

# The Effect of Benzyl Amino Purin and NaphtalenaAcetic Acid Applications on Direct Shoot Organogenesis in *Porang* (*Amorphophallus muelleri* B)

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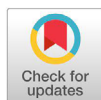
## ABSTRACT

*Porang* (*Amorphophallus muelleri* B.) is a tuberous plant with the potential to be the main source of carbohydrates and is rich in benefits. *Porang* proliferation is limited by a 6-month dormancy period per year, and generative propagation is unlikely due to the seeds being apomictic triploid. The aimed of my research to analyze the application of BAP and NAA in culture media for direct propagation of *porang* shoots. The explant used in this research was young leaves. The research was arranged in a completely randomized design with a combination of BAP and NAA hormones added to the MS medium. There were three BAP treatments, namely 1.0 mg/L, 2.0 mg/L, and 3.0 mg/L, while NAA treatments consisted of 2 levels, namely 2.0 mg/L and 4.0 mg/L. The addition of 1.0 mg/L BAP combined with 4.0 mg/L NAA was the best treatment that produced seven shoots with an average shoot length of 2.14 cm and root length of 3.6 cm, with the earliest bud emergence (9.7 weeks after planting).

**Keywords:** BAP; NAA; *Porang*; Shoot Organogenesis

## INTRODUCTION

The *porang* plant (*Amorphophallus muelleri* B.) belongs to the Araceae or taro group. *Porang* is one of the suppliers of carbohydrates with a polysaccharide called glucomannan (Sari & Suhartati, 2019). The content of glucomannan in *porang* plants is 55%. The glucomannan content in *porang* plant carbohydrates is very rich in benefits, such as in the functional food industry, namely lowering blood lipids, lowering blood glucose levels, obesity solutions, and ingredients for the cosmetic industry, rubber, airplane frames, and bioethanol (Supriati, 2016). Glucomannan plays a role in improving insulin sensitivity and reduces insulin requirements by helping tissue insulinization. Glucomannan will increase gastric viscosity and reduce the rate of glucose absorption (Anggraito et al., 2018). In the food industry, *porang* is used as a modified flour with the physical characteristics of a white-brown color, a distinctive flour aroma, a powder form, and a slightly salty taste (Ferdian and Perdana, 2021).



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In general, the *porang* can be grown under trees in the forest as one of the agroforestry commodities ([Dermoredjo et al., 2021](#)). The best *porang* production is under 90% shade conditions using soil, rice husks, and manure as a growing medium ([Mita et al., 2023](#)). Indonesia produces *porang* in Madiun, East Nusa Tenggara, South Sulawesi, and West Nusa Tenggara. The processor and exporter sell *porang* as chips, powder, and flour ([Dermoredjo et al., 2021](#)). Data from the Central Statistics Agency ([BPS, 2012](#)) shows that the production capacity of *porang* in Indonesia is only 600 tons/year, while demand for the export market reaches 3,000 tons/year. This lack of fulfillment is due to the dormancy of tubers and frogs/bulbil as the main seeds with 6-month dry intervals in a 3-year planting cycle to reach maximum weight ([Supriati, 2016](#)). Meanwhile, seed propagation has little opportunity because the seeds are apomixic triploid ([Jansen et al., 1996](#)).

*Porang* plants experience dormancy in the dry season, characterized by pseudo stems and leaves drying out for 5-6 months. *Porang* plants have two life cycles, namely the vegetative and generative cycles. In the generative cycle, *porang* plants produce fruit and seeds ([Hidayah, 2016](#)). *Porang* plants can produce bulbils, which can be used as planting material. Utilizing *porang* bulbils and seeds as planting material takes a long time. Therefore, the propagation of *porang* through tissue culture is more effective and faster. According to [Khozin & Restanto \(2022\)](#), the regeneration of *porang* by in vitro propagation is one alternative to producing *porang* seedlings and breeding with biotechnology. Planting through tissue culture techniques can solve the mass supply of *porang* seeds. The totipotent nature of plant cells is the basis theory for the application of tissue culture planting carried out on sterile culture media so that cells can grow and regenerate to form new plants. Young tissue in plants tends to be used for planting explants with tissue culture because it has meristematic tissue that easily regenerates new cells and has the opportunity to make complete new plantlets or individuals ([Irawati et al., 2017](#)). Young leaf explants have meristematic tissue appropriate for *porang* culture ([Zhao et al., 2012](#)). According to [Khozin & Restanto \(2022\)](#), leaves explants could grow callus at 28 days and shoot at an emergence time of 35 days. According to [Triharyanto et al. \(2022\)](#), bulbil explants could grow to shoot at 35 dap or 5th week after planting.

