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INOCULATION OF ENDOPHYTIC BACTERIA FOR INCREASING PLANT HEIGHT AND NUMBER OF RICE ROOTS (*Oryza sativa*)

Fari Fadilah[1], Triastuti Rahayu[2], Putri Agustina[3], Kistantia Elok Mumpuni[4], Listy Hasti Mandiri[5], Trio Ageng Prayitno[6], & Yasir Sidiq[7]*
[1,2,3,5,&7]Department of Biology Education, Faculty of Teacher Training and Education, University of Muhammadiyah Surakarta, A. Yani Street, Sukoharjo, Central Java 57162, Indonesia
[4]Department of Biology Education, Faculty of Teacher Training and Education, University of Sebelas Maret, Ir. Sutami Street Number 36, Surakarta, Central Java 57126, Indonesia
[6]Department of Biology Education, Faculty of Exact Sciences and Sports Education, Budi Utomo Institute of Education, Simpang Arjuno Street Number 14B, Malang, East Java 65119, Indonesia
**Email: ys120@ums.ac.id*
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ABSTRACT: Four endophytic bacteria have been isolated from banana kluthuk plants and banana ambon. Previous study showed that they produce Indole Acetic Acid (IAA). This study aimed to analyze the effect of endophytic bacterial inoculation on plant height and the number of rice root. For this reason, two endophytic bacteria of each klutuk banana and ambon banana plants were cultured and examined for growth rate using a 600 nm spectrophotometer. Isolate growth was calculated every two hours for 16 hours of bacterial growth in NB media. Bacterial inoculation experiments on rice plants were carried out with five replications. This inoculation begun with germination of rice seeds until the radicle was observed. Then germinated rice seedlings were soaked in a bacterial suspension for 1 hour at room temperature. The results showed the exponential phase of all isolates was observed at 6 hours with shaking. Inoculation using A22 and A51 isolates significantly increases plant height, number of leaves. While, A22 isolate significantly increased the number of roots compared to the uninoculated plants (control). It supports the potential of endophytic bacteria from banana kluthuk and ambon plants.

Keywords: Endophytic Bacteria, Root Inoculation, Rice Growth.

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INTRODUCTION

Microorganism exploration has been widely developed, not only for rhizosphere microorganisms, but also for microorganisms in plants (endophytes) that function as plant growth boosters (Hidayati et al., 2014). Endophytes include fungi or bacteria that part of their life cycle attacks living plant tissue and is asymptomatic entirely in plant tissue (Aziez, 2022). Endophytic bacteria are beneficial to their host plants, including stimulating plant growth, inducing plant resistance to pathogens, and fixing nitrogen (S & Sopialena, 2022). Endophytic

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bacteria are isolated or extracted from healthy plant parts, the process of colonization of plant tissues by endophytes through complex stages which include adaptation, spore germination, penetration and colonization is able to produce bioactive compounds that have similar or the same characters as their hosts (Pratiwi, 2021). This is due to the genetic exchange that occurs between the host and endophytic bacteria evolutionarily (Lestari, 2021).

The information growth rate of the endophytic bacteria is crucial prior the root inoculation. This is because the bacterial inoculation is effective at the exponential growth phase. Purnama (2023) emphasizes that bacterial growth can be monitored through a growth curve using a spectrophotometer. The main purpose of this curve is to identify the optimal time for bacterial cell division. The growth curve consists of four distinct phases, namely the lag phase, the exponential phase, the stationary phase, and the death phase (Risna et al., 2022). The lag phase is the adjustment phase of a microbial activity in its new environment (Purnama, 2023). The exponential phase is influenced by temperature, pH, nutrient conditions in the media and microbial genetic properties (Risna et al., 2022). The stationary phase when the nutrients in the medium are getting thinner, in this phase there is no significant decrease or increase in the number of cells (Wahyuningsih & Zulaika, 2019). In the death phase occurs when the amount of nutrients decreases and cell viability decreases, the death phase can also occur environmental changes such as increased accumulation of toxic substances in the growth medium (Novanti & Zulaika, 2019). However, the information of growth phase of the four isolate was unknown. Thus, this study examined the growth phase of the bacteria prior to root inoculation.

