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Evaluation of Seed Viability and Quality for Santigi (*Pemphis acidula* J.R. Forst and G. Forst) Conservation: Assessing the Effect of Chemical and Physical Scarification

Shakira Chandra Putri Datumaya¹, Melisnawati H. Angio², Balqis Balqis¹, Dewi Ayu Lestari², Esti Endah Ariyanti², Febrina Artauli Siahaan², Dian Latifah², Andik Wijayanto^{1*}

¹ Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Malang, Indonesia

² Research Center for Applied Botany, National Research and Innovation Agency, Indonesia

*Corresponding author: andik.wijayanto.fmipa@um.ac.id

Pemphis acidula J.R.Forst. and G. Forst. or santigi is a plant native to Indonesia, widely recognized for its high economic value. Although listed as Least Concern by the International Union for Conservation of Nature (IUCN), its existence in the wild is increasingly threatened by land degradation, climate change, and it suffers from low natural seed germination rates. This study aimed to evaluate the seed viability and quality of *P. acidula* and assess the effectiveness of chemical and physical scarification techniques on the viability of santigi seeds. Six scarification treatments were evaluated: control, 24-hour room temperature water immersion (24.9°C), 6-hour 80°C hot water immersion, 1-minute 10% H₂SO₄ immersion, 24-hour 250 ppm GA₃ soaking, and seed coat piercing. Data were analyzed using the Shapiro-Wilk test for normality and Kruskal-Wallis ($p > 0.05$) for non-parametric comparison. The results showed that none of the scarification treatments significantly affected the germination rate of *P. acidula* seeds. This lack of response was primarily attributed to poor internal seed quality rather than the treatments themselves. The cut test revealed that the majority of seeds were nonviable or empty, lacking both embryos and endosperm. However, tetrazolium testing confirmed that the filled seeds remained viable, indicating

that while some seeds possessed life potential, their quantity within the lot was insufficient. These findings suggest that the low germination rate resulted from a combination of poor seed quality and deep physical dormancy protected by a woody, spongy structure. Consequently, this study concludes that internal seed factors were the limiting constraints for this specific lot. Future conservation and propagation efforts must prioritize rigorous seed sorting and viability testing prior to applying scarification protocols.

Keywords: *Pemphis acidula*, conservation, germination, scarification, seed.

Introduction

Pemphis acidula J. R. Forst. and G. Forst., commonly referred to as santigi, is a species native to Indonesia with a small tree form and sympodial branching that is specifically adapted to coastal areas, either on seashores or coral reefs (Goutham-Bharathi et al., 2015; Baderan et al., 2024). Besides the knee-root system that strengthens its structure in coral substrates, the colonization success of this species along the Indonesian coastline is supported by a hydrochory mechanism that demonstrates specific reproductive adaptation to coastal ecosystems (Jian and Ren, 2019). The seeds of *P. acidula* possess spongy tissue that provides buoyancy and dispersal through ocean currents (Guja et al., 2010), which explains the wide geographical distribution of this species in the Indo-Pacific region (POWO, 2023). *P. acidula* is widely recognized for its high economic value, particularly for its wood used in bonsai and crafts. Its ethnobotanical applications include the use of leaves and bark as traditional medicinal ingredients to treat bacterial infections and inflammation (Untu, 2019; Ap et al., 2022; Page and Batmomolin, 2022). Furthermore, in certain regions, the species holds cultural significance and is believed to possess specific mystical properties (Utami et al., 2017). *P. acidula* is widely recognized for its high economic and ethnobotanical value in Indonesia, ranging from its use in bonsai and crafts to its application as medicinal ingredients and its believed mystical properties (Untu, 2019; Ap et al., 2022; Malik, 2022; Page and Batmomolin, 2022).

Despite its widespread use and recognized benefits, santigi faces significant threats in its natural habitat. The International Union for Conservation of Nature (IUCN, 2024) lists this species as Least Concern, but notes a declining population trend. This decline is considered widespread, including in Indonesia (Baderan et al., 2022). The dependence of this species on coastal habitats is not only due to external threats (e.g., climate

change, overexploitation) but also because of specific germination requirements. Seeds of *P. acidula* require substrates with extreme drainage and specific salinity tolerance that are difficult to satisfy in non-littoral environments, which explains its strict ecological specialization. Wijayasinghe (2023) reported that only 60% of *P. acidula* seeds are capable of germinating, indicating barriers during the regeneration phase. This low rate is not only caused by non-viability factors or the presence of empty seeds frequently found in coastal species, but is also strongly suspected to be influenced by seed dormancy mechanisms. Given the spongy and woody structure of the *P. acidula* seed coat, there are indications of physical dormancy that limits water imbibition. Research findings from the Purwodadi Botanic Garden Seed Research Team reported that the *P. acidula* germination rate is extremely low at 5.33% (Purwodadi Botanic Garden Seed Research Team, 2023, unpublished data). Based on interviews with local communities around the coast of Baluran Beach (East Java) and Tomini Bay (Sulawesi) where *P. acidula* is located, it is also stated that this species is difficult to germinate outside its natural habitat and no one has yet succeeded. This situation creates a gap between seed production and the generation of new individuals, which is caused by the slow growth rate of the species. Without intervention in seed-based regeneration mechanisms, *P. acidula* populations are at risk of local extinction, even while mother trees remain in their habitats. Consequently, *P. acidula* faces a silent threat of reproductive failure, highlighting the critical need for effective conservation strategies.

