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Growth optimization of white turmeric (*Curcuma zedoaria)* plantlets with growth regulators gibberellins

Abstract. The addition of cytokinin to the multiplication of white turmeric (*Curcuma zedoaria*) seedlings in vitro proved effective in shoot multiplication, but the addition of high cytokinin concentrations could cause stunted shoots and stunted growth. The addition of the hormone gibberellins (GA_3) is often used in tissue culture for shoot elongation, so increasing the viability of plantlets. The experiment aims to determine the effect of GA_3 and obtain the best concentrations of GA_3 consisting of 0.50, 1, and 1.5 ppm GA_3 on optimizing the growth of zedoary plantlets. The experimental parameters included the number of shoots, roots, leaves, plantlet height, and chlorophyll content observed at 6 WAP after subculturing. The results showed that giving 1 ppm GA_3 had the best effect on optimizing plantlets' growth, namely the growth component of the number of shoots and plantlets' height.

Keywords: *Curcuma zedoaria* · Plantlets height · Thidiazuron

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Introduction

White turmeric (*Curcuma zedoaria*) is an herbal plant of the Zingiberaceae family that has the potential to be cultivated. The content of sesquiterpenes, curcuminoids, and ethyl p-methoxycinnamate in white turmeric has cytotoxic activity against cancer and tumor cells by inhibiting cell proliferation and cell colony formation (Sagita et al., 2022). Therefore, white turmeric is widely used as an industrial raw material, especially in the biopharmaceutical industry.

In 2021, the production of the rhizome plant found, one of which is white turmeric has decreased to 203.74 tons, which previously reached 213.39 tons in 2020 (Statistics Indonesia, 2021). The decline in production certainly impacted the supply of industrial raw materials. According to Salim & Munadi (2017) there are 112 traditional medicine industries and 828 small traditional medicine businesses in Indonesia, 94% of these industrial raw materials come from within the country. Still, the supply of these industrial raw materials cannot be fulfilled due to the limited availability of simplicia.

The limited availability of white turmeric rhizome can be caused by harvesting the rhizome, which can only be done once a year so that the availability of simplicia and seed sources for the cultivation of white turmeric is increasingly limited (Puspita et al. 2019).

One way to overcome this problem is through tissue culture techniques. Tissue culture is a technique for isolating plant parts in the form of cells, tissues, organs, protoplasm, and other parts, which are cultured aseptically in a medium containing several nutrients (Ziraluo, 2021). This technique allows seed production to be done quickly and produces large quantities of seeds (Fong & Sani, 2019). According to Ziraluo (2021) seed production through tissue culture techniques is carried out under more controlled environmental conditions so that the seeds produced will be free from disease attacks.

The success of the growth and development of an explant through tissue culture techniques is influenced by the components contained in the culture media. Plant Growth Regulator (PGR) are one of the media components that affect the morphogenesis and organogenesis activity of a cell, besides that, the addition of ZPT affects the growth rate of tissue until it forms a complete plant (Rahman et al. 2021).

Growth regulators commonly used in seed production through tissue culture are cytokinins. Thidiazuron (TDZ) is a type of cytokinin most often used in seedling propagation through tissue culture because it accelerates cell division (Kholifah et al. 2022). However, giving TDZ in high concentrations can cause short shoots and abnormal growth because it affects the morphogenesis and organogenesis of an explant (Deepa et al. 2018).

Stem elongation occurs due to the process of cell division, elongation, and enlargement of cells in the stem tip meristem, resulting in an increase in height. The stem elongation activity can be induced by administering the growth regulator gibberellins (GA_3) (Bagale et al. 2022). Therefore, giving GA_3 can be used to optimize the growth of stunted shoots due to the addition of TDZ in high concentrations.

The effect of GA_3 on optimizing the growth of white turmeric through tissue culture has not been widely carried out. Therefore, this research was conducted to determine the effect and obtain the best concentration of GA_3 for optimizing the growth of white turmeric (*Curcuma zedoaria*) plantlets.

Materials and Methods

The research was conducted at the Tissue Culture Laboratory of the Agrotechnology Study Program, Faculty of Agriculture, Padjadjaran University. The research was conducted from November 2020 - April 2021.

Media preparation. The media used were Murashige and Skoog's media consisting of 30 g/L sugar, 2 g/L gelzan, and gibberellin growth regulator consisting of 0.50 ppm, 1 ppm, and 1.5 ppm.

