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Application of Growth Regulatory Substances CPPU and GA3 on the Growth of Porang Plants from Bulbil

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ABSTRACT
Porang is known as an alternative food source. The main obstacle for porang production is the long harvest period with growth time needed ranging between 4-5 months and 7-8 months of dormancy. The application of plant growth regulators (PGR) such as CPPU and GA3 could be used to break porang's dormancy. This study aimed to determine the effect of the application of CPPU and GA3 on the growth of porang plants from bulbil. Best CPPU and GA3 concentration were determined to be 60 ppm and 100 ppm, respectively. The PGRs were used to treat the bulbils by soaking them for 15 minutes. The bulbils then planted on planting media containing soil:husk charcoal:compost (1:1:1) for 1 month. After 1 week of PGR treatment, the bulbils showed an increase in the number of shoots, bulbil diameter, and bulbil weight when compared to the control. Then after two weeks of PGR treatment, an increase in shoot length, root length, and the number of roots only occurred in the GA3 treatment while CPPU treatment showed different response. At 1 month of PGR application, all treatments showed a lower result in plant height, number of leaf, leaf area, and stem diameter when compared to control. This study showed that while CPPU and GA3 are able to induce shoot propagation, it negatively affected the development of the organs such as roots, stem, and leaves.

Keywords: *Bulbil, CPPU, GA3, Growth Regulatory, Porang*

1. INTRODUCTION

Porang is widely used in the food, beverage, and medicine industry [1]. Every year porang demands are always increasing. Therefore, it is necessary to cultivate porang widely, intensively, and sustainably. Porang can be propagated through tubers, seeds, and bulbil. Bulbil is one of the organs used for the vegetative propagation of porang plants [1, 21].

The main obstacle to accelerate porang production is the time it takes to harvest. The time needed to reach harvest time is 4-6 months [2]. This is caused by porang growth is ranging between 4-5 months of an active period and 7-8 months of dormancy [3]. This makes it difficult to increase productivity. One of the efforts made is the application of plant growth regulators (PGR) such as CPPU and GA3. CPPU (N-(2-Chloro-4-pyridinyl)-N-phenyl urea) is a group of cytokinin that is effective in inhibiting aging and accelerating cell division. Meanwhile, GA3 is a gibberellin group that is often used as a dormancy breaker [4, 11].

CPPU plays a role in the induction of plant cell division and enlargement, while increasing the intensity of cell division by shortening the interphase phase, especially in the G1 and G2 phases [26, 27]. CPPU as a synthetic cytokinin can also increase endogenous cytokinins [11]. This is supported by research on the application of synthetic dormancy breaking substances using chemical substances such as thiourea 0.5% and KNO_3 40 g/L, and 2 synthetic hormones such as CPPU 5 ppm and Dormex 3% on the mangosteen plant [28]. The result showed that CPPU with a concentration of 5 ppm can shorten the dormancy period compared to the application of other dormancy-breaking substances.

This shows that CPPU is effective in breaking dormancy by accelerating the active period and shortening the dormancy period [29].

According to the research on seed germination and breaking dormancy in *Ocrhadenus arabicus*, maximum results showed with the use of GA3 with a concentration of 100 µM compared to other treatments [14]. Indicating the role of GA in breaking dormancy so that it can lead to the germination phase of plants [24]. Seed germination is a complex process and GA3 plays a very important role in controlling and encouraging germination in many plant species [31]. Therefore, it is necessary to conduct research on porang cultivation in vivo with the application of CPPU and GA3 dormancy breaker.

2. METHODOLOGY

2.1. Bulbil Screening

In our research, the planting material used is bulbil. The bulbils were obtained from porang farmers in Kepel Village, Kare District, Madiun, East Java Province, Indonesia. The bulbil's candidate used in this research must pass the bulbil weight screening stage first to obtain a relatively uniform bulbil. Bulbils selected as good materials were ranged from 8-10 g in weight [8].

2.2 Determination of Plant Growth Regulator's (PGR) Concentration

Determination of PGR concentration was conducted to determine the best concentration of CPPU and GA3. The results of determination were obtained based on the measurement of the percentage of shoot emergence. The determination of PGR concentration began by preparing the CPPU (Raffatih Farm) in 20, 40, 60, and 80 ppm while for the GA3 (Phytotech Labs, USA) in 20, 40, 60, and 80 ppm. The planting medium used for the concentration determination was a mixture of soil: husk charcoal: compost (1:1:1) was placed in a polybag. Then the bulbils were soaked in each all of the PGR treatments for 15 minutes and each treatment was repeated 5 times, while the controls were soaked distilled water for 15 minutes as well. Then the bulbils that have been soaked were planted in the planting medium, then every 2 days observed the emergence of shoots on bulbil.

