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The effectiveness of plant growth-promoting rhizobacteria (PGPR) on the growth of pepper (*Piper nigrum* L.) cuttings

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Abstract
One of the efforts to increase the growth of pepper cuttings (*Piper nigrum* L.) and encourage environmentally friendly and sustainable agricultural development (SDGs) is by utilizing Plant Growth Promoting Bacteria (PGPR). This study aims to determine the effect of PGPR concentration on the growth of pepper cuttings. The research design used in this study was a Completely Randomized Design with four PGPR concentration treatments, namely 0 $g.L^{-1}$, 0.5 $g.L^{-1}$, 1.0 $g.L^{-1}$, 1.5 $g.L^{-1}$. Data obtained from observations and measurements were analyzed statistically using analysis of variance and Duncan's multiple range test. The results showed that PGPR treatment can increase the vegetative growth of pepper cuttings. PGPR concentration of 1.0 $g.L^{-1}$ gave more effective and significant results compared to treatment without PGPR in stimulating the growth of shoots, leaves, and roots of pepper cuttings.

Keywords: Pepper (*Piper nigrum* L.), stem cutting, plant growth promoting rhizobacteria, vegetative growth

Introduction
Pepper (*Piper nigrum* L.) is a type of plant from the Piperaceae family which has high economic value and is used in the pharmaceutical, cosmetic and food industries (Takooree *et al*., 2019; Joshi *et al*., 2018) [21,8]. The seeds and fruit of the pepper plant have been traded throughout the world for thousands of years. South Sumatra, Indonesia, has been a pepper producer since the 17th century, when the pepper trade was monopolized by the Dutch East India Company (Verenigde Oostindische Compagnie - VOC) (Farida, 2009) [3]. To this day, South Sumatra Province is one of the largest pepper producers in Indonesia.

Vegetative propagation by cuttings is a common method used by pepper farmers. Plant propagation by cuttings has several advantages, including its practicality, ease of use, and the lack of special techniques. Furthermore, the resulting new plants have characteristics similar to those of their parent plants (Sarkar *et al*., 2022) [17]. The success of cuttings is influenced by several factors, including plant growth regulators (Hassanein, 2013; Sarkar *et al*., 2022) [5,17]. Plant growth regulators are needed by plant cuttings to induce and stimulate root and shoot growth (Quan *et al*., 2022) [15]. During the cutting growth process, the level of endogenous plant growth regulators in the cuttings changes (Hu *et al*., 2019) [6], so that they may not be sufficiently available to stimulate cutting growth, therefore the addition of exogenous plant growth regulators is necessary. So far, pepper farmers generally use synthetic plant growth stimulants that act as exogenous plant growth regulators.

Efforts to improve the sustainability of agricultural production systems include reducing the use of synthetic chemicals and shifting to the use of environmentally friendly natural biostimulants. Rhizobacteria can be used as biofertilizers and natural biostimulants. Rhizobacteria that play a role in plant growth are known as plant growth-promoting rhizobacteria (PGPR) (Goswami *et al*., 2016) [4]. Rhizobacteria that live in symbiosis with plant roots can produce phytohormones such as endogenous plant growth regulators synthesized by plants, such as gibberellins, auxins, and cytokinins. (Backer *et al*., 2018) [1]. PGPR produces and induces the phytohormone auxin, especially indole-3-acetic acid (IAA) thereby significantly increasing plant growth (Kaymak *et al*., 2021; Pantoja-Guerra *et al*., 2023) [10,12]. Auxin is a group of plant growth regulators that plays a role in shoot and root growth in cuttings (Sarkar *et al*., 2022; Wardana *et al*., 2025) [17,22]. In addition, PGPR can increase the availability of nutrients for plants and also increase the total nitrogen and phosphorus content in plant tissues (Song *et al*., 2021) [20]. Insoluble potassium present in the soil can be converted into soluble potassium by PGPR, thereby increasing nutrient uptake and plant growth (Parmar and Sindhu, 2019) [14].