This study aims to diagnose the regeneration barriers in *P. acidula* through an integrated approach that distinguishes between viability constraints and dormancy mechanisms. Specifically, the research evaluates initial seed quality using cut-tests and tetrazolium (TTZ) assays to identify the proportion of empty seeds and verify embryo viability status. Research into effective dormancy-breaking techniques is necessary to

enhance seed viability. The physical and chemical dormancy-breaking methods applied in this study were selected based on species with similar seed characteristics. The seeds of this species possess a distinct spongy and woody seed coat, which typically acts as a physical barrier to germination. While the woody exterior requires robust treatments to increase permeability, the spongy tissue can lead to rapid heat or acid penetration. Physical treatments, including mechanical scarification and thermal applications, are employed to break dormancy by altering seed coat permeability (Hsiao and Hanes, 1983; Hidayatulah et al., 2019; Chhaya, 2020). However, to address potential internal constraints, Gibberellic Acid (GA₃) and sulfuric acid (H₂SO₄) were applied as physiological triggers. GA₃ is essential in breaking physiological dormancy by stimulating the production of hydrolytic enzymes that mobilize seed reserves and enhance the growth potential of the embryo, allowing it to overcome the mechanical resistance imposed by the woody integument (Shreelalitha et al., 2015; Siregar et al., 2022). By evaluating these various techniques, this study seeks to determine the optimal protocol for enhancing both the germination percentage and rate of *P. acidula* as an effective conservation strategy.

Materials and Methods

Place and time of research

This research was conducted at the East Nursery of the Purwodadi Botanical Garden, National Research and Innovation Agency (BRIN) for seed planting, the BRIN Laboratory at the Purwodadi Botanical Garden for the preparation of treatment solutions, and the Laboratory of Ecology and Environment, Department of Biology, State University of Malang for the observation of seed morphology and structure. The research was conducted from September 2023 to May 2024.

Experimental design

This study is classified as an experimental study with a quantitative approach because it was conducted to determine the effect of the independent variable (dormancy-breaking treatment) on the dependent variable (viability of *P. acidula* seeds) under controlled conditions. This study used a non-factorial completely randomized design (CRD) because the factors used did not

have combinations, and the study was conducted in a greenhouse. The factors were as follows:

S1 = Control

S2 = Soaking in water at room temperature (24.9°C) for 24 hours

S3 = Soaking in hot water at 80°C for 6 hours until it cooled down

S4 = Soaking in 10% H₂SO₄ for 1 minute

S5 = Soaking in gibberellic acid (GA₃) 250 ppm for 24 hours

S6 = Seed coat piercing (antiraphe side)

In this study, 3 replicates (R1, R2, and R3) were used, resulting in 18 experimental units. Each experimental unit used 25 seeds (Utami et al., 2020), for a total of 450 seeds.

Seed collection and post-harvest handling

P. acidula seeds were collected by BRIN Purwodadi Botanical Garden from exploration in Baluran National Park on September 30, 2022, by the Purwodadi Botanical Garden (PBG) Seed Conservation Team. The collection was conducted in strict compliance with conservation ethics and regulations, with the necessary permit secured from the Baluran National Park administration prior to the expedition (Permit Number: Sl. 599/T.37/TU/KSA.6/9/2022). To achieve sample homogeneity, only seeds from physiologically mature fruits (natural dehiscence) were harvested. The extracted seeds were sorted using graded sieves to separate viable, filled seeds from empty ones. The seeds were stored in a refrigerator at a temperature of -14.8°C and a humidity of 46% at the Purwodadi Botanical Garden Laboratory. Seeds were stored at -14.8°C based on the presumed orthodox nature of the species, following established seed bank conservation standards. After a storage period of approximately 12 months, the seeds were then utilized for the treatments and observations mentioned above. To evaluate the success of this storage regime and its impact on embryo integrity, post-storage viability was assessed using tetrazolium tests.

Procedure

One hundred seeds were randomly selected and weighed using a Mettler Toledo AL204 analytical balance. Observations of one hundred seeds included shape, color, surface, size (length, width, thickness),

and any special characteristics, with the results expressed as Mean $\pm$ Standard Deviation (SD) to account for the sample. The seed size was observed and measured using an Olympus SZ61 stereo microscope and an Olympus EP50 digital microscope camera. The shape and surface of the seeds were examined descriptively. The color of the seeds was determined using the Royal Horticultural Society (RHS) Color Chart 6th Edition. After that, longitudinal sections were prepared from 30 seeds to examine the internal structure (Shalimu et al., 2015; Rahmawati et al., 2022).

Tetrazolium test

The tetrazolium test was initially conducted prior to scarification treatment as a rapid preliminary indicator of seed viability. *P. acidula* seeds were transversely cut and soaked in a 1% solution of 2,3,5-triphenyltetrazolium chloride for 24 hours at 30°C (Corsino et al., 2024). The tetrazolium staining pattern on *P. acidula* seeds was observed using a Dinolite AM3113T digital microscope.

Seed sowing

P. acidula seeds, scarified according to the experimental design, were sown in a prepared planting medium composed of burnt rice husks, Malang sand, cocopeat, humus, manure, mahogany andam, humus soil, and bamboo leaves, at a depth of approximately 2.5 to 3 cm. The number of seeds sown was observed daily, and watering was done whenever the planting medium appeared dry. Temperature, humidity, and light intensity in the nursery environment were measured daily as supporting data. Temperature and humidity were recorded using a humidity and temperature data logger Elitech RC-4HC, while light intensity was measured with a digital luxmeter peakmeter PM6612.