*Porang* regeneration could be done through organogenesis and somatic embryogenesis ([Khozin & Restanto, 2022](#)). Direct propagation of shoots aims to carry out an aseptic culture of a meristematic organ to become a new individual in the form of a complete plant in large quantities that takes place on growth culture media without going through the subculture process to grow callus and then the process of organogenesis in different media ([Singh et al., 2011](#)). Shoot population directly provides media and time efficiency because it is done in one planting process until the shoots grow proportionally.

The role of hormones or growth regulators (PGR) is very important and affects the growth of in-vitro plants or tissue culture techniques. PGR is an organic compound obtained from synthesizing plant parts, which is then transported and expressed in certain parts, causing physiological effects on plants, such as tending to grow certain parts of the plant ([Karjadi & Buchory, 2008](#)). Auxin and cytokinin are major PGR widely used in *porang* shoot propagation. Regeneration of *porang* is done on murashige skoog (MS) medium with 0.1 mg/L Naphtalena Acetic Acid (NAA) and 3 mg/L Benzylaminopurine (BA) ([Widoretno et al., 2023](#)). Cytokinins play a role in regulating cell

division, stimulating shoot growth, and can activate dormant cells with the help of auxins. Auxin, if synthesized by plants, will produce expression in the form of cell elongation and is more likely to form adventitious roots ([Santosa & Sugiyama, 2007](#)).

The use of Benzyl Amino Purin (BAP) with a concentration of 2.0 mg/L, which was applied to *porang* plant (*Amorphophallus muelleri* B.), resulted in shoot growth with a total of 19 shoots within 100 days after planting ([Imelda et al., 2008](#)). According to [Lailani & Kuswandi \(2023\)](#), adding two mg/L BAP to MS media resulted in faster callus induction of *porang* with an average explant bending time of 1.4 weeks and an average callus emergence time of 2.6 weeks. Benzyl Amino Purin 2 mg/L has been shown to give a lot of shoot response but with a stunted morphology, so a combination with other PGR is needed ([Imelda et al., 2008](#)). The use of NAA has a quite helpful response, in which, at low concentrations, it produces quite proportional shoots, but the number of shoots decreases drastically ([Imelda et al., 2008](#)). Using NAA in high concentrations can also produce embryonic calluses with green spots and potentially growing shoots proportionally ([Zhong et al., 2017](#)). Therefore, it is necessary to have in-depth research on the right combination to obtain optimal shoot propagation. This study aimed to analyze the interaction response of BAP and NAA treatment combinations to direct shoot propagation and to determine the best concentration for *porang* shoot propagation.

## **MATERIALS AND METHODS**

### **Experimental Design**

The research was carried out at the Ecophysiology and Plant Tissue Culture Laboratory of the Program Study of Agronomy, Agriculture Faculty, University of Jember, from May 2021 to February 2022. The research was arranged in a completely randomized design (CRD) with a combination of BAP and NAA hormones added in the murashige Skoog (MS) basal medium. There were 3 levels of BAP, namely 1.0 mg/L, 2.0 mg/L, and 3.0 mg/L, and 2 levels of NAA, 2.0 mg/L and 4.0 mg/L. Each treatment consisted of 3 replications.

### **Explants Preparation**

The explants were prepared by washing (with detergent), rinsing young leaves, and sterilizing them in the LAF by shaking them with 70% alcohol and Clorox ([Adawiyah et al., 2021](#)). Leaves explants were cut around 1 cm and planted on the media. The results of the planting were then stored in an incubation room for 13 weeks at a temperature of 22° C in bright conditions or illuminated with an LED lamp (3000 lux) ([Girsang et al., 2023](#)).

### **Observation**

The variables observed in this study include the early emergence of buds, calculated by the age of the explants' emerging buds. Early emergence was observed every day, and units of measurement were converted to weeks after planting. The number of shoots formed was calculated in the 13<sup>th</sup> week. The length of the shoots and roots was calculated using a ruler at the end of the observation (13<sup>th</sup> week).

