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Research article

Seed Morphology and Adverse Effect of Seed Drying on Endemic and Endangered *Pinanga arinasae* Morphometric and Viability

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Abstract

Pinanga arinasae is an endemic and endangered palm from Bali, Indonesia. Despite its enormous traditional use, ornamental potential, and confined distribution, the study of this species, seed biology, still needs to be explored. This study aimed to describe seed morphology, morphometrics, and viability in fresh and dried *P. arinasae* seeds. The palm seeds were collected from the Bali Botanic Garden. Seed morphology description, morphometric measurement, and tetrazolium viability assay were conducted on fresh and dried *P. arinasae* seeds. The results indicated that *P. arinasae* obovoid shape corresponded with the species original description. The fresh seeds and embryos had length and width of 1.70 and 1.19, and 0.41 and 0.18 cm, respectively. The seed morphology was similar to palm seeds, with reticulating endosperm and a germinative button during early germination. Fresh seeds showed perfect viability on the tetrazolium test. However, the study highlighted the adverse effects of extensive drying on the *P. arinasae* seed morphometric parameters and zero viability after the tetrazolium test. This study provides baseline data for further research on *P. arinasae* seed biology to support its conservation and domestication effort.

Keywords: biometric; desiccation; palm; seed; TTZ

1. Introduction

Palms are highly diverse plants widely distributed in tropical and subtropical regions (Rizmasari et al., 2023; Couvreur et al., 2024). The diversity of palm species makes this plant group necessary for many aspects of human life, from utensils to construction (Dennehy & Cámara-Leret, 2019). Palm species such as date palm (*Phoenix dactylifera*) and *Arenga pinnata* are also used as human food sources (Zuhud et al., 2020; Al-Karmadi & Okoh, 2024). Numerous other palm species are also considered to be commercial ornamental plants (Spennemann, 2021).

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Despite their high diversity, distribution, use, and potential, palm species also face the threat of extinction. Previous studies found that hundreds of palm species are threatened with extinction (Bellot et al., 2022). Habitat loss, over-exploitation, and climate change are causes of palm extinction (Blach-Overgaard et al., 2015). This threat of extinction is magnified in endemic palm species as its low distribution makes it more susceptible to extinction. *Pinanga arinasae* Witono is an example of this endemic and threatened palm species.

Pinanga arinasae is a Bali Island endemic palm locally known as Nyabah and is considered an endangered plant on the IUCN Red List (Sutomo & Chadburn, 2021). Despite its endangered status, studies on this species are still very limited. Previous studies of *P. arinasae* focused only on the species distribution and ecology (Kuswantoro et al., 2018; Yudaputra et al., 2022), taxonomy, and genetic (Witono, 2003; Witono et al., 2024). In previous study, *P. arinasae* seed and germination traits were overlooked, and only one study mentioned the germination and seedling functional type of this palm species (Kuswantoro & Oktavia, 2019).

Seed biology is an essential subject of study as it can provide key information about palm propagation, including knowledge of palms such as *P. arinasae*. Seed traits such as seed and embryo biometrics and desiccation-tolerance are crucial to study. However, these two subjects still need to be popularly studied for many endangered species. Biometric study, for example, was conducted on ornamental plant *Dypsis decaryi* (Souza et al., 2023). Meanwhile, desiccation-tolerant studies were conducted in some commercial plants such as *Bactris gasipaes* and *Euterpe edulis* (De Andrade, 2001; Rodrigues et al., 2024).

The main aim of this study is to determine the morphological features of *P. arinasae*. The next objective is to measure the morphometrics and viability of fresh and dried *P. arinasae* seeds and embryos. This study will provide essential data for *P. arinasae* seed properties that support stakeholders in the plant species conservation and domestication effort through generative propagation and seed storage.

2. Materials and Methods

2.1 Study site

The seed collection and laboratory trials were conducted in Bali Botanic Garden from March to August 2024. The study site was located in Bedugul, Bali, Indonesia, at 8°16'42"S and 115°09'21"E, with an altitude of about 1,200 m above sea level (Figure 1A). Administratively, the study site was located in Tabanan Regency, Bali Province, Indonesia.

