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The Effects of Scarification on Seed Germination of Porang (*Amorphophallus muelleri*)

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Abstract. Porang is a member of the genus *Amorphophallus* that has high economic value because of its glucomannan content. Glucomannan is a fiber carbohydrate that is beneficial for health, for example, it can be used to control obesity. Currently, the cultivation area of porang is increasing. However, the availability of seeds is not sufficient for land expansion. Planting materials that may be supplied in large quantities are seeds rather than bulbil or bulbs. Disadvantages of seeds are less uniformity and the long time for its germination. Therefore, it is necessary to have germination by scarification and non-scarification treatments simultaneously. Scarification includes physical (sandpaper or nail clippers) and chemicals (1% H2SO4) scarifications. Non-scarification treatments include a soak in 1% KNO3, 0.1% GA3 or 1% Ethrel and exposure to light (7 x 24 hours) or dark (7 x 24 hours). The experiment was designed using Randomized Controlled Designs (RCD), with three replicates per experiment. Each replicate consisted of 25 seeds. The parameters included percent germination, hypocotyl length, and number of rooted sprout. The data were analyzed using ANOVA and LSD (0.05). The results showed that physical scarification (nail clippers) resulted in 100% germination, highest hypocotyl length, and highest rooted root. Dark and light treatment gave almost equal results in a percentage of germination, hypocotyl length and number of rooted sprout. Under the treatment of GA3 and Ethrel, Ethrel gave all the highest observed parameters.

Keywords: *Amorphophallus*, ethrel, glucomannan, porang

INTRODUCTION

Porang (*A. muelleri*) is a forest tuber that has high economic value, 1 kg of fresh *A.muelleri* was 4000 IDR in 2017. Tubers that are ready to harvest weigh arround 3 kg. The yield of porang per hectare is a minimum of about 6 tons,[1] calculation per gross hectare from the sale of *A. muelleri* is 24,000,000 IDR or $1755.67 US ($1 = 13670 IDR). To date, *A. muelleri* tubers have been in high demand by Australia and East Asian countries including Japan, China, Taiwan and Korea because *A. muelleri* contains nutraceutical compounds, e.g., glucomannan. Glucomannan is known to control obesity and type 2 diabetes,[2] lower total cholesterol, triglycerides and LD cholesterol help with weight loss and overcoming constipation by decreasing fecal transit.[3-5] According to *A. muelleri* collectors, the demand for *A. muelleri* increases from year to year. To meet market demand, the forest service, as the official representative of the government, allowed the use of secondary forest land to be planted by farmers with a resource sharing scheme. Unfortunately, the expansion of the land was not matched by an adequate amount of seed material. Seeds were chosen as a starting material with the consideration that seeds can be provided in large quantities in a short time. Seed materials of *A. muelleri* include tuber, bulbil (aerial tuber) and seed. Among the three seed

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materials, seeds are more beneficial as planting material because of number; one individual plant can produce about 300 seeds.[6]

A.muelleri is a unique plant, it has a resting period from collapse time until the beginning of the rainy season, with a duration of 4-6 months.[7,8] The rest period applies to tubers, bulbil, and seeds. It is known that some treatments can shorten the rest period or dormancy of the seed or tuber. Physical treatment by immersion in hot water, 80°C, for three days resulted in increased germination compared to controls for palm kernels.[9] The combination of scarification treatment using sandpaper and 60°C immersion for 10 minutes produced 100% germination of palm seeds.[10] Applied a chemical treatment in the form of 1% KNO3 for 48 hours to hasten germination of gogo paddy seed varieties Kalimutu, Gajah Mungkur and Way Rarem, showing it appears to be a sufficient concentration for germination.[11] This was also shown in the 100% germination of Angsana seeds after the seeds were soaked for 24 hours in 1% KNO3 while soaking in 1% H2SO4 required a shorter soak (10 minutes) to achieve 100% germination.[12] Gibberellin or ethylene phytohormone treatment has a positive effect on germination. The commonly used gibberellin group is GA3, while ethylene groups can be Ethrel, calcium carbide (CaC2) or Ethephon. Giving 500 ppm of GA3 for 24 hours resulted in 57.33% germination of *Calopogonium caeruleum* while the control was 44.00%.[13] While soaking teak seeds in 10 ppm GA3 for 24 hours resulted in 60% germination.[14] Treatment with GA3 (500 ppm) or Ethrel (25 ppm) on sunflower seeds produced different germinations, resulting in 80% and 55% germination, respectively. In promotion of germination, GAs act by activating hydrolytic enzymes,[15] while ethylene generally encourages germination by lowering the reactive oxygen species in stress-bearing seeds.[16] For certain seeds derived from the forest, light is needed to encourage germination. The percent germination of forest seed varieties depends on seed size.[17] Also, seeds of the prairie in the mid-continental USA require light to germinate.[18] Because using seed materials of *A. muelleri* has a constraint, i.e., a prolonged resting phase, in this study a treatment was sought that affected the germination of *A. muelleri* seeds.

