Effects of Seed Storage Duration and Several Germination Media on Germination Rate of *Pinanga javana Seeds*

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ABSTRACT

The IUCN red listed *Pinanga javana* Blume, also known as Javanese Pinang, as a protected and threatened species that is endemic to Java island, Indonesia. This research focused on study of the germination and growth process of *P. javana* seedlings using four types of planting media: cocopeat, husks, sand, and sawdust. The results showed that *P. javana* seed water content slowly decreased from 33.96% to 24.45% during the storage period from day 0 to day 21. The planting medium had a significant influence on the germination rate, with sand exhibiting the highest value (2.34) and husks providing the best seedling growth. Cocopeat had the highest moisture level (90%), while sand generated the lowest moisture level. The pH of all media was within the normal range (6-7), which supports optimal germination. The analysis of various parameters concluded that sand was the optimal planting medium for *P. javana* in this study.

Keywords: germination, planting media, Pinanga javana, viability, water content

Introduction

Pinanga javana Blume, or Pinang Jawa, is an endemic palm species from Java island, Indonesia. This palm is known by the local names Njawar in Javanese and Hanjawar in Sundanese (Mogea, 1991; Alandana, 2015). The plant is usually found on hillsides and natural forests (Zulkarnaen *et al.* 2019; Zulkarnaen *et al.* 2020) and is often used as an ornamental plant. *P. javana* is included in the list of protected species by the Indonesian government (PP No. 106 Tahun 2018) and is listed as an endangered species according to the IUCN Red List 1994 (Khoshbahkt and Hammer, 2007).

The growth pattern of *P. javana* in its natural habitat follows a group distribution pattern, with morphological developments parallel to the Corner architectural model (Zulkarnaen et al. 2020). P. javana seeds are classified as vigorous seeds and exhibit hypogeal germination, with dispersal facilitated by civets (Zulkarnaen et al. 2020). In addition, the population structure of P. javana in Mt. Slamet, Central Java, shows dominance by adult individuals, with a preference for hillsides and specific classes of height and trunk diameter (Zulkarnaen et al. 2019). This species faces threats during the nursery stage, especially from predators, which has an impact on recruitment despite continuous seed production (Zulkarnaen et al. 2019). Understanding the morphology and ecological dynamics of P. javana is essential for effective conservation efforts

to conserve this unique species of palm. Conservation strategies of *P. javana* include obtaining information about seed germination and understanding the cultivation process and its natural life cycle

Pinanga javana is spread naturally through seeds. Seeds are the easiest natural medium to use to propagate common palm species (De Leon, 1958). The natural vegetative propagation in palms is rare (Silva et al., 2014). The germination process of *P. javana* takes 45 to 60 days. The long germination time is due to the hard endocarpal layer of palm kernels, thus inhibiting the germination process (Ferreira *et al.* 2021).

Conservation of *P. javana* needs to be carried out to protect its population in natural habitat through various conservation efforts. This research aimed to find the best germination media for *P. javana* to support its conservation.

Research Methods

Research on seed viability, germination speed, and growth of P. javana seedlings was carried out at the Biji Bank Greenhouse, Bogor Botanical Garden, Bogor, West Java, Indonesia. The greenhouse is located at S: 06 0 36'155", E: 106 0 47'825" with a location altitude of approximately 290 meters above sea level. Seedlings were taken from P. javana collection in Baturaden Botanical Garden, Central Java, Indonesia. Four media used in this study were cocopeat, husk, sand, and sawdust. The observation took four months.

Moisture Content Measurement

The moisture content of *P. javana* was measured before and after the storage process. In this study, the seeds were stored at a temperature of 103°C and dried for 17 hours to determine the moisture content. After the drying process, the seed sample was divided into three replicates, each consisting of 5 grams. The chamber was stored in a desiccator for 30-45 minutes for cooling, then the beans were weighed with the container (ISTA, 1993).

