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Response of Shoots from Porang Leaf Bulbs to Cytokinins and IAA in Shoot Multiplication In Vitro

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Abstract

The growth regulators in media have the potential to significantly influence the process of in vitro plant regeneration. The response of explants in forming shoots also depends on the type and the combination of growth regulators. This research aimed to identify the most optimal type and concentration of cytokinin for the multiplication of in vitro porang shoots. Additionally, it determined whether the addition of cytokinins should be accompanied by auxin/IAA for porang shoot multiplication. To achieve these objectives, a completely randomized design with six replications was employed, followed by a 5% LSD. The tested treatments consisted of various combinations of growth regulators. Several observations were recorded, including the speed of explants forming prospective shoots/shoots (days), the age of the explants forming prospective shoots/shoots, the number of prospective shoots and shoots produced per explant, and shoot height (cm). The results showed that 1) Tdz 2 and IAA 0.5 mg.l-1 formed the highest shoots candidates and shoots in porang multiplication in vitro, and 2) the addition of BAP and Tdz 2 mg.l-1 should be combined with IAA 0.5 mg.l-1, while BAP and Tdz 3 mg.l-1 did not require IAA.

Keywords: BAP, IAA, multiplication, porang, thidiazuron

1 Introduction

Porang (*Amorphophallus muelleri* Blume), also recognized as iles-iles, is one of the three varieties of tubers cultivated for commercial purposes, improving significant economic value [1]. According to [2], the tuber has a high selling price due to its main compound, glucomannan [3], functioning as a cholesterol-lowering agent [4], anti-diabetic [5], anti-colorectal cancer [6], and anti-inflammatory [7].

According to [8], the propagation of porang plants can be accomplished generatively or vegetatively. Furthermore, the seeds are not always available [9] and planting with corms or bulbils takes a long time due to dormancy in the bulbs. To support the availability of seedlings, the propagation is obtained using tissue culture methods since it can produce uniform plantlets in large quantities within a relatively short time and free from diseases [10].

The response of plant tissues/parts cultured in vitro shoot formation depends on the type of media [11]. As stated by [12] the response depends on the combination of growth regulators, including auxin [13], cytokinin [14], and the genotype (type and cultivar) of the plant [15]. Several research [16-18] stated that the highest number of shoots produced in porang multiplication was achieved using MS media with the addition of BAP without auxin. According to [19] showed that there was an interaction between IAA and kinetin (Kin) in shoot multiplication and the best combination was obtained with 0 mg.l-1 IAA and 2 mg.l-1 Kin. The research conducted by [20] on *Prunus duicis* Mill. (Almond) showed that thidiazuron (Tdz) was more effective than the other cytokinins. Application of Tdz at a concentration of 1 mg.l-1 significantly increased shoot proliferation. According to [21] on Rhododendron plants, the frequency of shoot regeneration and the number per explant increase with the concentration of Tdz. Therefore, this research aimed to determine the effect of cytokinin types (with or without combination with IAA) and their concentrations in promoting axillary shoot growth, particularly in the shoot multiplication stage.

2 Material and Methods

This research was conducted between May and October 2022 at the Tissue Culture Laboratory of Lampung State Polytechnic. The materials used consisted of propagules obtained from the previous year's initiation, along with Murashige and Skoog [22] basal media. This was supplemented with vitamins (thiamine-HCl, pyridoxine-HCl, nicotinic acid), and myo-inositol, as well as growth cytokinin (BAP, Kin, and Tdz), and auxin (IAA). Additionally, HCl and NaOH, agar, sugar, rubber, plastic, and aluminum foil were employed. The tools also used encompassed an

autoclave, laminar air flow cabinet, hand sprayer, and dissection equipment (forceps, scalpel, petri dish).

The experiment was conducted using a completely randomized design (CRD). The treatments tested were BAP 2 mg.l-1 + 0 mg.l-1 IAA (P1), BAP 2 mg.l-1 + 0.5 mg.l-1 IAA (P2), BAP 3 mg.l-1 + 0 mg.l-1 IAA (P3), BAP 3 mg.l-1 + 0.5 mg.l-1 IAA (P4), Kin 2 mg.l-1 + 0 mg.l-1 IAA (P5), Kin 2 mg.l-1 + 0.5 mg.l-1 IAA (P6), Kin 3 mg.l-1 + 0 mg.l-1 IAA (P7), Kin 3 mg.l-1 + 0.5 mg.l-1 IAA (P8), Tdz 2 mg.l-1 + 0 mg.l-1 IAA (P9), Tdz 2 mg.l-1 + 0.5 mg.l-1 IAA (P10), Tdz 3 mg.l-1 + 0 mg.l-1 IAA (P11), and Tdz 3 mg.l-1 + 0.5 mg.l-1 IAA (P12). The data were subjected to analysis of variance, and differences between treatments were tested using the LSD test at the 5% level.