MATERIALS AND METHODS

Materials And Preparation Research

Porang (*A. muelleri*) seeds used in this researh were freshly obtained directly from *A. muelleri* fruits. The seeds were taken from fruit, flesh of the fruit was removed and they were air dried. Seeds of equal size were choosen as planting material. The germination tray was washed, air dried and wiped out with 70% ethanol. Straw paper used as germinating media was cut to a size of the equal width of the tray. The paper was sterilized using an autoclave at 1 atm for 15 minutes. Aquadest, which was used to moisturize the germination media, was autoclaved as well.

Germination

The germination method used was the test method on paper. Twenty-four germination trays (17.5 X 22.5 cm) were prepared. Each tray was layered with eight sheets of straw paper. Each tray was filled with 25 sterilized seeds to get treatment (Table 1). To maintain moisture conditions, each tray was covered with plastic wrap.

TABLE 1. Treatment that was applied to the A.muelleri seed

Kind treatment	Duration/ amount treatment	Incubation after treatment
H_2SO_4 1%	10 minute	RT
Sandpaper	10 x rubs, at the distal region	RT
Nail clipper	10 x cuttings, at the distal region	RT
GA3 0.1%	10 minute	RT
Ethereal 1%	10 minute	RT
KNO_3 1%	10 minute	RT
Light	24 hours 7 days	RT
Dark	24 hours 7 days	RT

Seed Sterilization

Dried fresh seeds were soaked with chlorox (commercial sodium hypochlorite) for five minutes then rinsed twice with sterile water for five minutes each. The next step was a short soak with 70% alcohol followed by a rinse with sterile water two times for five minutes each. At this point, the seeds are ready to be treated.

Experimental Design And Data Analysis

This study was performed using a completely randomized design with three replicates of each treatment. Each replicate consisted of 25 seeds, which represents one tray. The data obtained was analyzed by variant. If the ANOVA result was real/significant, then LSD test (0.05) was used to determine differences between treatments. Parameters measured included: germination power, expressed in percent (Formula 1); the number of sprouts that produce roots (Formula 2); and the length of the coleoptile. Before calculating the germination power, the germination criterion was determined. Germinated seeds were characterized by the appearance of a coleoptile at least 1 mm long (Fig. 1A), whereas sprout growth was measured by measuring the length of the coleoptile. The length of the coleoptile was measured from the 'crown' area to the end of the coleoptile (Fig. 1B).

FIGURE 1. Germinated seed with 1 mm coleoptile (A), Length of coleoptile was measured from crown until coleoptile tip (B*)

$$\text{percent germination } (\%) = \frac{Germinated\ seeds}{Total\ seeds}\ x\ 100\% \quad (1)$$

$$\text{rooted sprout } (\%) = \frac{Number\ of\ rooted\ sprouts}{Total\ no.of\ germinated\ seeds}\ x\ 100\% \quad (2)$$

RESULTS AND DISCUSSION

Apparently, in this study *A. muelleri* seeds did not show a dormancy phenomenon, the seeds began to show signs of germination on day three after they were planted in germination trays. The number of seeds that showed germination marks varied in each tray and one tray never showed 100% germination. Finally, percent germination was counted three weeks after planting in germination tray. The results were not significantly different (Figure 2). Scarification treatments (H_2SO_4, sandpaper or nail clipper) produced more sprouts than non-scarification treatment (Fig. 2). By applying scarification the seeds can access more water and oxygen, or in other words, the seed coat is more permeable to water and gaseous oxygen.[19,20]

