

## Rhizosphere Bacteria *Plumeria acuminata* Increases Growth of *Zea mays* After Root Inoculation

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**Abstract.** The bacteria found in the rhizosphere are called *plant growth promoting rhizobacteria* (PGPR), namely bacteria that can stimulate plant growth by producing growth hormones, one of which is the hormone *indole acetic acid* (IAA) which plays a role in regulating plant growth and development. Five isolates of rhizosphere bacteria were isolated on frangipani roots (P26, P31, P36, P37 and P38). Previous research showed that five bacterial isolates were capable of producing the hormone IAA. However, evidence of its potential as a PGPR has not been obtained. Therefore, the aim of this study was to test the effect of inoculation of rhizosphere bacteria on maize plants. The research method uses a quantitative experimental method, namely 15 repetitions for each treatment. Data collection was carried out 1 day after planting (HTS) until the 14th day. Data were analyzed using a 95% confidence student t-test or with a significance level of 5% ( $\alpha=0.05$ ). This inoculation begins with germinating maize seeds and observing the roots. The germinated maize seeds are then soaked in the bacterial suspension for one hour at room temperature. The results showed that inoculation with isolates P36, P37 and P38 significantly increased the height of maize plants, while isolate P37 significantly increased the number of leaves and root length of corn plants, isolate P38 could significantly increase the number of roots of corn plants. Thus, these results support the evidence that rhizosphere bacteria from frangipani have an effect on increasing the growth of maize plants.

**Keywords:** Rhizosphere bacteria, indole acetic acid, growth, maize plants

## Introduction

Rhizobacteria provide food which are located in the rhizosphere of plant roots and have an important role for plants (Tarigan & Jamilah, 2023). Plant-growth-promoting rhizobacteria (PGPR) are a group of bacterial species that colonize the plant root zone

(rhizosphere) and thus have a positive influence on plant growth and health. PGPR is an agricultural biological agent that stimulates plant growth and productivity. They also cause plant resistance to various phytopathogens in many plants, including vegetables, fruits, and some trees (Babalola et al., 2020). Further, PGPR promote the production and oxidation of methane and acetone and increase soil pH, water content, organic carbon content, and porosity (Keswani et al., 2019; Ju et al., 2020). Soil moisture and plant root conditions influence rhizosphere bacterial populations. A high bacterial population indicates the need for energy, appropriate temperature, and sufficient water availability for bacterial growth (Kadeawi et al., 2021).

In addition, several facts show the superiority of rhizosphere bacteria in promoting plant growth. Rhizobacteria isolated from natural grasslands can increase plant growth in vitro and in greenhouse conditions in maize plants (Khambani et al., 2019). Rhizobacteria from soil in Jimma, Ethiopia can significantly increase seed germination, root and shoot length, root fresh and dry weight, and corn shoots in field trials compared to controls (Waday et al., 2022). In addition, bacteria isolated from the rhizosphere of protected soil can significantly increase corn growth (Chen et al., 2021). Some rhizobacteria can increase the growth of corn plants, but no bacteria have been isolated from frangipani plants in graves.

Rhizosphere bacteria synthesize the growth hormone, indole acetic acid, which plays a role in regulating plant growth and development. Apart from producing IAA, rhizosphere bacteria are also able to solubilize phosphate and act as biocontrol agents by inducing the plant immune system (Marista et al., 2013). IAA is a major member of the auxin family that controls many important physiological processes including cell enlargement and division, tissue differentiation, and response to light and gravity. IAA is known to produce more lateral roots, root hairs, and root hair branches (Rini et al., 2020). However, the abilities of rhizosphere bacteria can vary depending on the biotic and abiotic conditions where the bacteria originate (Lengkong et al., 2022). The presence of certain bacteria that can produce IAA causes increased plant growth (Putra & Advinda, 2022).