This research started by preparing the treatment media and the chemical materials of Murashige and Skoog basal media were prepared as stock solutions. The media were prepared by pipetting each stock solution and adding the growth regulators according to the treatments. Subsequently, 30 g.l-1 of sugar and distilled water were added, and the pH was adjusted to 5.7 by adding HCl 1 N or NaOH 1 N. The media were sterilized at a temperature of 121°C for 20 minutes, then incubated for 3-5 days. Apart from the treatment media, MS0 media (media without growth regulators) were also prepared to grow to shoot candidates into shoots used as explants. The planted explants were maintained in a room with a temperature of 25°C - 27°C, with 16 and 8 hours of light and darkness. The observations were made on the following variables 1) the speed of explant forming shoot candidates/shoots (days), 2) the age of explants forming shoot candidates/shoots, 3) the number of shoot candidates produced per explant, and 4) shoot height (cm).

3 Results and Discussions

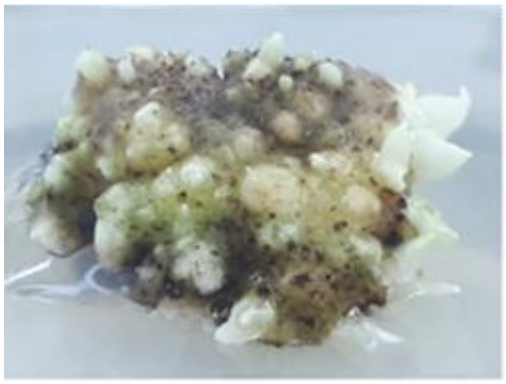
The shoot candidates were grown into shoots by subculturing the propagules on Murashige and Skoog media until proliferation occurred, which were used as explants in the multiplication stage (Fig. 1).

Shoot proliferation for the explants occurred 3-5 weeks after subculturing. The shoots, which had reached a size of 1 cm, were isolated and planted on the treatment

media. Generally, the explants showed a response in multiplication with a speed of forming shoot candidates/shoots ranging from 10 to 20 days after being subcultured on the treatment media. Meanwhile, the number of shoots on the explants ranged from 1-2 shoots per explant with a shoot height of 0.6-0.8 cm, as shown in Table 1.

The analysis of variance showed that the treatment of cytokinin with or without auxin significantly influenced the number of shoot candidates produced on the explants. According to [23], shoot meristem formation requires an increase in cytokinin and auxin levels. Furthermore, cytokinin and auxin are needed for cell division and play a role in meristem formation and activity. Cytokinin also plays a crucial role in shoot organogenesis when the ratio to auxin increases.

Explants planted on treatment media showed different rates of forming shoot candidates. The LSD test at a 5% significance level showed that explants cultured on media with the addition of BAP 2 IAA 0,5 mg.l-1, BAP 3 IAA 0 mg.l-1, Tdz 2 IAA 0,5 mg.l-1, and Tdz 3 IAA 0 mg.l-1+ exhibited the fastest growth response in shoot candidate and took 10-10.2 days (Table 2). Meanwhile, explants cultured on media with the addition of BAP 3 IAA 0.5 mg.l-1, Kin 2 IAA 0.5 mg.l-1, and Kin 3 IAA 0.5 mg.l-1 showed the slowest growth response in shoot candidate and formation, taking 19.7-20.2 days. The LSD test at a 5% significance level showed that the explants cultured on media with the addition of Tdz 2 IAA 0.5 mg.l-1 (P10) produced the highest number of shoot candidates and shoots (31.33), followed by the treatment of BAP 3 IAA 0 mg.l-1 (P3) at 22.00 (Table 2). The lowest number of shoot candidates and shoots were obtained from the explants cultured on media with the addition of Kin 3 IAA 0.5 mg.l-1 (P8) at 6.33 (Fig. 2).



Propagules of shoot candidates

Proliferation of shoots on propagules

Porang shoot explants

Figure 1. The stage of preparing shoot explants for multiplication

Tdz 2 IAA 0.5 mg.l^{-1} (P10)

BAP 3 IAA 0 m g.l^{-1} (P3)

Kin 3 IAA 0.5 (P8)

Figure 2. Growth of prospective shoots and shoots on explants cultured on treatment media

The research [16-18] on porang showed that the use of a single cytokinin can increase the number of formed shoots. Furthermore, [19,24] stated that the combination of cytokinin with low auxin can improve the percentage of shoot formation and multiplication. According to [25], the response of plants to the use of specific types and concentrations of auxin and cytokinin in organ regeneration is species-specific, depending on the genotype of the cultured plant. [26] stated that the use of Tdz combined with IAA has been widely reported to promote shoot regeneration in many plant species. The combination of these two growth regulators with a lower concentration of IAA plays a crucial role in morphogenesis, such as inducing and proliferating axillary shoots.