The moisture content was calculated in percent weight with the following formula:

$$Moisture \ content = \frac{M2 - M3}{M2 - M1} X100\%$$

Note:

M1: chamber weight with lid M2: chamber weight with lid and contents

before drying in the oven M3: chamber weight with lid and contents after drying in the oven (Draper *et al.* 1985)

Moisture content (MC) measurement was done every 2 days. The moisture content of 10 samples was measured with each sample containing 20 seeds. The average weight (X), standard deviation (S), variance (R), and coefficient of variance (KK) of the sample were calculated by the following formula:

Average weight (X)

$$X = \frac{\sum x_i}{n}$$

Standard Deviation (S)

$$S = \sqrt{rac{\sum (x_i - X)^2}{n - 1}}$$

Variance (R)

$$R = S^2$$

Coefficient of Variance (KK)

$$KK = rac{S}{X} imes 100\%$$

(Draper et al., 1985; Pramono, 2002)

n : the number of samples

Xi : the ith sample

Table 1 Weasurement results of various germination parameters in various media								
Various media	Sand	Sawdust	Cocopeat	Husk				
Total germination (DKT) (%)	23.33	18.33	5.00	18.33				
Normal germination (DKN) (%)	23.33	18.33	5.00	18.33				
First germination day	34	34	34	34				
The last germination day	64	58	48	60				
Germination rate coefficient	2.34	2.25	1.69	1.27				

Table 1 Measurement results of various germination parameters in various media

Seed germination rate

The sample seeds came from mature fruits, characterized by a hard structure and a black outer layer of seeds. The seed's length and diameter were measured using caliper. The outer layer of the fruit was discarded, and the same treatment was performed on all sample fruits. Observation of seed embryos was carried out using an optical microscope combined with the SZ-CTV optical camera. A total of 240 seeds were soaked in Dithane solution for 10 minutes and then planted in germination medium. The germination media used in this study were husk, sawdust, cocopeat. and sand. The seeds were divided into 3 trays, each consisting of 20 seeds with each replicate.



Figure 1 Reduction in moisture content (%) of *P. javana* seeds during storage.

Observations were carried out every day to record the germination process in various media. The total germination rate (DB) and germination rate coefficient were calculated using the following formula: Total Germination Rate (DB)

$$DB = \frac{\text{Total germinating seed}}{\text{Total seed}} \ x \ 100\%$$

Germination Rate Coefficient (KLP)

$$KLP = rac{\sum (G_i/T_i)}{\sum G_i} imes 100\%$$

Germination Coefficient (KP)

$$KP=rac{\sum (G_i/T_i^2)}{\sum G_i} imes 100\%$$

Information:

G1 : Number of seeds germinated at the third time

T1 : Time of the third day since planting

Observations were made to record seed germination in several media every day.

Results and Discussion

Moisture content measurement is an important factor in seed treatment. Moisture content affects physiological and biochemical processes (Stubsgaar, 1990, Poulsen, 1994). The initial moisture content of *P. javana* was 33.96%. During the storage process, the moisture content of the seeds slowly decreases from day 0 to day 21 (Figure 1). The moisture content dropped to 24.45% at the end of storage.

The moisture content of *P. javana* may play an important role in their survival and storage potential. Research on various palm species such as *Euterpe edulis*, *Pritchardia remota*, and palm oil (*Elaeis guineensis*) showed that different levels of moisture affect germination yield and seed storage. For example, *E. edulis* seeds retained viability when dehydrated to 35-30% moisture content, while further drying up to 24-18% led to loss of germination (Andrade, 2001). Embryo *P. remota*

Table 2 Data analysis of P. Javana Germination						
Source of variation	Free degree	Sum of squars	Mean squares	F calculate	F Table	
Block	2	2,5272	1,2636	0,18	5,21	
Media	3	106,7815	35,5938	4,94**	4,34	
Error	28	201,6177	7,2006			
Total	38	446.6944	,			