Previous research has isolated 43 isolates of rhizosphere bacteria from frangipani trees (*Plumeria acuminata*) in the cemetery area. Among them, 5 isolates were selected which were proven to produce high IAA, namely P26, P31, P36, P37 and P38 with IAA production of 9.81; 28.14; 12.21; 83.09 and 65.50 (Putra et al., 2023). However, its effect on plant growth has not been tested. Therefore, 5 isolates were selected and inoculated on maize roots and then observed maize growth 14 days after inoculation (dai). Maize plant was employed as the experiment object since its prominent crop for supporting food security. In this study, growth differences were analyzed and compared with controls (plants that were not inoculated). This study aims to test the effect of inoculation of rhizosphere bacteria on Maize plants using 5 isolates proven to have high IAA content. Through this research, it is expected to obtain information about rhizosphere bacterial isolates that affect the growth rate of Maize plants.

## Methods

The used materials in this experiment were rhizosphere bacterial isolates isolated from frangipani roots with codes P26, P31, P36, P37, and P38. Bacterial isolates were grown on nutrient agar (NA) and nutrient broth (NB) for culture preservation and inoculation. Plant growth inoculation was carried out using maestro rajawali maize (*Zea mays*) seeds. Meanwhile, other materials used were 70% ethanol, erlenmeyer, sterile Petri dishes, sterile tweezers, sterile water, laminar air flow, test tubes, and plastic polybags, soil media, spirit burner, markers, labels.

The starter was made using bacterial isolates from frangipani roots P26, P31, P36, P37, and P38. Then the bacterial isolate was taken using a loop needle from the agar slant which was inoculated into a test tube containing 5 mL of NB media (aseptic). Do the same thing with the other isolates, then put the test tube containing the NB media that has been inoculated with the isolate into plastic to make it safer when homogenized using a shaker. Incubate for 6 hours at room temperature with a shaker. Then proceed by using 5% bacterial suspension (0.5 mL) into a test tube containing 10 mL NB media (aseptic). Do the same with all other bacterial isolates. The test tube containing NB media is put in plastic to make it safer. Incubate for 6 hours at room temperature with a shaker then store in the refrigerator.

Maize kernels are soaked in warm water for 6 hours. The seeds chosen for sowing are sunken seeds (Mustaqimah et al., 2020). Then discard the soaking water. After that, the Maize seeds are put into a petri dish which has been lined with sterile cotton and moistened with distilled water. Close the solder container then wrap it with plastic wrap so that it is tight. Store in a dark place for approximately 3 days.

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Maize seeds from which roots have emerged are inoculated into a bacterial suspension whose concentration is known in line with research (Lopes et al., 2021), namely by soaking the seeds in a solution of microorganisms whose concentration is known. Inoculation was carried out by placing Maize seeds in a bacterial suspension in a stained dish for 1 hour. Comparison of 10 ml bacterial suspension: 10 seeds (Putra & Advinda, 2022). Maize seeds are sown in polybags measuring 20x20 cm. Each polybag contains 3 seeds. Maize seeds sown in polybags are watered every day and their height and number of leaves are measured. Treatment was stopped when the plants were 14 days old, by recording: plant height (cm), number of leaves (strands), number of roots (total) and root length (cm).

The plant growth research data collection technique uses a quantitative experimental method, namely by rolling 15 times for each treatment ( $n = 15$ ). Data collection was carried out from day 1 to day 14 after planting. Data obtained from observing plant growth parameters was then analyzed using a 95% confidence student t-test or with

a significance level of 5% ( $\alpha = 0.05$ ). The variables observed in this research include: plant height, number of leaves, root length and number of roots.

## Results and Discussion

Germination is the process by which a seed begins its growth into an adult plant. Germination involves four main processes, namely imbibition, enzyme system formation, initiation of organ formation and shoot formation until it emerges from the soil surface (Steinbrecher & Leubner-Metzger, 2017). There are two factors that influence germination, namely internal (internal factors) such as genetics and hormones, while external factors (external factors) such as nutrition, light, humidity, water, temperature (Maghfiroh, 2017).

