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Growth responses of white turmeric (*Curcuma zedoaria* Rosc.) rhizome on plant growth promoting rhizobacteria treatment

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Abstract
One of the efforts to increase the growth of white turmeric (*Curcuma zedoaria*) rhizomes and promote environmentally friendly, sustainable agriculture is to utilize Plant Growth Promoter Rhizobacteria (PGPR). The present research aimed to determine the effect of PGPR concentration on the growth of white turmeric rhizomes. The research was carried out in the physiology and development laboratory, department of biology, faculty of mathematics and natural sciences, Sriwijaya University. The research design used in this study was a Complete Randomized Design with four treatments of PGPR concentration (0, 62.5, 125, and 187.5 ppm) and five repetitions. The data obtained from observations and measurements is statistically analyzed using the analysis of Variance and Duncan's multiple range test. The results showed that PGPR treatment could increase the growth of white turmeric rhizomes. Concentration of PGPR at 125 ppm gave the best results and had a significant effect compared to no PGPR treatment in stimulating the growth of white turmeric rhizomes on the parameters of time of shoot emergence, shoot height, number of shoots, number of roots, and root length. The PGPR concentration of 62.5 ppm gave a significantly significant result compared to the average number of leaves without PGPR treatment.

Keywords: White turmeric rhizome, plant growth promoting rhizobacteria, growth responses

Introduction

The genus Curcuma of the Zingiberaceae family is a group of plants that produce curcumin. Curcumin is a bioactive compound that has strong antioxidant and anti-inflammatory effects (Ayati *et al*., 2019) [1]. Curcumin has an anti-cancer effect on various types of cancer (Yang *et al*., 2022) [22]. One species of the genus Curcuma is white turmeric (*Curcuma zedoaria*). The rhizomes of white turmeric are used in cooking spices, flavoring food, and traditional medicine, such as to treat menstrual disorders, gastric diseases, and stimulants. White turmeric contains bioactive compounds that are anti-allergic, analgesic, and anti-nociceptive, as well as anti-cancer (Lobo *et al*., 2009) [12], anti-tumor effect on murine and human cancer cells (Lakshmi *et al*., 2011) [11], anti-filarial activity (Senathilake *et al*., 2016) [18].

White turmeric is widely cultivated and traded in traditional markets in Indonesia. White turmeric cultivation is still conventionally done using chemical fertilizers and synthetic growth hormones. Excessive and continuous use of chemical fertilizers in agriculture results in a large number of environmental problems, such as ecosystem damage and unbalanced soil fertility, and can lead to eutrophication (Pirttilä *et al*., 2021; Thorat and More, 2022) [16, 21]. Currently, innovation in environment-friendly technology to increase soil fertility and plant growth in the framework of sustainable agriculture is the application of beneficial microorganisms as biological fertilizers (Pirttilä *et al*., 2021; Kumar *et al*., 2022) [16, 9].

Beneficial microorganisms, including plant growth promotion rhizobacteria (PGPR). PGPR is a group of beneficial plant bacteria that promotes plant growth (Goswami *et al*., 2016) [5]. Rhizobacteria, which are symbiotic with plant roots, can produce plant hormones similar to those synthesized by plants, such as gibberellin, auxin, and cytokinin. (Backer *et al*., 2018) [2]. PGPR can increase the availability of nutrients to plants and also increase the content of total nitrogen and phosphorus in plant tissues (Song *et al*., 2021) [20].

The benefits of PGPR in stimulating plant growth and development by producing phytohormones, increasing nutrient availability, and increasing nutrient uptake have been reported. Experimental treatment of PGPR strain Pseudomonas fluorescens on blackberry plants can increase the number of fruits and plant growth, as well as the content of bioactive compounds flavonols, phenolics, and catechins (Garcia-Seco *et al*., 2015) [4]. Pseudomonas fluorescens K-34 produces the phytohormone indole acetic acid (IAA) and plays a role in the release of phosphate for plants (Parani and Saha, 2012) [14]. According to observations on soybean plants, PGPR strain *Bacillus aryabhattai* SRB02 produces the phytohormones ABA, GA, and IAA and increases the growth of shoots and roots (Park *et al*., 2012) [15].