 Table 2 Data analysis of P. javana Germination

**Significantly different at 0.01 level test (F.hit > F Tabel

survived drying down to 0.16 g g–1 (–49 MPa) but perished with further drying (Pérez et al. 2012). If stored at room temperature or in a place with a lower moisture content after the harvesting process, the moisture content of *P. javana* seeds decreases naturally. A decrease in moisture content can cause the embryos in the seeds to die and the percentage of germination to reduce. In oil palm, moisture at 10-12% maintains seed viability during storage, while drier seeds experience a decrease in viability at colder temperatures (Ellis *et al.* 1991).

According to Sadjad (1980), the seed germination process is influenced by internal factors such as seed metabolism and seed composition, as well as external factors, namely environmental conditions. Seedlings that have high viability tend to produce saplings with a good level of adaptation to the surrounding environment. The seed germination process is often influenced by the size of the seeds and the type of medium in which the seeds are planted (Hendromono, 1996). Nurhasybi (1997) explained that the quality of the beans can be seen from their viability. The viability of seeds in the germination process greatly affects the number or percentage of saplings that can grow (Syamsuwida, 1997).

The highest germination power coefficient was found in the sand medium with a value of 2.34. Sand has a relatively low moisture content, which is likely to allow the seeds to absorb water more effectively. Sand can help stabilize the moisture content of seeds, which is important for the germination process (Sartika *et al.* 2023). Sand is considered important because of its role in improving soil structure, water retention, and nutrient availability, which are important for the early stages of plant growth and formation (Rofiq and Murniati, 2008).

The length of seed storage will have an impact on the speed of germination. The longer the storage, the germination power tends to decrease because the seed metabolism is slow. This results in a lack of energy and nutrients necessary for growth embryonic (Sutopo, 1984; Suripatty, 1993). Byrd (1983) stated that storing seeds for a long period can lead to degeneration of seed organs, which ultimately reduces their vigor and can even lead to seed death.

Prolonged storage can also reduce the survival of seeds because it can damage the plasma membrane, cause ion leakage, and interfere with the biochemical processes of the seeds (Hanson, 1983). In the context of this study, it is important to understand how each type of media affects seed germination and seedling growth of P. *javana*. The results of this study can be seen in Table 1, which presents a medium that had a significant effect on the seed germination rate of P. javana with an F value of 4.94. Observation of seedling growth started from the time of seed germination or occurrence of prospective shoots until the 100th day of observation (Fig. 2).

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Figure 2. Increase in the height of *Pinanga javana* seedlings in different planting media.

Based on Figure 2, the seedling height of *P. javana* shows a consistent increase over time on all types of media used. However, husk is the medium that provides the best seed growth. Planting media has an important role in supporting plant growth properly, especially if it can provide enough nutrients. Husks have several advantages, such as the ability to efficiently remove excess water, provide a soft soil structure, store water, have an ideal neutral pH for plant growth (6-7), and are rich in potassium (Fahmi, 2010).



Figure 3 Measurement results of (A) pH and (B) Humidity in various planting media *of Pinanga javana*

Figure 3A shows that the pH of the growth medium is not significantly different and remains within the normal range between 6 to 7, which corresponds to the germination needs of oil palm seeds. Different plant species exhibit varying responses to pH stress, with optimal germination rates often observed at neutral to slightly acidic pH levels (Wang et al. 2022). For example, Swiss radish (Beta vulgarise L.) seeds showed the highest germination rates at pH 6 and 7, while pH 2 significantly inhibited germination (Sisay and Fentaye, 2023). In addition, a study of Acinos alpinus subsp. meridional seeds revealed that a pH of 7 produced the best germination rate, while an extreme pH level below 3.5 inhibited germination altogether (Cherrate et al. 2023). Furthermore, the impact of acid rain on seed germination varied among tree species, with a pH of 3.5 identified as the threshold level for adverse effects on germination and emergence of seedlings (Gilani et al. 2021). These findings underlined the important role of pH in seed germination and highlighted the importance of maintaining appropriate pH conditions for optimal seedling growth and plant production.

