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Full Length Research Article

Effects of Initial Water Temperature and Soaking Duration on the Germination of Canarium (*Canarium indicum*) Seeds

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ABSTRACT

Canarium (*Canarium indicum*) seeds have mechanical dormancy, i.e., seeds have a hard outer seed coat, which causes inhibition of germination. Mechanical dormancy can be broken by mechanical and chemical scarification. One way of chemical scarification is water soaking. This study aimed to analyze the initial effect of temperature of the soaking water and duration of soaking on the germination of canarium seeds. The research design used was a two-factor experiment in a completely randomized design. The first factor is the different initial water temperature and the second factor is soaking duration. The initial water temperature consists of 60°C, 70°C, 80°C, 90°C, and 100°C, while the soaking duration consists of 0 h, 24 h, 48 h, and 72 h. The results showed that the initial water temperature had a very significant effect on germination percentage, peak value, mean daily germination, and germination value. The soaking duration did not significantly affect germination percentage, mean daily germination, and germination value, but it significantly affected the peak value. The interaction of initial water temperature and soaking duration significantly affected the germination percentage, mean daily germination, peak value, and germination value. The initial water temperature and soaking duration affected the germination of canarium seeds.

1. Introduction

Canarium (*Canarium indicum*) is a native Indonesian plant that grows in the eastern part of Indonesia (Rahman et al. 2019; Rashid et al. 2015). North Maluku is a province in East Indonesia where canarium trees can grow well (Tamaleme et al. 2016). The leaves, stems, and fruits of canarium have many benefits (Siahaya et al. 2020), and it also contains antioxidant (Cajuday et al. 2017). Canarium seeds contain omega 3, omega 6, and omega 9, which are very good for health (Hadipoentyanti 2012). The local name of canarium is kenari (Indonesia), pili (Philippines), kernel, and walnuts (English) (Bone et al. 2013; Silalahi and Wakhidah 2021). Canarium tree propagation could be occurred using vegetative or generative propagations (Masarip et al. 2020).

The problem in the propagation of canarium plants through seeds is that the seeds have a very hard outer shell resembling a coconut shell, which inhibits water and oxygen from entering the seeds. Hence, the ability to germinate is very low. The type of canarium dormancy is seed coat dormancy (Anozie and Oboho 2019; Yuniarti et al. 2016). Seed coat dormancy, also known as mechanical dormancy, occurs when the seed coat physically inhibits the embryo's growth. This

seed coat is generally thick, hard, and impermeable to water and gas, thus inhibiting growth during germination (Widajati 2013).

For dormant seeds to germinate, dormancy can be broken through pretreatment (Anozie and Oboho 2019). This dormancy can be broken by scarification treatment, such as physical scarification of seeds by soaking the seeds in cold or hot water (Hadijah 2013; Rusdy 2014). The water immersion treatment aims to facilitate water absorption into the seeds (Sutopo 2012). Imbibition of water into the seed in a certain amount will stimulate the physiological activity of the seed at the beginning of germination (Yuan and Wen 2018). Water absorption by the seed is influenced by the characteristics of the seed, especially its protective shell, as well as the amount of water available around the seed (Costa et al. 2019).

A study of pretreatment of canarium seeds with pre-sowing treatment involving soaking in water at ambient temperature for 12-32 h has been reported (Nyamukuru et al. 2014), with low germination. To increase germination, it is necessary to carry out other treatments, such as hot water soaking. The use of hot water for pretreatment of seed germination can increase the germination of seeds (Romdyah et al. 2017). However, studies on the pretreatment of canarium seeds with hot water soaking on germination are still lacking. Hence, it is necessary to do research related to this. This study aimed to analyze the effect of the initial water temperature and soaking duration and their interaction on the germination of canarium seeds.

