Effect of 6-benzyl amino purine on the multiplication ability of shoots of various sizes of porang (Amorphophallus muelleri Blume) bulbils

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Abstract. Porang plant is very prospective and developed because it has high economic value. The high demand for exports has resulted in increased needs for seeds. Tissue culture using plant regulator 6-Benzyl Amino Purine (BAP) has been widely used for seed propagation. This study aimed to examine the effect of adding BAP on the multiplication of shoots at different bulbil weights. Bulbils (corm leaf) were used as explants and cultured on Murashige and Skoog medium containing BAP (according to treatment), 30 g L⁻¹ sucrose, and 2.5 g L⁻¹ phytagel. This study used two factors Completely Randomized Design with five replications. The first factor was concentrations of BAP i.e., 0.0, 1.0, 2.0 and 3.0 mg L⁻¹ and second factor was bulbil weight i.e., 0.25, 0.50 and 1.00 g. There was significant interaction at the number of buds and shoots, but not for other parameters. The results showed that bulbil weight had a significant effect on the number of buds, shoots, and plantlet height, while the concentration of BAP had a significant effect on all parameters. Bulbil size < 1 gram can reproduce seeds with the highest multiplication ability when 3 mg L⁻¹ BAP was given.

Keywords: in vitro culture, corm leaf, plantlet, propagation, seed

1. Introduction

Amorphophallus spp., a plant belonging to the family of Araceae, is currently in high demand for its glucomannan content so that it potential to be developed. The genus Amorphophallus in the world consists of approximately 200 species, but only three species have been cultivated in Indonesia. One of them is A. oncophyllus Prain ex Hook. f. synonym A. moelleri Blume [1]. Amorphophallus moelleri Blume, known as porang has the highest glucomannan content compared to the other two species. Glucomannan flour is potentially used as an essential material in food and industries.

The high demand on porang export products in recent years needs serious attention. To fulfill this demand requires support especially adequate planting material and improved cultivation technologies. Currently, the availability of planting material is the main problem in the expansion program. Due to the scarcity of planting material, the price of seeds and bulbil porang has increased considerably [2, 3].

Porang propagation is usually done both vegetatively (stem tubers and bulbil) and generatively (seeds). However, vegetative propagation using tubers need to be considered carefully because it can interfere with the production process. In addition, one stem tuber only produces one plant [2].

Propagation using bulbils (corm leaf) is very limited because each plant produces not many bulbils. Another problem in using bulbils is the non-uniformity of weight. Different sizes of the bulbs will also
lead to a different growth rate of the seeds; larger bulbil grows faster. Previous researches suggested the use of bulbils measuring more than 5 grams for seeds [4, 5].

The use of seeds is an alternative to getting adequate seeds. However, seeds are produced by plants that are older than three years old (Year 4), while most plants are harvested at 3 years old. Thus, seeds are rarely obtained from farmers [6].

In vitro culture techniques are an alternative solution to produce porang seeds in large quantities with good qualities to overcome these problems. The vegetative parts of plants are grown under aseptic conditions to reproduce and grow into complete plants. This technique is fast, the seeds produced are uniform, free of pest and disease, do not interfere with the crop yields, and can meet demand in large quantities.

In technique in vitro culture, plant growth regulators have a very important role. The use of growth regulators in vitro culture depends on the intended purpose or direction of plant growth. 6-Benzyl Amino Purine (BAP) is the most widely used to stimulate shoot multiplication because it has a strong activity, making it more effective for in vitro shoot production.

Porang plant propagation using in vitro culture has begun to be widely studied. Some sources of explants that can be used to reproduce porang plants include: bud (young shoots from stem tubers), stem tubers (tuber cuttings), petioles, leaves, bulbils, and seeds [2]. The use of bulbils smaller than 1 gram as explants in porang tissue culture or conventional propagation has not been reported. This study examined the effect of adding plant regulator BAP on the multiplication of shoots at different bulbil weights. The results of the study are expected to overcome the limitations of porang seeds by using bulbil < 1 gram, which are not commonly used by farmers.

2. Materials and methods

2.1. Materials
The Porang plant used in this study (Accession number: Amul01) was the germplasm collection of the Indonesian Industrial and Beverage Crops Research Institute (IIBCRI) grown in the greenhouse. Bulbils (corm leaf) were used as the explant (Figure 1A). The study was conducted in the Tissue Culture Laboratory, IIBCRI. Murashige and Skoog (MS) medium was used as a basal medium.

