The effect of various storage condition to maintain Macadamia (Macadamia integrifolia) seeds viability

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Abstract. Macadamia integrifolia is an exotic species in Indonesia. However, based on planting trials in Lake Toba, this species also well adapted on critical land dominated by Imperata cylindrica and produce high economic value fruits, so that potential as a forest and land rehabilitation tree species and community development. Unfortunately, the information on its seed handling for propagation still very limited. Therefore, our research was conducted to determine the effect of various storage condition to maintain the seeds viability. Research was designed using factorial in complete randomized design with three factors and 36 treatments. Each experimental unit consisted of 15 seeds. Results on variance analysis showed that, interaction of Natrium benzoate and charcoal powder treatment with different storage periods over 3 months (12 weeks) was significant on germination value and growth rate. The interaction effects with different storage conditions and different storage periods over 3 months was significant on 50 % limit of germination. During storage of seed, storage treatment and storage period play an important role in determining seed viability.

1. Introduction

Non-timber forest products (NTFPs) play an important role for the community’s forest income. However, NTFPs utilization in sustainable forest management schemes is still not optimal [1] one of which is macadamia. Macadamia (Macadamia integrifolia), has not been utilized optimally in Indonesia, whereas macadamia nut have high price for direct consumption and many utilization. Macadamia can be utilized as food combination, fuel, tannins, lipids, apiculture purpose, and essential oils. Macadamia oil also containing high antioxidant which is good for the skin [2]. Macadamia oil has a high value for cosmetics and can be used safely for cosmetic products [3]. The high economic and ecological values, fast growing character of this species, was potential to be developed for critical land rehabilitation, target trees for agroforestry schemes, including for bare lands around the Lake Toba catchment area North Sumatra, Indonesia [4].

Based on planting trials in Sipiso-piso Forest Research, Tanah Karo district (1,200 m.a.s.l), this species well adapted in imperata lands with low nutrient availability, high temperature, low humidity, and high light intensity. This tree also does not require intensive maintenance, not many leaves fall, do not easily die during a fire and easily germinate again after burning. Tolerance to fires is an important character considering that fires are events that often occur in this region. This species is also classified into non invasive trees, favored by the community and of high economic value [5]. Besides being adaptive on critical land, macadamia known as also edible fruit producer with high economic value.
However, to support macadamia development the information regarding on the characteristics of seeds and the their viability, especially its growth character in Indonesia was needed. Fresh macadamia nuts also have high water content which increases susceptibility to damage [6]. The seed viability is also easily lost in storage. This semi-recalcitrant seed germination is very erratic, low, and not simultaneous [7]. This study aims to determine the effect of various storage conditions to maintain the viability of Macadamia seeds. The data and information is expected to increase the successfulness of makadamia seedling supply for both forest and land rehabilitation programs and the development of seed sources.

2. Material and Methods

Fresh mature seeds were obtained from identified trees Macadamia at the Sipiso-piso Seed Orchard belongs to Aeknauli Forest Research Agency, Merek Subdistrict, Karo District, North Sumatra. The initial step of this research were selecting trees using to identified the fruiting trees. The fruit was directly harvesting from identified trees by threshing fruit, picking fruit, and collecting fruit on the forest floor. The research was conducted at the silviculture laboratory and seed germination tests were carried out in greenhouse of FERDA.

2.1 Seed extraction

Seed extraction is the process of removing seeds from fruit or other seed wrapping materials. Macadamia seed extraction process in the form of the activities of the release of the flesh and rind, cleaning, and drying.

2.2 Seed storage treatments

Fresh seeds without soaking and giving charcoal treatments (control) were put into airtight plastic. While the seeds treated with Natrium benzoate were first soaked in 3 g/l Natrium benzoate solution (3 g of Natrium benzoate dissolved in 1 liter of water) for 3 hours. After soaking the seeds were dried, then put in airtight plastic. The seeds with the charcoal powder storage treatment get 60 g of charcoal powder into it in an airtight plastic container. Furthermore, the seeds were stored in the storage room (room temperature and refrigerator) and various storage periods, namely 2, 4, 6, 8, 10, and 12 weeks.

