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ENDOPHYTIC BACTERIA FROM BANANA PLANTS IMPROVES THE GROWTH OF RICE (*Oryza sativa*) AND MAIZE PLANTS (*Zea mays*)

BAKTERI ENDOFIT DARI TANAMAN PISANG DAPAT MENINGKATKAN PERTUMBUHAN TANAMAN PADI (*Oryza sativa*) DAN JAGUNG (*Zea mays*)

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Abstract

Five isolates of endophytic bacteria from the roots of klutuk and ambon banana plants produce indole acetic acid (IAA) hormone. However, the evidence of its potential as Plant Growth Promoting Bacteria (PGPB) has not been observed. Therefore, this study aims to determine the effect of bacterial isolate to increase the growth of rice and maize plants. The growth rates of bacterial isolates were measured before the inoculation into the plant root. The root inoculation was performed following a complete randomized design. Root inoculation was carried out using 5 isolates of endophytic bacteria in rice and maize plant seedlings, and the process was repeated 15 times (n=15). The growth of rice and maize plants was monitored from 1st day after planting (dap) to the 14th dap. The collected data were analyzed by student t-test with a significant level of 5% (a= 0.05). The results showed that the logarithmic phase of all isolates was reached at 4 to 16 hours and K10, K25, K35, K111, and A41 isolates increased the height of plants while the K111 isolate can significantly increase the plant height compared to the control. This experiment revealed that the endophytic bacteria from banana plants increase the rice and maize plant's growth.

Keywords: Endophytic bacteria; Growth rate; Indole acetic acid; PGPB

Abstrak

Lima isolat bakteri menghasilkan hormon indole acetic acid (IAA) yang berpotensi memacu pertumbuhan tanaman. Namun, bukti empiris potensinya sebagai Plant Growth Promoter Bacteria (PGPB) belum didapatkan. Penelitian ini bertujuan untuk mengetahui pengaruh isolat bakteri terhadap peningkatan pertumbuhan padi dan jagung. Penelitian ini menggunakan metode eksperimen dengan desain rancangan acak lengkap dan setiap perlakuan diulang 15 kali (n= 15). Hasil pertumbuhan tanaman yang diinokulasi dengan isolat bakteri masing-masing dibandingkan dengan perlakuan kontrol. Sebelum perlakuan, tingkat pertumbuhan bakteri endofit diukur sehingga didapatkan fase logaritmik yang cocok untuk dilakukan inokulasi ke akar tanaman. Setelah perlakuan, pertumbuhan tanaman padi dan jagung dipantau dari hari ke-1 setelah tanam (hat) sampai dengan ke-14 hat. Data dianalisis dengan uji t dengan taraf signifikan 5% (α= 0,05). Hasil penelitian menunjukkan bahwa 5 isolat yang berasal dari tanaman pisang mengalami fase logaritmik pada masa inkubasi 4 hingga 16 jam dan semua isolat bakteri meningkatkan tinggi tanaman serta isolat K111 dapat meningkatkan tinggi tanaman secara signifikan dibandingkan dengan kontrol. Penelitian ini mengungkapkan kemampuan bakteri endofit dari tanaman pisang untuk meningkatkan pertumbuhan tanaman.

Kata Kunci*: Bakteri endofit; Indole acetic acid; Laju pertumbuhan; PGPB*

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INTRODUCTION

Endophytic bacteria in the plant's tissues commonly have many benefits in improving plant quality, including plant growth, productivity, and plant resistance against disease-causing organisms (Ayilara et al., 2023; Ali et al., 2022; Eid et al., 2021). An endophytic bacteria *Bacillus subtilis* improves seedling growth and reduces the effect of oil pollution on the oxidative stress response of wheat plants (Kuramshina et al., 2023). Other reports showed that single and consortium inoculation using three isolates of endophytic bacteria from the klutuk banana plant in the root of rice plants increases plant growth and production 40 days after inoculation (Rahayu et al., 2023). Additionally, two endophytic bacteria from stress-tolerant plants, *Pelomonas aquatic* and *Solibacillus silvestris* have plant growth-promoting properties and potential to reduce plants' multi-stress (Kaur & Karnwal, 2023). It is believed that other reports showing the great potential of endophytic bacteria to improve plants' growth have been published (Zhao et al., 2024).