2.2 Seed collection procedure

Pinanga arinasae seeds were collected from fallen but intact fruits scattered under four plant specimens within Bali Botanic Garden in March 2024. The seeds were then transported to the botanic garden laboratory for cleaning and processing. On the same day of the collection, seeds were cleaned to remove debris, washed under running tap water several times, and dried using a clean cloth.

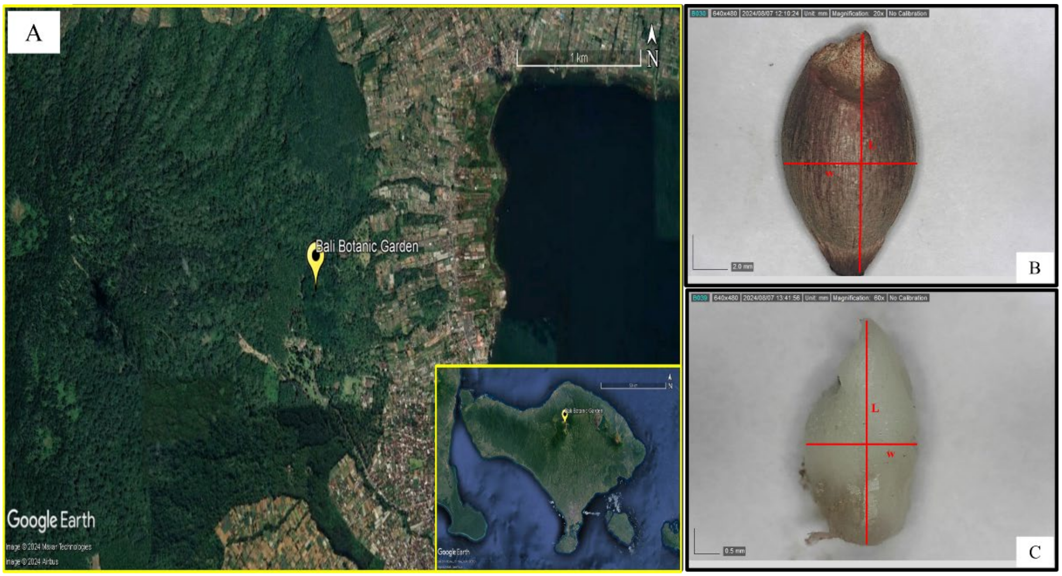

Figure 1. Study site and morphometric measurement of *P. arinasae* seed. A. Bali botanic garden location. Base map from Google Earth. B. Morphometric of *P. arinasae* seed and C. Morphometric of *P. arinasae* embryo (Note: L: Length and W: Width).

2.3 Descriptive analysis of *P. arinasae* seed

A descriptive analysis of *P. arinasae* seed was conducted by observing the morphological features of *P. arinasae* seed. Internal morphology was also observed by cutting the seed in half using a pruner. Documentation was performed using a digital camera and digital microscope (Dino-Lite AM3111/3113 Series). This work was conducted with ten seeds with no replication.

2.4 Seed morphometric measurement

The clean seeds were promptly de-husked on the same day as the harvest. These seeds were then separated into two groups of fresh and dried seeds. Morphometric measurements were conducted directly on the fresh seeds. However, due to seed number limitation, fresh seed moisture content was not measured. Meanwhile, the dried seeds underwent a drying process by placing the seeds in the laboratory for three weeks at 24˚C and 65% (±10%) relative humidity using a dehumidifier machine (OASIS D165 dehumidifier). The dried seeds were stored in seed envelopes at ambient temperature under laboratory conditions (23˚C±1˚C and 60%±5% relative humidity) for five months to study the effect of drying on seed viability. It was assumed in this procedure that seed viability would not decrease during the storage period, as a previous study reported that palm seeds started to lose their viability after six months of storage (Oliveira et al., 2015). After the storage period, seed morphometric measurement was conducted for the dried seeds. The morphometric measurement involved the collection of data on seed length and width (Figure 1B) using ImageJ software. Meanwhile, fresh and dry seed weights were measured using an analytical balance (RAPTOR SY-204). The seed morphometric measurement was conducted on a completely randomized design of three replications of twenty seeds.