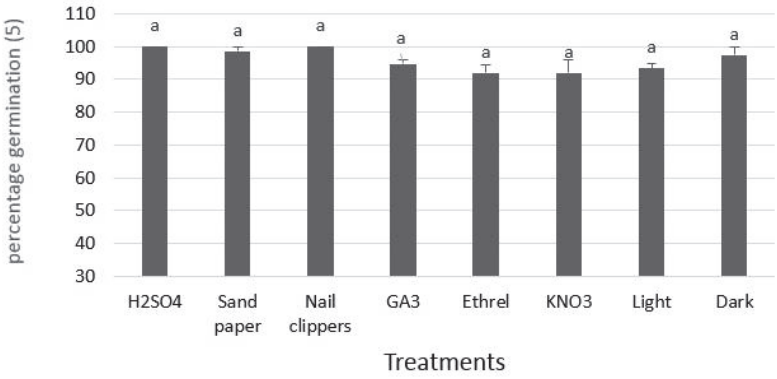

FIGURE 2. Percentage germination of A.muelleri seeds that were treated with scarification and non-scarification treatments.
note: The same letters show no significant difference based on LSD 0.05 test

With adequate nutrients from the degradation of food reserves, the growth of sprouts becomes more rapid than that of limited nutrient supply. Nutrient adequacy is reflected in the growth of the coleoptile and the appearance of the root sprouts. It turned out that from eight treatments, the treatment of cutting the seed coat with nail clippers resulted in the most extended coleoptile growth and most root sprouts (Fig. 3, 4 and 5A).

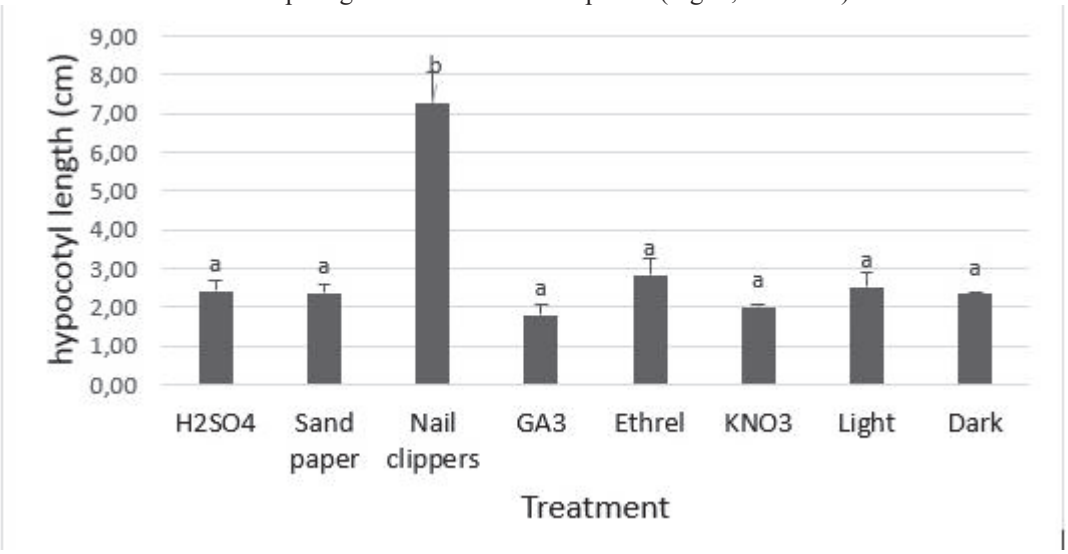

FIGURE 3. The effect of 8 treatments to hypocotyl length.
note: The same letters show no significant difference based on LSD 0.05 test