2.3 Cultivation of porang plants in seedling media

Cultivation was conducted in the greenhouse of Biology Department, Institut Teknologi Sepuluh Nopember, Surabaya. Cultivation began with preparing bulbil with a relatively uniform weight in the range of 8-10 g. The planting media was prepared by mixing soil: husk charcoal: compost in a 1:1:1 ratio and then put in a polybag. CPPU was prepared with a concentration of 60 ppm (Figure 1) while GA3 was prepared with a concentration of 100 ppm (Figure 2). The bulbils were then soaked in distilled water (control), CPPU, and GA3 for 15 minutes, followed by planting in the medium for 1 month. The observation was conducted after the first week, second week and 1 month. One-week-old plants were measured for average weight, diameter, and a number of buds, two weeks-old plants were measured for average shoot length, root length, and a number of roots, and 1 month-old plants were measured for height, a number of leaves, leaf area, and stem diameter.

2.4 Statistical Analysis

The data obtained in this research was expressed as mean ± SD. All data were statistically analyzed using one-way ANOVA (analyses of variance) followed by Post-Hoc DNMRT and Tukey test. The *P*-value of less than 0.05 was adopted as statistically significant.

3. RESULTS AND DISCUSSION

The results of determination concentration of CPPU and GA3 hormones were shown by the percentage of shoot emergence on bulbil that had been treated with a concentration of CPPU and GA3 as much as 20, 40, 60, 80, and 100 ppm for 8 days (Figure 1 and 2).