Endophytic bacteria as Plant Growth Promoting Bacteria (PGPB) are used as inoculants to increase plant growth and yield (Ji et al., 2014). PGPB is found in the soil around plant roots, vegetal tissues, and leaf or stem surfaces (Andana et al., 2023; Orozco-Mosqueda et al., 2020). Endophytes affect plant growth and biomass by producing phytohormones such as Indole Acetic Acid (IAA) (Raimi & Adeleke, 2023). The IAA hormone is a key hormone for various aspects of plant growth and development (Tanjung et al., 2015). Several endophytic bacteria have been isolated from rice, sugarcane, sorghum, grass, and corn. As many as 4 out of 8 bacterial isolates showed the ability to spur the growth of plant height and the number of roots of rice plants. This is because some isolates are able to stimulate the production of the IAA hormone. The isolates used are, A22, A51, K1, and K28. Therefore, this study aimed to investigate the effects of inoculation of endophytic bacteria on plant height and the number of roots of rice plants (*Oryza sativa*). This study sought to uncover new insights into their potential as agents to enhance plant growth.

METHOD

This research was carried out in April - June 2023 at the Tissue Culture Laboratory and Research Laboratory of the Department of Biology Education, Faculty of Teacher Training and Education, University of Muhammadiyah Surakarta. The methods implemented include bacterial growth analysis and

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bacterial inoculation in rice plants on several isolates that have successfully measured bacterial growth curves.

Tools and Materials

This study used one endophytic bacterial isolated from the root of banana klutuk, one endophytic bacteria from the midrib of banana kluthuk and two endophytic bacteria from the root of banana ambon. The two endophytic bacteria from banana klutuk are coded K1, K28, while endophytic bacteria from banana ambon are coded A22, A51. These bacteria are grown in Nutrient Agar (NA) and Nutrient Broth (NB) for preservation and culture inoculation respectively. The concentration of bacteria was measured using the Shimadzu 1280 UV-Vis spectrophotometer. Initial inoculation of plant growth was carried out on rice plants (*Oryza sativa* L.) cultivar Inpari-32. Seeds are obtained from the public market.

Trial Design and Procedure

The experimental design used in this study is an experimental method with three repetitions (n = 3) for bacterial growth analysis. While five repetitions (n = 5) for bacterial inoculation in rice plants for each selected isolate.

Bacterial Growth Analysis

Growth curves are needed to determine the optimum incubation time for endophytic bacteria isolated from banana plants. The growth curve in this study was created by counting the number of bacterial cells during incubation at specific time intervals (Candrawati et al., 2018). The calculation of the number of bacterial cells to create a growth curve begins with preculture, in which bacterial isolates from oblique agar are inoculated into NB with a volume of 6 mL and incubated for 8 hours at room temperature at a speed of 100 rpm. After that, 1.5 mL of preculture is poured into 50 mL NB then incubated by agitation. Optical Density (OD) observation using UV-Vis spectrophotomoter at 600 nm wavelength (Novanti & Zulaika, 2019). On non-implanted NB media, bacterial isolates are prepared for blanks. After growing, the Optical Density value was measured until it showed an absorbance value of 1 (Respati et al., 2017). Optical Density (OD) measurement at intervals every 2 hours until the 16th hour. The Optical Density data obtained is then made a growth curve with the x-axis as time (t) and the y-axis as a result of the results of Optical Density (Novanti & Zulaika, 2019).

Bacterial Suspension Preparation

The starter is made with bacterial isolates of kluthuk K1 banana root, K28 kluthuk banana sheath, and A22, A51 ambon banana root. Bacteria were taken 1 ose on NA media and transferred into 6 mL NB media aseptically. Do the same with other bacterial isolates, NB media is inserted into a test tube then inserted into plastic to make it safer when incubated on a rotary shaker at a speed of 100 rpm at room temperature for 6 hours. Then proceed with the preparation of bacterial suspension from the starter as much as 5% (0.5 mL) into NB 10 mL media aseptically. Do the same with other bacterial isolates, NB media is put into a test tube then put into plastic to make it safer when incubated on a rotary shaker at a speed of 100 rpm at room temperature for 6 hours then stored in the refrigerator.

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Rice Seed Sterilization

Rice seeds are sterilized by soaking with warm water for 5 minutes. The seeds chosen for sowing are sunken seeds (pithy seeds) (Mustaqimah & Nurhatika, 2019). After that seed sterilization is carried out in LAF with stages soaked in 70% alcohol for 1-2 minutes with a ratio of 1 : 1, alcohol is removed and rinsed with sterile aquades, then soak the seeds using 1% NaOCl solution for 1 minute then removed and rinsed with sterile aquades 3 times. Seeds that have been sterilized are then put into culture bottles that are given sterile aquades in a ratio of 1 : 1. The culture bottle is then covered with aluminum foil and plastic wrap and placed at room temperature for up to 24 hours (Lisdyayanti, 2019).