The first step is seed preparation, this preparation can be done by soaking the seeds in water, usually bad seeds will float on the surface of the water. During this preparation, the process of water imbibition into the seed also occurs which causes the seed to expand, breaks down the skin covering the seed and triggers changes in metabolites in the embryo which causes the seed to continue growing (Shofi, 2017). The following figures are the results of rhizosphere bacterial inoculation in the maize root at 14 days after inoculation.

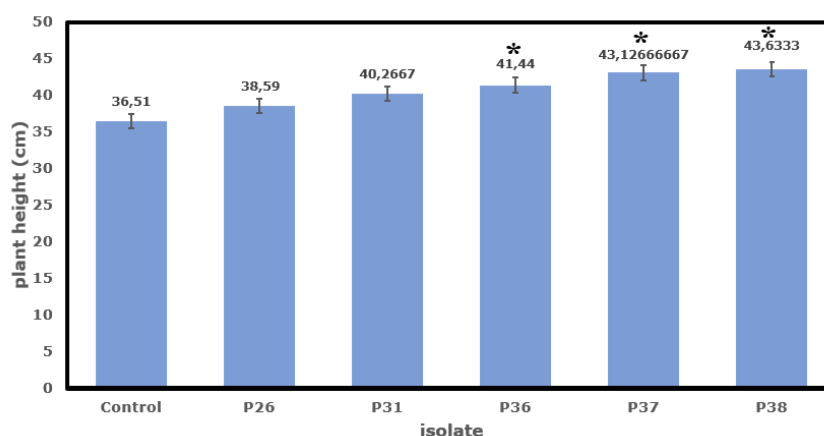


Figure 1. T test results on isolates P36, P37 and P38 showed a significant differences (\*)

Based on data from research conducted on Maize plants, P36, P37 and P38 isolates inoculated on Maize plants showed significant differences in plant height compared to the control group (Figure 1). Other isolates such as P26 and P31 also increased the height growth of Maize plants compared to the control treatment but were not significantly different from isolates P36, P37 and P38. Rhizobacteria encourage plant growth both directly and indirectly (Sembiring & Sumanto, 2021). The bacteria found in the rhizosphere are called PGPR, namely bacteria that can trigger plant growth by producing growth hormones, dissolving phosphate and becoming biocontrol agents against plant pathogens (Nuraini et al., 2020).

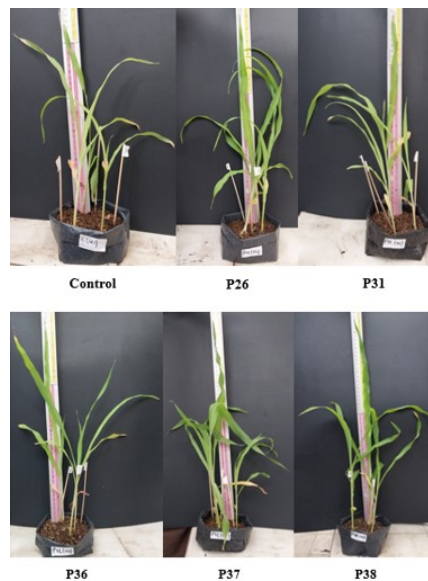


Figure 2. Representative photos of all Maize plant treatments after 14 days.