Plant growth promoting rhizobacteria can be used to enhance rhizome growth. Research information on the use of PGPR in white turmeric is currently limited. Information about PGPR studies that have been carried out on Zingiberaceae, especially on red ginger (*Zingiber Officinale* var. Rubrum) and ginger (*Zingiber Officinale*). PGPR was proven to significantly increase the growth of red ginger rhizome at a 25% PGPR concentration (Kurniahu *et al*., 2017) [10]. PGPR increased the growth of ginger rhizomes, which increased plant height, leaf length, and number of leaves. (Jabborova *et al*, 2021)) [6].

Based on the description above, it is necessary to conduct research on the effect of PGPR on the growth of white turmeric rhizomes. The present study aimed to determine the effect of PGPR concentration on the growth of white turmeric rhizomes. The results of this study are expected to be further developed so that they can be applied to increase the growth and production of white turmeric. In addition, this research is also an effort to increase soil fertility within the framework of eco-affable, sustainable agriculture.

Materials and methods

The research was carried out in the physiology and development laboratory, department of biology, faculty of mathematics and natural sciences, Sriwijaya University. The white turmeric rhizome used is selected it has 3 buds and weighs about 20 g. Then the rhizomes are washed and dried. The research design used in this study was a Complete Randomized Design with four treatments and five repetitions. The commercial PGPR used contains *Bacillus subtilis* and *Pseudomonas fluorescens*. The concentrations of PGPR treatment were 0, 62.5, 125, and187.5 ppm.

The washed rhizomes were then put each into a plastic beaker containing the prepared PGPR solution and soaked for 1 hour. Planting media in the form of rice husk charcoal and polybags were prepared, then rice husk charcoal was moistened with water until the humidity reached 70% which was measured using a soil tester, then put into polybags of 1000 g each. The rhizomes that have been soaked with PGPR for 1 hour then air-dried for 15 minutes, after that the rhizomes are planted in planting media to a depth of ± 3 cm.

Variable observations carried out in this study were the time of emergence of shoots on rhizomes, percentage of rhizomes growing shoots and roots, number of shoots, shoot height, number of roots, root length, and number of leaves. The data obtained from observations and measurements is statistically analyzed using the analysis of Variance and Duncan's Multiple Range Test.

Results and discussion

1. Time of shoot emergence, percentage of sprouted rhizomes, and rooted rhizomes

The time of emergence of shoots on the rhizomes was observed every day until the end of the observation, while the percentage of sprouted rhizomes and rooted rhizomes was carried out at the end of the observation (8 weeks). Table 1 shows that PGPR concentrations of 125 ppm and 187.5 ppm resulted in an average shoot emergence of 5.60 and 6.60 days after planting (DAP), respectively, with a significant impact compared to without PGPR treatment, namely 9.40 DAP, in the development of white turmeric rhizome shoots. According to Kanimozhi and Jeyanthi (2018) [7], growth-promoting rhizobacteria (PGPR), which are bacteria that dwell in the rhizosphere environment, have a vital role in affecting plant growth. PGPR not only ensures the supply of vital nutrients for plants, but it also promotes the efficiency of nutrient usage.

Tabel 1: The effect of PGPR treatment on the average time of shoot emergence on rhizomes, the percentage of sprouted rhizomes, and rooted rhizomes.

PGPR treatment (ppm)	Shoot emergence (DAP)	Rhizomes grow shoots (%)	Rhizomes grow roots (%)
0	9.40 ^{a}	100	100
62.5	7.20 ^{ab}	100	100
125	5.60 ^{b}	100	100
187.5	6.60 ^{b}	100	100

Note: Different superscripts in the same column mean significantly different at the DMRT test level of 0.05.

Based on Table 1, it is known that the percentage of rhizomes grow shoots and rhizomes grow roots in the PGPR treatment and without the PGPR treatment for 8 weeks of observation is 100%. It is suspected that the endogenous hormones present in white turmeric rhizome are able to induce shoot and root growth, and the effect of adding exogenous hormones produced by rhizobacteria present in PGPR. PGPR stimulates plant growth by increasing nutrient uptake by plants and augmenting growth by regulating phytohormone levels such as auxin, cytokinin, gibberellin, abscisic acid, and ethylene (Basu *et al*., 2021) [3]. The development of shoots on the rhizome will be followed by root growth. The bacteria present in PGPR are able to have a positive influence on the growth of rhizomes, one of which is producing auxin as a growth hormone. According to Kanimozhi and Jeyanthi (2018) [7], auxin production is an important factor in promoting plant growth in PGPR treatment, where the percentage of rhizosphere bacteria tends to synthesize auxin as a secondary metabolite because of the rich root exudate supply.