The role of PGPR in enhancing the growth of plant cuttings has been reported in several studies, including PGPR stimulating root initiation and increasing adventitious root growth in kiwi plant (*Actinidia deliciosa*) cuttings (Erturk *et al*., 2010) [2], and *Ficus benjamina* cuttings (Sezen *et al*., 2014) [18]. PGPR increased root and shoot growth in *Epipremnum aureum* cuttings (Kaymak *et al*., 2021) [10]. PGPR treatment increased the percentage of root growth and root length in *Euphorbia pulcherrima* cuttings (Karagöz, 2023) [9]. Research utilizing rhizobacteria to enhance the growth of pepper cuttings is still limited. This study aimed to determine the effectiveness of PGPR at different concentrations on shoot and root growth responses in pepper (*Piper nigrum* L.) cuttings. The results are expected to provide an alternative method for environmentally friendly pepper cultivation.

Materials and Methods
This research was conducted in the greenhouse of the Biology Department, Faculty of Mathematics and Natural Sciences, Sriwijaya University. The greenhouse maintained a temperature of 26°C to 29°C throughout the day and night, with an average relative humidity of 65% to 70%. The

mother plants used as cuttings came from a pepper plantation in Banyuasin, South Sumatra, Indonesia.

The parent plant is selected in healthy condition and in vegetative growth, not flowering or fruiting. Cuttings are obtained by cutting branches about 20 cm from the top, having 3 nodes and 3 leaves. Next, the leaves are cut approximately half of the full leaf size to reduce the evaporation process. The planting medium used is a mixture of soil and rice husk charcoal with a ratio of 1: 1. in a 1 kg polybag. The PGPR used is a commercial PGPR containing a consortium of rhizobacteria *Azospirilium* sp., *Bacillus subtilis*, *Azotobacter* sp, and *Pseudomonas flourescens* each 10^{12} Cfu/gr.

This study used a Completely Randomized Design (CRD) with four PGPR concentration treatments, namely; 0 g.L^{-1}, 0.5 g.L^{-1}, 1.0 g.L^{-1}, 1.5 g.L^{-1}, with 10 replications. The data obtained were analyzed using analysis of variance (ANOVA) and continued with Duncan's multiple range test (DMRT) at a 95% confidence level ($\alpha = 0.05$).

The prepared pepper cuttings were soaked in PGPR suspension according to the treatment for 45 minutes, except for the control treatment (0 g.L^{-1}) which was soaked in distilled water (Sezen *et al*., 2014) [18]. Next, the pepper cuttings were planted in polybags containing planting media. Cutting care was carried out by watering three times a day using a hand sprayer. Observations and measurements including shoot growth time (days), number of shoots, shoot length (cm), number of leaves, number of roots, and root length (cm) were carried out at the end of the experiment (60 days), except for the shoot growth time observed from the first day until the shoots grow.

Result and Discussion

The growth response data of pepper cuttings in all treatments are shown in Table 1, while the visual morphology of shoots and roots of cuttings from the control treatment and the best PGPR treatment are shown in Figure 1.

Table 1: Growth response of pepper cuttings (*Piper nigrum* L.) to PGPR treatment (60 days).

PGPR Treatments (g·L^{-1})	Shoot Emergence (days)	Number of Shoots	Shoot Length (cm)	Number of Leaves	Number of Roots	Root Length (cm)
0.0	40.00^{a}	1.00^{a}	6.60^{a}	2.20^{a}	16.80^{a}	12.40^{ab}
0.5	36.00^{ab}	1.40^{ab}	10.50^{ab}	6.20^{b}	26.60^{ab}	8.60^{a}
1.0	31.80^{b}	2.40^{b}	14.20^{b}	6.80^{b}	30.80^{b}	14.20^{ab}
1.5	34.40^{ab}	1.60^{ab}	11.00^{ab}	4.40^{ab}	26.40^{ab}	16.80^{b}

Note: Different superscripts in the same column indicate significant differences at the DMRT test level of 0.05.