2.2. Methods
Bulbils of various sizes were grouped based on fresh weight, i.e., 0.25; 0.50, and 1.00 g. The study used smaller bulbil (< 1 g) because it is not commonly used by the farmers and is more available. Bulbils were cleaned under running water, soaked in a mixture of 0.2% fungicide and 0.1% bactericide solution for one hour, then rinsed with aqua dest. The fungicide used in this study had an active ingredient of 0.2% mancozeb, while the bactericide was 15% streptomycin sulfate and 1.5% oxytetracycline. Then, the bulbils were sterilized by immersion for twenty minutes in 70% alcohol and shaken into 0.35% and 0.25% sodium hypochlorite solutions for 30 minutes, respectively. For the last, the bulbil was rinsed with sterile aqua dest water three times. The treated bulbils were then put into the treatment media.

Based on treatment, the medium MS was supplemented with 30 g L\(^{-1}\) of sucrose, phytagel 2.5 g L\(^{-1}\), and BAP (0.0 mg L\(^{-1}\), 1.0 mg L\(^{-1}\), 2.0 mg L\(^{-1}\), and 3.0 mg L\(^{-1}\)). First, the pH of the medium was adjusted to 5.7. Then the medium was sterilized using an autoclave at a temperature of 121ºC and a pressure of 1.5 psi for 20 minutes.

The cultures were placed in the darkroom at 25 ºC ± 0.2 and relative humidity ± 60%. After forming shoots, the cultures were transferred to the room at 16 hours in bright and 8 hours in the dark at the same temperature and relative humidity.

2.3 Data analysis
Growth parameters, namely percentage and number of buds developed, number of shoots and root formed, and plantlet height were observed every month until six months of culture. The collected data were then analyzed using Analysis of Variance (ANOVA) followed by Duncan Multiple Range Test at 5% level.
3. Results and discussion
The results of morphological observations in the first month showed an increase in the size of the bulbils. Microscopic observation also showed many tubercles were found on the bulbil peel. The tubercle scattered throughout the bulbil peel (at different bulbil weights) and became a bud growth locus.

Figure 1. (A) Bulbil was used in the study. (B) Nodules and emerged buds at 8 weeks. (C) Bulbil (0.25 g) in BAP 3 mg L\(^{-1}\) at 3 months. (D) Bulbil (0.5 g) in BAP 2 mg L\(^{-1}\) at 3 months. (E) Bulbil (1 g) in BAP 3 mg L\(^{-1}\) (Adaxial section). (F) Bulbil (1 g) in BAP 3 mg L\(^{-1}\) (adaxial section) at 3 months. (G) Bulbils (1 g) in BAP 2 mg L\(^{-1}\) at 3 months. (H) Bulbil (1 g) in BAP 1 mg L\(^{-1}\) at 4 months. (I) Bulbil (1 g) in BAP 3 mg L\(^{-1}\) at 6 months.

Two months after culture, observations under a microscope showed nodules (buds) and small shoots that were visible on almost the entire bulbil surface (Figure 1B). The appearance of buds indicates that the bulbil has germinated. At the end of the dormancy period, the tubercle in the adaxial part will be transformed into buds [11]. This means that bulbil grown in vitro culture medium has a shorter dormant period than conventional cultivation. In Conventional cultivation, bulbil has a 3 - 4 months dormancy period after planting. While using in vitro culture techniques, the dormancy period was 2 months after culture.

In the third month, the buds increased in the adaxial and abaxial sections (Figures 1E and 1F). These results show that using bulbil explants in in-vitro culture can grow shoots in the adaxial and in the abaxial. That is different from bulbil that is grown conventionally, where shoots were only formed on the adaxial part. This statement has been proven by a performed histological study of Shoot Apical
Meristem (SAM) on conventionally grown Bulbil porang. The results observed at day 130, the presence of SAM does not form a dome in the abaxial section [11].