2. Growth of shoots and roots in rhizomes

The growth of shoots and roots, including the number of shoots, shoot height, number of leaves, number of roots, and root length, was observed up to 8 weeks (Table 2). Based on Table 2, it is known that the 125 ppm PGPR treatment had a better effect on the number of shoots and shoot height on white turmeric rhizome, namely for the number of shoots of 2.40 shoots and for shoot height of 15.80 cm. The results at this concentration also showed significant differences compared to the treatment without PGPR (0 ppm). Jabborova *et al*, (2021) [6] stated that the bacteria present in PGPR function to mass produce growth hormones such as IAA. According to Singh (2018) [19], indole-3-acetic acid, or IAA is one of the essential auxins included in phytohormones. IAA plays a major role in organ development, cellular responses such as differentiation, expansion, division, and gene regulation.

Tabel 2: The effect of PGPR treatment on the growth of shoots and roots of white turmeric rhizomes.

PGPR treatment (ppm)	Number of shoots	Height of shoots (cm)	Number of leaves	Number of roots	Root length (cm)
0	1.00 ^{a}	11.02 ^{a}	2.40 ^{a}	3.80 ^{a}	13.46 ^{a}
62.5	1.40 ^{ab}	13.04 ^{ab}	4.00 ^{b}	6.00 ^{ab}	17.00 ^{ab}
125	2.40 ^{b}	15.80 ^{b}	3.40 ^{ab}	6.40 ^{b}	20.26 ^{b}
187.5	1.60 ^{ab}	13.44 ^{ab}	3.00 ^{ab}	5.40 ^{ab}	16.12 ^{ab}

Note: Different superscripts in the same column mean significantly different at the DMRT test level of 0.05.

Based on the results of Table 2, it is known that the highest average number of leaves is at a concentration of 62.5 ppm, which is 4.00. This result has a significant effect compared to the treatment without PGPR, which has the smallest average yield of 2.40. The increase in the number of leaves is related to the availability of nitrogen in plants. It is known that PGPR is able to increase the availability of nutrients, one of which is nitrogen. Based on the results of the study, a concentration of 62.5 ppm is the optimal concentration to support the availability of nitrogen nutrients in white turmeric rhizomes forming leaves. Plants that get a efficient supply of N will form broad leaves with high chlorophyll content so that plants can produce assimilate in sufficient quantities to support vegetative growth (Masclaux-Daubresse *et al*., 2010) [13].

Based on Table 2, it can be seen that white turmeric rhizomes with a PGPR concentration of 125 ppm obtained an average root number of 6.40 and a root length of 20.26 cm. These results had a significant effect compared to those without PGPR treatment, namely 3.80 for the number of roots and 13, 46 cm for root length. Rhizobacteria, which are symbiotic with plant roots, can produce plant hormones similar to those synthesized by plants, such as auxins (Backer *et al*., 2018; Jabborova *et al*., 2021) [2, 6]. The phytohormone auxin plays a central role in almost every aspect of root development, from the cellular level to the root system as a whole (Roychoudhry and Kepinski, 2022) [17]. White turmeric rhizome without PGPR treatment, even though the growth was not better than the concentration of 125 ppm, it can be seen that root growth can also develop even though it is not optimal. This is because nutrients and endogenous hormones that exist even without PGPR can also induce root growth.