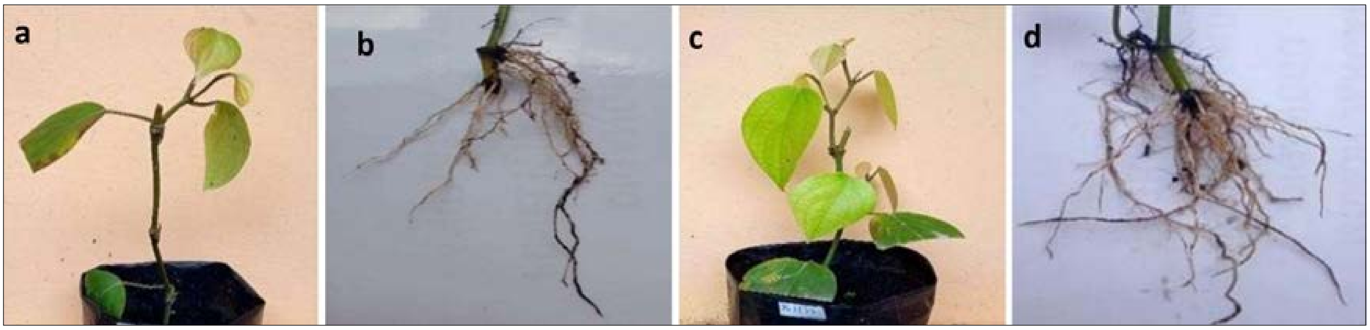

Fig 1: Growth response of pepper cuttings (*Piper nigrum* L.). Shoots and roots without PGPR (a and b), shoots and roots with PGPR 1.0 g·L^{-1} (c and d).

Based on the observation data in Table 1, PGPR at a concentration of 1.0 g.L^{-1} was more effective in increasing shoot and root growth in pepper cuttings. The effectiveness of PGPR at a concentration of 1.0 g.L^{-1} indicates that the rhizobacterial consortium contained in the PGPR used worked synergistically in supporting shoot and root growth in pepper cuttings. PGPR is known to have the ability to synthesize and secrete plant hormones (phytohormones) including cytokinins, gibberellins (GA), and auxins (IAA), which are utilized by plants to stimulate plant growth (Backer *et al*., 2018) [1]. The rhizobacterium Bacillus subtilis in PGPR is able to produce the phytohormone IAA and increase shoot and root growth in ginger rhizomes (Jabborova *et al*., 2021) [7]. The rhizobacteria Pseudomonas fluorescens is known to be able to produce the phytohormone IAA and also plays a role in the availability of phosphate for plants (Parani and Saha, 2012) [13]. Auxin (IAA) and cytokinins play a major role in organ development, cellular responses such as cell differentiation, cell expansion, cell division, and gene regulation (Singh, 2018) [19]. In addition, auxins produced by rhizobacteria in PGPR play 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) [16].

Shoots, leaves, and roots are parts of plants whose formation process cannot be separated from the role of nutrients such as nitrogen (N) and phosphorus (P), which are absolute nutrients needed by plants because these two elements play an important role in the vegetative growth of plants. The increase in vegetative growth (shoots and roots) in pepper cuttings in the PGPR 1.0 g.L^{-1} treatment is related to the availability of nitrogen in plants. It is known that PGPR can increase the availability of nutrients, one of which is nitrogen. Plants that receive sufficient N supplies will encourage the growth of shoots and leaves with high chlorophyll content so that plants can produce sufficient assimilates to support vegetative growth (Masclaux-Daubresse *et al*., 2010) [11]. PGPR is a biofertilizer containing microorganisms that play a role in increasing plant growth and yield through direct mechanisms, namely by fixing nitrogen and solubilizing phosphate, or increasing phosphate availability to plants. PGPR can increase nutrient availability to plants and also increase the total nitrogen and phosphorus content in plant tissue (Song *et al*., 2021) [20].

Conclusion

Based on the research that has been conducted, it can be concluded that PGPR treatment of 1.0 g.L^{-1} provides more effective results and has a significant effect compared to treatment without PGPR in stimulating the growth of shoots, leaves and roots in pepper cuttings (*Piper nigrum* L.). The use of PGPR can be the right choice in efforts to encourage environmentally friendly and sustainable agricultural development (SDGs).

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