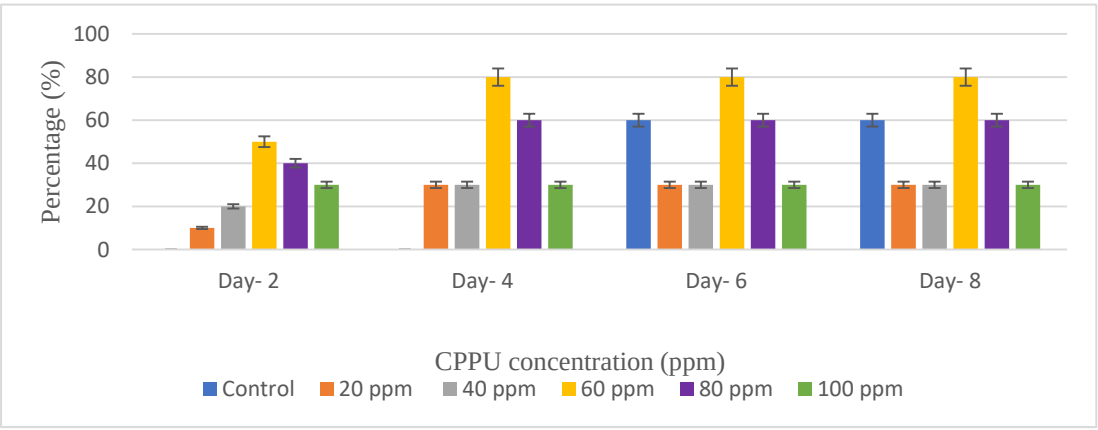

Figure 1. Percentage of Shoot Emergence on Porang Plants Treated with CPPU

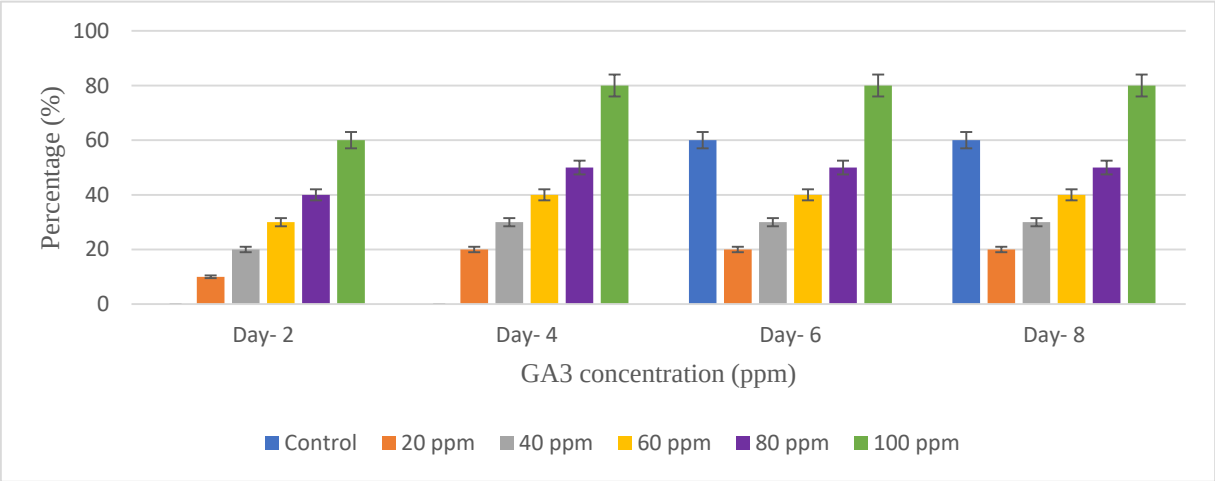

Figure 2. Percentage of Shoot Emergence on Porang Paalants Treated with GA3

Based on Figure 1, bulbils given different CPPU concentrations showed different responses. Bulbil treated by CPPU with a concentration of 60 ppm showed the best response in which the percentage of shoot emergence was higher than the control by 20%. Giving CPPU with different concentration levels will give a different increase in reaction to the observed variables [3]. The benefits of cytokinin are highly dependent on the concentration given. If the dose is right, it will bring up more shoots [15]. The role of CPPU as cytokinin can function as a substance that breaks dormancy and increase cell division, cell growth, and development in plants [15]. While in Figure 2, bulbil given different concentrations of GA3 showed different responses. Bulbil treated with GA3 with 100 ppm concentration showed the best response resulted in the shoot emergence higher than control by 60%. Plants treated with increasing concentration of GA3 will also increase the number and growth of shoots [16]. This showed the role of gibberellins as essential plant regulators for plant developmental processes such as seed germination, stem elongation, leaf extension, and flowering induction [17].

Our research showed that PGR applications on the bulbil can spur growth (Table 1). Treatment with CPPU (60 ppm) and GA3 (100 ppm) showed shoots appearance on day 2. In addition, PGR application also increased dormancy breaking faster than control [22]. The control treatment showed shoots appearance on day 6 in average with at most 4 shoots (Table 1). Plant development is a combination of several complex processes namely growth and differentiation [6]. Growth and differentiation are stimulated by growth regulators such as GA3 and CPPU. GA3 can control the synthesis of hydrolytic enzymes in seed germination [7,8]. Giving GA3 at the right concentration can help increase cell activity in seeds. This is following with [9] statement that the gibberellin hormone plays a role in encouraging the formation of α-amylase and hydrolytic enzymes that enter the cotyledon or endosperm of seeds to produce energy for cell activity. GA3 can break dormancy through GA3 by metabolizing starch and sugar and activating several genes such as *AMY*, *BMY*, *TPS*, and *BGs*) into glucose, fructose, and trehalose which are used for another metabolic process [37]. Breaking dormancy is also influenced by several genes activities *FT*, *EBB1*, *EBB3*, *CYD* which up-regulated due to the addition of GA3 [30] in the cells. Higher availability of gibberellins in seeds influence the ability of seeds to germinate [10]. Likewise, the effect of CPPU as a cytokinin group also stimulates the emergence of shoots earlier and increases the number of shoots formed [15]. This is because cytokinin can affect various physiological processes, biochemical metabolism, and plant development such as cell division and cell enlargement [4]. In addition, cytokinin is effective in inhibiting aging and accelerating cell

Table 1. Effect of PGR treatment on the growth of bulbil

1	Control	6±5,2^{b}	2,172±0,56^{a}	15,90±0,23^{a}	4±2,07^{a}
2	CPPU	2±0,33^{a}	2,582±0,22^{a}	17,96±0,51^{a}	12±7,23^{b}
3	GA3	2±0,33^{a}	2,374±0,18^{a}	17,40±0,73^{a}	24±8,34^{c}

Note: Numbers followed by the same letter in the column show no significant difference based on 5% DNMRT

division, so cytokinin can affect development and grow periods [11].