2. Materials and Methods

2.1. Materials

This research was performed in the greenhouse of the Faculty of Agriculture, Khairun University, Ternate, which is located in Fitu Village, South Ternate District, Ternate City (0°45'34.3" N, 127°20720.0" E). This research was conducted from November 2019 to January 2020. The equipment used in this study were shovel, sprayer, thermometer, ruler, writing instrument, treatment nametag, soaking container (16 cm × 11 cm × 5 cm), and germination container (16 cm × 11 cm × 5 cm). The materials used were planting media (100% soil), water, and canarium seeds. The canarium seeds used in this study were obtained directly from the Kota Village, Makian Island District, South Halmahera Regency, North Maluku (0°21'56.3" N, 127°25'01.4" E) (**Fig. 1**).

2.2. Research Design

The research design used was a two-factor experiment in a completely randomized design. The first factor is the different initial water temperature (S) which is modified (Hadijah 2013; Romdyah et al. 2017) and the second factor is soaking time (T) (Lubis et al. 2014). The initial water temperature (S) consists of S1 (60°C), S2 (70°C), S3 (80°C), S4 (90°C), and S5 (100°C), while the soaking duration (T) consists of T0 (0 h), T1 (24 h), T2 (48 h), and T3 (72 h). The replication used in the experimental units, namely the temperature factor × the soaking time factor × replications (5 × 4 × 3), a total of 60 experimental units (**Fig. 2**).

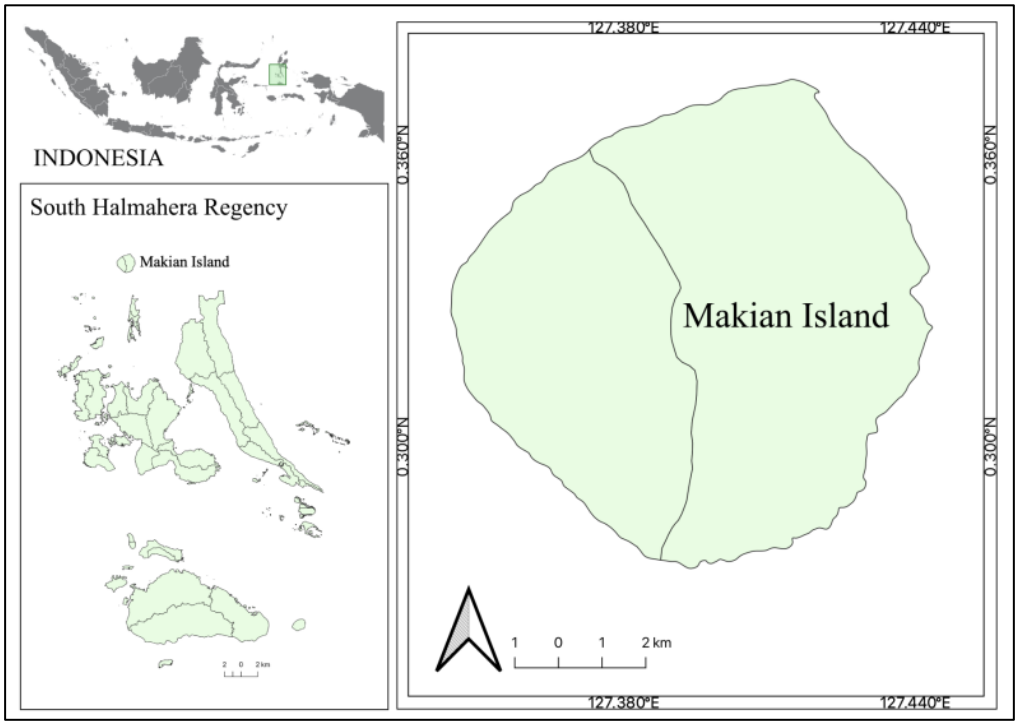

Fig. 1. Study site in Makian Island District, South Halmahera Regency, North Maluku.