Bacterial Inoculation in Rice Plants

Rice seeds are germinated by soaking sterile aquades for 6 hours, then placed on a petri dish that has been given filter paper for 48 hours until the seeds grow roots. This inoculation is done by soaking rice seeds in a bacterial suspension. Rice seedlings that have emerged roots are then transferred to sterile petri dishes using sterile tweezers. Inoculation of rice seeds by bacterial suspension as much as 10 mL is poured on rice seeds and soaked deeply on a petri dish for 1 hour. Rice seeds are put into plastic pots measuring 8 cm. Each pot contains 3 seeds as many as 5 repetitions. Rice seeds that have been planted in pots are watered with water as much as 50 mL per pot every day. The treatment was stopped after the plants were 14 HST by observing: plant height (cm), number of leaves (strands), number of roots (total), and root length (cm).

Data Analysis

The amount of bacterial growth was qualitatively analyzed based on the sample absorbance results from the spectrophotometer. Meanwhile, the observation data of plant growth parameters were analyzed with a 95% student t-test confidence test or with a significance level of 5% ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Endophytic Bacteria Growth Test

A total of 4 different isolates were obtained from endophytic bacteria isolated from banana klutuk root, kluthuk banana midrib, and ambon banana root. Bacterial population growth is studied by observing growth curves in bacterial cultures.

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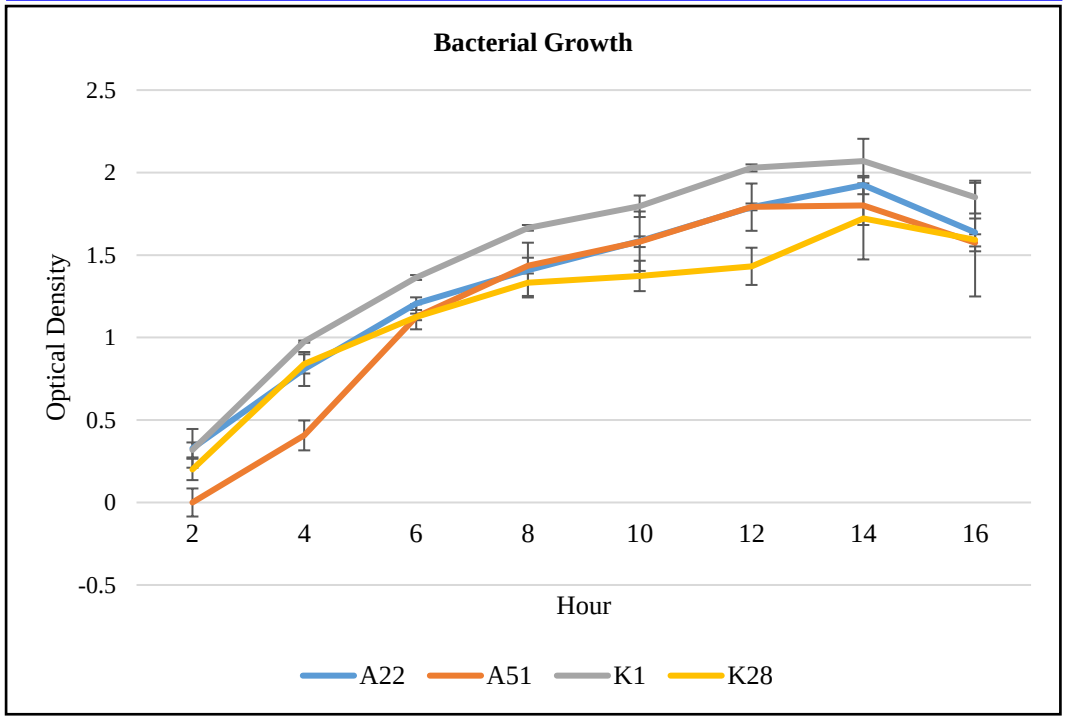

Figure 1. Growth Curves of A22, A51, K1, and K28 Endophytic Bacteria.

