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RESEARCH PAPER

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Germination sensitivity of Candlenut (*Aleurites moluccana* Willd) on burning, sowing depth, and positions of seeds in the field

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Abstract

The objective of the research was to assess the germination sensitivity of candlenut on burning, sowing depth, and seed positions in the field. The method used was a factorial in a randomized complete block design. With the least significant difference level of 5%, the results showed that germination rates were significantly sensitive on the interaction of sowing depth and seed positions. The interaction of sowing depth of 4 cm and the face up position yielded the highest germination rates with the average of 36 days. The lowest ones were the seeds sown at the depth of 2 cm with the face-up position, on average of 58 days. The cotyledon opening rates were significantly sensitive on the interaction of the sowing depth and seed positions. The sowing depth of 6 cm with the face-up position yielded the highest cotyledon opening rates, on average of 46 days. The lowest ones were the seeds sown at the depth of 2 cm with the face-up position, on average of 64 days. The seeds that germinated earlier did not automatically open their cotyledons earlier. The highest seed germination percentage was yielded by the sowing depth of 2 cm, on average of 47%. The lowest one was the seeds sown 6 cm deep, on average of 34%. Recommended treatment was the combination of the seeds sown 4 cm deep with the horizontal position. Treatment of seed burning should be done before sowing the seeds or sowing the seeds at 0 cm from the soil surface.

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Introduction

Candlenut is one of the most domesticated multipurpose tree species throughout the tropics. The species can be used for various purposes; the seeds can be used as material for lighting, cooking and pharmaceuticals, and the trunk is used for timber (Krisnawati et al., 2011). In addition, the waste of candlenut from extracted oil is good for fertilizer because it contains 8.86% Nitrogen (N₂), 1.67% Kalium (K2O), and 1.02 Phosphate (P2O5) (Atjung, 1981). Besides, candlenut trees are useful for silvicultural practices. The candlenut trees that have reached five years old were effective (62%) to suppress the growth of weeds like alang-alang (Imperata cylindrica) (Agriculture Department, 1983). In agricultural systems, candlenut trees can be used as windbreaks, borders, shade, soil stabilization, and fallow improvement. In urban areas, the trees make attractive shade with their large leaves and white flowers (Krisnawati et al., 2011). Based on the information above, candlenut trees are very important to cultivate.

One of main problems of cultivating the candlenut trees is germination. This is because candlenut seeds have thick and hard seed coats that they are very difficult to germinate. According to Ochse et al. (1961), candlenut seeds have thick and hard seed coats, while Sin not and Wilson (1955) explained that one of factors causing dormancy is the hardness of seed coats that inhibits oxygen and water to infiltrate the seeds, and the seeds are impossible to germinate before absorbing water for metabolism and oxygen for respiration. O'Brien et al. (2013) found that seed coat thickness and toughness were two unmeasured traits that likely affected germination. The species with the longest days to germination regardless of seed mass had the thickest and most lignified seed coats. So, the species needed a softening or breaking for the radical to emerge. Li et al. (2010) found that the coat-imposed dormancy played a more important role in determining changes in seed germination among zovsia grass strains than physiological factors.

In this case according to Harjadi (1979), germination of seeds that have thick and hard seed coats can be triggered with scarification by means of softening the seed coats to enable gases and water to penetrate. In addition, Rosman and Djauhariya (2006) explained that to hasten the candlenut seed germination, the seeds were pressed gently into the soil and then covered by a layer of dried leaves or grass up to 3-10 cm thick. The grass was then burnt for about 3 minutes. Immediately after burning and whilst seeds were still hot, they were thrown into cold water, which made the hard shells crack. But again, the treatment did not include the sowing depth and positions of seeds in the soil. Also Tohir (1981) explained that for relatively big seeds, it is very important to consider the sowing depth and seed positions when sowing the seeds. Big seeds relatively need much water and oxygen in the soil, meanwhile positions of seeds affect the difficulties or the ease of the main radicle to reach the soil.

Based on the description above, it was presumed that there was interaction effect among seed burning, sowing depth of seeds, and seed positions on the candlenut seed germination. This interaction effect was not sufficiently studied particularly in the field, and therefore it was challenging to study the interaction effect. Main effect was analyzed except there was no interaction effect on a variable. In addition, this information will be very important to farmers for planting candlenut trees through direct seeding in the field.