Several reports stated that indole acetic acid (IAA)-producing-bacteria potentially improves plant growth (Aji & Lestari, 2020; Akhdiya et al., 2014; Homthong et al., 2022; Nuriyanah et al., 2023). The sensitivity of plant tissue can be affected by IAA production by bacteria (Moliszewska et al., 2020; Herlina et al., 2016). This hormone stimulates root growth and promotes plant growth including plant height and number of leaves (Zuhra, 2017). Additionally, Backman and Sikora (2008) reported that the isolated endophytic bacteria from plant tissues might be beneficial to improve plant growth by production of growth-promoting substances, nitrogen fixation, phosphate mobilization, and playing a role in plant health.

The previous report of Rahayu et al. (2021a) isolated 93 endophytic bacteria from klutuk (*Musa accuminata colla*) and ambon banana plants growing in regosol and grumusol soils. The klutuk banana is well known as a relatively disease-resistant cultivar compared to Ambon banana plants (Rahayuniati & Subandiyah, 2022). This disease resistance and susceptibility of the two cultivars are influenced by the core endophytic bacteriome (Rahayu et al., 2021b). Thus, these two banana cultivars are expected to have potential endophytic bacteria to improve plant growth or health. Among 93 isolated endophytic bacteria, five isolates namely A41, K10, K25, K35, and K111, exhibited IAA production (Rahayu et al., 2021a; Table 1).

Although the five isolates have the potential to improve plant growth, conclusive evidence has not yet been obtained. Therefore, this study aimed to evaluate plant growth after endophytic bacterial inoculation. The five isolates from klutuk banana plants were employed in this study. A potential bacterial isolate from a plant may have specific compatibility only with the endogenous plant or could also be compatible with other plants (Mushtaq et al., 2023; Wu et al., 2021). In this study, endophytic bacterial isolates from banana plants were applied to the rice (*Oryza sativa*) and maize (*Zea mays*) plants. Besides rice and maize being the main crops supporting food security, the application of five isolates to non-endogenous plants evaluated the compatibility. In addition, bacterial growth was also investigated to consider optimal times for plant inoculation. This study revealed the tangible benefits of endophytic bacteria from klutuk banana plants in improving the growth of rice and maize plants.

MATERIALS AND METHODS

This study used 5 endophytic bacteria from banana plants. The five endophytic bacteria have the codes namely A41, K10, K25, K35, and K111. Each of these bacteria is grown in Nutrient Agar (NA) and Nutrient Broth (NB) media. Then the concentration of population colonies is measured using a Shimadzu UV Vis Spectrophotometer UV-1280.220–240. There were five isolates selected based on moderate to high IAA yield content. The isolates are, two isolates derived from klutuk banana roots in sand clay (LM) namely K10 and K111 with IAA content respectively, 73.45 (+++++) and 72.60 (++++++), then one isolate was from klutuk banana roots in silt clay (SL) namely K35 with an IAA content of 59.80 (+++), then one isolate was from the leaves of klutuk bananas in LM soil, namely K25 with an IAA content of 62.90 (+++) and one isolate came from ambon banana roots in SL soil is an isolate with code A41 with an IAA content of 44.60 (+++). These isolates produce IAA during observation (Table 1).

Table 1. IAA produced by three used isolates of this study

Organ origin	Organism of origin	Isolate code	IAA production
Root	Ambon banana	A41	+++
Root	Klutuk banana	K10	+++++
Petioles	Klutuk banana	K25	+++
Root	Klutuk banana	K35	+++
Root	Klutuk banana	K111	+++++

Note: += have low ability; ++= have medium ability; +++= have a rather high ability; ++++= have high abilities; +++++= have very high abilities

Seed Sterilization

Seed inoculation begins by soaking rice and maize seeds for 5 minutes with warm water. The seeds chosen for sowing are sunken. Then the water is removed and 70% alcohol in a ratio of 1:1 and shaken for 1 minute. Alcohol is removed and the seeds are with sterile aquades. After that, a 1% NaOCl solution is added and left for 1 minute and then rinsed with aquades 3 times. Put the seeds into a sterile clock bottle given aquades in a ratio of 1:1, after which it is allowed to stand for 24 hours.

The Growth of Isolates and Inoculum Preparation

Measurement of bacterial growth began with the preparation of isolates starter. It was conducted by inoculating one end of ose bacterial isolate from oblique agar into 6 mL of NB liquid media in a reaction tube (aseptic) and then incubated for 8 hours at a speed of 100 rpm. Afterward, 1.5 mL of the starter was added to 10 mL of NB media. To investigate the bacterial growth, the optical density of 600 nm measurement was carried out by inserting NB in the cuvette and measured by spectrophotometer every 2 hours for 16 hours. The inoculum was prepared from the respectively reaching 0.8 OD namely A41 and K35 at 2 hours after shaking, K10 and K25 at 3 hours after shaking and K111 isolate at 8 hours after shaking.