1 S2TO	5 S1T3	9 S2T0	13 S4T0	17 S5T0	21 S4T1	25 S5T3	29 S4T0	33 S3T2	37 S1T2	41 S1T3	45 S2T1	49 S5T3	53 S3T3	57 S2T1
2 S4T2	6 S1T0	10 S3T1	14 S4T0	18 S3T2	22 S3T3	26 S2T2	30 S2T1	34 S1T1	38 S5T1	42 S1T1	46 S1T2	50 S2T3	54 S4T3	58 S4T2
3 S5T1	7 S3T1	11 S3T1	15 S3T3	19 S1T0	23 S1T1	27 S3T0	31 S4T3	35 S5T0	39 S2T2	43 S2T2	47 S1T2	51 S5T2	55 S2T0	59 S4T2
4 S3T0	8 S2T3	12 S5T3	16 S4T1	20 S5T2	24 S3T0	28 S1T0	32 S5T1	36 S4T1	40 S5T2	44 S5T0	48 S4T3	52 S2T3	56 S3T2	60 S1T3

Fig. 2. The layout of the experimental plots.

2.3. Research Procedure

2.3.1. Preparation of seeds, germination sites, and planting media

The first step in preparing canarium seeds was to extract their seeds. Extraction is carried out by wet extraction, namely by manually peeling the fruit, then the flesh of the fruit is cleaned that is attached to the seed using running water. After that, the seeds were aerated at room temperature (27°C) (Yuniarti et al. 2016). Preparation of the germinations site was carried out by cleaning weeds around the greenhouse. The soil media was cleaned of dirt and put into the germination container one week before germination. Each germination container was marked with a treatment label. There was no special preparation for seeds, only stored at room temperature (27°C).

2.3.2. Seed soaking, seeding, and germination observation

Canarium seeds soaking was carried out by heating the water until it reached the initial temperature of 60°C, 70°C, 80°C, 90°C, and 100°C and poured into the soaking container. The seeds were then soaked in hot water for 24 h, 48 h, and 72 h. The containers were then placed in a shaded room at room temperature. All the seeds that had been soaked were then sown on the germination media. Control seeds were not soaked and germinated directly on the germination media. Soil moisture in germination media is maintained by watering in the morning (07.00 am) and evening (05.00 pm). Every two days in the morning, the germination media were cleaned due to the growth of the weed. This cleaning is to avoid the nutrition competition between seedlings and weeds. The variable observed in this study was the number of germination. Germination was observed every day for two months.

2.4. Data Analysis

The number of germination data was analyzed to obtain germination parameters, namely: germination percentage (GP), peak value (PV), mean daily germination (MDG), and germination value (GV) (Caliskan 2014; Gairola et al. 2011; Mente et al. 2020) using following equations:

$$GP = \frac{NG}{NS} \times 100\% \quad (1)$$

where GP is germination percentage (%), NG is the number of germinated seeds, and NS is the number of seeds.

$$PV = \frac{GP\text{-}i}{ND} \quad (2)$$

where PV is peak value (%/day), GP-i is germination percentage on day-i (%), and ND is the number of days needed to reach GP-i (day).

$$MDG = \frac{GP\text{-}e}{OD} \quad (3)$$

where MDG is mean daily germination (%/day), GP-e is germination percentage at the end observation (%), and OD is observation duration (day).

$$GV = PV \times MDG \quad (4)$$

where GV is germination value ($[\%/day]^2$), PV is peak value (%/day), and MDG is mean daily germination (%/day).

The results of germination parameter data were then analyzed using the Analysis of Variances (ANOVA). If there was a treatment that had a significant effect, it was continued with Duncan's Multiple Range Test (DMRT) (Mattjik and Sumertajaya 2013; Mira et al. 2017).

3. Results and Discussion

3.1. Effect of Water Temperature on the Germination of Canarium Seeds

Germination of the canarium was observed every day for two months. Canarium seeds began to germinate on the 21st day (**Fig. 3**). Germination observations were stopped when no additional seeds germinated on the 63rd day (**Fig. 4**).

Fig. 3. Canarium seeds begin to germinate.

Fig. 4. Canarium germination at the end of observation.

Germination parameters of seed are usually measured by germination percentage (GP), peak value (PV), mean daily germination (MDG), and germination value (GV) (Irmayanti et al. 2015; Tang et al. 2019). Germination percentage (GP) describes the percentage of seeds that germinate from seeds that were seeding. The high GP value in the study was obtained at the initial temperature treatment at 60°C and 70°C (**Table 1**). The peak value (PV) is the percentage of germination when the increase in germinating seeds is the greatest (Gairola et al. 2011). The peak value in the observations was also the highest at the initial temperature treatment at 60°C and 70°C.