Cut test

This test was conducted on seeds that did not germinate until the end of the observation period. The test was conducted by digging up the planted seeds, cutting them with a razor blade, and categorizing them as follows: full seeds, empty seeds, moldy seeds, insect-infested seeds, and abnormal seeds (Davies et al., 2015).

Observation parameters

Several parameters were observed to evaluate the effectiveness of the scarification treatments and the overall seed quality. These parameters included

germination rate, plant height, and number of leaves, which are detailed below.

Germination rate

Germination rate is calculated based on the number of seeds that germinate normally. According to Sadjad (1999) in Suita and Bustomi (2014), the formula for germination rate is as follows:

$$DK = \frac{(\sum KN)}{N} \times 100\% \quad (1)$$

where *DK* – germination rate, *KN* – number of seeds that germinated normally until the last day, *N* – number of seeds sown.

Plant height (cm)

Plant height was measured once a week until the end of the study using a ruler. Plant height was measured from the base of the stem to the tip of the tallest leaf (Tri et al., 2022).

Number of leaves

The number of leaves was counted on each seedling that grew. The leaves counted were those that had fully opened and were green. The leaves were counted at weekly intervals until the end of the study (Tri et al., 2022).

Data analysis

Data on *P. acidula* seed characteristics were analyzed descriptively, including the calculation of mean values and standard deviations for each observed parameter. The staining pattern of the tetrazolium data was calculated using Microsoft Excel and illustrated in the figure. Data on the effects of scarification treatment on germination rate, plant height, and number of leaves were analyzed using the Shapiro-Wilk test for normality and the Kruskal-Wallis test for non-parametric data with a significance level of 0.05, both performed with RStudio 2025.09.2+418 “Cucumberleaf Sunflower” Release statistical software. The effect of scarification treatment on germination rate was presented in a bar chart, while the effects on plant height and number of leaves were illustrated using box plots. Additionally, data on environmental conditions and germination rate were analyzed using the Pearson correlation test in RStudio. Cut-test data is presented in the table and figure with various categories.

Results and Discussion

Seed characteristics

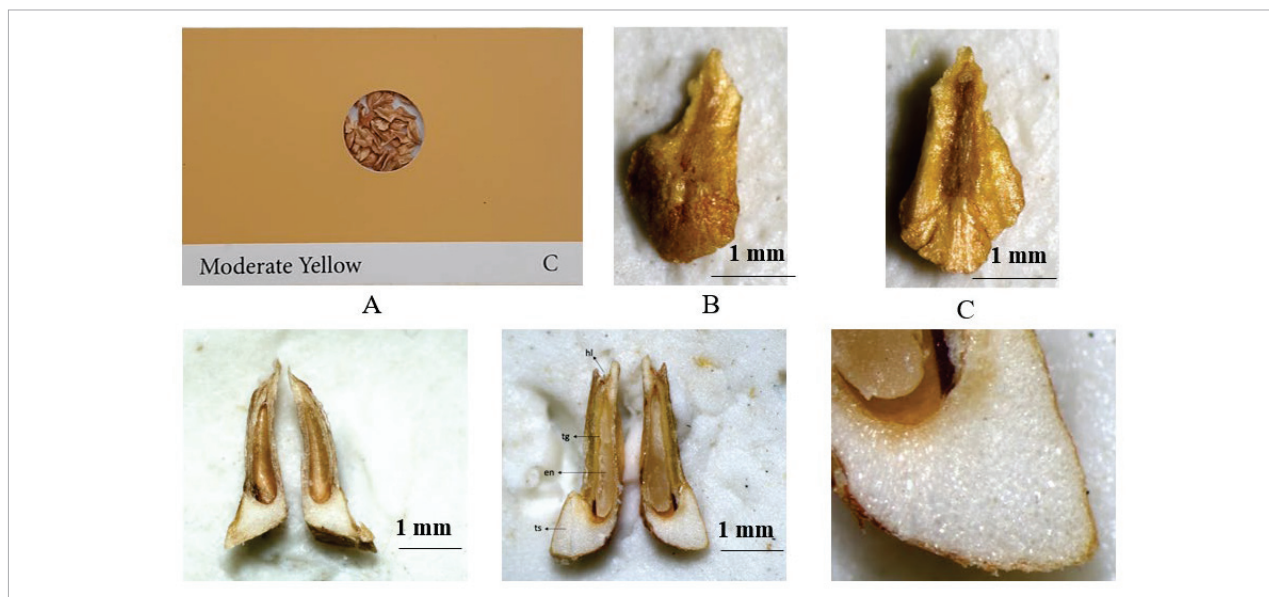
Observations of seed characteristics show that the outer structure of santigi seeds is Moderate Yellow 163-C in color, with a shiny, smooth surface and an irregular shape (Fig. 1A). The measurements obtained from the observation were an average length of 3.18 ± 0.17 mm, a width of 1.85 ± 0.15 mm, a thickness of 0.82 ± 0.04 mm, and an average weight of 1.05 ± 0.003 mg (Fig. 1B, 1C). These findings are consistent with Graham and Graham's (2014) research, which also found that santigi seeds have a golden yellow color, a shiny surface, and an irregular shape.