Inoculation of Bacteria into Plant Roots

Inoculation of endophytic bacteria into the root of rice (*Oryza sativa*) and maize plants (*Zea mays*) was carried out. It began by soaking rice and maize seeds for 24 hours in sterile water, then placed on a petri dish with filter paper for 2 days. Maize's seeds germinated after 6 days of incubation. The germinated seeds were then transferred to a petri dish using sterile tweezers. Then, it was incubated by laying the seeds on a bacterial inoculum suspension (OD 0.8) for an hour. Rice and maize seedlings were then sowed in a 20 cm polybag. Each pot contains 3 seedlings and this experiment used 5 pots for each treatment (five replications). This was an experimental study with a complete randomized design with 15 replicates for each treatment. The growth of rice and maize seedlings was observed for 14 days after plantation (dap) and the growth measurements were conducted at 14 dap.

Data Analysis

The obtained data of growth parameters (plant height, leave number, root number, and root length) were tabulated and analyzed as the average and standard deviation of 15 replicates. The recapitulated data then were analyzed using the 95% student t-test confidence test or with a significant level of 5% (a= 0.05). T-test was done by comparing each treatment to the control, uninoculated plants. A P-value less than 0.05 was designated as significantly different while a p-value more than 0.05 was indicated as no significant difference between treatment and control. The significant differences are indicated by an asterisk (*).

RESULTS

Growth Rates of Endophytic Bacterial Isolates

The growth rate of endophytic bacterial populations derived from banana klutuk and banana ambon can be studied by observing the growth curve in bacterial culture. Observation of population changes and optical density values (Optical Density= OD) is carried out to observe the growth phase of bacteria. The average data from the study of the growth rate of endophytic bacteria can be seen in Figure 1.

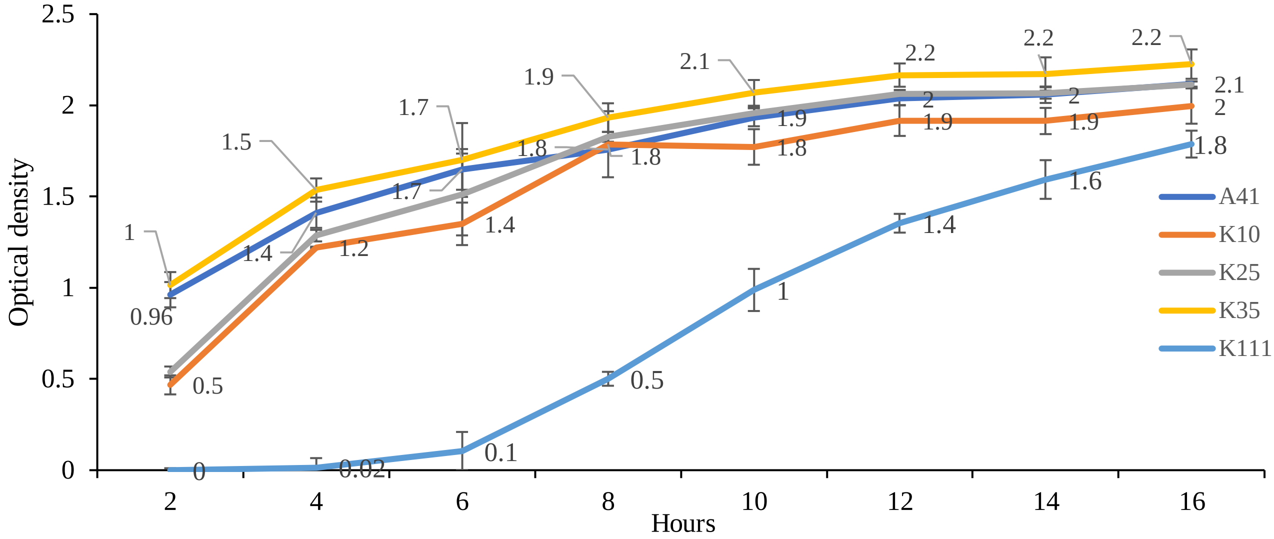
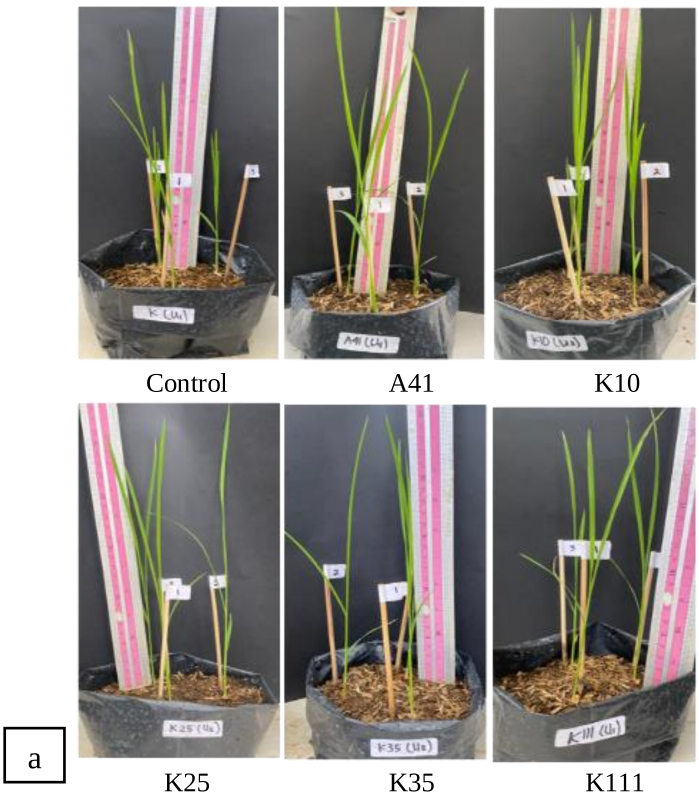