## Statistical Analysis

Data obtained from observations were analyzed using analysis of variance (ANOVA), followed by the Duncan multiple range test (DMRT) at a 95% confidence level. Data analysis was carried out using the SPSS 26 statistical application.

## RESULTS AND DISCUSSION

### Early Emergence of Buds

Observation of the early emergence of buds in each treatment, given the combination of BAP and NAA hormones, showed significantly different results. The combination of BAP and NAA hormones affected a fast explant response to emerging buds. Earlier bud emergence is indicated as the better response of the explant to the given hormone combination. Based on the results of the observations, the emergence of buds began in the 9<sup>th</sup> week and was observed until the 13<sup>th</sup> week. The combination of 1.0 mg/L BAP and 4.0 mg/L NAA was the best treatment that produced the earliest bud emergence at 9 WAP (Table 1). In contrast, the combination treatments (BAP 1.0 mg/L + NAA 2.0 mg/L), (BAP 2.0 mg/L + NAA 2.0 mg/L), and (BAP 3.0 mg/L + NAA 4.0 mg/L) showed the longest emergence time, which was 11.7 WAP. The previous study reported that adding 3 mg/L BAP could increase the number of shoots in *porang* multiplication in vitro (Wardana et al., 2017). The addition of 2 mg/L BAP resulted in the best effect on the regeneration of *porang* by indirect organogenesis (Ferziana et al., 2021).

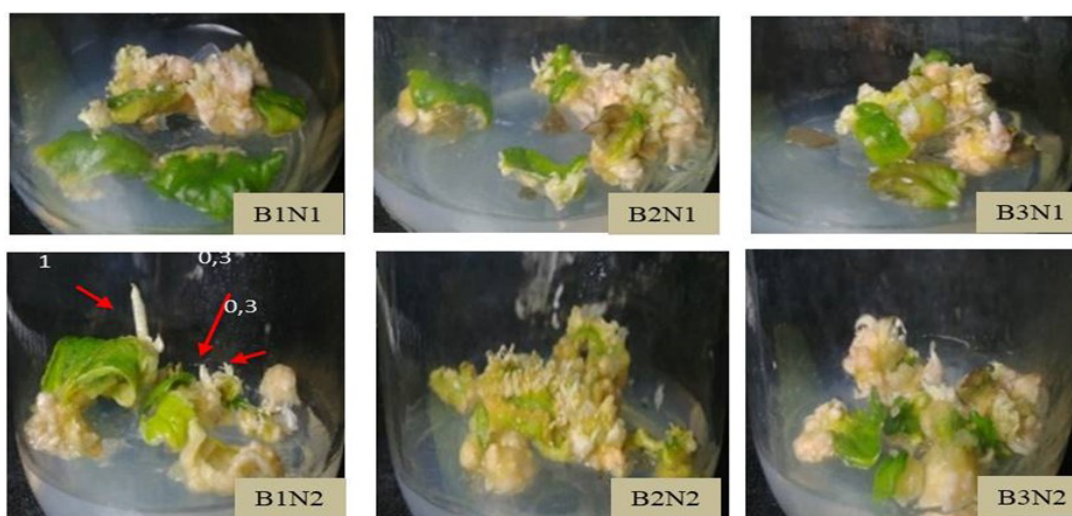
**Table 1.** The average time of bud emergence (week after planting)

Treatment	Early bud emergence		
	BAP 1 mg/L	BAP 2 mg/L	BAP 3 mg/L
NAA 2 mg/L	11.7 ± 0.00 <sup>b</sup>	11.7 ± 0.00 <sup>b</sup>	11.0 ± 0.00 <sup>ab</sup>
NAA 4 mg/L	09.7 ± 0.00 <sup>a</sup>	11.0 ± 0.00 <sup>ab</sup>	11.7 ± 0.00 <sup>b</sup>

Each treatment consisted of 3 replications; Values followed by the same lowercase letters are insignificantly different based on DMRT at a level of  $\alpha$  0.05

The combination treatment of BAP and NAA affects the micropropagation of *porang* leaf stalks, especially in the formation of shoots (Prayana et al., 2017). The early emergence of buds significantly affects the efficient growth of plant explants because the earlier the buds are formed, the faster the growth period for other organs. The early emergence of buds highly affects the growth efficiency of plant explants because it can escalate additional shoot formation; the accelerated growth period for other organs is promoted by the reaction of photosynthetic carbon, which helps the formation of other organs so that they can better utilize the hormones contained in the tissue culture media. Shoots appear on organogenic calluses formed from leaf explants (Figure 1).