Table 1. Average age of explants forming shoot candidates/shoots, number of shoots, and shoot height (cm)

Treatment	Speed of Explants Forming Shoot Candidates and Shoots (days)	Number of Shoots	Shoot Height (cm)
P1 (BAP 2 + IAA 0 mg.l-1)	15	1	0.6
P2 (BAP 2 + IAA 0.5 mg.l-1)	10	1	0.7
P3 (BAP 3 + IAA 0 mg.l-1)	10	1.2	0.8
P4 (BAP 3 + IAA 0.5 mg.l-1)	20	1	0.6
P5 (Kin 2 + IAA 0 mg.l-1)	14.7	1	0.7
P6 (Kin 2 + IAA 0.5 mg.l-1)	19.7	1	0.7
P7 (Kin 3 + IAA 0 mg.l-1)	15	1	0.7
P8 (Kin 3 + IAA 0.5 mg.l-1)	20.2	1	0.8

P9 (Tdz 2 + IAA 0 mg.l-1)	13.2	1	0.8
P10 (Tdz 2 + IAA 0.5 mg.l-1)	10	1	0.7
P11 (Tdz 3 + IAA 0 mg.l-1)	10.2	0.8	0.6
P12 (Tdz 3 + IAA 0.5 mg.l-1)	11	1	0.7

Table 2. LSD test at the 5% significance level for the variable of the speed of explant forming shoot candidates and shoots, as well as the number of shoot candidates and shoots formed on the explants

No.	Treatment	Average Speed of Explants to Form Shoot Candidates & Shoots (days)	Average of the Number of Shoot Candidates and Shoots
1.	P1 (BAP 2 + IAA 0 mg.l^{-1})	15.0 C	13.33 E
2.	P2 (BAP 2 + IAA 0.5 mg.l^{-1})	10 .0 A	20.00 B C
3.	P3 (BAP 3 + IAA 0 mg.l^{-1})	10.0 A	22.00 B
4.	P4 (BAP 3 + IAA 0.5 mg.l^{-1})	20.0 D	12.50 E
5.	P5 (Kin 2 + IAA 0 mg.l^{-1})	14.7 C	11.00 E
6.	P6 (Kin 2 + IAA 0.5 mg.l^{-1})	19.7 D	14.00 D E
7.	P7 (Kin 3 + IAA 0 mg.l^{-1})	15.0 C	17.67 C D
8.	P8 (Kin 3 + IAA 0.5 mg.l^{-1})	20.2 D	6.33 F
9.	P9 (Tdz 2 + IAA 0 mg.l^{-1})	13.2 B C	10.33 E
10.	P10 (Tdz 2 + IAA 0.5 mg.l^{-1})	10.0 A	31.33 A
11.	P11 (Tdz 3 + IAA 0 mg.l^{-1})	10.2 A	18.50 C
12.	P12 (Tdz 3 + IAA 0.5 mg.l^{-1})	11.0 A B	12.00 E

Even though the use of a single cytokinin was the primary factor for inducing and proliferating shoots, this research showed that the addition of both auxin and cytokinin to the shoot induction media resulted in a higher number of shoots. The explants showed a faster ability to form shoots compared to the treatment with a single cytokinin.

The effectiveness of Tdz as cytokinin in shoot induction and multiplication may be due to its ability to stimulate endogenous cytokinin biosynthesis, which will alter the metabolism of endogenous cytokinins. The compound is also highly resistant to degradation by cytokinin oxidase enzymes in plants. Therefore, Tdz exhibits strong cytokinin-like activity that surpasses other commonly used adenine types such as benzylaminopurine or Kin [27].

IAA as auxin also affected on number of shoots. This may due of its activity on cell division and cell differentiation that makes shoots proliferation higher. [28] stated

that effectivity on cell division of IAA is more powerful than another auxin such as NAA and IBA.

Based on the results, the response of porang shoot explants to growth regulators in forming shoot candidates and shoots is best observed with Tdz (Tdz 2 IAA 0.5 mg.l-1), which outperforms BAP and Kin. This was consistent with [21] in Sutsukui Azalea (*Rhododendron indicum*), where Tdz optimally induced shoots compared to BA and Kin. It also aligned with [20] that Tdz was more effective than the other cytokinins in enhancing shoot proliferation. According to [29], the use of Tdz in Gymnocladus assamicus resulted in higher shoot induction and proliferation compared to BA.

4 Conclusions

Based on the research on the effects of cytokinin and IAA treatments in media, the following conclusions were drawn:

1. The application of Tdz 2 + IAA 0.5 mg.l^{-1} resulted in the highest formation of shoot candidates and shoots in *in vitro* multiplication of porang shoots.
2. The addition of BAP and Tdz 2 mg.l^{-1} should be combined with IAA 0.5 mg.l^{-1}, while BAP and Tdz 3 mg.l^{-1} did not require IAA.

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