2022; Monpara *et al*., 2019). Kinetin combined with benzyl adenine can break seed dormancy of *Sorbus aucuparia* L. in vitro, increasing shoot length and proliferation (Dincer, 2023). The mean value of the dormancy intensity effect of the chemicals-immersed treatment to seed presented in Table 3.

Table 3. Seed dormancy-intensity after chemical-immersed treatment

Chemical-immersed treatment	Seed	
	Unstored	Three-months stored
Control	53.0 ± 0.8 a	18.0 ± 2.8 fgh
H_2SO_4	25.0 ± 3.5 cd	10.0 ± 2.9 i
Acetone	29.0 ± 1.2 b	18.5 ± 1.9 fgh
GA_3	25.5 ± 1.0 cd	20.0 ± 2.3 efg
KNO_3	19.0 ± 1.1 fgh	16.0 ± 2.3 h
H_2SO_4-GA_3	23.0 ± 1.4 de	20.5 ± 1.9 efg
Acetone-GA_3	19.5 ± 1.0 fg	21.5 ± 1.0 ef
H_2SO_4-KNO_3	21.0 ± 1.1 efg	20.5 ± 1.9 fg
Acetone-KNO_3	27.0 ± 2.6 bc	12.0 ± 2.2 i

Mean values in percent (%) ± standard deviation; numbers followed by the same letter show no significant difference based on the DMRT test at $P < 0.05$

The dormancy-intensity decreased after the chemicals-immersed treatment on unstored seed (Table 3). It also occurred in three-months stored seed, except for the acetone-GA_3 treatment. H_2SO_4 treatment reduced the dormancy-intensity of both unstored and three-months stored seed from 53% to 25% and 18% to 10%, respectively. After chemicals-immersed treatment to three-month stored seed, the intensity was lower than unstored except for the KNO_3, H_2SO_4-GA_3, acetone-GA_3 and H_2SO_4-KNO_3 treatments.

A single chemical treatment of GA_3, KNO_3 and a combination of H_2SO4-GA_3, acetone-GA_3, H_2SO_4-KNO_3, and acetone-KNO_3 reduced the dormancy intensity of unstored seed (Table 3). The GA_3 treatment to three-months stored seed did not reduce the dormancy-intensity as well as other chemicals-immersed treatments were not significantly different from the control of three-month store seed (Table 3). Combination of chemicals-immersed treatment was suspected to cause a phytotoxic effect because the decrease in dormancy intensity was not accompanied by increasing germination. The chemical combination treatment resulted in more abnormal than normal seedlings. Unstandardized rinsing of seeds after immersing with chemicals was assumed to obtain a different reaction resulting in low germination. Yuniarti and Djaman (2015) explained that seeds that have been immersed with chemicals need to be rinsed with running water for 5-10 minutes to clean the remaining chemicals and growth inhibitors on the seed coat.

Dormancy-breaking method

The results showed that germination substrate and dormancy-breaking methods significantly affected germination both as a single factor and as an interaction between the two. The mean value of the germination percentage affected by germination substrate and dormancy-breaking methods is presented in Table 4.

Table 4. Effect of germination substrate and dormancy-breaking methods on germination percentage

Dormancy-breaking methods	Germination substrate	
	Filter paper	Cocopeat
Control	78.5 ± 1.9 d	43.5 ± 1.9 h
Aquades	78.0 ± 1.6 d	27.0 ± 1.1 j
H_2SO_4	82.0 ± 1.6 c	18.5 ± 1.0 k
UFB	75.0 ± 2.0 e	27.50 ± 1.9 j
−80 °C	78.0 ± 1.6 d	32.0 ± 2.8 i
50 °C	93.0 ± 1.1 a	56.0 ± 2.3 g
70 °C	88.0 ± 0.0 b	69.0 ± 2.5 f

Mean values in percent (%) ± standard deviation; numbers followed by the same letter show no significant difference based on the DMRT test at P < 0.05

Germination percentage on cocopeat substrate ranged from 18.5-69.0% and was lower than germination on filter paper which ranged from 78.5-93.0% (Table 4). The low germination of *R. fraxinifolius* seeds on cocopeat is thought to be due to the acidity of the cocopeat substrate. Cocopeat having the pH of 4–5, is not suitable for some types of plants. In contrast, filter paper has a pH of 6-7.5, commonly used in laboratory seed testing, because it is porous, and free of fungi, bacteria, and toxic materials (Yuniarti *et al*., 2017; Haraz *et al*., 2020). Rosaceae seeds germinate at optimum substrate pH 6-8 (Kołodziejek *et al*., 2019). pH values and H+ concentrations can destabilize enzyme activity during the germination process. Using filter paper makes it easier to observe the germination of small seeds such as *R. fraxinifolius*.