2.3 Experimental design

The factorial with three factor was designed in completely randomized design (Table 1). The first factor was storage room (factor A), included storage in the refrigerator 4-8 °C (A1) and room temperature 17 - 25 °C (A2). The second factor was preservative (factor B) with 3 treatments including control (B0), charcoal powder (B1), and Natrium benzoate (B2). The third factor was storage period (factor C), with six level treatments those were 2 (C1), 4 (C2), 6 (C3), 8 (C4), 10 (C5), and 12 weeks (C6).

| Table 1. Matrix of experimental treatments |
|-------------------------------------------|
|   | C1    | C2    | C3    | C4    | C5    | C6    |
|---|-------|-------|-------|-------|-------|-------|
| B0| A1B0C1| A1B0C1| A1B0C3| A1B0C4| A1B0C5| A1B0C6|
| A1| A1B1C1| A1B1C2| A1B1C3| A1B1C4| A1B1C5| A1B1C6|
| B2| A1B2C1| A1B2C2| A1B2C3| A1B2C4| A1B2C5| A1B2C6|
| A2| A2B0C1| A2B0C2| A2B0C3| A2B0C4| A2B0C5| A2B0C6|
| B1| A2B1C1| A2B1C2| A2B1C3| A2B1C4| A2B1C5| A2B1C6|
| B2| A2B2C1| A2B2C2| A2B2C3| A2B2C4| A2B2C5| A2B2C6|

2.4 Seed germination

The seeds were germinated in polybags. For this purpose, basic media those were sand and soil was used. Before germinating process, medium sterilized firstly by roasting. The media was quantified
using 1:1 v/v ratio between soil and soil. The media were also spraying with fungicide *Dithane M-45* with a dose of 1 g/l.

### 2.5. Germination Assessment

Direct measurement and observation was conducted during germination process. The seeds have observed every day since germinating until all seeds germinate. The variables observed were germination rate, maximum growth potential, growth rate, T50 limit and germination value.

### 3. Result and Discussion

Macadamia fresh seeds (without storage and soaking) require 20 to 115 days to reach 100% germination. While the seeds stored for 2 to 12 weeks periods take a longer time, 23 to 150 days. This shows that *M. integrifolia* seeds need a long time to germinate. Other studies show that the germination process of macadamia seeds without scarification takes 45 to 140 days to achieve stable germination with a percentage of 71.5% [8].

The germination rate (GR), maximum growth potential (MGP), growth speed (GS), and germination value (GV) are not affected by a single factor of storage room (Table 2). This means that storage room does not affect seed viability. Storage conditions do not affect the germination of seeds for up to 12 weeks. This is due to macadamia seeds character with thick and strong shells so that seeds are not influenced by environmental conditions in certain periods. Furthermore, the factors that affect seed viability during storage are divided into two factors, namely internal and external factors. Internal factors include genetic traits, growth and vigor, skin conditions and initial seed content. External factors include seed packaging, gas composition, storage temperature and humidity [9]. The shells and skin thickness of the macadamia seeds are an advantage to survive before being planted.

#### Table 2. Variance analyses of storage parameter

| Factors                      | GR     | MGP    | GS     | T50    | GV     |
|------------------------------|--------|--------|--------|--------|--------|
| Environment of storage (A)   | 0.704* | 0.432* | 0.469* | 0.042  | 0.492* |
| Treatments of storage (B)    | 0.003* | 0.157* | 0.001**| 0.017* | 0.000**|
| Periods of Storage (C)       | 0.000**| 0.000**| 0.000**| 0.000**| 0.000**|
| A*B                          | 0.703ns| 0.500ns| 0.396ns| 0.020* | 0.639ns|
| A*C                          | 0.960ns| 0.592ns| 0.443ns| 0.612ns| 0.456ns|
| B*C                          | 0.183ns| 0.483ns| 0.003* | 0.207ns| 0.000**|
| A*B*C                        | 0.390ns| 0.549ns| 0.089ns| 0.067ns| 0.163ns|

Description:

****: very significant at 99% confident level  
*: significant at 95% confident level  
ns: not significant at 95% confident level