The cocopeat medium shows the highest humidity level (90%), while the sand medium shows the lowest humidity level. The humidity level of the media might be affected significantly by the composition of that medium. Cocopeat has a high water storage capacity, allowing it to retain moisture for a longer period compared to the other media. On the other hand, sand has a high porosity so it cannot store water for a long period.

In seeds, maintaining optimal humidity levels during germination is essential for successful growth (Ahmed *et al* 2018; Fitriyah *et al.* 2021). Different weed plants and seeds showed varying responses to moisture levels, with some showing increased germination under certain humidity conditions (Das *et al.* 2020). The water content in the seeds, vacuole biogenesis, and the role of aqueducts in membrane water transport are key factors affecting seed viability and germination success (Obroucheva *et al.* 2017).

Conclusion

Moisture content in P. javana seeds plays a crucial role in physiology and biochemistry, as evidenced by the decrease in initial moisture content by 33.96% to 24.45% after several storages. During this process, the decrease in seed moisture content occurs slowly from day 0 to day 21. The importance of moisture content in seed management has been indicated by studies on various other palm species, such as E. edulis, P. remota, and oil palm, which showed that the moisture content affected viability and germination yield. Internal factors such as seed metabolism and composition, as well as external factors such as environmental conditions and planting media, play important roles in the germination process. Different growing media showed a significant effect on germination rates, with sand media standing out for its ability to maintain moisture content stability, while husk media offered ideal conditions with soft soil structure good and water storage capabilities.

Conflict of Interest

We confirm that we have no conflict of interest regarding financial, personal, or other affiliations with individuals or organizations related to the subject matter discussed in the manuscript.

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References

Ahmed S, Ullah Z, Zia-ul-Haq, Siddiqui MR, Latif S. 2018. Effect of moisture stress on seed germination and early seedling growth of pulse crops. *Pesquisa Agropecuaria Brasileira*. 7(2):775-782. doi: 10.19045/BSPAB.2018.70096

- Andrade, ACS. 2001. The effect of moisture content and temperature on the longevity of heart of palm seeds (*Euterpe edulis*). Seed Science and Technology. 29(1), 171–182.
- Alandana, IM, H. Ristiami, P. Widodo.
 2015. Inventarisasi palem di hutan bodogol taman nasional Gunung Gede Pangrango. Buletin Kebun Raya, Vol. 18 No. 2 halaman 81-98. e-ISSN: 2460-1519/p-ISSN: 0125-961x.
- Cherrate M, Radouane N, Ezrari S, Echchgadda G, Maissour A, Makroum Plavan G, Abd-Elkader OH. Κ. Bourioug M. 2023. Effects of Temperature, pH, and Salinity on Seed Germination of Acinos alpinus subsp. Meridionalis and FTIR Analysis of Composition Molecular Changes. Sustainability. 15(6):4793-4793. doi: 10.3390/su15064793
- Das MBB, Acharya BD, Saquib M, Chettri MK. 2020. Seed germination and seedling growth of some crops and weed seeds under different environmental conditions. *Journal of Research in Weed Science*. 3(3):363-381. doi: 10.26655/JRWEEDSCI. 2020.3.8
- Ellis R, Hong TD, Roberts EH, Soetisna U. 1991. Seed storage behaviour in Elaeis guineensis. *Seed Science Research*. 1(2):99-104. doi: 10.1017/S0960258500000726
- Fahmi, Z, I. 2010. Media Tanam Untuk Faktor Eksternal Yang Mempengaruhi Pertumbuhan Tanaman. Balai Besar Perkebunan Biji dan Perlindungan Tanaman Surabaya.
- Fitriyah H, Nugroho H, Widasari ER, Hahmanda H, Kusuma L, Chairurrafi M. 2021. Moisture Control using Linear Regression for Automatic Seed Germination Box on Rockwool. *Proceedings of the 6th International Conference on Sustainable Information*