Making a bacterial growth curve based on the absorption value seen on the spectrophotometer. The wavelength used is 600 nm. This test aims to determine the growth of selected isolates from kluthuk banana plants and ambonese bananas (A22, A51, K1, and K28) on NB media for 16 hours, with observations every 2 hours. The choice of NB media because it contains complete nutrients for bacterial growth (Purnama, 2023). The results of the observation of the growth of selected isolates can be seen in Figure 1.

The growth pattern of bacterial isolate consists of 4 phases, namely the adaptation phase, exponential phase, stationary phase, and death phase. The growth curve shows that the selected 3 isolates have a timed adaptation phase that is in the first 2 hours of incubation. However, A51 isolate undergoes an adaptation phase at an incubation time of 4 hours. Because A51 isolate experiences slow cell growth due to isolate adapting to the environmental conditions of the growing media. The adaptation phase indicates the initial growth of the bacterial culture after inoculation on the new medium. Indirect growth occurs because it requires a period of time to adapt (Novanti & Zulaika, 2019). In this phase, slow growth occurs influenced by the process of adjusting to environmental conditions such as pH, temperature, and nutrients (Risna et al., 2022). The adaptation phase occurs at the incubation time of the first two hours of the initial growth period, then there has been an exponential phase in the next two hours (Nurhajari et al., 2016).

The exponential phase in all isolates occurs at 6-10 hours. The highest exponential phase in isolate K1 occurs at the 10th hour with an absorbance of 1.841. On average, all four isolates after reaching the exponential phase experienced a decrease in the growth curve. This event is possible because many

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bacteria that have died and then settle to the bottom of a liquid medium that is less homogeneous due to lack of shaking before the isolate is measured turbidity value. This causes the measured OD value on the spectrofotmeter to be very low (Respati et al., 2017). The stationary phase occurs at different hours of isolation from one another. The duration of the stationary phase is between 2-3 hours. In this phase the metabolism of bacteria begins to slow down due to a decrease in the amount of nutrients needed in their growth. The stationary phase occurs at 10-14 hours. In this phase, the number of bacteria that grow is balanced with the number of dead bacteria (Risna et al., 2022). This phase is caused by reduced sources of nutrients, the formation of inhibitory compounds, and unfavorable environmental factors (Purnama, 2023). Endophytic bacteria will experience a lysis phase or death phase after passing through the stationary phase (Candrawati et al., 2018). The lysis phase occurs in all isolates after the 16th hour of incubation.

Effect of Endophytic Bacteria Inoculation on Rice Plants

Examination of endophytic bacteria is carried out whether it affects the growth rate of plants. The results of research on the growth of rice plants inoculated with endophytic bacteria isolated from kluthuk banana root, kluthuk banana frond and ambon banana root are seen in Figure 2, Figure 3, Figure 4, and Figure 5.