Materials and methods

The research was done in the field, i.e. at the Seed Stand Development site in Takalar District South Sulawesi Province for four months. The observation was ended when there was no more seed germinating within the last two weeks. The research was conducted with materials and methods below.

Materials

Materials used were candlenut seeds from newly fallen ripe fruits collected at local community farming areas in Camba subdistrict, Maros district, South sulawesi province.

Another material used was grass (dominated by alang-alang) needed for burning seeds, while pesticide was for preventing germinants (geminating seeds) from pests like ants and mice.

Tools used were thermometers for measuring the air and soil temperature; hoes and crowbars for making planting holes; barbed wire and poles for fencing; scales for weighing dry grass/alang-alang; and hand sprayer for pesticide application.

Methods

Factors applied were seed burning (A) that consisted of 0.5 kg (A₁), 1 kg (A₂), and 1.5 kg (A₃) dry grass; sowing depth of seeds (B) that consisted of 2 cm (B₁), $4 \text{ cm } (B_2)$, and $6 \text{ cm } (B_3)$; and seed positions (C) that consisted of the face-up position (C1), horizontal position (C_2), and face-down position of seeds (C_3). The face-up position of seeds means the micropyle (the thin part of seeds) is facing up. Seed positions can be seen in Fig. 1. There were 27 treatment combinations, and each combination consisted of 10 seeds, and three replications.







Fig. 1. Seed positions.

Parameters observed were (1) seed germination rates, (2) cotyledon opening rates, and (3) seed germination percentage with a formula (Hartmann and Kester, 1978):

1. Seed germination rates (mean days)

- N1T1 + N2T2 + NxTx Total number of seed germinating
 - Nx: Number of seeds germinating at certain time. Tx: Time needed by a seed to germinate.
- 2. Cotyledon opening rates applied the same formula with the seed germination rates.
- 3. Germination percentage
- $= \frac{\text{Number of germinants}}{\text{Number of germinants}} \times 100\%.$ Total seeds sown

Procedures

Research procedures were started from laying down the candlenut seeds in planting holes with certain sowing depth and positions according to each treatment. Each hole was filled with 10 seeds with spacing of 5 cm x 10 cm. Number of holes were 81. The seeds that had been sown were burnt using dry grass above the holes.

Dry grass volume used depends on the weight of dry grass applied for each treatment. Each treatment was marked with a label.

Analysis

The experimental design applied was a factorial 33 in a randomized complete block design using the Statistical Package for the Social Sciences (SPSS) software. Significance effects among treatments were analyzed with the least significant difference (LSD) test at critical level of 5%.

Results

Seed germination rates

Based on the test between-subject effects (the F-test), seed germination rates were significantly sensitive on the interaction of sowing depth and seed positions (pvalue 0.00 < 0.05).

To search for which the treatment interactions that had significance effects on seed germination rates, they were tested with the LSD test as shown in Table 1.

Table 1. Germination rates of interactions of sowing depth and seed positions.

| Interaction | Germination Rate (day) | Mark |
|-----------------|------------------------|------|
| B2C2 | 35.78 | a |
| B2C1 | 41.56 | b |
| B3C3 | 41.70 | b |
| B3C1 | 41.99 | b |
| B1C3 | 43.56 | b |
| B2C3 | 45.57 | bc |
| B3C2 | 46.18 | bc |
| B1C2 | 50.17 | c |
| B1C1 | 58.08 | d |
| LSD 0.05 = 4.84 | | _ |

Note: different letters indicated significance differences among treatments.

Table 1 showed that the seeds that had the highest germination rate were the seeds sown at the depth of 4 cm with the horizontal position (B₂C₂). This treatment effect was significantly different from that of other treatments (LSD 5%). The lowest one was the seeds at the depth of 2 cm with the face-up position (B₁C₁).

Cotyledon opening rates

Based on the test between-subject effects (the F-test), cotyledon opening rates were significantly sensitive on the interaction of sowing depth and seed positions (p-value 0.00 < 0.05).

To search for which the treatment interactions that had significance effects on cotyledon opening rates, they were tested with the LSD test as shown in Table 2.

Table 2 showed that the highest cotyledon opening rates were obtained from the seeds sown at the depth of 6 cm with the face-up position (B_3C_1) . This treatment effect was significantly different from that of other treatments (LSD 5%). The lowest ones were the seeds sown at the depth of 2 cm with the face-up position (B_1C_1) .