Based on Table 1, it is known that the application of growth regulators CPPU and GA3 did not significantly affect the average bulbil weight and diameter. This is because the bulbils have a relatively uniform initial weight. The uniformity of bulbil size will provide uniform plant growth due to having the same amount of food reserves to support early vegetative growth [9]. In addition, the effect of giving PGR has not been seen because the growth regulators that have been absorbed by plants are presumably still being processed to induce new plant organs.

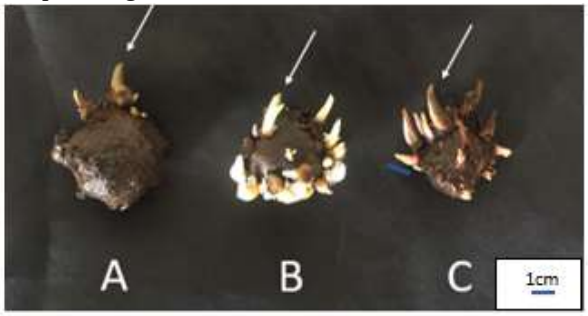

Figure 3. The appearance of shoots from bulbil on porang plants aged 1 week in the treatment: (A) Control; (B) CPPU and (C) GA3

It is also known that as a single factor, PGR (CPPU and GA3) showed differences in the number of shoots formed (Table 1). It can be seen that morphologically the shoots of bulbil were more numerous in plants treated with CPPU and GA3 compared to the control (Figure 3). This is because PGR application can extend the active growth period of porang. The application of CPPU is effective in increasing the cytokinin content so it can stimulate shoot emergence [7, 8]. Meanwhile, GA3 plays a role in germination with cell wall development, cell enlargement, and cell division [9]. The application of the GA3 hormone has benefits in breaking dormancy to induce the emergence of shoots [24].

Two weeks after PGR application, the plants were measured for various growth parameters (shoot length, root length, and a number of roots). The result showed that shoots developed with increased length but poor root development (Table 2).

According to Table 2, the application of CPPU and GA3 have significantly affected shoot length. CPPU which belongs to the cytokinin group stimulates cell division and inhibits the aging period of plants [5]. If the plant can grow well, the plant can maximize bulbil productivity. Furthermore, improvement of shoot growth by CPPU is supported by the opinion that CPPU can increase endogenous cytokinin content thus increasing shoot sink strength [11]. Another physiological effects of cytokinin are increasing the production of reducing sugar and sucrose and decreasing the osmotic potential of leaf cells [23]. More water will be absorbed by the cells, so the cells become turgid and enlarged [12]. Meanwhile, GA3 plays a role in influencing various plant physiological processes. The application of a given concentration of GA3 can stimulate plant growth by increasing shoot/stem height and leaf area [4]. An increase in stem or shoot length is the most specific response in most plants to external GA3 administration. This is due to an increase in the activity of apical cell division and elongation so that the cell size will increase [16].

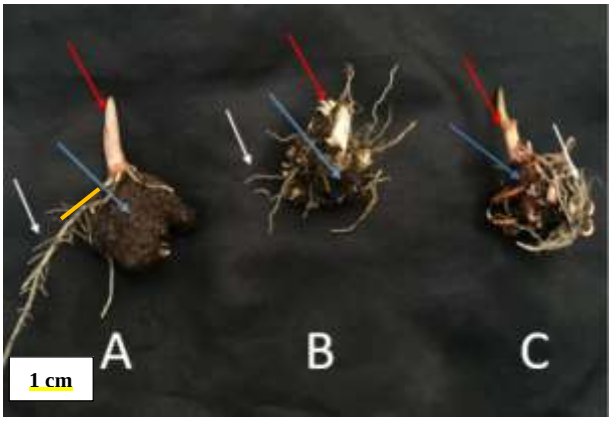

Figure 4. The appearance of bulbil shoots porang plants aged 2 weeks in the treatment: (A) Control; (B) CPPU and (C) GA3. Blue arrow: bulbil, White arrow: Root, Red arrow: shoot.

The number and length of the roots are another indicator of plant growth. Table 2 and Figure 4 showed that CPPU applications had lower root lengths than controls. CPPU can inhibit root growth by antagonizing auxin effect to modulate the rate of cell division and differentiation of root apical meristems [35]. An increase in cytokinin levels can cause inhibition of auxin signaling, and when auxin is low it can limit the action of cytokinins [36].