2.5 Embryo morphometric measurement

Morphometric measurement of *P. arinasae* embryo was also conducted in this study (Figure 1C). Fresh and dried *P. arinasae* seeds were cut in half using a pruner, and then the embryo was carefully removed from the seed. The intact embryo morphometrics were then measured using the same procedure previously mentioned in the morphometric measurement of *P. arinasae* seed. Embryo morphometric analysis was also conducted in a completely randomized design with three replications of ten embryos.

2.6 Tetrazolium viability test

This study also conducted a tetrazolium (TTZ) test to assess the viability of *P. arinasae* embryos. Carefully removed *P. arinasae* fresh and dried embryos were immersed in 2 mL of 0.5% tetrazolium (TTZ) solution in a closed porcelain container and stored in a dark cabinet at room temperature for 4 h (23˚C±1˚C and 60% ± 5%relative humidity) (Belniaki et al., 2020). After immersion, embryos were rinsed three times with tap water and observed under a digital microscope (Dino-Lite AM3111/3113 Series). The TTZ test was implemented in a completely randomized design of three replications with ten embryos per replication.

2.7 Data analysis

This study employed both descriptive and statistical analyses. Descriptive analysis was conducted to examine the morphology of *P. arinasae* seeds, while statistical analysis was carried out for seed and embryo morphometric and TTZ viability tests. Statistical analysis was performed using Student's t-test in JAMOVI 2.3.15 statistical software (The jamovi project, 2022).

3. Results and Discussion

3.1 *Pinanga arinasae* seed morphology

Palms seeds showed remarkably diverse seed shapes, sizes, and features (del Pozo et al., 2020). *Corypha umbraculifera* and *Astrocaryum aculeatum*, for example, showed rounded seeds, while *Attalea maripa* showed an ovoid seed shape (Salm, 2005; Viji et al., 2015). Other species, such as *Johannesteijsmannia altifrons* and *Mauritia flexuosa*, showed a squared seed shape (del Pozo et al., 2020).

Morphological observation in this study revealed an obovoid seed shape in *P. arinasae*. The obovoid seed shape was also in correspondence with the reported obovoid shape in the palm's original description (Witono et al., 2002). However, this obovoid seed shape did not correspond with the seed shapes of some other *Pinanga* species, such as the ovoid seed shape of *P. subterranea* (Randi et al., 2023) and *P. egregia* (Fernando, 1994) or the ovoid to ellipsoid seed shape of *P. coronata* (Witono et al., 2002; Lestari et al., 2024). This highlights the seed shape diversity in *Pinanga* species.

This study also found that the ripe *P. arinasae* seeds had a black epicarp. The blackish color resembled the seed color previously reported from *P. coronata* (Lestari et al., 2024). However, the observed epicarp color differed slightly from the blackish-red fruit color mentioned in another *P. arinasae* description (Witono et al., 2002). The observed color difference might be due to the overripening process that had already taken place in the collected seeds used in this study, which were from fallen seeds. Color change during different stages of fruit maturation is widely documented in many plant species, including palms.

The seed morphology observation also showed a thin, brown, fibrous mesocarp and a hard, white-brown endocarp. The observed mesocarp and endocarp corresponded with the report of a ribbed stringy and woody fibrous seed surface in *P. coronata* (Lestari et al., 2024), a condition that might be attributed to the presence of a fibrous mesocarp and woody epicarp, such as those in *P. arinasae*. Figure 2 shows the epicarp, mesocarp, and endocarp of the *P. arinasae* seed observed during this study.

