Effect of seed storage methods on germination growth of *Pericopsis mooniana* thw. through in-vitro technique

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Abstract. Nedun wood (*Pericopsis mooniana*) is one of the quality local wood species in the Wallacea region. The existence of kayu kuku in their natural habitat is vulnerable by overexploitation and habitat loss. The International Union for Conservation of Nature (IUCN) has declared kayu kuku as one of the endangered species. Tissue culture is one way to conserve genetic resources on micropropagation. The purpose of this study was to determine the effect of seed storage methods on nedun seed germination using in-vitro propagation. There are three methods of storage, which are stored with pericarp at room temperature (T0), storage with pericarp removal at room temperature (T1), storage with pericarp removal in 10°C (T2). The variables observed were germination rate, speed of germination, first day of root and sprouted, number of roots, length of root, and shoot. ANOVA analysis showed significantly different at shoot length. The duncant test results showed the highest shoot length at T2 (8.75 cm). The largest germination rate was found at T1 with an average of 82% and the highest germination speed was T1 (12.23% NG/etmal).

1. Introduction

Kayu kuku/nedun wood (*Pericopsis mooniana* Thw.) is considered as one of the quality and luxury wood products. This wood is a good substitution for teak wood due to the desirable physical and mechanical properties of the wood [1]. However, this high potential of nedun wood generates overexploitation that potentially decreases the population of nedun wood [2]. Categories nedun woods vulnerable species. Thus, the conservation efforts of nedun wood should be prioritized, like Jabon merah [3] and ebony [4].

Tissue culture is one of the notable techniques to conserve endangered and vulnerable species [5]. This technique is widely used in the conservation of endangered species since it can produce numerous seedlings in a short period [6] and preserve genetic materials (germplasm conservation) [7]. Material (seeds or non-seeds) storage is one of the essential aspects of tissue culture [8]. Seed germinations are necessary for growth and produce a new plant [9]. This is because the availability of seeds or genetic materials is infrequent in nature, especially for endangered species [8]. Further, the storage conditions, temperature, and moisture, also influences the seed viability [10], eventually affecting the success of in situ conservation. This study focused on the effect of seed storage methods on the successful of nedun germination through the in-vitro technique.
2. Materials and Methods
The study was conducted from February to April 2019 in Environment and Forestry Research and Development Institute of Makassar. Materials used in this study were nedun seeds that were stored for 3 years using 3 treatments, namely non-removal pericarp (T0), pericarp removal stored in room temperature (T1), pericarp removal stored at 10°C (T2).

In total, 120 nedun seeds were used, 40 seeds in each treatment. The seeds were sterilized and scarified using Nursyami procedure [11]. The seeds were cultured in Murashige and Skoog (MS) medium. The cultures were incubated at 25°C. Treatments were arranged in a completely randomized design (CRD) with forty replicates. The observation was made on germination rate, speed of germination, the first day of germination, first day of the rooted, first day of sprouted, number of roots, and length of shoot and root.

3. Results and Discussion

3.1. Effect of seed storage on germination rate
Seed storage with removal pericarp stored in room temperature (T1) showed the highest average in germination rate (Figure 1). In line with this result, the germination rate of Merbau seeds obtained the best result in seeds stored at room temperature [10]. Compared to T0 (non-removal pericarp), the average germination rate of seed storage with removal pericarp stored in room temperature (T1) increases 8%, whereas seed storage with removal pericarp stored in 10°C temperature decrease 2% in the average of germination rate. The lower germination rate in T0 might be driven by high moisture content due to the presence of pericarps. From moisture measurement, the moisture content of T0 (non-removal pericarp) was higher (10.75%) than T1 (6.07%) (Supplementary 1). A review [12] showed that low moisture storage is the best method to enhance longevity and the storability of orthodox seeds.

![Figure 1. The germination rate of nedun seed in three treatments](image)

3.2. Effect of seed storage on germination growth
The seed storage methods significantly affected the number of leaves and shoot length (P<0.05), Table 1). The seed storage with removal pericarp in 10°C showed the highest result in the shoot length (8.75...
cm) (Supplementary 2). This result might be driven by the difference in seed storage temperature. The low temperature could impede the decrease of amino acid content that is essential for the initial growth phase as accelerator seedling growth. A study [13] revealed that seed stored under room temperature had lower amino acid content compared to seed stored at low temperatures. Meanwhile, the lowest shoot length in T0 might be affected by high seed moisture content (Supplementary 1). A high concentration of moisture content triggers more intense metabolism activities [13] and consequently, more amino acid consumption as a source of energy [14]. Low amino acid content will decrease the germination ability since amino acids are an important factor to stimulate the growth of explants [15].

Table 1. Analysis of variance for the first day of germination, the first day of rooted, the first day of sprouted, number of roots, root length and shoot length for three seed storage methods.

| Source                        | Sum of Squares | df | Mean Square | F    | Sig. |
|-------------------------------|----------------|----|-------------|------|------|
| The first day of germination  | 20.272         | 2  | 10.136      | 3.025| 0.054|
| The first day of rooted       | 51476          | 2  | 25.738      | 2.147| 0.123|
| The first day of sprouted     | 70.707         | 2  | 35.353      | 2.455| 0.091|
| Number of Roots               | 43.299         | 2  | 21.650      | 0.624| 0.538|
| Root Length                   | 3.598          | 2  | 1.799       | 0.189| 0.828|
| Shoot length                  | 16.867         | 2  | 8.333       | 3.179| 0.046*|

Figure 2. Measurement of nedun shoot length

3.3. Effect of seed storage on germination speed

The speed of germination was recorded highest at T1 (12.23%) and lowest at T2 (7.22%) (Figure 3). This result might be driven by pre-treatment and moisture content. T0 pre-treatment, pericarp removal could drive the seed to uptake water rapidly from the media. This rapid absorption could decline the germination speed due to imbibitional injury [16]. Besides imbibition injury, moisture content and respiration also influence germination speed [17]. Meanwhile, the lowest germination speed of T2 was altered by the low seed moisture content (Supplementary 1). The study [17] showed that the germination speed of *Larix decidua*, an orthodox seed, decreases as a function of lowered seeds moisture content. Furthermore, the germination speed could also be influenced by seed extraction, storage condition, and shipment [17].
Figure 3. Average of germination speed in three treatments

Figure 4. Germination of nedun using micropropagation technique

4. Conclusion
The best method for storing nedun seeds was seed storage at room temperature. Seeds without pericarp stored at room temperature demonstrate the best germination rate and germination speed, 82% and 12.23, respectively.

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