Early bud emergence in the combination treatment of 1.0 mg/L BAP and 4.0 mg/L NAA had very efficient growth, growing three shoots (Figure 1). In contrast, other combination treatments had not elicited a shoot response even though they had responded to totipotency on the planted explants. These results are relevant to the study of Zhong et al. (2017), which stated that the combination of high concentrations of NAA, namely 4mg/L with the proper BAP concentration, could help the growth of explants into shoots because the character of the cytokinin hormone in BAP tended to



**Figure 1.** Early emergence of *porang* plant buds at the age of 9 weeks after planting (WAP) as affected by BAP 1 mg/L + NAA 2 mg/L (B1N1), BAP 2 mg/L + NAA 2 mg/L (B2N1), BAP 3 mg/L + NAA 2 mg/L (B3N1), BAP 1 mg/L + NAA 4mg/L (B1N2), BAP 2 mg/L + NAA 4 mg/L (B2N2), and BAP 3 mg/L + NAA 4 mg/L (B3N2).

cause meristematic swelling to encourage cell growth towards meristematic ([Restanto et al., 2023](#)), namely shoot, so that the green spot area on *porang* callus can be analyzed directly to shoot without subculture. Adding 0.1 mg/L NAA and one mg/L BAP showed the most significant number of shoots for micropropagation of *porang* ([Prayana et al., 2017](#)). According to [Hardjo et al. \(2023\)](#), 5.0 mg/L BAP and low concentrations of NAA (0.2 mg/L) showed optimal shoot induction growth and shoot height for regeneration of *porang*. The addition of BAP concentrations is proven to slow the reaction of shoot formation due to the saturation of the concentrations contained in the growth media, as in the study of [Ratnasari et al. \(2016\)](#) reporting that adding high concentrations of the BAP hormone will make shoot growth longer.

## Number of Shoots

The most significant shoots number formed at 13 wap was obtained in the combination treatment of 2.0 mg/L BAP and 4.0 mg/L NAA with 8 shoots (Table 2). This result differs from the research of [Mardhiyetti et al. \(2017\)](#), which reported more efficient results from administering a combination of 0.1 mg/L BAP and 0.08 mg/L NAA hormones to grow shoots in hummingbird plants. Proportionate shoots formed were obtained in the B1N2 combination treatment (BAP 1.0 mg/L and NAA 4.0 mg/L), with 7 shoots formed having long roots.

**Table 2.** The average number of *porang* plant shoots

Treatment	Number of Shoots		
	BAP 1 mg/L	BAP 2 mg/L	BAP 3 mg/L
NAA 2 mg/L	4.7 ± 0.58 <sup>b</sup>	2.0 ± 1.00 <sup>c</sup>	3.7 ± 0.58 <sup>b</sup>
NAA 4 mg/L	6.7 ± 1.53 <sup>a</sup>	7.7 ± 0.58 <sup>a</sup>	1.0 ± 0.00 <sup>c</sup>

Each treatment consisted of 3 replications; Values followed by the same lowercase letters are insignificantly different based on DMRT at a level of  $\alpha$  0.05



The addition, the combination of BAP and NAA hormones showed highly significantly different results. Still, there was a combination treatment that had no significantly different results, namely the 1 mg/L BAP + 4 mg/L NAA (B1N2) and 2 mg/L BAP + 4 mg/L (B2N2) NAA treatments, which had the highest average growth yield of 6.7 and 7.7 shoots, respectively. Meanwhile, the treatments of 2 mg/L BAP + 2 mg/L NAA (B2N1) and BAP 3 mg/L + NAA 4 mg/L (B3N2) had the lowest average growth yield of 2 shoots and 1 shoot, respectively. The result of this decrease in growth can occur because the explants' ability to absorb nutrients has reached its limit, as stated by [Mardhiyetti et al. \(2017\)](#), that there is an increase in callus initiation that forms shootled when the combination of 2.0 mg/L BAP and 0.08 mg/L NAA in hummingbird plants, but increasing the concentration to the next level will result in a decrease in shootled. This comparative study explains differences in the maximum effect of the concentration of BAP and NAA applications. Given three mg/L, BAP can increase the shoots number in *porang* multiplication in vitro ([Wardana et al., 2017](#)).