Figure 2. *Pinanga arinasae* seed external view showing Epicarp (left), Mesocarp (middle), and Endocarp (right)

The seed anatomical observation during this study also found a small, white, ruminate endosperm and a white, elongated, conical embryo in *P. arinasae* seeds (Figure 3). The observed endosperm rumination was consistent with the palm species original description, which mentioned that the palm seed was deeply ruminated (Witono et al., 2002). A ruminate endosperm is an irregular growth of endosperm tissue that occurs due to the seed coat's inward folds and invaginations growth (Norup et al., 2006; Gagul et al., 2018). Ruminate endosperm is widely observed in palm species, including *Pinanga,* such as *P. javana* and *P. coronata* (Witono et al., 2002; Norup et al., 2006). The ruminate endosperm is also observed in other plant groups, such as members of Elaeocarpaceae family (Gagul et al., 2018).

Pinanga arinasae embryo was attached to the operculum and protected by a brownish opercular tegument (Figure 3A). However, this study also observed that the opercular teguments in some seeds had already been removed by the growth of a germinative button through the operculum (Figure 3B). The germinative button is a part of the embryo that develops during the early germination process of palm species with adjacent germination types (Viji et al., 2015; Bastos et al., 2017). The presence of a germinative button in *P. arinasae* seeds was consistent with previous report of adjacent germination types of the plant species (Kuswantoro & Oktavia, 2019). Germinative button emergence in early palm germination was also reported in other species, such as *Oenocarpus bataua* (Bastos et al., 2017) and *Phytelephas macrocarpa* (Ferreira & Gentil, 2017).

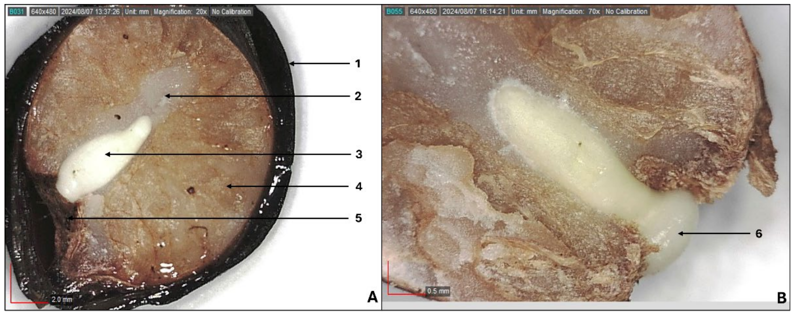

Figure 3. Longitudinal section of *Pinanga arinasae* seed. (A) Anatomical structure showing 1. Epicarp, 2. Endosperm, 3. Embryo, 4. Endocarp, 5. Opercular tegument, and 6. Button. (B) Close-up view of the germinative button

3.2 *Pinanga arinasae* seed and embryo morphometric

Morphometric measurements showed that fresh *P. arinasae* seeds were, on average, 1.70 cm long and 1.19 cm wide. Meanwhile, the average weight of 20 fresh seeds was 25.23 g. However, this study also found that drying significantly decreased *P. arinasae* seed morphometric parameters. After storage, measurement of dried *P. arinasae* seeds indicated that their average length and width were reduced to 1.50 cm and 0.88 cm, respectively. Seed weight reduction was also measured as the average weight of 20 dried seeds and was only 18.67 g. Statistical analysis of the comparison of the morphometrics of *P. arinasae* fresh and dried seed are presented in Figure 4.