Table 2. Cotyledon opening rates of sowing depth and seed positions.

| Interaction | Cotyledon Opening Rate (day) | Mark |
|-------------------------------|------------------------------|------|
| B ₃ C ₁ | 45.60 | a |
| B2C2 | 46.80 | ab |
| B3C3 | 48.67 | abc |
| B2C1 | 49.33 | abc |
| B ₃ C ₂ | 50.66 | bc |
| B1C3 | 52.20 | cd |
| B2C3 | 53.68 | de |
| B1C2 | 57.48 | e |
| B1C1 | 64.33 | f |
| LSD 0.05 = 5.05 | | · |

Note: different letters indicated significance differences among treatments.

Seed germination percentage

Based on the test between-subject effects (the F-test), there was no treatment interaction that significantly affected the sensitivity of seed germination percentage, but sowing depth treatment did it with p-value 0.00 < 0.05. To search for the treatments that had significance effects on the sensitivity of seed germination percentage, they were tested with the LSD test as shown in Table 3.

Table 3. Seed germination percentage of each sowing depth of seeds.

| Germination Percentage (%) | Mark |
|----------------------------|----------------|
| 47.00 | a |
| 45.11 | a |
| 34.13 | b |
| - | 47.00 45.11 |

Note: different letters indicated significance differences among treatments.

Table 3 showed that the highest germination percentage was gained from the seeds sown at the depth of 2 cm (B_1) that were not significantly different from the seeds sown at the depth of 4 cm (B_2) . The two treatments were significantly different (LSD 5%) from the seeds sown at the depth of 6 cm (B_3) .

Discussion

Based on Table 1, the seeds sown at the depth of 4 cm were quick to germinate because they might be quick to have favorable conditions of water and oxygen in the soil. According to Baker (1950), the seeds sown deeper would get better soil moisture because the effect of evaporation was relatively small. Furthermore, Baker (1950) explained there were some conditions that influenced seed germination, but the influence varied, depending on precipitation. Precipitation during observation varied from month to month. Precipitation at the first month after seed sowing was very low compared to the following months. As a result, soil surface was late to catch adequate water for seed germination. So it was considered that the seeds sown close to the soil surface (2 cm) were late to have favorable conditions to germinate. This was worsened by the face-up position of seeds, where the micropyle of seeds was very close to the soil surface. According to Wondimu et al. (2005), the rate and percentage of germination, and emergence and early seedlings growth of sorghum were significantly reduced by water deficit stress. Also, Severino et al. (2012) identified that germination percentage increased following the increments in soil moisture, and no evidence of caruncle influenced castor seed germination under low soil water content.

Likewise, the seeds sown too deeply (6 cm) also germinated late. It was considered that the germinants struggled to reach the soil surface. According to Harjadi (1979), the seeds with cotyledons emerge to soil surface (epigeous germination) like candlenut seeds should be sown more shallowly than the seeds with cotyledons remain in the soil (hypogeous germination). Furthermore, Kains and Mc Questen (1956), and

Christhoper (1958) stated that the seeds sown too deeply would cause the failure of germination because of limited oxygen, and inability of the germinants to penetrate the soil surface. Wilson and Loomis (1962) concluded that the failure of seed germination could be caused by the seeds sown at the soil surface did not absorb adequate water, and the seeds sown too deeply would have a food supply depleted before reaching the soil surface.

Besides the sowing depth of seeds, seed positions should also be considered. Karaeng (1982) have studied the effect of seed heating of 35°C and the way of laying the seeds in the soil on seed germination. Karaeng (1982) concluded that seed heat did not affect the seed germination solely, but it was supported by the presence of oxygen through the way of laying the seeds in the soil. In this point, it was considered that by sowing the candlenut seeds on different positions would affect the presence of oxygen around the seeds differently. Especially if the candlenut seeds were laid horizontally, this position would make the oxygen possible to be present around the seeds because the surface of candlenut seed coats has horizontal grooves. The horizontal grooves were not filled with soil but oxygen when candlenut seeds were sown at the horizontal position. That was the reason the candlenut seeds laid horizontally germinated earlier than those of other positions. This conformed to Liu et al. (2012) stating that removing pericarp of sharp tooth oak acorns could cause faster and simultaneous germination. Removing pericarp of the acorns also made the oxygen possible to be present around the acorns because the surface of the acorns without pericarp had also horizontal grooves.