Furthermore, these results complement the data reported by Baderan et al. (2024), who observed seeds from Tomini Bay, Sulawesi, using a similar methodology and reported that santigi seeds have lengths of 3.52–4.09 mm, widths of 2.28–3.32 mm, and an average weight of 3–7.2 mg. Consequently, this study identified differences in seed size and weight compared to previous research. This high variation in seed traits and morphological characteristics is aligned with the findings of Hantsch et al. (2013), who demonstrated that such differentiation across populations is mediated by genetic diversity and adaptation to different

environmental conditions. Since the santigi seeds in this study were collected from Baluran National Park, the differences in location likely contribute to morphological variations, as coastal plants adapt to their specific living environments (Lira-Medeiros et al., 2015). Additionally, seed characteristics are influenced by environmental factors such as altitude and temperature (Ouarda et al., 2014; Bareke, 2018).

Of the 30 seeds sliced lengthwise, 29 were empty or lacked embryos and endosperm; only one contained endosperm (Fig. 1D). The internal structure of santigi seeds consists of the testa, tegmen, endosperm, and embryo (Fig. 1E), each differing in texture and function. The testa, which protects the embryo and nutrient tissue, is dense, hard, and sponge-like (Fig. 1F) (Debeaujon et al., 2007), while the tegmen, covering the endosperm, has a smooth, shiny texture. At the top of the seed, the hilum forms a hole, marking the point of attachment to the fruit and serving as a pathway for nutrients and water during germination (Zhao et al., 2021). In sliced seeds, the tegmen measured 0.39 mm in length and 0.08 mm in width. The endosperm is white. The embryo, likely also white, generally includes a young root (radicle), an apical meristem (epicotyl), and one or more seed leaves (cotyledons); the transition region between root and stem is called the hypocotyl (Bareke, 2018).

Fig. 1. Seed characteristics. A. Colour; B. Raphe; C. Antiraphe; D. Empty seed; E. Full seed; F. Dense, hard, spongy texture of the testa. Notes hl: hilum; tg: tegmen; en: endosperm; ts: testa



Germination rate

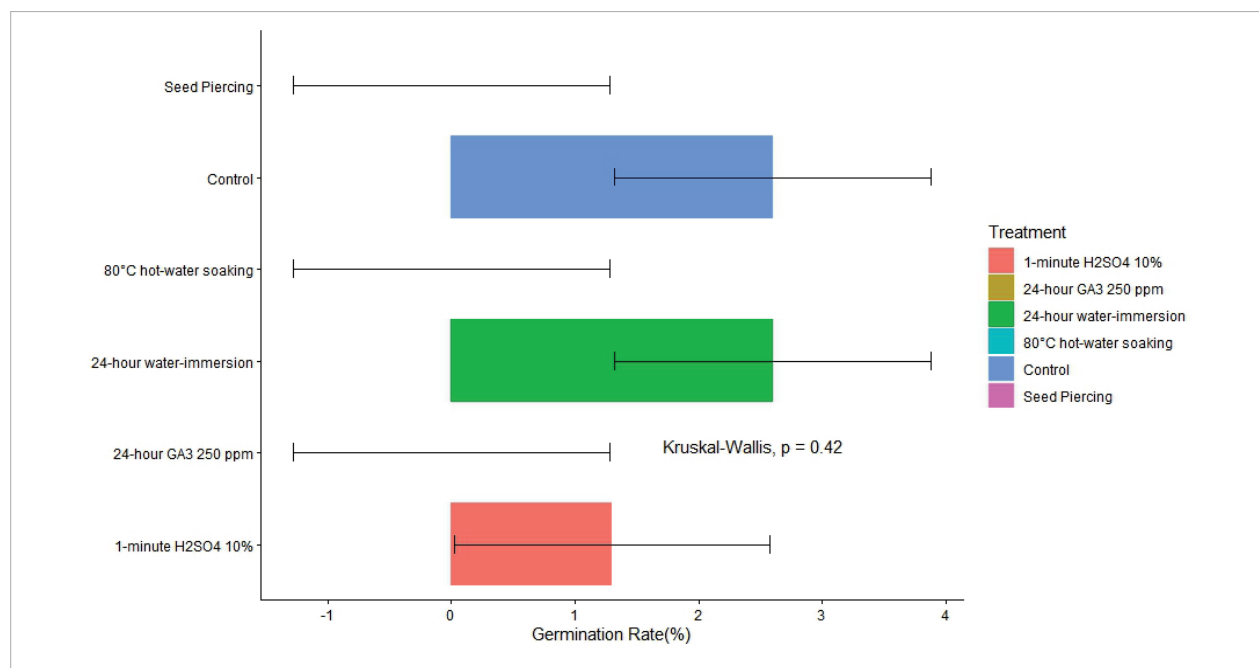
The internal structure of santigi seeds is characterized by a testa that is dense, hard, and spongy-woody in texture. This specialized anatomy, while providing robust protection for the embryo and nutrient tissues, acts as a significant physical barrier to water and oxygen. The woody nature of the seed coat contributes to its hardness, while the spongy internal layers may trap air or resist rapid liquid penetration. These findings align with Debeaujon et al. (2007), who noted that such dense structures are designed to protect the embryo from environmental stressors but often result in physical dormancy.

Both the control and 24-hour water-immersion treatments had a 2.6% germination rate, with only 2 of 75 seeds sprouting in each case (Fig. 2). Notably, in both treatments, groups control and 24-hour water immersion, germinated on the 14th day after planting. Thus, a direct comparison between the control and 24-hour water-immersion revealed no difference in santigi seed germination rate, suggesting that soaking at room temperature for 24 hours does not increase santigi seed permeability. Supporting this, Louf et al.

(2018) reported that soaking duration affects imbibition and seed coat permeability. For instance, in *Samanea saman*, soaking for 72 hours resulted in the highest germination rate compared with 24- and 48-hour treatments (Ardani Lubis et al., 2014).