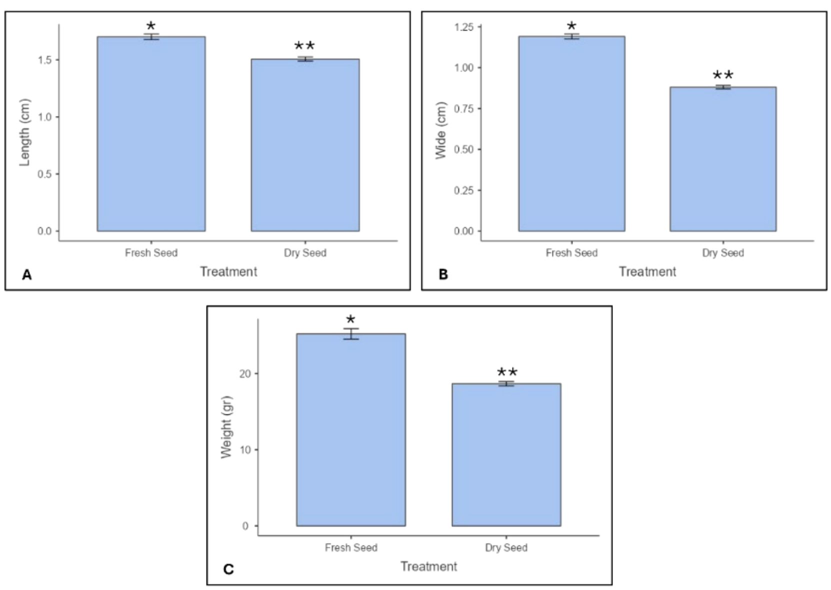

Figure 4. Morphometric measurements of *P. arinasae* fresh and dry seeds. A. Seed length; B. Seed width; and C. Seed weight. The graph represents the mean ± SE (n=3). Different asterisk numbers indicate statistical differences at $P < 0.05$ on the student's t-test.

This study's morphometric measurement also showed that, on average, *P. arinasae* embryos were 0.41 cm long and 0.18 cm wide. Meanwhile, the average weight of ten fresh embryos was 0.076 g. However, these morphometric parameters were significantly reduced in dried seeds. The dried seed's embryos were, on average, 0.21 cm long and 0.14 cm wide. Meanwhile, the dried seed ten embryo weight was, on average, 0.014 g. Statistical analysis of *P. arinasae* embryo morphometrics during this study are presented in Figure 5.

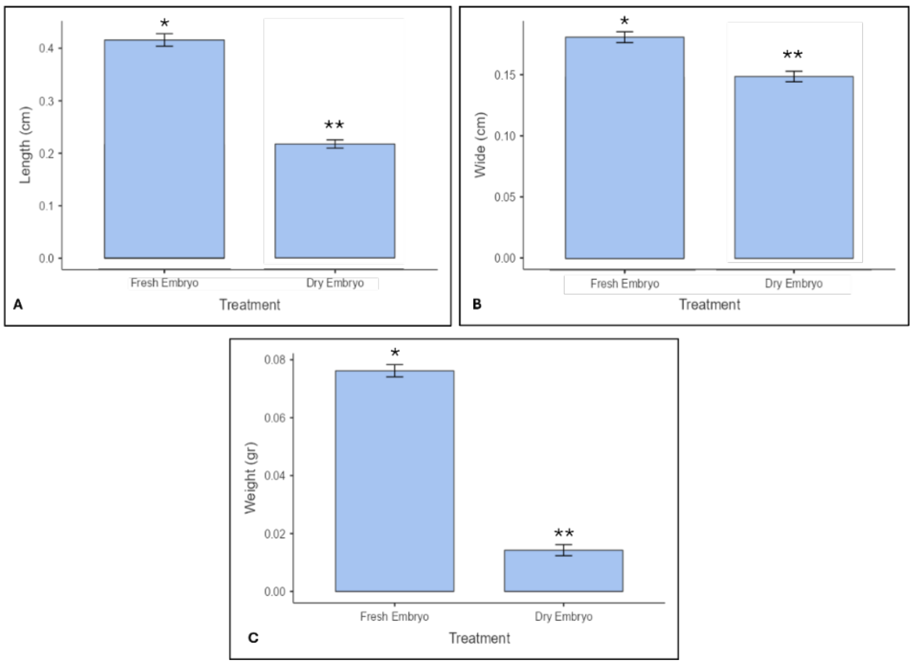

Figure 5. Morphometric measurements of *P. arinasae* fresh and dry embryos. A. Embryo length; B. Embryo width; and C. Embryo weight. The graph represents the mean±SE (n=3). Different asterisk numbers indicate statistical differences at $P < 0.05$ on the student's t-test.