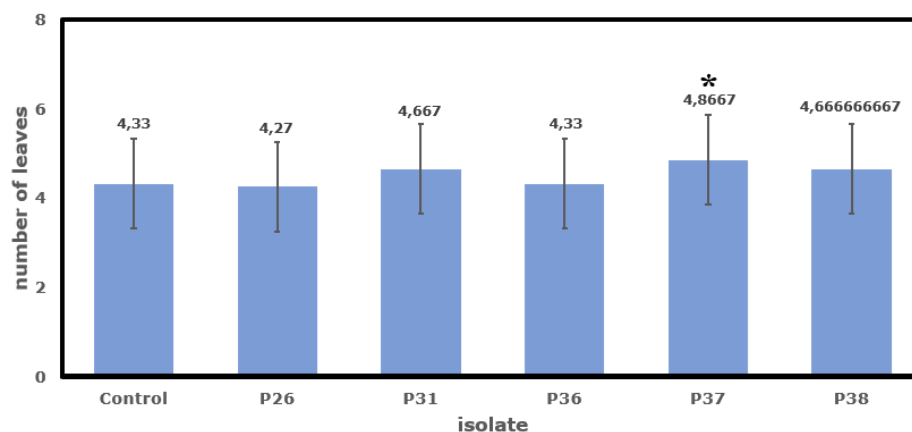


Figure 3. T test results on isolate P37 showed a significant difference (\*)

Based on research data conducted on Maize plants inoculated with isolate P37, it showed a significant difference in the number of leaves compared to the control group (Figure 3), and other isolates such as P26, P31, P36 and P38 also had an effect on the number of leaves compared to the control, but not significantly. This is in line with (Yulia et al., 2020) where in their research on Cymbidium orchid plants, the number of leaves was not influenced by IAA treatment. The increase in the number of leaves is also influenced by an increase in the rate of photosynthesis, where the rate of photosynthesis will be followed by the results of photosynthesis. The results of photosynthesis play a role in the formation of plant structures. Low plant height can affect the number of leaves. Plant height affects the number of leaves, namely the taller the plant, the greater the number of leaves (Suleman et al., 2019).

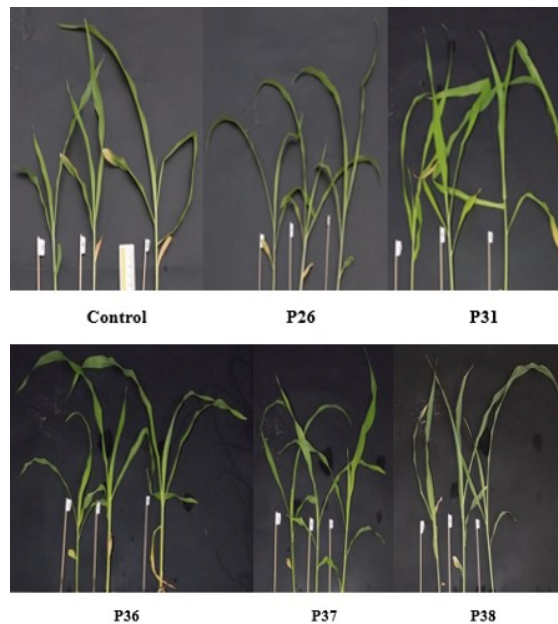


Figure 4. Representative photos of all Maize plant treatments after 14 days

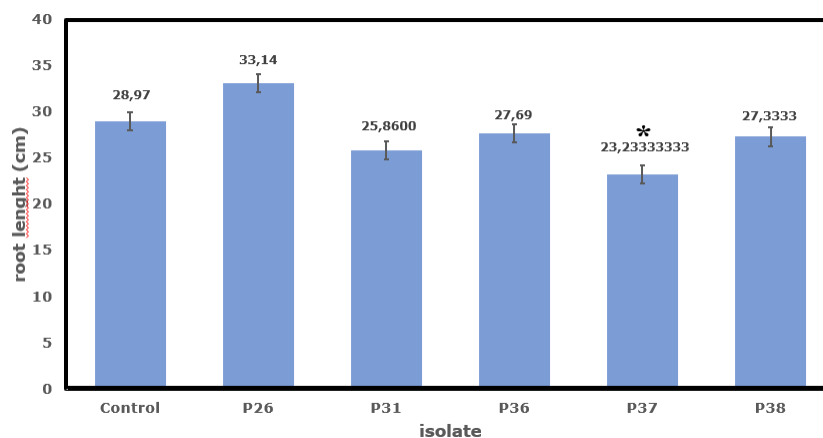


Figure 5. T test results on isolate P37 show a significant differences (\*)