Figure 1. The growth rate of potential isolates from banana plants. Bars indicate the standard deviation

Rice Growth Improvement after Endophytic Bacterial Inoculation

The potencies of isolated endophytic bacteria as PGPB were investigated in this study with 15 replications of the experiment. The measured plants' growth parameters were the plant heights, number of leaves, number of roots, and root length. The results showed some improvements in rice and maize growth after inoculation using endophytic bacteria. All five isolates except for K111 improved the rice height and specifically K25 and K35 isolates significantly enhanced the rice height at 14 dap (Figure 2). In addition, K10, K25, and K35 isolate relatively improved the number of leaves and root length without significant difference compared to control (Figure 3 & 4). Interestingly, K25, K35, and K111 isolated significantly increased the number of rice roots compared to the control (Figure 5).



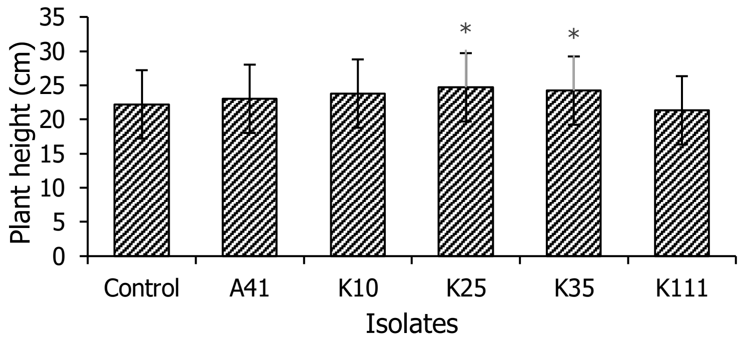


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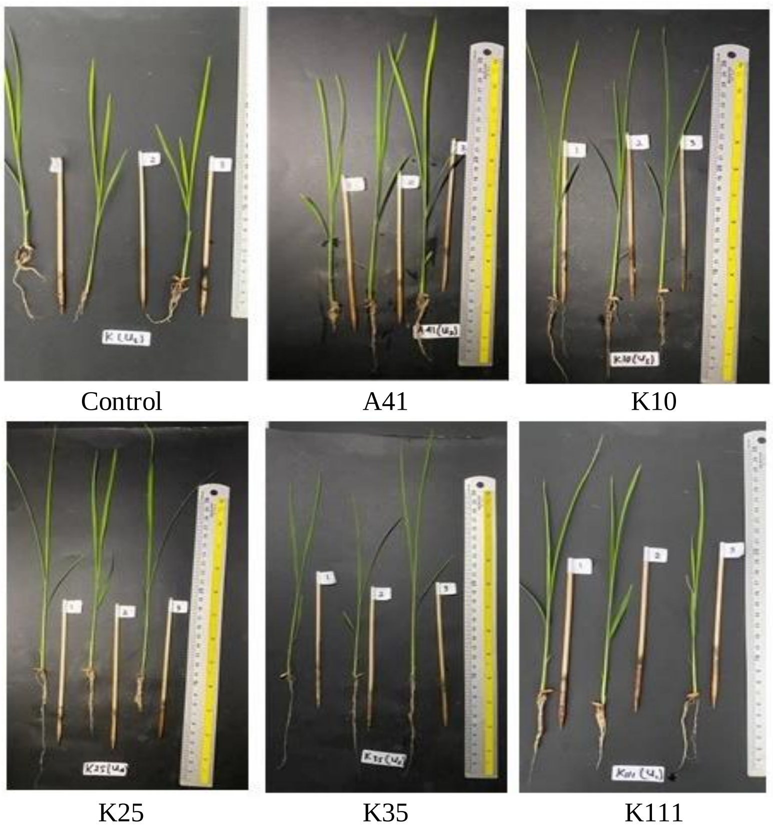
Figure 2. The plant height at 14 daps with inoculation treatment of endophytic bacterial isolates, Representative photographs of all treatments (a), and the measurement results of the t-student test showed that the treatment with K25 and K35 isolates showed a significant difference compared to the control (b). The asterisks (*) indicate a significant difference



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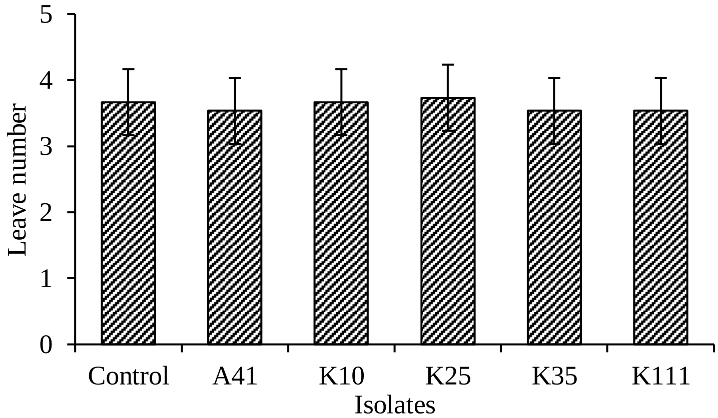

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Figure 3. The number of leaves of rice plants at the 14 daps with inoculation treatment of endophytic bacterial isolates, representative photographs of all treatments (a), and the measurement results of the t-student test showed that treatment with isolates A41, K10, K25, K35, and K111 did not show a noticeable difference in leaf numbers compared to the control (b)