Planting material and sterilization. The turmeric rhizome was obtained from the Biopharmaca Cultivation Conservation Unit of the Tropical Biopharmaceutical Center Institute for Research and Community Service, Bogor Agricultural University (IPB). The sterilization of white turmeric shoots was done twice, namely outside the Laminar Air Flow (LAF) and inside the LAF. Intersection white shoots are washed using running water until there is no dirt attached to the shoots. Shoots were soaked in

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detergent solution for 10 minutes and rinsed using sterile aquadest until clean. The shoots were soaked in a fungicide solution of 0.6 g/100 mL and bactericidal 0.1 g/100 mL for 24 hours and rinsed using sterile distilled water until clean.

Sterilization was continued in LAF by soaking the shoots in 70% alcohol for 5 minutes and rinsing with sterile distilled water three times; then, the shoots were soaked in 20% Clorox solution for 15 minutes and rinsed using sterile distilled water three times. The shoots were soaked again in 10% Clorox solution for 15 minutes and rinsed with sterile distilled water three times, then, the shoots were soaked in 0.1% $HgCl_2$ solution for 10 minutes, then rinsed using sterile distilled water until clean.

Planting. The explants used were the best plantlets aged 6 WAP with the highest shoot multiplication rate, namely those grown on TDZ media with a concentration of 0.3 ppm TDZ but with short plantlets with a height of 3.65 cm. Plantlets that are 6 WAP on TDZ media subcultured on GA_3 treatment media to see the optimization of the growth of white turmeric plantlets.

Data analysis. Analysis of the results of the study was carried out descriptively.

codes	GA_3 treatments
A	Without Plant Growth Regulator (Control)
B	0.50 ppm GA_3
C	1 ppm GA_3
D	1.5 ppm GA_3

Parameters observed included the number of shoots, roots, leaves, plantlet height, and total chlorophyll content, which were observed at 6 WAP after being subcultured.

Results and Discussion

The number of shoots. High shoot multiplication is an indicator of successful seedling production through tissue culture. One factor that affects the shoot multiplication rate is the use of appropriate growth regulators (Kajla et al. 2018).

Based on the research results, the addition of GA_3 growth regulator with several concentrations had an effect on the increase in the number of shoots at 6 WAP. Table 1 shows that treatment C, namely adding 1 ppm GA_3, has better potential in optimizing the formation of shoots, namely 2.22 pods, compared to treatments B (0.50 ppm GA_3) and D (1.5 ppm GA_3).

According to Rajagukguk et al. (2018) the ability of GA_3 to induce budding is due to the fact that GA_3 can act as a substitute for auxin in inducing shoot formation through cell division activity. Giving GA_3 can also increase the sugar content in plants to trigger growth which is used in the respiration process so that energy is formed and stimulates cell division (Asra et al. 2020)

Another thing that affects the increase in the number of shoots on GA_3 media is that the formation of meristemoids can cause on plantlets due to the use of the initial media, namely TDZ, for shoot multiplication so that the administration of GA_3 into the culture media affects the development and elongation of shoots (Rajagukguk et al. 2018).

The number of roots. Roots are an important part of a plant because they function to absorb water and minerals needed for plant growth. Roots are an important part of a plant because they function to absorb water and minerals needed for plant growth.

Table 1 shows that treatment C, namely 1 ppm GA_3, has better potential in root formation than other treatments. The number of roots formed in treatment C was 11.11. This condition is in accordance with the research of Padrón et al. (2020) on *Alpinia purpurata* plants which showed that giving GA_3 could increase the number of roots but not significantly different from the control treatment.

According to Triani et al. (2020) addition of gibberellins can stimulate the formation of roots due to the formation of proteolytic enzymes that can release tryptophan, namely the precursor of auxin so that gibberellins can increase the auxin content, which can induce rooting.

The number of leaves. Leaves are plant organs involved in several plant physiological activities such as photosynthesis, respiration, transpiration, synthesis, and provision of growth regulators. According to Novianto & Setiawan (2019), the number of leaves affects plant development; the more leaves that are formed, the more light that is captured by the leaves so that the process of photosynthesis will be faster. Based on the results of the study giving 1 ppm GA_3 showed a better effect than other treatments in leaf formation, with a total of 5.44 leaves.