Engineering and Technology. 162-167. doi: 10.1145/3479645.3479707

- Ferreira, KB, de Souza, AMB, Muniz, ACC, Pivetta, KFL. 2021. Germination of palm seeds under periods of rehydration. *Ornamental Horticulture*. 27(4): 446–452. doi: 10.1590/2447-536x.v27i4.2303
- Gilani MM, Tigabu M, Tigabu M, Liu B, Farooq TH, Haroon MU, Rashid, Ramzan M, Ma X. 2021. Seed germination and seedling emergence of four tree species of southern China in response to acid rain. *Journal of Forestry Research*. 32(2):471-481. doi: 10.1007/S11676-020-01102-0
- Kapasitas Hendromono. 1996. dan perkecambahan kecepatan biji Eucalyptus deglupta Blume pada berbagai ukuran butir sedang. Buletin Penelitian Hutan. 603/1996. Pusat Penelitian dan Pengembangan Hutan dan Konservasi Alam, Bogor.
- Mogea JP. 1991. Indonesia: Pemanfaatan dan Konservasi Sawit. WWF. Proyek 3325. Rotterdam- Belanda.
- Nurhasybi. 1997. Media kecambah dan cara penaburan damar (*Agathis loranthifolia* Salisb.). *Buletin teknologi biji*. Balai Teknologi Pembibitan vol.4 2 Bogor.
- Obroucheva NV, Sin'kevich IA, Lityagina SV, Novikova GV. 2017. Water relations in germinating seeds. *Russian Journal of Plant Physiology*, 64(4):625-633. doi: 10.1134/S102144371703013X
- Pérez HE, Hill LM, Walters C. 2012. An analysis of embryo development in palm: interactions between dry matter accumulation and water relations in Pritchardia remota (Arecaceae). *Seed Science Research*. 22(2):97-111. doi:10.1017/S0960258511000523

- Sadjad, S. 1980. Pedoman Perkecambahan Biji Tanaman Berkualitas Kehutanan di Indonesia. Dit. Jen. Kehutanan-Institut Pertanian Bogor. Bogor.
- Sartika S, Maemunah M, Adelina E. 2023.
 Pengaruh media perkecambahan dan sumber benih yang berbeda terhadap viabilitas benih manggis (Garcinia mangostana L.). Agrotekbis: Jurnal Ilmu Pertanian. 11(4): 938 947. https://doi.org/10.22487/agrotekbis.v11i 4.1816
- Sisay BT, Fentaye H. 2023. Effect of acid media on seed germination performance of Swiss-chard (*Beta vulgarise* L.). *Horticult Int J.* 7(1):9-12. doi: 10.15406/hij.2023.07.00269
- Wang Z, Tian S, Wang J, *et al.* 2022. Effects of pH and calcium salt stress on the seed germination performance of three herbage species. *Authorea* [preprints]. May 23,.
- Witono JR, Rondo K. 2006. Analisis Genetik Beberapa Spesies Pinaga (PALMAE) dengan Menggunakan Marka ISSR. *Berita Biologi* 8 (1):19-26.
- Zulkarnaen, RN, Nisyawati, N., & Witono, JR 2019. Studi populasi dan preferensi habitat Pinang Jawa (*Pinanga javana*) di Gunung Slamet, Jawa Tengah, Indonesia. *Biodiversitas Jurnal Keanekaragaman Hayati*, 20(3), 712-718.
- Zulkarnaen, RN, Nisyawati, & Witono, JR 2020. Pola pertumbuhan dan persebaran tanaman endemik Jawa (*Pinanga javana* Blume) di Gunung Slamet, Jawa Tengah, Indonesia. Dalam *Prosiding Konferensi AIP* (Vol. 2231, No. 1, hal. 040056). Penerbitan AIP LLC.