### Shoot Length

Observation of the shoot length in each treatment given the combination treatment of BAP and NAA hormones showed significantly different results. Shoot length is the main variable, where the measurement of shoot length results will be a benchmark for how effective the role of the combination hormone applied to achieve proportionally the shoots of *porang* plants by planting tissue culture. A combination of 1.0 mg/L BAP and 4.0 mg/L NAA (B1N2) produced the longest shoots with an average shoot length of 2.14 cm (Table 3). The combined treatments also had significantly different effects on shoot length.

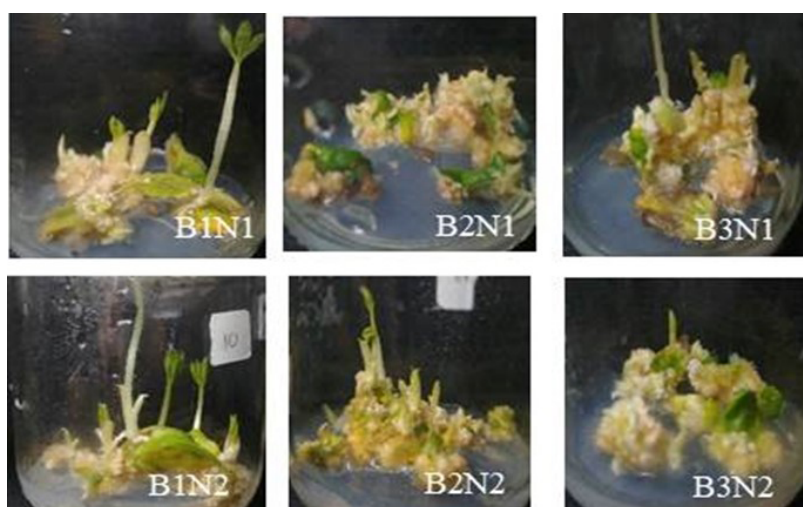
**Table 3.** Average shoot length (cm) of *porang* plants

Treatment	Shoot Length		
	BAP 1 mg/L	BAP 2 mg/L	BAP 3 mg/L
NAA 2 mg/L	1.8 ± 0.46 <sup>b</sup>	0.9 ± 0.10 <sup>c</sup>	1.3 ± 0.35 <sup>b</sup>
NAA 4 mg/L	2.1 ± 0.50 <sup>a</sup>	1.4 ± 0.20 <sup>a</sup>	1.0 ± 0.15 <sup>c</sup>

Each treatment consisted of 3 replications; Values followed by the same lowercase letters are insignificantly different based on DMRT at a level of  $\alpha$  0.05

Shoot growth in the 13th week showed a significant increase in height and number of shoots (Figure 2). The faster the shoots appeared, the taller the shoots produced. The shoots did not occur simultaneously and had different heights in one media treatment.

The best concentration for shoot length can be seen in the application of B1N2 while adding a higher concentration of BAP resulted in a decrease in shoot length. This can be observed in the B2N2 treatment, which had the largest shoots (Figure 2). This effect can occur because an increase in the number of shoots will reduce the ability to absorb nutrients by explants due to the division of the results of nutrient uptake expression on the number of shoots so that shoots are shorter, which can be seen clearly in Figure 2. This effect is also explained in the results of research by [Kasutjjaningati et al. \(2011\)](#), stating that in the induction process, the number of shoots will be inversely proportional to the process of shoot elongation due to the need for a balance of hormone supply between the right combination of cytokinins and auxins to grow shoots.



**Figure 2.** Shoot Growth 13<sup>th</sup> week on *Porang* Plants as affected by BAP 1 mg/L + NAA 2 mg/L (B1N1), BAP 2 mg/L + NAA 2 mg/L (B2N1), BAP 3 mg/L + NAA 2 mg/L (B3N1), BAP 1 mg/L + NAA 4 mg/L (B1N2), BAP 2 mg/L + NAA 4 mg/L (B2N2), and BAP 3 mg/L + NAA 4 mg/L (B3N2).