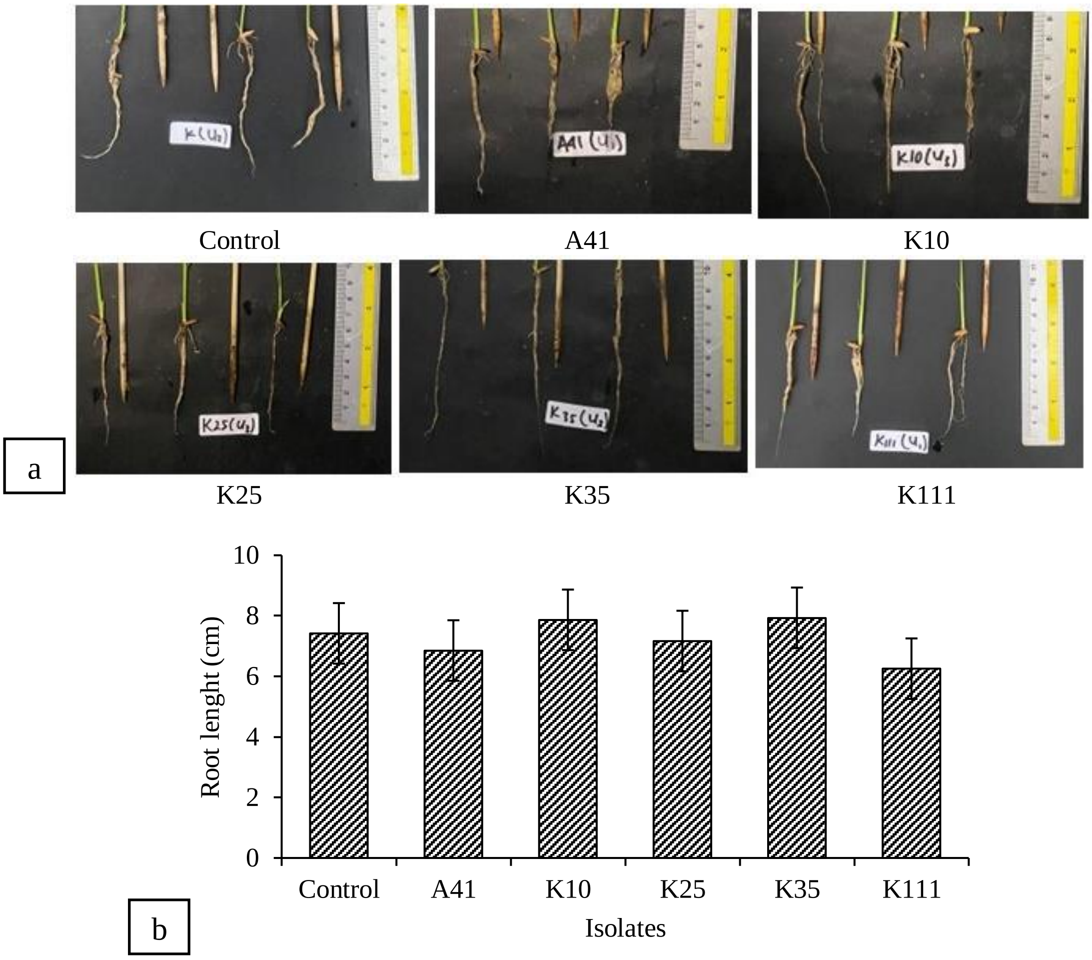
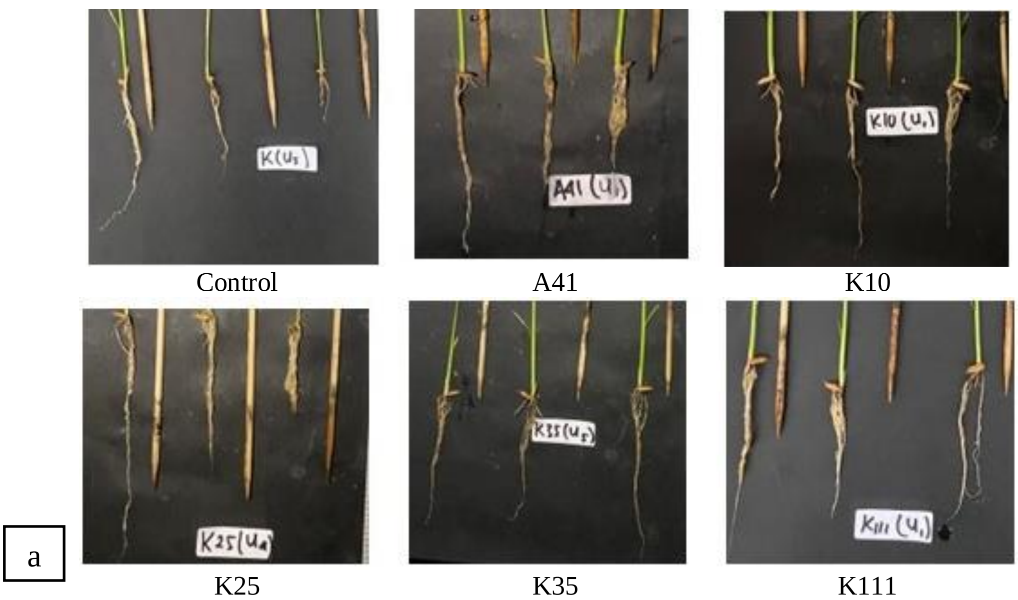


Figure 4. The root length of the rice plant at 14 daps with endophytic bacterial inoculation, representative photographs of all treatments (a), and the measurement results of the t-student test showed that the treatment with isolates A41, K10, K25, K35, and K111 did not show a noticeable difference in root length to the control (b)



