The effect of the container for tpm (top of paper method) test on germination and infection rate of porang (Amorphophallus muelleri) seeds

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Abstract. Now, the need for porang seedlings is very urgent, so it is necessary to provide seedlings in a short amount of time. Among the sources for propagation of porang, the use of seeds as planting material promises to supply large quantities of seedlings. The initial step of preparing the seedlings is germination. The success of germination determines the next step. The purpose of this study was to examine the effectiveness of using trays and jars in reducing infection rates and to determine the rate of germination of porang seeds. This study used a complete random design with 3 replications. Each replication consisted of 50 seeds. While data analysis used unpaired t-test. And the germination method used the top-on-paper method (TPM). The observed-parameters included germination rate at 7 days after planting (dap), 17 dap, and 30 dap; percentage of seed contamination at 7 dap and 11 dap; the percentage of the number of sprouts rooted at 28 dap and 42 dap. The results showed that the percentage of germination using trays and jars at 7 dap, 17 dap, or 30 dap were not significantly different. Seed contamination at 7 dap and rooted-sprouts at 28 dap were not significantly different between tray and jar. However, for seed contamination at 11 dap, the tray produced higher contamination than the jar and the results were significantly different. As for the rooted sprouts at 42 dap, the trays produced lower rooted sprouts than the jar, 44.67% and 89.33% respectively and significantly different. The conclusion of this research was that germination on top-paper method using a jar was better than a tray

Keywords: tray, jar, test on paper, sprouts, contamination, rooted

1. Introduction

Porang (Amorphophallus muelleri) is a native plant of Indonesia, family Araceae. The specialty of A.muelleri compared to other members of the genus Amorphophallus, besides A.konjac, is that it contains quite high glucomannan. Glucomannan is known to reduce body weight, decrease fasting blood sugar, triglycerides and LDL cholesterol[1-3]. As natural laxative, glucomannan improved colonic ecological constipated people, glucomannan can overcome constipation when glucomannan is reacted with alkali will produce film [4,5]. The glucomannan film is proven as wound dressing [6]. Because of a lot function of glucomannan in health, East Asian countries, such as Japan and China, look for alternative tubers of Amorphophallus besides A. konjac as a source of glucomannan. They chose A.muelleri because it is the only species from southeast Asia that has the highest glucomannan after A.konjac. From the Indonesian Quarantine Full Automation System (IQFAST) at Surabaya, export data from A.muelleri chips in 2017 and 2018 were 4.3 tons and 5.5 tons, respectively. Chips
exporter said that she can export 25-50 tons in one month to China [7]. Many requests from other countries came in for *A. muelleri* chips, but rejected by exporters because of lack of *A. muelleri* tuber supply. The high demand for tuber of *A. muelleri* caused extensive land expansion. Unfortunately the expansion was not balanced with the adequacy of the available seed-plant material.

Naturally, *A. muelleri* seed-plant material can be obtained from bulbil, small tubers, and seeds. Of the 3 sources of planting material, the most promising are seeds because the seeds provide the highest number of seed-plant material. However, the seeds cannot be planted directly on the land because of many disturbances such as birds and competition with weeds. Therefore, the seeds need to be germinated first to see the germination rate and seed health, followed by acclimatization to adapt the seedling to the near-field environment and get seedling with a certain plant height, and the last step is transfer to the field. Now we focussed on the first step i.e. germination. There are many methods of germination. For *A. muelleri* seeds, the appropriate germination method is on paper. That is because the seeds are of medium size and are numerous.

Research on the effects of paper types on top-on-paper tests has been widely carried out, for example testing the optimization of germination media in the viability test of lettuce and onion seeds, alternative paper substrates for testing seed viability by the method of testing on paper and research on the effect of tool or container has not been done yet [8,9]. On this occasion the effectiveness of the tray or jar against the germination and infection rate of seeds from *A. muelleri* will be tested as an initial step for the supply of planting material.

### 2. Experimental Details

#### 2.1. Materials and preparation research

*A. muelleri* fruit was obtained from farmer of Oro-Oro Waru sub-village in Sumberbendo village, Saradan sub-district, Madiun Regency, East Java Province, Indonesia. Each fruit was squeezed to get the seeds. The seeds were then washed, dried, measured by weight, length and diameter. The seeds had an average weight, length, and thickness of 190 mg, 10.78 mm, 4.19 mm, respectively. As a place to germinate, we use plastic jars with a diameter of 27 cm (diameter) X 6.5 cm (height) and plastic tray with and plastic trays of length, width and height of 30 cm, 23 cm, 4 cm, respectively. The germination plastic jar and tray were washed, air dried and then rubbed out with 70% ethanol. Straw paper that will be used as germination media was cut to the same size as the base of the jar or tray. The paper and plastic bags (which are used for bagging the tray) were sterilized using an autoclave at 1 atm for 15 minutes. Aquadest which will be used to moisturize germination media was autoclaved as well.