Table 2. Effect of PGR on porang phenotypes 2 weeks after treatment

No.	Treatment	Shoot Length (cm)	Root Length (cm)	Number of Root
1	Control	0,08±0,02^{a}	0,65±0,13^{c}	4±1,23^{b}
2	CPPU	0,45±0,06^{b}	0,20±0,06^{a}	2±0,58^{a}
3	GA3	0,83±0,35^{c}	0,38±0,55^{b}	4±1,91^{b}

Note: Numbers followed by the same letter in the column show no significant effect based on 5% DNMRT

Table 3. Effect of PGR treatment on porang phenotypes 1 month after treatment

1	Control	57,5±0,75[a]	6[a]	230,55±0,95[a]	5,8±0,14[b]
2	CPPU	34±0,72[b]	6±0,75[a]	167,14±0,70[b]	4,51±0,59[ab]
3	GA3	46,80±1,03[c]	6±0,92[a]	185,29±0,43[c]	5,09±0,24[ab]

Note: Numbers followed by the same letter in the column show no significant effect based on 5% Tukey

In the GA3 application, the number of roots were not significantly different from the control and the root length was shorter than the control shown in Figure 4. This is because GA3 can stimulate cell elongation. Root length treated with GA3 was shorter than control because auxin strongly promotes rooting and is thought to be essential for the differentiation of root primordia [32]. GA3 is acted as an inhibitor for rooting because GA does not enhance the expression of *Early Auxin-Induced* (*LPEA3*) gene in plant loblolly pine (*Pinus taeda* L.), which is thought to be involved in auxin-induced rooting above the basal level [33]. GA is suggested to be less required for rooting and rather inhibitory for auxin-promoted rooting [34].

The application of CPPU and GA3 caused a change in growth patterns in each parameter such as a number of leaves, leaf area, plant height, and stem diameter (Table 3). The results showed that several parameters such as plant height, leaf area, and stem diameter were significantly different, which the three parameters were lower in the treated plants compared to the control plants (Table 3 and Figure 5), while the number of leaves was not significantly different between treatment and control plants. Lower results on growth parameters contradicted with known effect of GA3 to stimulate plant growth by increasing shoot/stem height and leaf area [4] and known effect of cytokinin to stimulate the formation of buds and increasing the number of leaves, by encouraging lateral branching and increased lateral buds, also helped to increase the absorption of mineral elements from the soil to support plant growth [38]. Cytokinin can stimulate processes responsible for elongation and division of the cell so can stimulates new leaves and increase plant height [39].

On the bulbil skin, there are tubercles. When bulbil dormancy ends, some tubercles develop into buds and not all buds will develop into shoots. The success of growing shoots into mature plants begin with the supply of food reserves in the bulbil [18]. PGRs such as CPPU and GA3 usually used in breaking dormancy and producing shoots. However, not all shoots can successfully grow into mature plants. It depends on the supply of food reserves in the bulbil to be used for the formation of organs such as roots and shoots [19,20]. The number of shoots will trigger competition between shoots in obtaining nutrients and space to grow, so only single shoot can survive and develop into an adult plant (Figure 5) [40, 41].

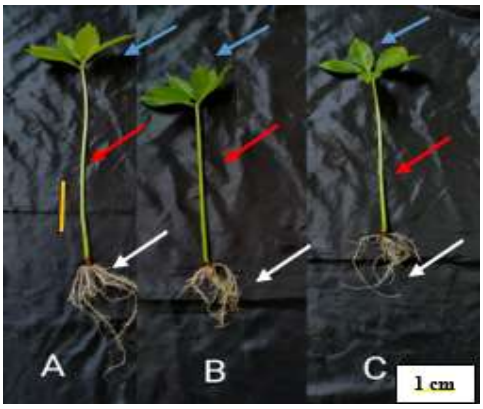

Figure 5. The growth of porang plant at 1 month of age: (A) Control; (B) CPPU and (C) GA3. Blue arrow: leaf, White arrow: Root, Red arrow: stem.

Lower roots length and plant height could be associated with the administration of exogenous PGRs. CPPU can inhibit root growth root growth by antagonizing auxin to modulate the rate of cell division and differentiation of root apical meristems [35] and GA3 is less required for rooting and rather inhibitory for auxin-promoted rooting [34]. Lower length and root number then affect the amount of nutrients that could be absorbed by the plant [42]. Resulting in the plant height of the treatments to be shorter than the control.

AUTHORS CONTRIBUTION

T.N as a main and correspondence author designed the research, analyzed the data, and wrote the manuscript, K.I.P; Z.F and F.F.N collected the data.

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