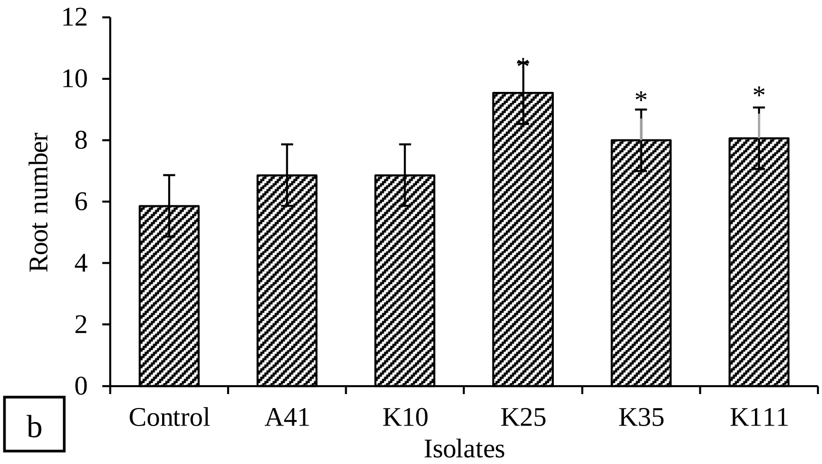
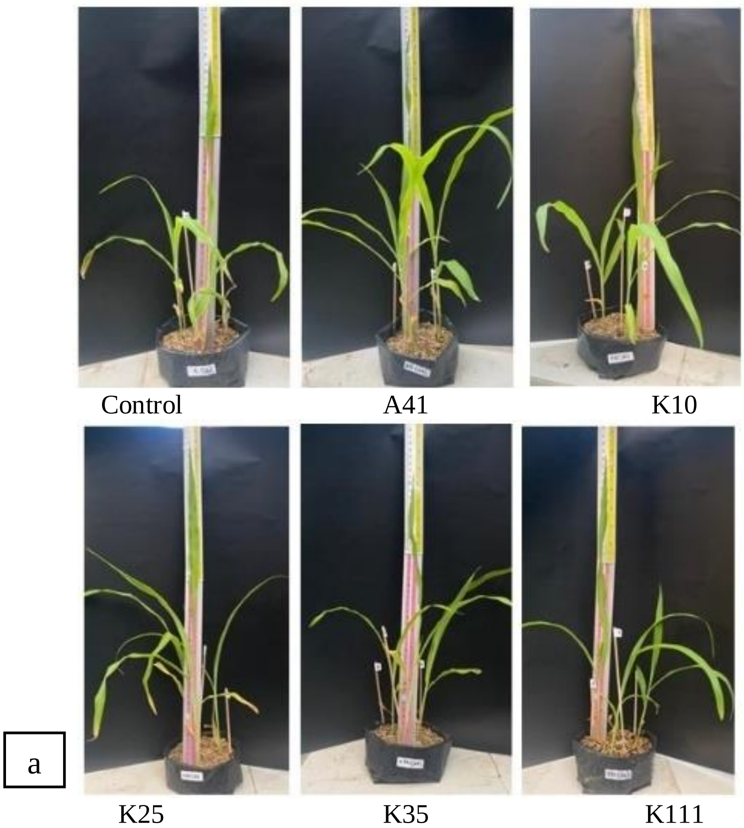


Figure 5. The number of roots of rice plants on the 14th day with inoculation treatment of endophytic bacterial isolates, representative photographs of all treatments (a), and the measurement results of the t-student test showed that treatment with K25, K35, and K111 isolates showed significant differences in control (b). The symbol "*" indicates a significant difference at the significant level of 5%

Maize Growth Improvement after Endophytic Bacterial Inoculation

Furthermore, most of the five isolates relatively improved the growth of maize plants except for A41 and K111 isolates which decreased the root length of maize plants. In particular, A41, K10, and K111 isolates significantly increased the plant's height (Figure 6). Even though statistical analysis of leaves number, root numbers, and root length showed insignificant improvement, most isolates slightly increased these growth parameters (Figures 7, 8, & 9).



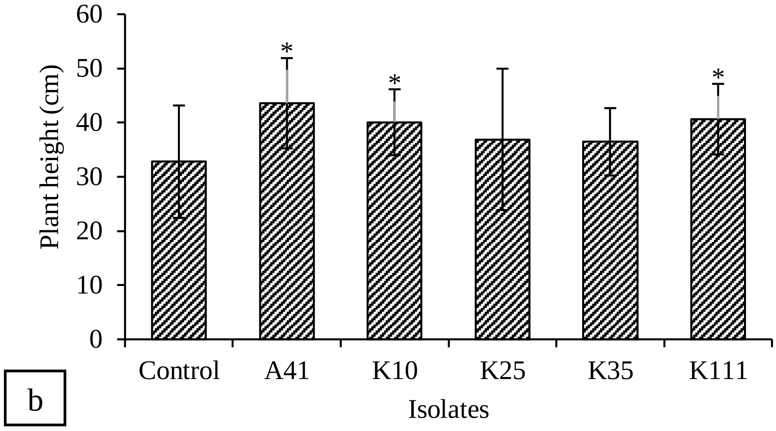


Figure 6. Maize plant height at 14 days with inoculation treatment of endophytic bacterial isolates, representative photographs of all treatments (a), and the measurement results of the t-student test showed that the treatment with A41, K10, and K111 isolates showed significant differences in plant height compared to control (b). The symbol "*" indicates significance