Table 1. Effect initial temperature soaking water on germination of canarium seed

Initial Temperature (℃)	GP (%)	PV (%/day)	MDG (%/day)	GV ([%/day]^2)
60	55.00^a	1.59^a	0.87^a	1.51^a
70	61.66^a	1.63^a	0.97^a	1.65^a
80	36.66^b	1.07^b	0.58^b	0.70^b
90	20.00^c	0.56^c	0.31^c	0.29^b
100	10.00^c	0.32^c	0.15^c	0.29^b

Note: Different letter notations show significant differences at $p < 0.05$. GP = germination percentage, PV = peak value, MDG = mean daily germination, and GV = germination value.

The MDG is the percentage of the last germination divided by the number of days to reach final germination (Gairola et al. 2011). The highest MDG in this study was also found in the initial

water temperature treatment of 60°C and 70°C. Germination value (GV) is the multiplication of PV and MDG. Hence, the highest GV in this study was also obtained at the initial water temperature treatment of 60°C and 70°C.

The results showed that the initial temperature of the soaking water had a very significant effect ($\alpha = 0.01$) on all parameters of canarium seed germination (**Table 1**). The initial water temperatures of 60°C and 70°C resulted in high values of all germination parameters. The high value obtained was most likely because of the temperature of 60°C to 70°C is the optimum temperature. The water temperature can soften the very hard shell of canarium seeds and not damage the embryo. The soft seed coat causes water and oxygen to easily enter the seed to activate metabolic processes in the seed. Temperatures of 60°C and 70°C are thought to accelerate the chemical and physiological stimulation of seeds throughout the seed tissue (Hadijah 2013).

The initial water temperatures of 80°C, 90°C, and 100°C had low values on germination parameters. It might be caused by the water temperature being too hot, so the entry of water into the seeds affects the condition of the embryo. The impact on the embryo could be even more significant, especially because the immersion is carried out for a long time. Soaking with hot water is intended to break the cuticle layer of the seed coat. However, immersion in very high temperatures makes the water dissolve the cuticle layer of the skin and break up or dissolve the embryo (Koller and Hadas 1982; Setiawan et al. 2021). Therefore, for the seeds to germinate properly, the water for soaking the seeds should be at a temperature of less than 80°C, with an optimal temperature of 60-70°C.

In addition, the low value of germination parameters in the initial water temperatures of 80°C, 90°C, and 100°C can also occur due to soaking the seeds in water with a high initial temperature for an extended period (more than 24 h). In such conditions, the seeds are exposed to high temperatures for a longer time so that the embryo is more susceptible to damage. Therefore, germination of canarium seeds soaked in high-temperature water (more than 80°C) may be better if the seeds are soaked for a shorter time. Several studies have carried the scarification process of seeds in hot water for a certain duration, such as the scarification of *Adenanthera pavonine* seeds in hot water for 5 min (Romdyah et al. 2017) and *Canarium resiniferum* seeds for 1 min (Hasnat et al. 2017), reporting that soaking the seeds at high temperatures for a shorter duration can help the scarification process without damaging the embryos inside.

3.2. Effect Soaking Duration on the Germination of Canarium Seeds

The soaking duration had no significant effect on GP, MDG, and GV but a significant effect on PV (**Table 2**). Based on **Table 2**, it was found that the germination parameters had values that were not significantly different at the $\alpha = 0.05$, except for the PV. The low value of the germination parameter was due to the duration of soaking of 24 h, 48 h, and 72 h, which were ineffective and inefficient for canarium seeds. The average parameter for each treatment of immersion duration showed a low value with a fairly low difference. However, a shorter soaking duration indicates a better PV. Similar studies on *Canarium schweinfurthii* (Anozie and Oboho 2019) and *Canarium resiniferum* (Hasnat et al. 2017) showed that soaking seeds for 24 h gave the most optimal results in seed germination. For *C. indicum*, based on this research, the soaking duration of 24 h showed promising results. However, it would be better if the duration of soaking the seeds was done in a much shorter time (less than 24 h) to prevent excess water absorption. Excess water will make the

seeds become saturated with water, impacting decreasing germination and germination percentage (Hadijah 2013).