Based on research data conducted on Maize plants, the P37 isolate inoculated on Maize plants showed a significant difference in root length compared to the control group (figure 5). Isolate P26 also had an effect on root length compared to the control, but not significantly. The elongation of the roots of this plant is caused by the IAA content in the isolate. The presence of the IAA hormone can stimulate plant growth by increasing root growth (Suwarni & Advinda, 2021). This is in line with (Ismawanti et al., 2022) that IAA produced by bacteria can increase the number of plant root branches in green beans. Good root growth by elongating the taproot and increasing the number of branches provides an advantage for the plant in absorbing water and nutrients from the environment and ultimately increases the plant's chances of survival. Root length is influenced by the role of IAA in stimulating the cell elongation process, influencing the elasticity and plasticity of cell walls. Root elongation increases wet and dry weight so that plant growth becomes better (Zuhra, 2017). However, this was different from isolates P31, P36, P37 and P38 (figure 5)

where the four plants inoculated with these isolates had shorter roots than control plants. This is in line with research (Herlina et al., 2016) that low IAA concentrations induce root and shoot elongation, while high IAA concentrations inhibit shoot and root elongation. Providing IAA-producing isolates does not affect the length of root germination because the presence of other hormones such as ethylene in roots which are produced in large quantities can inhibit root development (Suwarni & Advinda, 2021). One of the plant growth mechanisms is the production of the IAA hormone by microorganisms (Ismawanti et al., 2022). The metabolic process in the plant body takes place by utilizing IAA produced by bacteria so that it plays a role in the growth process in terms of height, stem diameter, number of leaves and plant seed area (Puspita et al., 2019). Apart from that, it is thought that Maize plants must be watered sufficiently to meet their water needs, so that they do not need to grow roots to expand the catchment area. Water needs are met so that increasing the roots to expand the absorption area is not needed. This is in line with (Solin et al., 2021) that mild to moderate drought increases root growth to expand the absorption area, and adequate water treatment produces a larger root area compared to less water treatment.

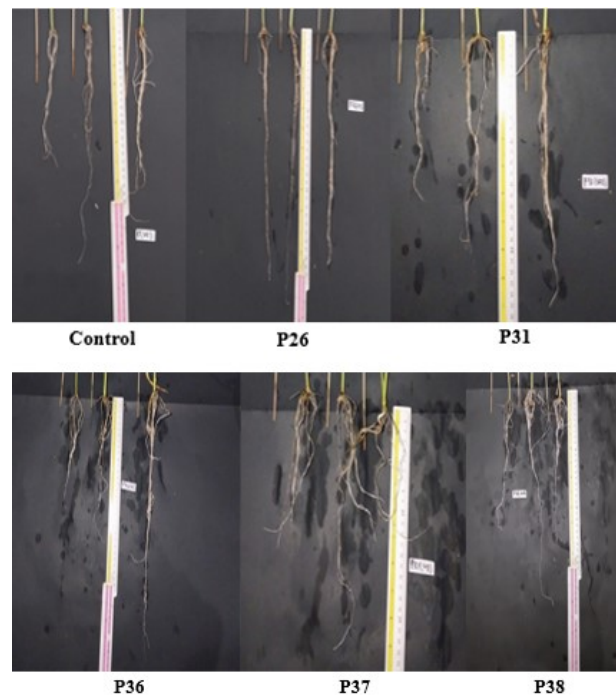


Figure 6. Representative photos of all treatments on Maize plants after 14 days

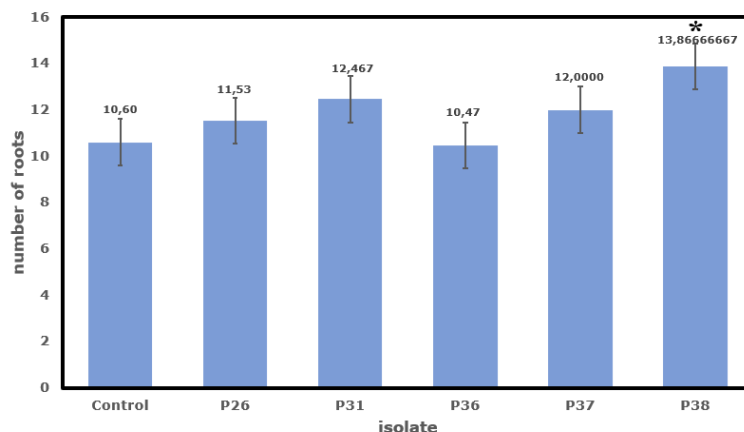


Figure 7. T test on isolate P38 showed a significant difference (\*)