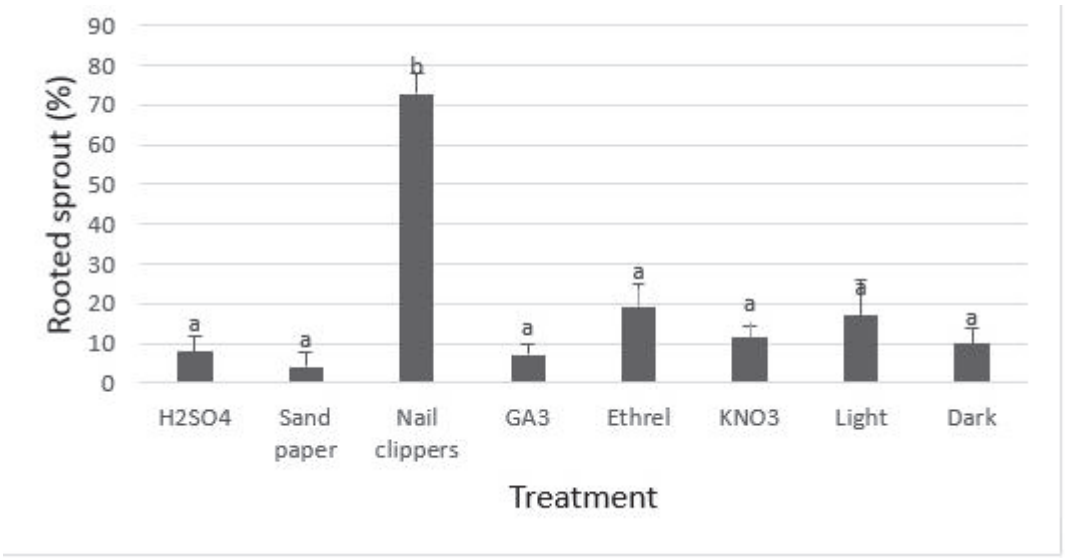

FIGURE 4. The effect of 8 treatment to rooted of sprout
note: The same letters show no significant difference based on LSD 0.05 test

From Fig. 3 and 4 above, we determined that germination of A.muelleri not be affected by light. Light and dark treatments gave similar effects. From these data, we can say that A.muelleri is not a photoblastic seed. Photoblastic seeds need light to germinate, in the dark, they do not germinate.[21] A.muelleri is a native forest plant. Forest plants that have small seeds need light to germinate.[17] The absence of light effects on A.muelleri seeds is an indicator that A.muelleri is a large-seeded plant.

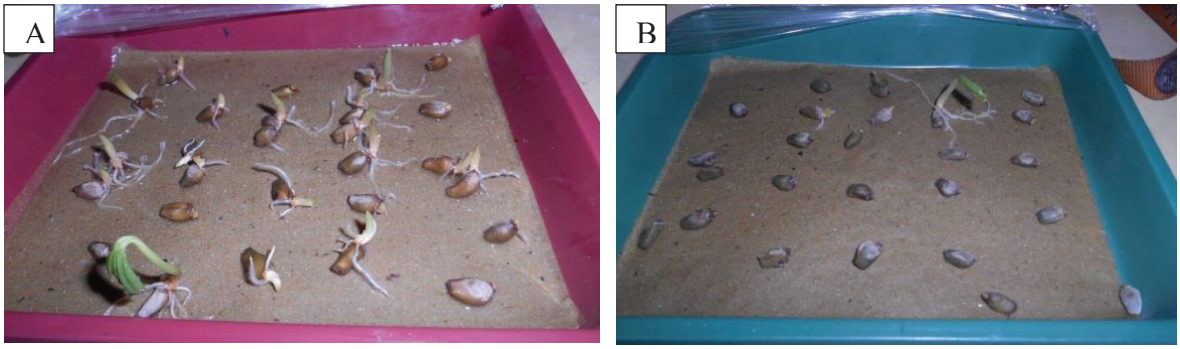

FIGURE 5. Effect cutting by nail clippe (A) dan sandpaper (B) to rooted sprout

In this experiment, commercial Ethrel, available in farm shops, had a positive effect on coleoptile elongation and ability to sprout roots than other physiological treatments. Ethrel is a chemical that can release ethylene, and usually is used to speed up the ripening of fruit.[22,23] A previous study successfully promoted ripening of guava with Ethrel up to a concentration of 1000 ppm.[24] Although the primary target of ethylene is to stimulate the ripening of fruits, another study showed that ethylene could stimulate germination (Lactuca sativa cv 'Grand Rapids').[25] Therefore, the results of research showing that Ethrel tends to hasten the germination of *A. muelleri* seeds can be accepted and understood. Furthermore, the results of another study showed that ethylene also plays a role in overcoming dormancy and working synergistically with kinetin to increase germination compared to the combined effect with GA3.[26]

All of the experiments previously done produced the idea to combine physical scarification (in this case the use of nail clippers) with the use of plant hormones (Ethrel) to encourage the germination and growth of *A. muelleri* seeds. The use of nail clippers produced high germination power, long coleoptiles, and almost all sprouts had roots (Fig. 3, 4 and 5A). The use of Ethrel provided a favorable physiological effect, a study showed that Ethrel could overcome obstacles of germination such as dry stress or high temperatures (outside the temperature of tolerance).[26]

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