Table 1. Number of Shoots, Number of Roots, Number of Leaves and Plantlet Height in GA_3 Media at 6 WAP

Treatments	Shoots number	Root number	Leaf number	Plantlet height (cm)
A = No treatment (control)	1.00	10.89	4.22	7.86
B = 0.50 ppm GA_3	1.55	6.55	3.78	9.06
C = 1 ppm GA_3	2.22	11.11	5.44	13.13
D = 1.5 ppm GA_3	1.66	7.33	4.33	9.50

Table 2. Leaf Chlorophyll Content in GA3 Media at 6 MST

Treatments	Chlorophyll content (mg/g) 6 WAP
A = No treatment (control)	0.66
B = 0.50 ppm GA_3	0.72
C = 1 ppm GA_3	0.57
D = 1.5 ppm GA_3	0.60

According to Farida & Rohaeni (2019) the formation of leaves due to the addition of the GA_3 hormone is due to the fact that the gibberellin hormone can stimulate cell division activity and increase phloem and xylem growth, so with this activity, the growth of the number of leaves increases.

Plantlet height. The increase in plant height is one indicator of plant vegetative growth. According to Do Vale et al. (2019), the height of plantlets resulting from better micropropagation when acclimatized is around 5cm – 15 cm, with a survival rate of up to 97%.

Based on the study's results, the addition of GA_3 with various concentrations affected the increase in plantlet height. Table 1. shows that treatment 1 ppm GA_3 has better potential in increasing plantlet height than other treatments. This is in accordance with the research of Behera et al. (2018), addition of 1 ppm GA_3 to Hedychium coronarium plants with stunted plantlet conditions due to too high TDZ concentrations showed the highest plantlet height compared to other treatments with a height of 5.2 cm at 4 WAP after subculture from 0.8 ppm TDZ media.

According to Noor et al. (2017) GA_3 can increase plant growth activities, such as stimulating cell division activity and cell elongation in stem meristems, so giving GA_3 can stimulate internode and stem elongation. The activity of cell elongation by GA_3 can be caused by increased cell wall plasticity followed by hydrolysis of starch into sugar. As a result, it can reduce water potential and allow water to enter cells, thus encouraging cells to develop (Rahman et al. 2019).

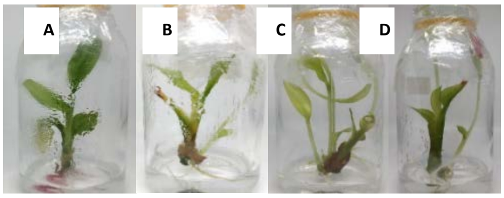

Figure 1. Plantlets Appearance for Each Treatment Age 6 WAP (A) 0 ppm GA_3 (B) 0.50 ppm GA_3 (C) 1 ppm GA_3 (D) 1.5 ppm GA_3

Total Chlorophyll Content. Chlorophyll is the most critical pigment associated with photosynthesis, absorbing energy from light, which is then used to convert carbon dioxide into carbohydrates (Zhao & Yaxin, 2014).

Table 2 shows that the 0.50 ppm GA_3 treatment has good potential in chlorophyll content compared to other treatments, which is 0.72 mg/g. According to Wen et al. (2018) GA_3 can increase the concentration of leaf chlorophyll, i.e., by increasing the number and size of chloroplasts and increasing plastid ultrastructural morphogenesis.

GA_3 influences plant biomass, especially in the vegetative parts, through the promotion of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), protein synthesis, ribose, and polyribosomes doubling, in addition to increasing enzyme activity and increasing membrane permeability which can facilitate absorption and use of mineral nutrition and transport of photosynthates (Miceli et al. 2019).

Wen et al. (2018) stated that the administration of exogenous GA_3 can affect source and sink relationships, including the strength of sources and sinks during the assimilation and partitioning of carbohydrates. The source is an organ or plant tissue that produces or exports photosynthate, while the sink is a photosynthate recipient (Mastur, 2015). Giving gibberellins can increase the power of the source by increasing the efficiency of photosynthesis and the energy of the sink by distributing photosynthetic assimilate.

Conclusion

Giving 1 ppm GA_3 gave the best effect for optimizing plantlet growth as indicated by certain variables of the number of shoots and plantlet height.

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