The 1-minute 10% H_2SO_4 soaking treatment yielded a germination rate of 1.3%, with only 1 of 75 seeds germinating on the 21st day after planting (Fig. 2). While this value is numerically lower than the control (2.6%) and the 24-hour water-immersion treatment (2.6%), statistical analysis using the Kruskal-Wallis test yielded a $p > 0.42$. This indicates that there are no significant differences in germination rates across all treatments. The short duration of acid exposure and room-temperature soaking were probably insufficient to soften or penetrate this resilient woody barrier (Barker and Abdi, 1987). Furthermore, the extremely low germination rates ($< 3\%$) across all treatments are fundamentally linked to the anatomical observation that 29 out of 30 seeds were empty or lacked embryos. This suggests that for santigi seeds in Baluran National Park, the primary constraint to regeneration is not only the physical dormancy imposed by the spongy-woody testa but also a high rate of seed emptiness.

Fig. 2. Germination rate (%) of *P. acidula* across different seed treatments. Error bars represent the standard error. Statistical analysis using the Kruskal-Wallis test ($p=0.42$) indicates no significant differences between the treatments and the control group, suggesting that the observed variations fall within the margin of sampling error due to low overall germination



The 80°C hot-water soaking treatment for 6 hours and GA₃ 250 ppm 24-hour soaking treatment both resulted in a 0% germination rate (Fig. 2), completely suppressing seed germination compared to all other treatments. Supporting this result, Haj Sghaier et al. (2022) stated that soaking seeds at temperatures above or below the optimum can decrease germination potential. For santigi seeds, either high water temperature or prolonged soaking was detrimental to seed quality (Fig. 1). Although hot water soaking can improve germination in some species, such as *Indigofera tinctoria*, where 80°C for 6 hours achieved 29% germination (Chhaya, 2020), it did not have the same effect on santigi. The failure of the piercing treatment (0% germination) also highlights the difficulty of manually scarifying such a spongy yet spongy-woody structure; the pressure required to break the woody exterior likely led to fatal embryo injury, as uncontrolled depth in hard-coated seeds often results in irreversible damage. Species responses to GA₃ vary, as noted by Cornea-Cipcigan et al. (2020); for example, in *Vigna mungo* and *Macrotyloma uniflorum*, higher GA₃ concentrations with 24-hour soakings correlated with lower germination percentages (Chauhan et al., 2009).

The extremely low germination rates observed in this study (max. 2.6%) are consistent with broader patterns of reproductive challenges in *P. acidula*. Previous reports by the Purwodadi Botanic Garden Seed Research Team (2023, unpublished data) also recorded a remarkably low germination rate of 5.33%, further validating the difficulty of propagating this species. This phenomenon is likely driven by the spongy-woody structure of the seed coat, which imposes a physical dormancy that severely limits water imbibition. As noted by Wijayasinghe (2023), even under favorable conditions, only 60% of *P. acidula* seeds may be capable of germinating, suggesting that the regeneration phase is hindered by both inherent non-viability, such as the high frequency of empty seeds found in this study.

Beyond physical barriers, the ecological specialization of *P. acidula* plays a decisive role in its recruitment failure. The species requires specific substrates with extreme drainage and precise salinity tolerances, conditions that are inherently difficult to replicate in non-littoral or controlled environments. This environmental sensitivity is reflected in local ecological knowledge; interviews with communities near Baluran Beach (East Java) and Tomini Bay (Sulawesi) confirm that *P. acidula*

is notoriously difficult to germinate outside its natural habitat, with no recorded success in local cultivation to date.

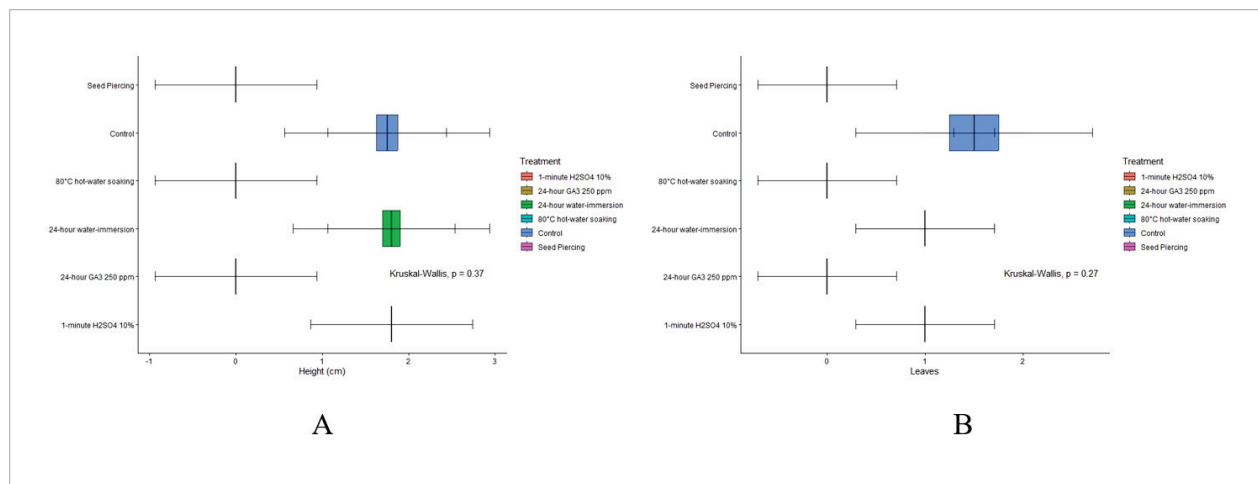
In the physical scarification treatment, piercing the seed coat resulted in a 0% germination rate, even lower than in any of the other treatments examined. Although piercing near the embryo is typically intended to facilitate water absorption, in this case, it may have been too deep, causing embryo damage and poor seed quality. This observation aligns with the findings of Hsiao and Hanes (1983), who noted that uncontrolled variation in piercing depth due to pressure differences can result in embryo injury.