3.1. The percentage of buds developed
The percentage of developing buds (sprouting) increased in time. After 4 months, the percentage of explants that sprouted had reached 100% except for 0.25 g bulbils (Table 1). Bulbils with 0.25 g in size did not sprout 100% due to the sterilization process that was too toxic. Therefore, need precaution in sterilization on < 0.25 g bulbils. The based on these results, we suggest modifying the sterilization process for bulbils weighing ± 0.25 g by reducing the concentration or soaking time.

The observation results at months 2, 3, and 4 showed that the heavier the tuber, the higher the percentage of shoots. That is presumably because the heavier seeds contain more carbohydrates. Carbohydrates are the main source of energy needed for germination. The percentage of explants forming shoots which reached 100% percent gives hope that small bulbils (< 1 g), which have not been used before, can be used as planting material in porang tissue culture.

Table 1. The percentage of buds developed following treatment combination of different BAP concentrations and weight of bulbils after 1 to 6 months in culture.

| Treatments | Percentage of buds developed (%) at (months) |
|------------|---------------------------------------------|
|            | 1   | 2   | 3   | 4   | 5   | 6   |
| Bulbils Weight (± 0.25 g) | | | | | | |
| BAP 0.0 mg L⁻¹ | 0% | 20% | 60% | 80% | 80% | 80% |
| BAP 1.0 mg L⁻¹ | 0% | 40% | 60% | 80% | 80% | 80% |
| BAP 2.0 mg L⁻¹ | 0% | 60% | 80% | 100% | 100% | 100% |
| BAP 3.0 mg L⁻¹ | 0% | 40% | 60% | 80% | 80% | 80% |
| Bulbils Weight (± 0.50 g) | | | | | | |
| BAP 0.0 mg L⁻¹ | 0% | 20% | 60% | 100% | 100% | 100% |
| BAP 1.0 mg L⁻¹ | 0% | 60% | 100% | 100% | 100% | 100% |
| BAP 2.0 mg L⁻¹ | 0% | 80% | 80% | 100% | 100% | 100% |
| BAP 3.0 mg L⁻¹ | 0% | 40% | 80% | 100% | 100% | 100% |
| Bulbils Weight (± 1.00 g) | | | | | | |
| BAP 0.0 mg L⁻¹ | 0% | 40% | 80% | 100% | 100% | 100% |
| BAP 1.0 mg L⁻¹ | 0% | 60% | 100% | 100% | 100% | 100% |
| BAP 2.0 mg L⁻¹ | 0% | 80% | 100% | 100% | 100% | 100% |
| BAP 3.0 mg L⁻¹ | 0% | 40% | 80% | 100% | 100% | 100% |

3.2. The Number of buds formed
Statistics analysis showed an interaction between bulbil weight and BAP doses on the number of budding parameters (Figure 2). The heavier the bulbils are cultured, the more buds are produced. The same thing was seen in the BAP treatment; the higher the BAP concentration, the more buds produced. The number of buds in this study was relatively high; the highest average was found in bulbil with 1 g weight, treated with BAP 3 mg L⁻¹, while the lowest was in bulbil 0.25 g in medium without BAP.

The use of tissue culture methods can produce much larger amounts than conventional planting, which produces an average of 1 bud. The one shoot produced in conventional propagation, because dominance apical. Apical dominance theories supported the emergence of shoots that if the apical shoots have grown, the other porang shoot candidates will be eliminated [11]. The apical dominance in the adaxial part of the bulbil can be eliminated by the addition of BAP at medium culture.
3.3. The number of shoots formed
The buds that are formed will grow into shoots. In the Araceae family, shoots will develop into pseudostem, so the number of shoots formed will be equal to the number of petioles. In line with the buds, the number of shoots formed also shows a real interaction. Statistical results showed that the number of shoots would increase as the weight of the bulbils cultured increased. The number of shoots also increased along with the BAP concentration given to the culture medium. These results are similar to the research by Chotigamas et al., added BAP 2 mg L$^{-1}$ also increased the average value on shoot multiplication 6.45 [8].

Figure 2. Interaction of BAP treatments and weight of bulbils on buds number at 4 months. The same letter at the top of the bar chart are not significantly different based on Duncan's Multiple Range Test at 5% level. Note. B = Bulbil weight.
In conventional cultivation, the number of shoots produced by bulbil weighs 9.10 g, and the number is less than 3 [5]. Meanwhile, in this research number of shoots of 1 g weight of bulbils could reach 13 shoots. The number of buds is very beneficial because one shoot can be made into one plantlet. Plantlets, after acclimatization, can be used as seeds.