Seed germination decreases with increasing storage period. In a storage period 12-week, seeds by storing charcoal powder have a growth rate of 0.75%/etmal, lower compared to seeds by soaking Natrium benzoate, with a growth rate of 1.13%/etmal (Table 3). Further, untreated seeds (control) having a higher growth rate other treatments, namely 1.19%/etmal. In all treatments, the seeds soaked in Natrium benzoate generated the highest average growth rate in the 4-week storage period with a growth rate of 1.73%/etmal. This value is not significantly different from the growth rate of Natrium benzoate treatment in a storage period of 2 weeks. This shows that soaking with preservatives is only able to influence the growth rate in the storage period of 2 weeks and 4 weeks.
Table 3. Summarized of Duncan’s multiple range test to the effect interaction between treatment and periods of storage on growth speed of *M. integrifolia* seeds

| Treatment | Growth speed (%/etmal) |
|-----------|------------------------|
| B2C1      | 1.729a                 |
| B2C2      | 1.727a                 |
| B1C1      | 1.536ab                |
| B0C2      | 1.516ab                |
| B1C2      | 1.416bc                |
| B0C3      | 1.380bcde              |
| B0C1      | 1.317bcde              |
| B1C3      | 1.268cdef              |
| B2C3      | 1.246defg              |
| B2C4      | 1.216defg              |
| B0C6      | 1.194defg              |
| B0C4      | 1.142efg               |
| B2C6      | 1.133efg               |
| B1C4      | 1.033fg                |
| B2C5      | 1.025fg                |
| B0C5      | 0.999g                 |
| B1C5      | 0.983g                 |
| B1C6      | 0.750h                 |

Note: values followed by the same letters in the same column are not significantly different at 95% confident level in accordance to the Duncan’s multiple range test.

Furthermore, Seed vigor also decreases with increasing storage period. The germination value at storage period of 2 weeks storage period (C1) decreased dramatically from the initial storage period (0 weeks -C0). The seeds which immediately germinated (0 weeks) had germination value of 1.24. This value declined sharply to 0.61 in a storage period of 2 weeks, then decreases to 0.54 in the storage period of 6 weeks.
period of 6 weeks, to 0.36 in the 8-week period, and 0.29 at 10 weeks, and 0.25 in the storage period 12 weeks. This shows that the more storage time, the value of seed germination decreases.

The highest germination value occurred in the storage period of 2 weeks on the seeds soaked in sodium benzoate, with an average germination value of 0.80. Meanwhile, the lowest germination value was found in the 12-week storage period with the treatment of storing charcoal powder, with an average germination value of 0.19. Natrium benzoate increases the value of germination in a storage period of 2 weeks and 4 weeks when compared to seeds without a stored treatment in the same storage period.

Decreasing the value of germination is in line with aging of seeds during storage. This is caused by disruption of endosperm and metabolic processes in the seeds. This is in accordance with about aging of seeds that affect seed viability. Seed aging affects the accumulation of toxins as waste respiration, food reserve (endosperm) depletion, inactivity of DNA, RNA, metabolic systems and the occurrence of protein denaturation.

Seeds stored in charcoal powder have lower seed germination values compared to stored seeds without storage in a storage period of 6 weeks, 8 weeks, 10 weeks, and 12 weeks. This is because the charcoal absorbs the water content and minerals - which found in the seeds, which causes inhibition of seed germination [9]. If the seeds lack water, the metabolism stops. Considering germination occurs due to metabolic activity, if there is a water stress when the seeds germinate, the metabolism of the seed is disturbed so that germination is inhibited.

Table 5. Summarized of Duncan’s multiple range test to the effect interaction between environment and treatment of storage on 50% limit germination of M. integrifolia seeds

| Treatment | T50    |
|-----------|--------|
| A1B2      | 64,176 |
| A1B0      | 69,500 |
| A2B0      | 73,611 |
| A2B1      | 77,833 |
| A2B2      | 78,722 |
| A1B1      | 80,889 |

The T50% limit shows the ability of the seeds to germinate 50% of the total seedling, a seed boundary is categorized as still having good viability. Table 5 shows that the limit of 50% germination is influenced by the interaction of storage space factor and storage period, where fresh seeds with a storage period of 6 weeks (C0) can reach a limit of 50% on day 45. Seeds stored at room temperature and soaked with Natrium benzoate require 64 days to reach a germination limit of 50%, while seeds stored in the refrigerator need 79 days. This indicates that the refrigerator temperature can inhibit germination. In this case the low temperature inhibits enzyme activity so that the seed metabolism slows down and therefore germination is delayed.

4. Conclusion

Soaking Natrium benzoate increases the growth and germination rate of Macadamia seed in the storage period of 2 to 4 weeks. The use of charcoal decreases growth and germination rate in the storage period 6, 8, 10 and 12 weeks. Storage period can reduce germination, maximum growth potential, growth rate, and germination value. Storage period duration increases the limit of 50% germination (T50) along with increasing storage period. The results also showed that the seeds could still germinate normally up to 68.52% after 3 months of storage.

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