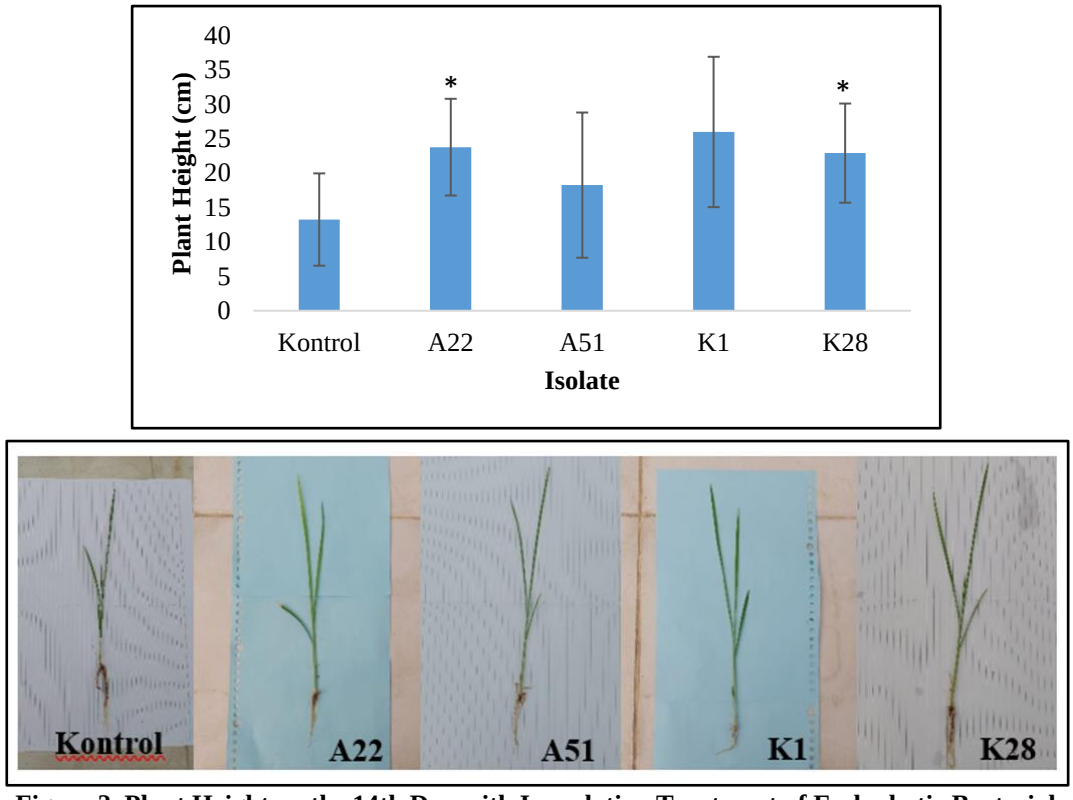

Figure 2. Plant Height on the 14th Day with Inoculation Treatment of Endophytic Bacterial Isolate. The Results of the Students' T-Test Showed that Treatment with A22 and K28 Isolates Showed a Noticeable Difference in Plant Height. The Bar Indicates the Standard Deviation. The Asterisk (*) Shows a Significant Difference Compared to the Control with a Confidence Level (α) of 0.05.

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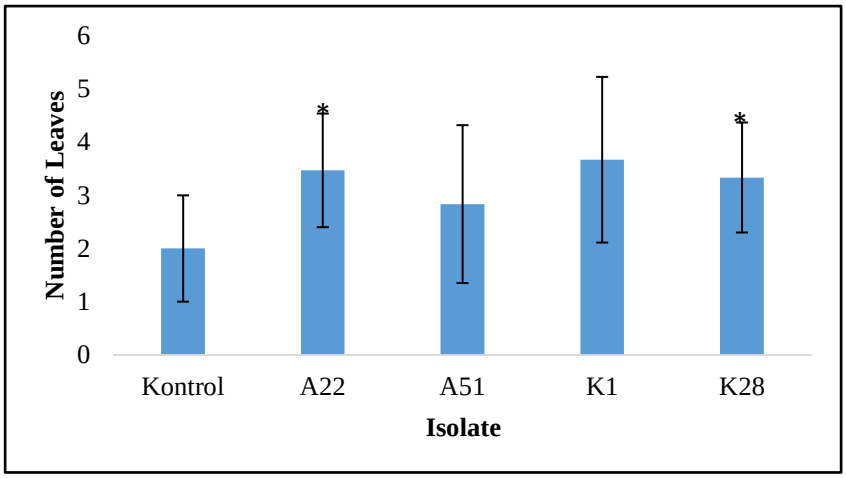


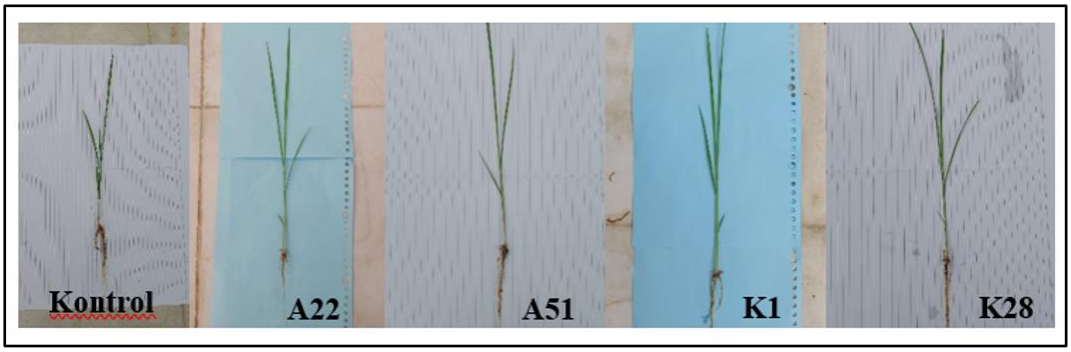
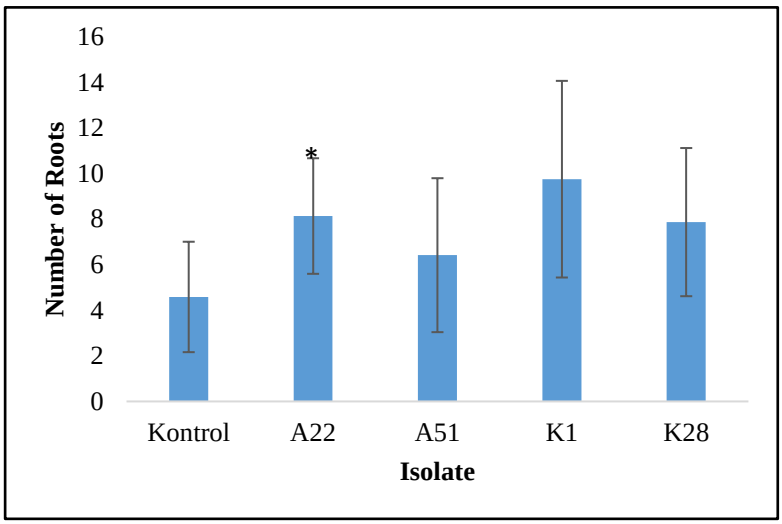