The dormancy-breaking methods with H_2SO_4 (50%), stratification at 50 °C and scarification at 70 °C resulted in higher germination rates than the control and other methods on filter paper substrates (Table 4). It showed 82%, 93% and 88%, respectively. H_2S0_4-immersed enhanced the germination of unstored and three-month stored seed and results in higher germination percentage than control and seed immersed with aquades and UFB water (Table 2, Table 4). It's a chemical scarification method to breaking physical-dormancy in due to hard-seed coat (ISTA, 2014). In this research, dry-heat physical scarification at 70 °C was higher than chemical scarification with H_2SO_4. Erickson et al. (2016) reported that dry-heat at 70 °C caused cracks in the testa of *Hibiscus haynaldii* seeds. Temperatures of 70 °C cause non-uniformity and damage to parenchyma cells in the seed coat and increase the permeability of the seeds to water (Chaodumrikul *et al*., 2016; Gbenou *et al*., 2021). A temperature higher than 70 °C can cause cracks in the seed coat, but the dehydration of the seeds is high, affecting enzyme activation and reducing germination (Musara *et al*., 2015). Germination substrate and dormancy-breaking methods affect dormancy-intensity is present in Table 5.

Table 5. Effect of germination substrate and dormancy-breaking methods on dormancy-intensity

Dormancy-breaking methods	Germination substrate	
	Filter paper	Cocopeat
Control	15.0 ± 1.1 f	44.5 ± 1.9 c
Aquades	13.5 ± 1.9 fg	59.5 ± 1.9 b
H_2SO_4	13.5 ± 1.0 fg	68.5 ± 1.0 a
UFB	12.0 ± 1.6 g	58.0 ± 1.6 b
−80 °C	13.5 ± 1.0 fg	59.0 ± 2.0 b
50 °C	6.5 ± 1.0 i	30.5 ± 1.0 d
70 °C	9.5 ± 1.0 h	23.0 ± 2.0 e

Mean values in percent (%) ± standard deviation; numbers followed by the same letter show no significant difference based on the DMRT test at P < 0.05

The dormancy-intensity of *R. fraxinifolius* under the influence of filter paper and stratification at 50 °C decreased significantly from 15 to 6.5% (Table 5). The results are in line with De-paula et al. (2012), dry-heat at 50 °C effectively broke dormancy in *Cassia leptophylla* Vogel and *Senna macranthera* DC. ex Collad seeds. Temperature activates seed metabolic activity thus germination occurs earlier (Gbenou *et al*., 2021). The germination substrates and dormancy-breaking affected GS as presented in Table 6.

Table 6. Effect of germination substrate and dormancy-breaking method on seeds speed gemination

Dormancy-breaking methods	Germination substrate	
	Filter paper	Cocopeat
Control	1.99 ± 0.02 de	0.92 ± 0.03 h
Aquades	1.88 ± 0.04 f	0.56 ± 0.02 j
H_2SO_4	2.15 ± 0.05 c	0.39 ± 0.02 k
UFB	1.88 ± 0.04 f	0.56 ± 0.03 j
−80 °C	2.03 ± 0.04 d	0.72 ± 0.01 i
50 °C	3.12 ± 0.07 a	1.27 ± 0.02 g
70 °C	2.87 ± 0.12 b	1.94 ± 0.03 ef

* Mean values in %NS.day^{-1} ± standard deviation; numbers followed by the same letter show no significant difference based on the DMRT test at $P < 0.05$

The speed of germination of *R. fraxinifolius* affected by filter paper and stratification at 50 °C was faster than the control and other dormancy-breaking (Table 6). It increased from 1.99 to 3.12 %NS. day^{-1} after 50 °C temperature. Stratification at 50 °C broke the physical dormancy of *Delonix regia* (Bojer ex Hook.) Raf seeds and its physiological dormancy as well as stimulated the germination of *Peltophorum dubium* (Spreng.) Taub. and *Mimosa bimucronata* (DC.) Kuntze (Jaganathan *et al*., 2016; Geisler *et al*., 2017).

Dormancy breaking with dry heat at 50 °C is the best method to overcome the physiological dormancy of hormonal regulation and physical hard seed coat on *R. fraxinifolius*. Temperature increases hydrogen peroxide (H_2O_2) in *Mesembryanthemum crystallinum* L. seeds, gene transcription, and protein oxidation increase embryo growth during rehydration (Visscher *et al*., 2018). Increasing the concentration of H_2O_2 during the seed imbibition process increases the rate of protein translocation and regulates the balance of gibberellins and abscisic acid (Farooq *et al*., 2021). GA and ABA in seeds affect the softening of the endosperm and testa micropillars (Leubner-Metzger, 2002).

Conclusions

Seed dormancy of *R. fraxinifolius* is found to have physical caused by hard seed coats and physiological dormancy due to low concentration of CKs. CKs in the form of kinetin increased after three-month stored from 9762.24 to 12067.43 pmol.mL^{-1} . The ABA:GA_3 ratio after three-month stored increased from 1.37 to 1.58. Chemical-immersed with H_2SO_4 (50%) for 2 hours can break physical dormancy. It's resulted in 82% - 90% of germination percentage. Physical hard seed-coat and physiological dormancy can be broken by stratification at 50 °C for 48 hours. It effectively overcomes two types of dormancies in *R. fraxinifolius* seeds. The stratification at 50 °C for 48 hours increased germination from 78.5 to 93%, reduced dormancy intensity from 15 to 6.5% and increased growth rate from 1.99 to 3.12 %NS.day^{-1} on filter paper substrate.

Authors' Contributions

Conceptualization: HR, AQ, MS and MIS; Laboratory work and data curation: HR, Technical Supervision: AQ, MS, MIS; Data analysis and interpretation: HR, AQ, MS, MIS; Manuscript writing: HR; Manuscript review: AQ, MR, 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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