### Root Length

Observation of the root length in each treatment given the combination of BAP and NAA hormones showed significantly different results. Root length was the last variable to be observed, measured in the 13<sup>th</sup> week after planting the explants through tissue culture techniques. Root length measurement was carried out to find the combination of treatments that can bring out the root system so that the plant growth can be better and ready for acclimatization as a substitute for nursery material. The 1.0 mg/L BAP and 4.0 mg/L NAA combined produce the longest roots, 3.6 cm (Table 4). The growth Regulatory Substance of NAA has the characteristics to grow roots precisely where each concentration and combination given will have a different effect on the results of the roots that appear. The combination treatment of BAP and NAA at high concentrations, namely 3.0 mg/L BAP and 4.0 mg/L NAA, gave insignificantly different results, which could not grow the root system.

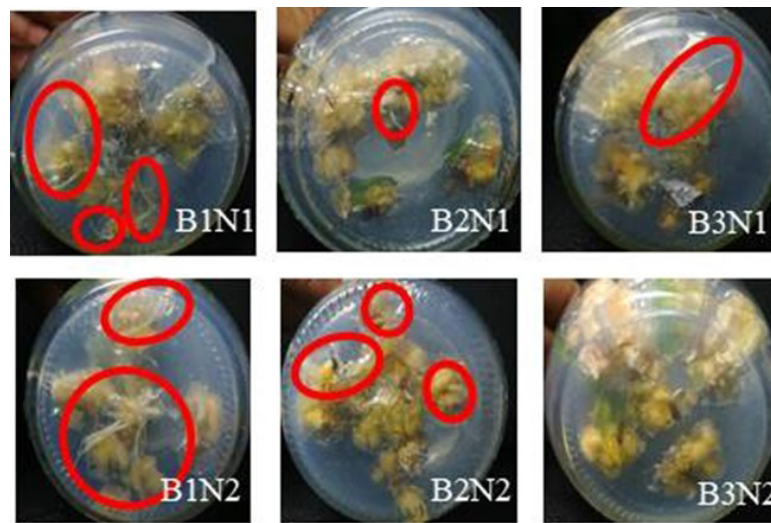
**Table 4.** Average root length (cm) of *porang* plants

Treatment	Root Length		
	BAP 1 mg/L	BAP 2 mg/L	BAP 3 mg/L
NAA 2 mg/L	2.6 ± 0.23 <sup>b</sup>	0.6 ± 0.10 <sup>c</sup>	2.3 ± 0.12 <sup>b</sup>
NAA 4 mg/L	3.6 ± 0.22 <sup>a</sup>	2.7 ± 0.06 <sup>a</sup>	0.0 ± 0.00 <sup>c</sup>

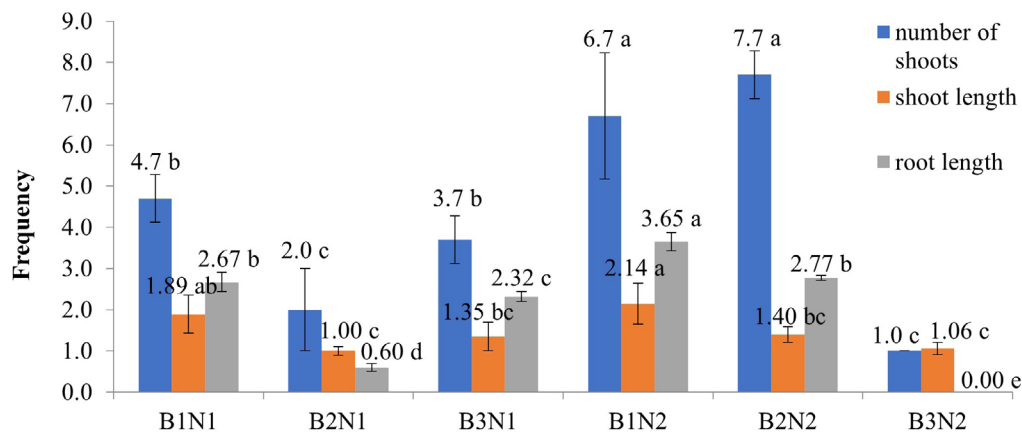
Each treatment consisted of 3 replications; Values followed by the same lowercase letters are insignificantly different based on DMRT at a level of  $\alpha$  0.05

Root growth in the 13<sup>th</sup> week showed significant differences (Figure 3). Observations of all treatments showed root growth except for the addition of 3 mg/L BAP + 4 mg/L NAA (B3N2). The proper concentration of BAP and NAA hormones can induce optimal shoots and roots.