Descriptive observations on initial seedling growth

Due to the limited number of surviving individuals (n=2), the data on plant height and leaf development are presented here strictly as marginal descriptive observations. Statistical analysis using the Kruskal-Wallis test showed no significant differences in height ($p = 0.37$) and the number of leaves ($p = 0.27$), suggesting that the observed variations likely reflect individual seed quality rather than treatment effects (Fig. 3). The low survival rate is attributed to the inherent physical characteristics of santigi seeds, which possess a thick, woody, and spongy outer layer. This structure serves as a formidable mechanical barrier that hinders water imbibition, leading to the deep physical dormancy observed. These preliminary findings suggest that conventional scarification may be insufficient to overcome the structural defenses of this species.

The germination trials were conducted under controlled greenhouse conditions. Environmental monitoring throughout the study period recorded an average temperature of $26.8 \pm 0.8^\circ\text{C}$ (ranging from 25.0°C to 28.3°C), a relative humidity of 74.6% to 89.1%, and a photoperiod of 1507–25683 lux. The environmental monitoring during the germination test showed that abiotic factors did not significantly influence the germination rate (GR). Pearson's correlation analysis revealed that temperature ($r = 0.13$, $p > 0.05$), humidity ($r = -0.49$, $p > 0.05$), and light intensity ($r = -0.34$, $p > 0.05$) had no significant correlation with GR (Fig. 4). These results demonstrate that the greenhouse conditions remained within a tolerable range for *P. acidula* and were not the cause of the low germination success. Instead, the poor germination is statistically

Fig. 3. Observation on initial seedling growth. A. Plant height; b. Number of plant leaves per two stems for each treatment during 60 days after planting



confirmed to be independent of environmental fluctuations, further supporting the conclusion that internal factors, specifically the high proportion of empty seeds and low viability within this seed lot, were the primary constraints.

The decision to store the seeds at sub-zero temperatures was based on the physiological characteristics of *P. acidula*. The species produces dry, non-fleshy fruits, and the seeds can reach a low moisture content of approximately 10%. These traits are indicative of orthodox seed behavior, which allows seeds to tolerate desiccation and remain viable during long-term storage at low temperatures. Therefore, the storage conditions used in this study were intended to maintain seed longevity rather than compromise it, although the inherent quality of the specific seed lot remained a primary constraint as identified by the cut test.

Seed quality analyst (cut test and tetrazolium test)

Cut tests involving the dissection of ungerminated seeds were conducted throughout. In the control treatment, 73 of 75 seeds did not germinate; 64 of these were found empty or lacked embryos and endosperm, and 9 were missing from the medium (Table 1). In the 24-hour room-temperature water-soaking and the 6-hour 80°C hot-water treatments, almost all seeds failed to germinate, and several were missing from the medium. The majority of recovered seeds from both treatments, as well as the piercing treatment, were

empty or lacked embryos and endosperm (Table 1). In the GA₃ 250 ppm 24-hour soaking, all 75 seeds failed to germinate. Of the seeds, 68 were retrieved and 7 were absent; cut tests found 66 empty or lacking embryos or endosperm and 2 complete (Table 1). In the 10% H₂SO₄ 1-minute soak, 67 seeds were retrieved, with 66 empty or lacking embryos or endosperm and 1 complete (Table 1). Seventy-four of 75 seeds failed to germinate, with 7 seeds absent from the medium.

The cut tests confirm santigi seeds exhibit poor germination across all treatments and in nature (Fig. 5A) (Manek and Puay, 2020). Although santigi seeds appear identical, empty seeds cannot be distinguished visually from full seeds. Empty seeds are non-viable because they lack food reserves and embryos. Endosperm supplies nutrients to the embryo during germination (Lafon-Placette and Köhler, 2014). Missing endosperm and embryo are likely due to developmental defects after fertilization. In angiosperms, seed development begins with double fertilization, resulting in a diploid embryo and a triploid endosperm (Song et al., 2021). Embryo and endosperm development rely on parental genomes and coordinated genetic regulation (Li and Berger, 2012). Additionally, diplospore-type apomixis can produce clonal seeds without fertilization, leading to empty seeds due to a lack of recombination and meiotic reduction (Cornaro et al., 2023). In the 250 ppm GA₃ 24-hour and 10% H₂SO₄ 1-minute treatments, some ungerminated seeds were intact or contained endosperm (Fig. 5B). Failure of intact seeds to germinate

Fig. 4. Heatmap of Pearson's correlation analysis between environmental parameters (temperature, humidity, light intensity) and germination rate (GR). Values indicate correlation coefficients (r), where 'ns' denotes non-significant relationships ($p > 0.05$)