The number of shoots formed in this study was not as many as the number of buds produced. This is presumably because to grow and develop, the buds require adequate nutrition. To solve this problem, in the future the research was recommended to separate the buds and shoots. After being separated, buds and shoots are subcultured into new medium. This method expected the bud can develop into shoot.

![Average Shoots Number](image)

**Figure 3.** Interaction of BAP treatment and weight of bulbils on shoots number at 6 months. The same letters at the top of the bar chart are not significantly different based on Duncan's Multiple Range Test at 5% level.

### 3.4. The plantlet height (pseudostem length)

In the family Araceae, the stem of the plant is a pseudostem. Results of statistical analysis on plantlet height showed there was no interaction between bulbil weight and BAP concentration. Figures 4 and 5 showed that the plantlet height is affected by the concentration of BAP addition and the weight of bulbils used on a single factor basis. The higher the concentration of BAP given to the medium, the lower the plantlet height. Meanwhile, the heavier the bulbils used, the taller the plantlet. Results of statistical analysis on plantlet height showed there was no interaction between bulbil weight and BAP concentration. Results statistical analysis single factor showed that the plantlet height is affected by the concentration of BAP addition. The higher concentration of BAP given to the medium, the lower the plantlet height (Figure 4), while the mean plantlet height was higher as bulbil weight increased (Figure 5).

In this study, bulbil with greater weight resulted in better height. The same results were also reported in the research done in the greenhouse [12] and experimental gardens at the Indonesian Industrial and Beverage Crops Research Institute [13]. Bulbil contains the main energy source for growth in the early stages of porang growth. As a result, larger bulbil will lead to better growth. The results of previous studies also showed that larger bulbils provide better growth in the early stages [14]. Porang was also reported to produce better bulbs when using larger bulbs [15].
Figure 4. The averaged height of plantlets at different BAP treatments after 6 months. The same letters at the top of the bar are not significantly different based on Duncan's Multiple Range Test at 5% level.

Figure 5. The averaged height of plantlet at different bulbils weight after 6 months. The same letters at the top of the bar are not significantly different based on Duncan's Multiple Range Test at 5% level.

3.5. The number of roots formed
The statistical analysis results on the number of roots parameters showed no interaction between bulbils weight and BAP concentrations. Figures 6 and 7 show that the addition of BAP concentrations affected the number of roots formed, but it was not the case for bulbils weight. The highest number of roots was found in the control treatment (without BAP). As the concentration of BAP in the treatment medium increases, the number of roots will decrease. This was also found in petiole explants treated with BAP [10].

The results showed that applying a high concentration of BAP reduces the formation of the number of roots during the initiation stage shoots. Incorporation of auxin into medium culture was required to
grow the roots. It is recommended to do subculture on the root media before the plantlets are acclimatized.

The results of this study provide information that small bulbils that have not been utilized so far can be used for in vitro propagation. Furthermore, the high percentage of bud formation (up to 100%) and shoot multiplication make propagation using in vitro culture techniques very promising.

This study recommended BA 3 mg L\(^{-1}\) treatment to get the highest number of shoots, but the number of roots formed in this treatment was very small, so further research is needed to get the best rooting medium. Plantlets with good roots will facilitate the acclimatization process.

**Figure 6.** The averaged roots number at different BAP treatments after 6 months. The same letters on top of the bar are not significantly different based on Duncan's Multiple Range Test at 5% level.

**Figure 7.** The averaged roots number at different weights of bulbils after 6 months.
4. Conclusions
The bulbils grown in vitro had a shorter dormant period than conventional cultivation. The bulbils explants developed shoots in the adaxial and in the abaxial. The percentage of shoots developed in small bulbils (<1 g) was 100%. There were interactions at the number of buds and number of shoots parameters, but not for the others. The heavier the bulbils, the higher the number of buds, shoots, plantlet height, and roots will be produced. The number of buds and shoots increases with BAP concentration, but not for plantlet height and number of roots. Bulbil with the size < 1 g can be used for seed propagation, with the highest number of seeds (approximately 11.2 seeds) produced when 3 mg L\(^{-1}\) BAP was given.

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