Table 2. Effect duration of soaking on germination of canarium seed

Soaking Duration (h)	GP (%)	PV (%/day)	MDG (%/day)	GV ([%/day]^2)
0	41.33^a	1.18^a	0.65^a	0.93^a
24	36.00^a	1.10^{ab}	0.57^a	0.92^a
48	37.00^a	1.10^{ab}	0.59^a	0.92^a
72	32.00^a	0.76^b	0.51^a	0.79^a

Notes: Different letter notations show significant differences at $p < 0.05$. GP = germination percentage, PV = peak value, MDG = mean daily germination, and GV = germination value.

Soaking the seeds in water resulted in rapid imbibition and absorption of water due to low water potential (Shiddique and Kumar 2018), which increases the dimensions of the embryo (Bradbeer 1988). In addition, it causes the seed coat to soften and activates enzymes that trigger germination (Anozie and Oboho 2019). Seeds require optimal amounts of water to hydrate the tissues in the seeds to germinate (Koller and Hadas 1982), but soaking for too long makes the seed moisture content too saturated so that seed viability decreases (Hutasoit et al. 2017). Excess water content can also trigger decay due to an increase in turgor pressure in the seed's cells which causes damage to the cell membrane (Solichatun et al. 2016).

3.3. Interaction Effect of Water Temperature and Soaking Duration on the Germination of Canarium Seeds

The results showed an interaction between the initial temperature of the soaking water and the duration of soaking of the canarium seeds. The effect of the interaction between the initial temperature of the soaking water and the duration of soaking the canarium seeds on germination was analyzed by variance (**Table 3**). Based on **Table 3**, it can be seen that the interaction of initial temperature of soaking water and duration of soaking had a very significant effect on GP and MDG, and a significant effect on PV and GV.

Table 3. Analysis of variance of effective interaction of initial temperature soaking water and duration of soaking on germination of canarium seeds

Source	p<0.05			
	GP	PV	MDG	GV
Initial temperature	**	**	**	**
Duration of soaking	ns	*	ns	ns
Replicates	ns	ns	ns	ns
Interaction between initial temperature and duration of soaking	**	*	**	**

Notes: ** = very significant, * = significant, ns = not significant.

Dormancy in canarium seeds was caused by a thick and hard seed coat that prevents imbibition. Therefore, the treatment was intended to create gaps or destroy the seed coat. The treatment of soaking the seeds in water with a certain initial temperature and duration can accommodate this. Soaking the seed in hot water can tear the seed coat walls (Tiwari et al. 2018) and make the seed coat more permeable (Lambers et al. 2008). The soaking duration facilitates the seeds to absorb water until it reaches the optimal amount and softens the coat. Seed soaking also

allows water to penetrate the seed tissue and physiological trigger changes that result in rapid embryo germination (Tiwari et al. 2018). In addition, the seed coat is no longer a mechanical barrier for the embryo to germinate because it has softened after soaking in water (Lambers et al. 2018). Thus, the roots of the embryo can easily penetrate the skin and initiate germination. The findings in this study are also supported by results from other studies, which state that soaking seeds at a specific initial temperature and duration affect the germination of the seeds of *Samanea saman* (Lubis et al. 2014), *Annona muricata* (Gea et al. 2018), and *Paraserianthes falcataria* (Marthen et al. 2013).

4. Conclusions

The initial water temperature and soaking duration affected the germination of canarium seeds. The initial temperature of soaking water had a very significant effect on germination percentage, peak value, mean daily germination, and germination value. Duration of soaking had no significant effect on germination percentage, mean daily germination, and germination value, but it significantly affected the peak value. The interaction of the initial water temperature and soaking duration significantly affected the germination percentage, mean daily germination, peak value, and germination value. The best result in canarium seed germination could be obtained at the initial water temperatures of 60-70°C for 24 h.

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