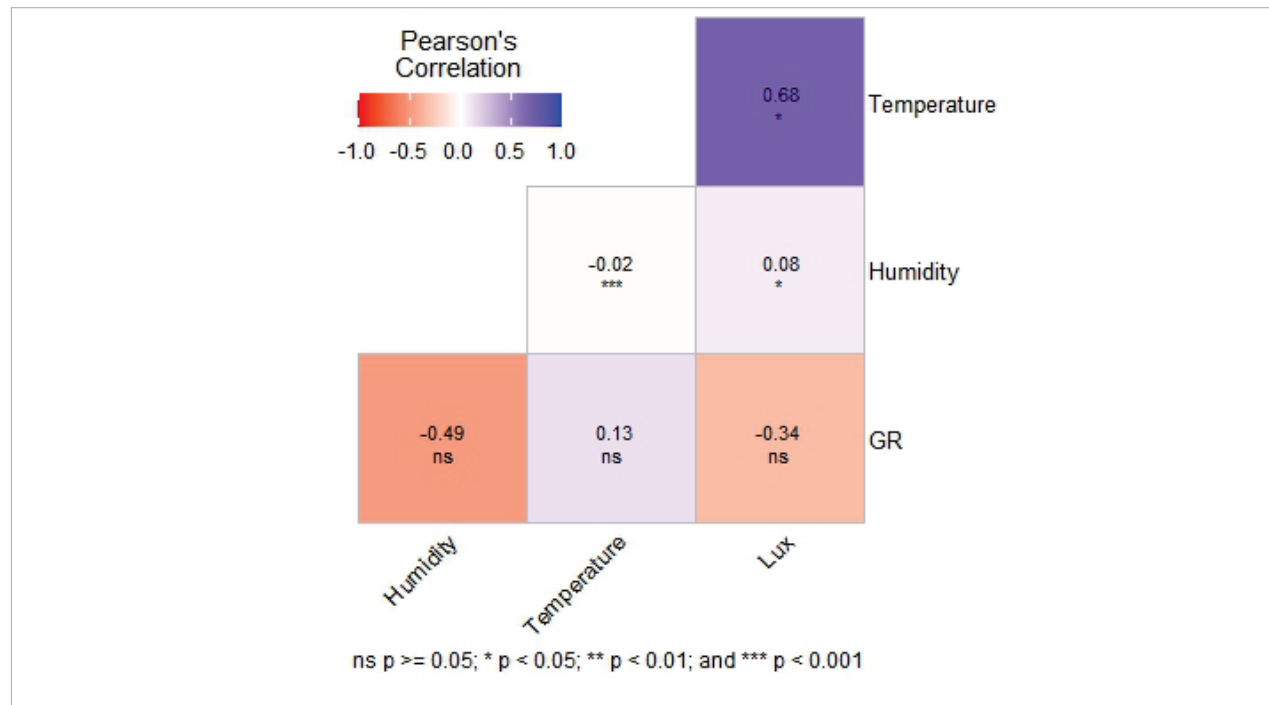


Table 1. Cut test result after planting

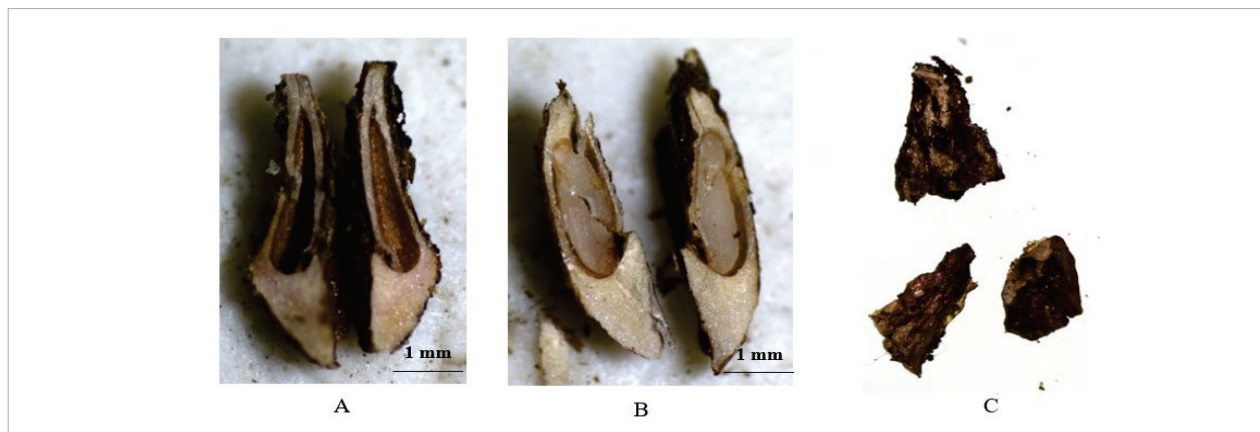
Treatmen	Category				
	Full	Moldy	Empty	Insect-infested seed	Abnormal
Control	0	0	64	0	0
Room Temperature Water for 24 hours	0	0	68	0	0
Hot Water at 80°C for 6 hours	0	0	71	0	0
10% H ₂ SO ₄ for 1 minute	2	0	66	0	0
GA ₃ 250 ppm for 24 hours	1	0	68	0	0
Seed coat piercing	0	0	72	0	0

seems linked to treatments that affect seed quality or to unsuitable germination conditions. Non-viable seeds with dead tissues do not germinate. Hartmann et al. (1997) in Bareke (2018) outline three germination prerequisites: (1) seed viability; (2) dormancy release; and (3) optimal environmental conditions, including water, temperature, and oxygen.