#### 2.2. Seed preparation and Germination.

Before the seeds were organized on paper test, the seeds were first sterilized using 0.5% Fungicide (score250EC, Syngenta) for 15 minutes, 25% commercial bleaching for 5 minutes and washed using sterile distilled water for 5 minutes each. This sterilize method was carried out based on preliminary experiment. 50 seeds were arranged aseptically in each tray or jar. Then the tray was put into a sterile plastic bag and sealed using a sealer, while the jar was closed using the lid and sealed using sticky tape. Seeds are ready to be observed. Each treatment (tray or jar) had three replications.

#### 2.3. Variable and analysis data

As free variable was tray and jar. While fix variable include Germination rate (%) at 7 - 21 - 30 dap (day after planting); number of contaminated seeds (%) at 7 dap and 11 dap; Number of rooted sprouts (%) at 28 dap and 42 dap. Germination (%) = (number of seeds germinated / total seeds) * 100%; Number of contaminated seeds = (number of contaminated seeds / total number of seeds) * 100%; Number of rooted sprouts (%) = (number of seeded sprouts / number of seeds) * 100%. The data obtained were analysed using unpaired t-test with 99% confidence level (P <0.05).
3. Result and discussion

*A. muelleri* seeds germinated starting 7 days after planting (dap) in both the tray and jar. The seeds were considered to germinate after appearing at least 1 mm of coleoptile length. The germination of 7 dap, 21 dap and 30 dap on the tray and jar was not significantly different (Figs 1A, B, C).

![Germination rate at 7 dap](A)
![Germination rate at 21 dap](B)
![Germination rate at 30 dap](C)
![Germinated seed](D)

**Figure 1.** Germination at 7-30 dap (A, B, C) and germinated seed (D). Note: Letters on the same image showed no statistically significant difference in the unpaired t-test α0.05.

*A. muelleri* seed germination with an average value of almost 100 percent indicated that the germinated seeds had reached physiological maturity [10].Although sterilization has been carried out, the seeds were still attacked by fungus that was marked white. The white indicated from hyphae on the surface of the skin of the seed coat (Figs 2A, B). Fungus attacks was occurred higher in the tray than in the jar (Figs 2 C, D). Fortunately, the fungus that attacks was not a pathogenic fungus. It seems that this fungus was a beneficial fungi. Ghorbanpour *et al.* (2018) stated that the function of beneficial fungi is by increasing induced systemic resistance (ISR) [11]. The evidence that attacks was not pathogenic i.e the seeds germinate close above 90% (Fig 1C.). Suppose the attack was a bacterium that causes rot, the seeds will experience softening and death. Such as *A. muelleri* tubers rot caused by *Bacillus altitudinis* and *Pseudomonas stutzeri* [12].
The success of a sprout to carry on its life is if the sprout has roots. Without roots, the sprouts will have difficulty carrying out their lives when transferred to the acclimatization media before being planted in the field. Of the two containers tested, it turned out that the jar produced rooted sprouts almost twice compared to container tray at 42 dap (Fig. 3B), while at 28 dap, the jar and tray were not significantly different, but the jar tends to produce more rooted sprouts.

It should be suspected that the jar was able to retain media moisture and oxygen higher than the tray, also technically the jar was easier to be maintained a sterile level. Bewley et al (2013) state that adequate water and oxygen are needed to continue the germination process, for example, root
lengthening. In addition, sprouts in the jar produced roots earlier than the tray. Sprouts in the jar produced roots at 24 dap, while a tray at 28 dap. Overall there were interesting things in the process of germination of *A. muelleri* seeds. *A. muelleri* seed was not marked by radicle production first but was marked by the appearance of coleoptile. In this study coleoptile came out at 7 dap, while radicles at 24 dap.

4. Conclusion

Jars and trays have almost the same effect in producing sprouts. However, the jar produces more rooted sprouts than the tray and encourages the emergence of roots earlier. The jars also allow microfungus hyphae to develop lower than the tray. Therefore to germinate *A. muelleri* seeds you should use a jar. And is wide opened to identify beneficial fungi that grew on the surface of the seeds.

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