Before this study, seed and embryo morphometric studies on *P. arinasae* were still absent in the literature. However, the palm's original description mentioned 1.2-1.7 cm long and 1.3-1.4 cm wide fruit measurement (Witono et al., 2002). The difference in fruit and seed measurement between this study and *P. arinasae*'s original description might be due to the seed's epicarp and mesocarp exclusion during this study. The measurement of *P. arinasae* fruit in the species original description possibly included these two fruit parts, resulting in slightly larger measurement values.

Embryo properties, including their measurement, are essential for seed viability and germination (Forbis et al., 2002; Perea et al., 2018). The close relationship between embryo properties and germination capacity is essential for plant propagation as plant germinability determines their seedling establishment. Thus, the provided data on *P. arinasae* embryo measurement is critical to supplementing palm seed biology data and supporting the species propagation effort.

The significant reduction in seed and embryo morphometrics after drying was due to the treatment's ability to remove water content. Water content is closely related to moisture content and critical for seed viability and storage (Ali et al., 2018; Paravar et al., 2023). Seed weight is a standard parameter used to assess seed moisture status (Hay et al., 2023). This study's findings aligned with previous concept of weight as a parameter to determine seed and embryo moisture content. Furthermore, the substantial seed length and width reduction observed in dried seeds suggest that these morphological traits could serve as complementary parameters to seed weight in the assessment of palm seed moisture content.

3.3 *Pinanga arinasae* embryo viability

The result of the TTZ test revealed that fresh *P. arinasae* embryos had 100% viability. However, the drying treatment completely reduced the embryo viability, resulting in zero viability after the TTZ test. The TTZ viability test results from this study are shown in Figure 6.

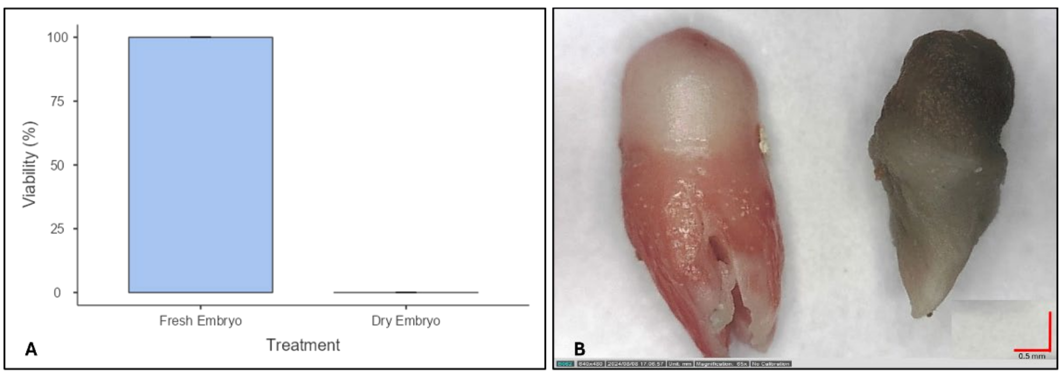

Figure 6. Viability test results of *Pinanga arinasae* embryos. (A) Viability percentage of fresh and dry embryos. (B) Morphological appearance of fresh (left) and dry (right) embryos after TTZ (triphenyl tetrazolium chloride) staining. Statistical analysis was not performed due to the lack of variation between treatments.

The complete viability loss in dried *P. arinasae* embryos was concerning. Biological and technical factors should be considered to explain this condition. Seed storage behavior involves a seed-drying-related biological factor that significantly affects seed viability. Three seed storage behaviors are recognized. These storage behaviors are the seed that can withstand and prolong its viability after drying (orthodox), the seed that cannot withstand and significantly lose its viability after drying (recalcitrant), and the seed in between (intermediate) (De Vitis et al., 2020). Meanwhile, technical factors such as the ideal moisture content to maintain *P. arinasae* seed viability and storage temperature and condition should also be considered. The loss of viability after drying highlights the desiccation sensitivity of *P. arinasae* seeds, indicating the recalcitrant storage behavior of this palm species. However, further study is needed to confirm this proposed storage behavior type.