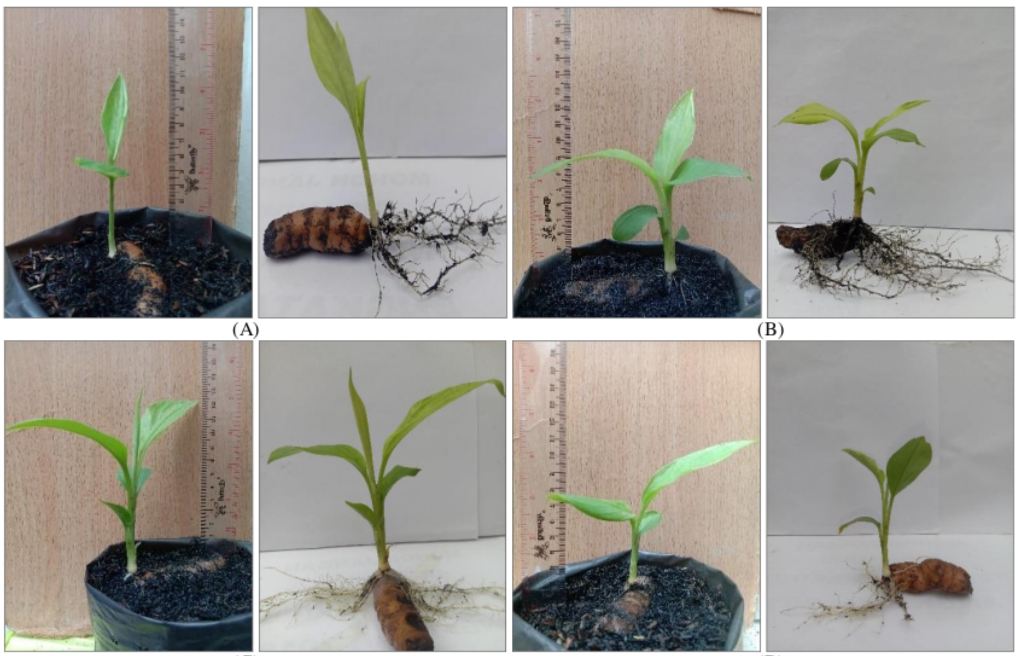

Fig 1: Growth of white turmeric rhizome at PGPR concentration treatment for 8 weeks. (A) 0 ppm, (B) 62.5 ppm, (C) 125 ppm, (D) 187.5 ppm

Based on Figure 1, it can be seen that treatment with PGPR resulted in better shoot and root growth compared to treatment without PGPR (control treatment). In addition, it was also seen that the PGPR treatment at a concentration of 62.5 ppm and 125 ppm showed relatively better growth of shoots and roots than at other concentrations. Based on the results of the analysis of Variance, the concentrations of 62.5 ppm and 125 ppm also showed no significant effect. It is suspected that the concentrations used both give good results in meeting the nutritional needs to form shoots and roots on white turmeric rhizomes.

The PGPR treatment showed better rhizome growth than the treatment without PGPR (Table 2, Figure 1). The growth of shoots, leaves, and roots in rhizomes is influenced by phytohormones produced by rhizobacteria. Jabborova *et al*. (2021) [6] reported that the rhizobacteria *Bacillus subtilis* produces the phytohormone IAA, which is utilized by ginger rhizomes to increase their growth. According to Singh, 2018) [19], IAA is a plant hormone of the auxin group that plays a role in stimulating the growth of shoots and roots in plants.

Shoots, leaves, and roots are part of the plant whose formation process is inseparable from the role of nutrients such as nitrogen and phosphorus, which are absolute nutrients needed by plants because these two elements can affect the vegetative growth of plants. According to Krouk and Kiba (2020) [8], the availability of nitrogen and phosphorus elements in soil and plants are the two elements that can increase leaf growth and play a role in the formation of new cells. They are also the main components of organic compounds in plants, including amino acids, nucleic acids, chlorophyll, ADP, and ATP. If the plant is deficient in these two nutrients, then the plant's metabolism is disrupted, so that the process of leaf formation is hampered.

PGPR is a biological fertilizer containing microorganisms that are involved in increasing plant growth and yield through direct mechanisms, namely as nitrogen fixation as well as phosphate solubilization or increasing the availability of phosphate for plants. PGPR can increase the availability of nutrients to plants and also increase the content of total nitrogen and phosphorus in plant tissues (Song *et al*., 2021) [20].

The symbiotic mechanism is through microbes that live symbiotically by fixing free nitrogen in the soil, which is reduced from free nitrogen in the air, while non-symbiotically it will convert air-free nitrogen into ammonia due to the nitrogenase enzyme, which is only owned by certain microbes, namely bacteria, which are free-living or non-symbiotic nitrogen fixers. While the main phosphate dissolution mechanism used by PGPR is through phosphate solubilizing bacteria excreting a number of organic acids that will react with phosphate-binding agents to form stable organic chelates so as to free-bind phosphate ions that can be utilized by plants.

Conclusion

Based on the research that has been carried out, the conclusions are as follows:

1. PGPR treatment had a better effect on white turmeric rhizome growth than without it.
2. Concentration of PGPR 125 ppm gave the best results and had a significant effect compared to no PGPR treatment in stimulating the growth of white turmeric (*Curcuma zedoaria*) rhizome on the parameters of time of shoot emergence, shoot height, number of shoots, number of roots, and root length. The PGPR concentration of 62.5 ppm gave a significantly significant result compared to the average number of leaves without PGPR treatment.

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