Based on data from research conducted on Maize plants, isolate P38 had a significant effect on the number of roots in Maize plants compared to the control group (Figure 7). IAA application affects the number of lateral roots, but does not affect shoot length (Herlina et al., 2016). IAA content affects root length, root area and number of root tips (Murthi et al., 2015). IAA production encourages cell elongation and the growth of root hairs and lateral roots of plants is encouraged by IAA production, so that the availability of nutrients and water is sufficient (Nugraheni et al., 2022). The results of the analysis (Pangestika et al., 2015) showed that the administration of IAA and Paklobutrazol showed a real effect on the number of garlic cloves. Growth slowed at higher values of IAA with paclobutrazol administered. Growth is strongly influenced by auxin, auxin stimulates and accelerates root growth and improves root quality and quantity. A study (Wuriesyliane & Sawaluddin, 2022) also states that the level of endogenous auxin content in plants affects the number of roots. Higher hormone levels can stunt growth and even poison plants.

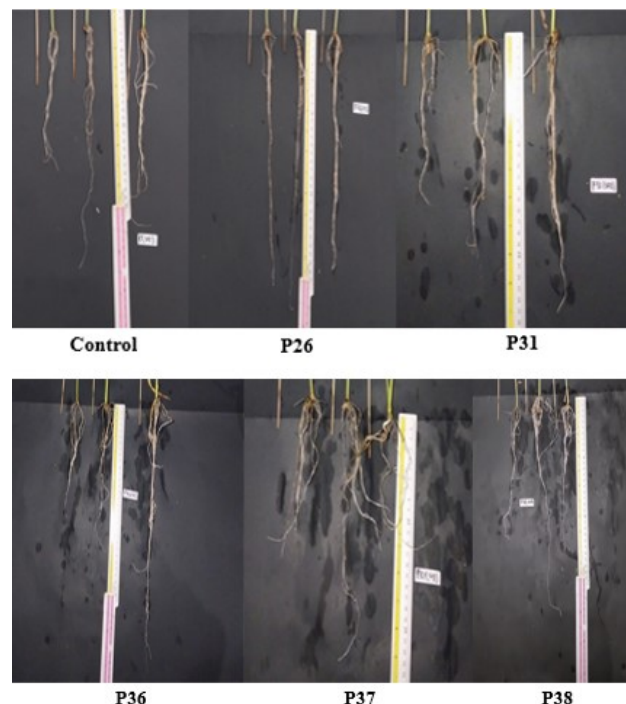


Figure 8. Representative photos of all Maize plant treatments after 14 days

The Auxin plays an important role in stem elongation, apical dominance, lateral root initiation, cell elongation, cell division, cell differentiation, etc. In monocot plants, auxin plays a role in the formation of adnaxal roots, while in dicot plants it plays a role in the formation of adnaxal roots. lateral root formation (Aji & Lestari, 2020). Abiotic factors such as soil (nutrients, heavy metals, pH, and salinity), water availability, light intensity, and temperature can influence plant-microbial interactions because they can change plant metabolism (Lopes et al., 2021).

## Conclusion

Rhizosphere bacterial isolates from frangipani can increase the growth of Maize plants. Maize plants treated with isolates P36, P37 and P38 can increase the height of Maize plants, isolate P37 can increase the number of leaves and root length of Maize plants, isolate P38 can increase the number of roots of Maize plants. Thus, these results support the evidence that rhizosphere bacteria from frangipani have an effect on increasing the growth of Maize plants.

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