Figure 3. The Number of Leaves on the 14th Day with Inoculation Treatment of Endophytic Bacterial Isolate. The Results of the Students' T-Test Showed that Treatment with A22 and K28 Isolates Showed a Noticeable Difference in the Number of Leaves. The Bar Indicates the Standard Deviation. The Asterisk (*) Showed a Significant Difference Compared to the Control with a Confidence Level (α) of 0.05.



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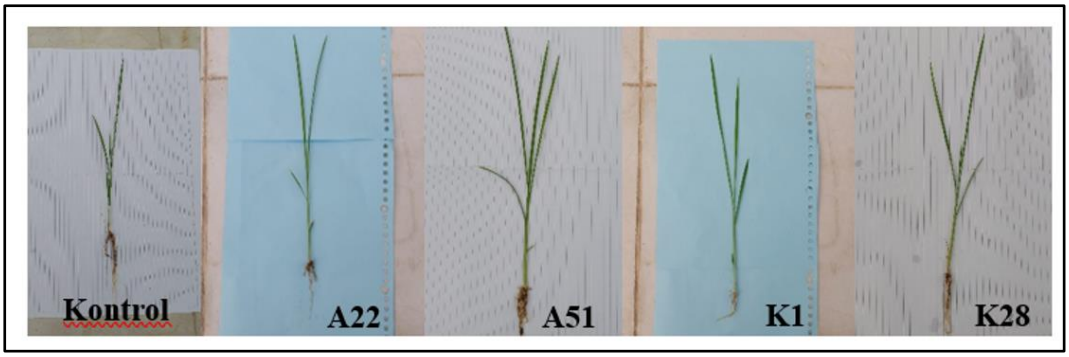

Figure 4. The Number of Roots on the 14th Day with Inoculation Treatment of Endophytic Bacterial Isolate. The Results of the Students' T-Test Showed that Treatment with A22 Isolate Showed a Noticeable Difference in the Number of Roots. The Bar Indicates the Standard Deviation. The Asterisk (*) Showed a Significant Difference Compared to the Control with a Confidence Level (α) of 0.05.