Studies reported the presence of various seed storage behavior in palm species but agreed that palm seeds were drying-sensitive (Lan et al., 2014; Corbineau et al., 2023). Variation in optimum moisture content for seed viability conservation in different palm species was also reported. *Bactris gasipaes,* for example, shows 45% moisture content (Rodrigues et al., 2024), while *Euterpe edulis* shows 40% moisture content (De Andrade, 2001). The mentioned condition and the result of this study suggest the desiccation-intolerant nature of *P. arinasae* seeds and the need to determine an optimum drying method and moisture content for the seeds to prolong the seed viability as the implemented seed drying method during this study was inappropriate to achieve the goal.

3.4 Applied consequences of the study

The results of this study on seed morphology and morphometrics of *P. arinasae* provide us with further knowledge on this subject. Seed biometry is essential for understanding plant genetic and environmental variability (Almeida et al., 2024). This fact is relevant for *P. arinasae*, which, despite its restricted distribution in Bali, is also found in different locations at various altitudes (Yudaputra et al., 2022). This information is vital for propagation efforts to support *P. arinasae* conservation and domestication.

This study highlights the adverse effect of uncontrolled drying on *P. arinasae* viability. This condition allows further research on seed drying-related studies to maintain *P. arinasae* viability. Determining the palm seed storage behavior is the most pressing subject for this palm species and can be achieved using the 100-seed test method (Wardani & Mimin, 2020). However, because previous studies found varied optimum moisture content for palm seed viability conservation during storage, future research should also aim to determine the *P. arinasae* seed's optimum moisture content along with optimum temperature, oxygen levels, initial seed quality, and genetics as a fundamental consideration for developing seed conservation protocol of the palm species.

Along with a continued study on palm genetics and ecology, the proposed research on *P. arinasae* seed biology, concerning drying procedure, morphology, and morphometrics, is vital to the plant's future. Knowledge of seed biology is key to improving plant conservation both in in-situ and ex-situ conservation sites such as botanic gardens and seed banks. This knowledge is also essential for plant domestication to harness the plant potential beyond its traditional use, for example, as an emerging commercial ornamental palm.

4. Conclusions

This study confirmed the obovoid seed shape, reticulated endosperm, length (1.70 cm) and width (1.19 cm) as previously described in *P. arinasae*. Notably, it also provided the first recorded data on embryo morphometrics, with a length of 0.41 cm and a width of 0.18 cm. Drying could significantly decrease the palm seed and embryo morphometric parameters, including its ability to obliterate the palm seed viability. This result improved information regarding palm seed diversity, especially within the genus *Pinanga.* The provided data also supported *P. arinasae* propagation as generative propagation, which was heavily linked to seed characteristics, and was the primary propagative method of palm species.

The seed biology data in this study is integral to *P. arinasae* conservation and domestication. Seed biology data is essential for in-situ and ex-situ conservation of a plant species as it provides data on the plant propagation syndromes such as dispersal and dormancy. Furthermore, data on seed drying sensitivity is fundamental for seed banking conservation of the palm species. This data could also support palm domestication in enhancing its economic value by enabling efforts to provide robust, uniform, and easily accessible seed and plant material.

5. Acknowledgements

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6. Authors' Contributions

Dewi Nur Fauziyyah: Validating the methodology, conducting data acquisition, and writing the manuscript. Farid Kuswantoro: Conceptualizing the research, conducting data acquisition and statistical analysis, and writing the manuscript. Cokorda Istri Meyga Semarayani and Muhammad Bima Atmaja: Writing the manuscript.

7. Conflicts of Interest

We stated that no conflict of interest is present in this study.

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