The cut test began with dismantling the planting medium. During this process, several seeds were absent from the medium, while others had broken or split shells (Fig. 5C). The missing seeds are believed to

have decomposed in the planting medium due to soil microbial activity. According to Greenfield (1999), microbial decomposition of seeds begins about 30 days after planting, with variation by species. Key decomposition factors include the type and size of organic matter, microbial composition, levels of carbon (C), nitrogen (N), phosphorus (P), potassium (K), as well as soil moisture, temperature, pH, and aeration (Rao and Subba, 1994; Fanin et al., 2016). Decomposition entails fragmentation, structural breakdown, and enzymatic conversion, transforming organic material into

Fig. 5. Result of the santigi seed cut test. A. Empty seeds after planting; B. Full seeds after planting; C. Split shells of santigi seeds in piercing treatments



inorganic compounds. The process starts with scavengers physically breaking down organic matter, leaving residues such as litter or debris. As a result, some seeds are crushed and difficult to find (Kirchman, 2012; Saptiningsih and Haryanti, 2015).

During the cut test, some seeds were suspected of containing fungi (Fig. 6). Seeds may carry pathogens from their place of origin, transmitting them to growing plants when conditions are suitable. This process is known as seed-borne disease. Pathogenic microorganisms, such as fungi, bacteria, and viruses, can reside on the seed surface or inside the seed. During harvesting, pathogens may transfer to seeds via contaminated tools or surfaces, such as nets or the ground, if seeds are allowed to fall before collection (Amza, 2018). “Field fungi” are those that infect seeds before harvest, either while seeds develop on the parent plant or after maturation. These fungi cause seeds to turn black and may weaken or kill the embryo (Martín et al., 2022). According to Burdon (1987) in Leishman et al. (2000), temperature and humidity are the two most critical factors influencing seed-borne fungal growth. High air and soil humidity, combined with warm temperatures, promote fungal survival and encourage pathogen movement from seeds to plants.

The cut test provided a primary finding in this study, demonstrating that a significant majority of the seeds were empty or lacked functional embryos. To further validate these findings, the tetrazolium test (TTZ) was employed to assess the physiological state of the remaining filled seeds. The results showed that only a small fraction of these individuals were metabolically

active, as evidenced by the red-stained embryos (Fig. 7). This combined evidence explains the low germination success and confirms that the study’s outcomes were primarily dictated by the poor quality of the seed lot.

The low initial germination observed was not solely a result of dormancy; our cut-test and TTZ assays revealed that 26.6% of the seeds were either empty or non-viable, thus establishing a baseline for true embryo viability before evaluating dormancy-breaking techniques. The overall low germination rate was significantly influenced by a high incidence of empty seeds, as revealed by cut-tests. When excluding these empty seeds, the viability of the filled seeds remained high (as evidenced by TTZ staining), confirming that the primary biological challenge for *P. acidula* is a combination of poor seed filling and deep dormancy.

Conclusions

Based on the descriptive observations and statistical results (Kruskal-Wallis, $p > 0.05$), concludes that neither chemical nor physical scarification treatments significantly increased *Pemphis acidula* seed germination in this specific seed lot. The primary constraint was poor seed quality, as the cut test revealed a high frequency of empty seeds. Furthermore, the TTZ test showed that only a small fraction of the filled seeds were viable (stained), while the majority remained unstained. This indicates that the low germination rate was caused by a combination of low seed viability and deep physical dormancy protected by the seeds’ woody, spongy structure. These findings suggest that for this

Fig. 6. Santiği seeds suspected of being affected by fungus. A. Grayish black fungus; B. Blackened testa; C and E. white fungus; D. pink fungus)

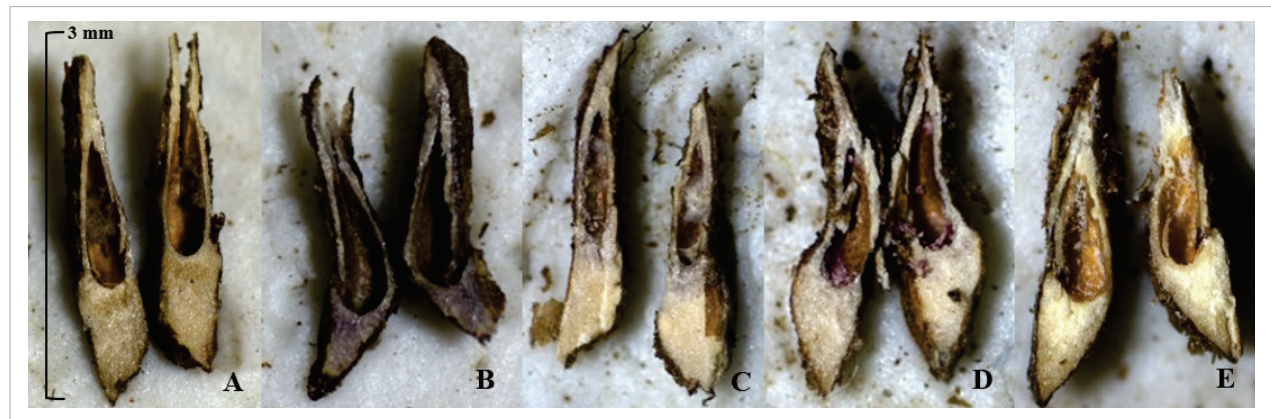


Fig. 7. Viability assessment tetrazolium test (TTZ). The staining confirms metabolic activity in a few viable seeds



specific batch, the lack of success was due to internal seed factors that the treatments could not overcome. Consequently, future conservation efforts must prioritize rigorous seed sorting and viability testing before applying propagation protocols.

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