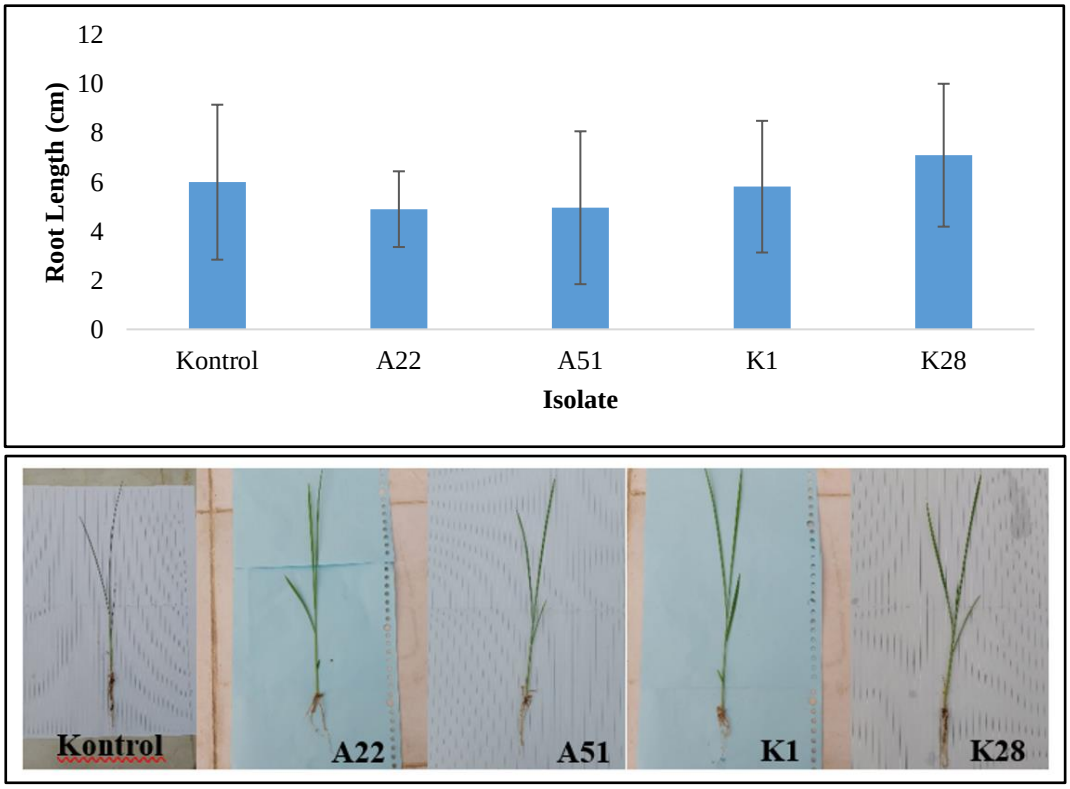

Figure 5. Root Length on the 14th Day with Inoculation Treatment of Endophytic Bacterial Isolate. The Results of the Students' T-Test Showed that Treatment with A22, A51, K1, and K28 Isolates Did Not Show a Noticeable Difference in Root Length. The Bar Indicates the Standard Deviation.

Based on the results of research that has been conducted (Figure 5) from four isolates inoculated into rice plants showed a noticeable effect with control plants on plant height growth, number of leaves and number of roots. However, some bacterial isolates increased plant height in A22 and K28 isolates (Figure 2), the number of roots in A22 isolates (Figure 4), and the number of leaves in A22

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and K28 isolates (Figure 3) compared to the control treatment. Plant growth is an event of increasing plant size as measured by size and diameter. The increase in plant size is the result of increasing the number and size of cells. The role of endophytic bacteria is influenced by the suitability of certain host plants to certain endophytic bacteria (Wulandari et al., 2023). In isolate A22 and A51 significantly increase plant height, number of leaves. In the number of roots that show significant is found in the A22 isolate. It supports the potential of endophytic bacteria from banana kluthuk and ambon plants. Endophytic bacteria can promote plant growth by providing plants with nutrients such as nitrogen, phosphates, and other minerals and producing growth hormones such as ethylene, auxin and cytokinins (Murthi et al., 2015).

Testing the potential of endophytic bacteria producing IAA hormones needs to be done so that it is known which bacterial isolate has the potential to produce the highest IAA hormone (Arifiani & Lisdiana, 2021). K1 isolate was detected to produce IAA of 37.26 or moderate concentration compared to K28, A22, and A51 isolates. The higher the level of IAA used, the better the effect on plant growth. Inoculation of IAA-producing endophytic bacteria can function to increase height and encourage early growth of rice plants. At low IAA concentrations cause root and shoot elongation, the higher the IAA concentration, the elongation of shoots and roots becomes inhibited (Herlina et al., 2016). It can be seen in (Figure 5) that treatment with A22, A51, K1, and K28 isolates showed no noticeable difference in root length. There is a real influence on the length of plant shoots and the number of leaves of potato plants due to the administration of auxin, which in this case in low conditions IAA is able to stimulate elongation of the roots, while at high levels IAA can inhibit the elongation of the roots (Amri et al., 2019).

CONCLUSION

A22 and A51 isolates significantly increase plant height, number of leaves. In the number of roots that show significant is found in the A22 isolate. It supports the potential of endophytic bacteria from kluthuk and ambon banana plants.

SUGGESTION

Endophytic isolate has the potential as a biological agent to promote plant growth is recommended to be tested further for identification to determine the cause of growth differences.

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