However, the seeds sown at 6 cm in depth, dry endosperm covering cotyledons did not emerge from the soil, so that the cotyledons opened earlier than the seeds sown shallower. And this was supported by the face-up position of seeds. With this position, the cotyledons were easily released from the endosperm through the micropyle. Micropyle is the thin part of seeds where the hypocotyl pulled out cotyledons to the soil surface.

This conformed to Baker (1950) reporting that the germination of epigeous pine seeds sown shallowly, testae covering the seed coats were lifted to soil surface, but the seeds sown deeply, the testae were left in the soil. Based on these findings, it was concluded that the candlenut seeds that germinate earlier will not automatically open their cotyledons earlier, and this will retard the seedling to do photosynthesis. The reason was the cotyledon opening was determined by the ease of the cotyledons released from the soil and micropyle through sowing depth of seeds and positions of germinants in the soil.

Daniel, et al. (1979) explained that the need of seeds for oxygen varied, yet a problem often emerges if seeds were sown at the soil with poor pores or with high moisture. Might be oxygen was available sufficiently, but it would be limited if the seeds were sown too deeply. In addition, according to Baker (1950), germination was a result of great respiration. Without oxygen, mostly seeds were not able to germinate. This might happen if seeds were sown too deeply, particularly in wet soils. Moreover, precipitation during observation was getting higher from month to month. As a result, soil pores were filled with water, and oxygen became limited. At this situation, according to Harjadi (1979), a shallow seed sowing was needed.

In addition, the soil types at the research location are association of reddish-brown and dark-brown mediterranean soils with texture of silty clay loam. And according to Soegiman (1982), if the soil types contain much water, the soils become very sticky. In this case, Benvenuti (2003) identified that clay content of soils inhibited buried-seed germination. And Tohir (1981) stated that at sticky soils, seeds were not able to be sown deeply because generally the deeper the layer in the soil, the less air available. So it was considered that the seeds sown at the shallow layer (2 cm) absorbed sufficient water and air, but the seeds sown at the deeper layer (6 cm) did not absorb adequate air. This was supported by Awan et al. (2014) and Lu et al. (2012) stating that the deeper the seeds sown in the soil, the less the seeds to germinate. On the other hand, Tsuyuzaki (2006) stated that with increasing burial depth, diurnal temperature fluctuations decreased and seed survival increased. But Tsuyuzaki (2006) did not explained about the texture and water content of the soils.

From the three factors (A, B, and C), factor A (seed burning) did not affect the candlenut seed germination significantly. But Moreira and Pausas (2012) found that seed dormancy in all populations of all woody species tested was able to be stimulated with a single high temperature peak of 100°C, 120°C or 150°C through fire treatments for 5 minutes. Also, Gashaw and Michelsen (2002) identified that germination of most of the studied plant species in wooded savanna grasslands was able to be stimulated and enhanced with frequent and light burning. For the candlenut burning, it was considered that the heat from the burnt grass did not optimal to soften and crack the candlenut seed shell. It was presumed that the soil layer of 2-6 cm thick insulated the heat. Moreover, the candlenut seeds have thick, hard, and rough shell that made the seeds difficult to crack (Elevitch and Manner, 2006). In addition, Hanley et al. (2003) found that the larger seeds sown deeper than the smaller ones were likely to be insulated by a deeper soil covering against high above-ground temperature.

Conclusions and recommendations

Sowing candlenut seeds at the depth of 4 cm with the horizontal position yielded the highest germination rate. Sowing candlenut seeds at the depth of 6 cm with the face-up position yielded the highest rate of cotyledon opening. This meant that the seeds germinated earlier did not automatically open their cotyledon earlier. Sowing candlenut seeds at the depth of 2 cm yielded the highest germination percentage. For planting candlenut seeds directly in the field, it is recommended to apply planting depth of 4 cm with a horizontal position.

For sowing candlenut seeds at a nursery, it is recommended to sow the seeds at the depth of 2 cm with a horizontal position because soil water and other conditions can be controlled.

Because seed germination was not sensitive enough on seed burning treatment, it is recommended to burn the seeds before being sown or to sow the seeds at soil surface (o cm deep). Water content of the dry grass/alang-alang should be measured before being applied for burning the candlenut seeds. It is also possible to use duration of seed burning instead of dry grass weights for seed scarification. For comparison with the research conducted in the field, it is suggested to do the same research in a nursery or in a greenhouse.

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