Based on the observation, treatment with high concentrations of hormone combinations does not necessarily give good results in plant growth because there is a limited ability to absorb hormones in plant media. [Nuryadin et al. \(2017\)](#) explained that properly applying a combination of hormones



**Figure 3.** Root cross section for each treatment 13th week after planting as affected by BAP 1 mg/L + NAA 2 mg/L (B1N1), BAP 2 mg/L + NAA 2 mg/L (B2N1), BAP 3 mg/L + NAA 2 mg/L (B3N1), BAP 1 mg/L + NAA 4 mg/L (B1N2), BAP 2 mg/L + NAA 4 mg/L (B2N2), and BAP 3 mg/L + NAA 4 mg/L (B3N2).



**Figure 4.** Growth variables including the number of shoots, shoot length (cm), and root length (cm) as affected by BAP 1 mg/L + NAA 2 mg/L (B1N1), BAP 2 mg/L + NAA 2 mg/L (B2N1), BAP 3 mg/L + NAA 2mg/L (B3N1), BAP 1 mg/L + NAA 4 mg/L (B1N2), BAP 2 mg/L + NAA 4 mg/L (B2N2), and BAP 3 mg/L + NAA 4 mg/L (B3N2).

auxin and cytokinin in plant explants would result in root growth, which can help supply plant nutrients and accelerate proportional shoot propagation. According to [Hardjo et al. \(2023\)](#), low concentrations of NAA (0.1 mg/L) resulted in earlier root induction, and the rooting rate reached 100% for regeneration of *porang*. The concentration and type of auxiliary hormone influence the response of the root system. Giving IAA at a concentration of 0.4 mg/L can increase root induction in *porang* multiplication in vitro. The interaction of BAP and IAA has no significant effect on *porang* multiplication in vitro ([Wardana et al., 2017](#)). Adding one mg/L IBA can induce the best rooting of *porang* plants in vitro ([Ibrahim, 2019](#)).

The research on the combined treatment of BAP and NAA hormones for *porang* direct shoot propagation was conducted to determine the most effective treatment to achieve research objectives.





**Figure 5.** Result of B1N2 (BAP 1.0 mg/L and NAA 4.0 mg/L) treatment on porang shoot propagation.

Almost all combination treatments of BAP and NAA showed a significant effect on each variable, especially the number of shoots (Figure 4) that increased to the peak of the average shoot of 8 shoots in the B2N2 treatment and tended to decrease when the last treatment (B3N2) was applied, which produced only one shoot (Figure 4).

This result is a maximum interaction reaction, and each interaction has a saturation point experienced by explants where the explants are unable to express high interaction concentrations, causing osmotic pressure on the cells to inhibit growth and remove the rest of the reaction, which usually becomes browning on the explants (Nuryadin et al., 2017). The combination of BAP and NAA has a good response for direct shoot propagation, which can be seen from the overall response to the observed variables. The histogram (Figure 4) of the hormone treatments shows that the best treatment for each observation variable is found in the B1N2 treatment, namely the combination of 1.0 mg/L BAP and 4.0 mg/L NAA. The treatment of 1.0 mg/L BAP and 4.0 mg/L NAA (B1N2) showed optimal growth, as seen in Figure 5.

The B1N2 combination treatment had the most significant data for each observation variable for direct shoot propagation, and an efficient reaction to the expression of explants in the treatment applied supported the data. According to Nuryadin et al. (2017), who researched tropical pitcher plant explants to grow shoots even though the concentration of the NAA hormone used was one mg/L, lower than the treatment applied to *porang* research. These data were supported by shoot growth image data applied to a combination of 1.0 mg/L BAP and 4.0 mg/L NAA (Figure 5).

## CONCLUSION

The combination treatment of 1.0 mg/L BAP combined with 4.0 mg/L NAA is the best treatment that induces direct shoot organogenesis, showing earliest bud emergence in 9.7 wap, producing shoots with an average shoot length of 2.14 cm and resulting in the longest extended system of 3.6 cm. The addition of the maximum treatment concentration, namely 3.0 mg/L BAP combined with NAA 4.0 mg/L BAP, decreased the growth rate in each observation variable due to saturation of hormone absorption by plants. The results of this research can be used as a reference in propagating *porang* to produce seedlings. It is necessary to know more about the success rate of acclimatization of *porang* seedlings resulting from in vitro propagation.

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