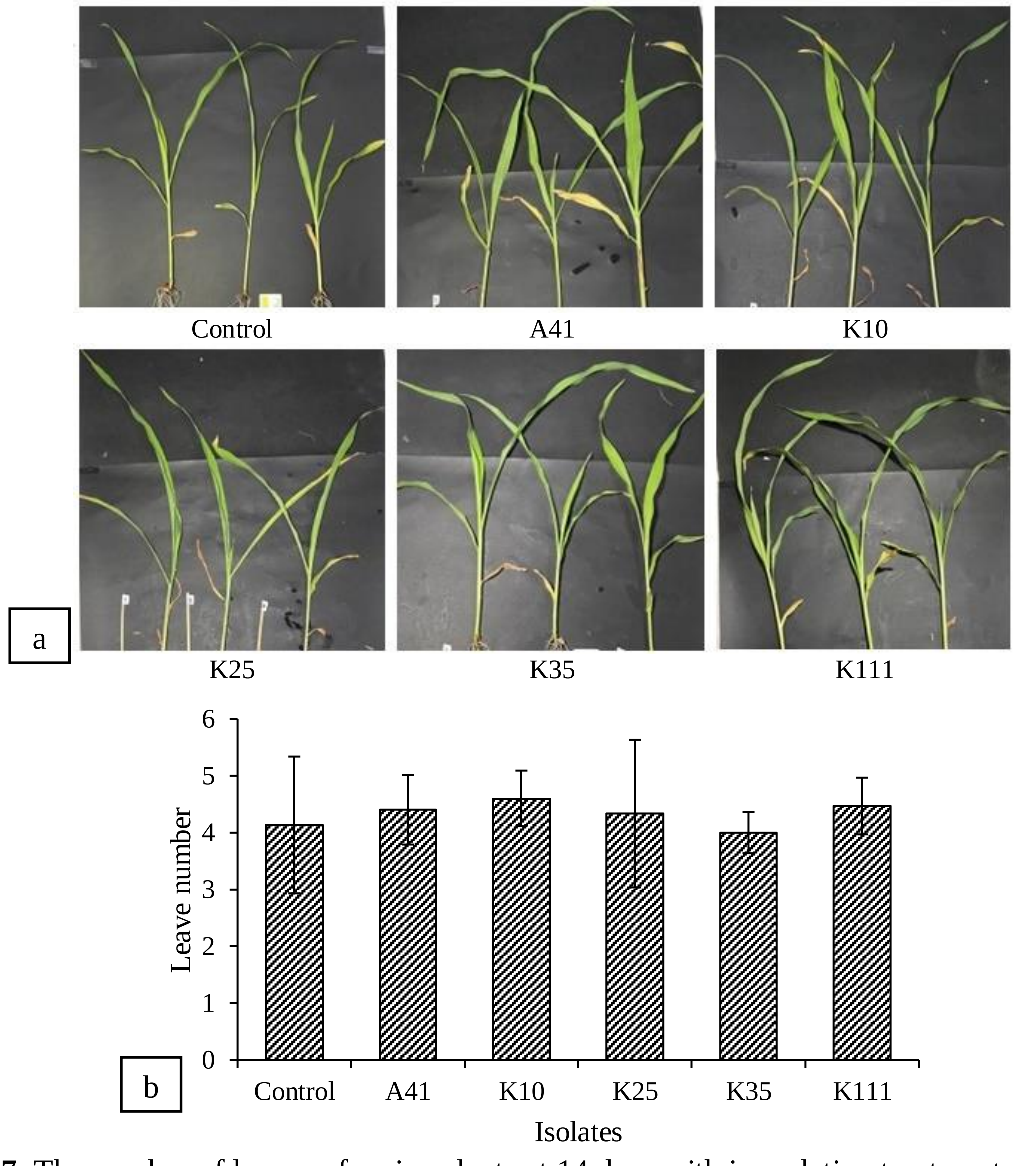


Figure 7. The number of leaves of maize plants at 14 days with inoculation treatment of endophytic bacterial isolates, representative photographs of all treatments (a), and the measurement results of the t-student test showed that the treatment with isolates A41, K10, K25, K35, and K111 did not show significant differences in control (b)

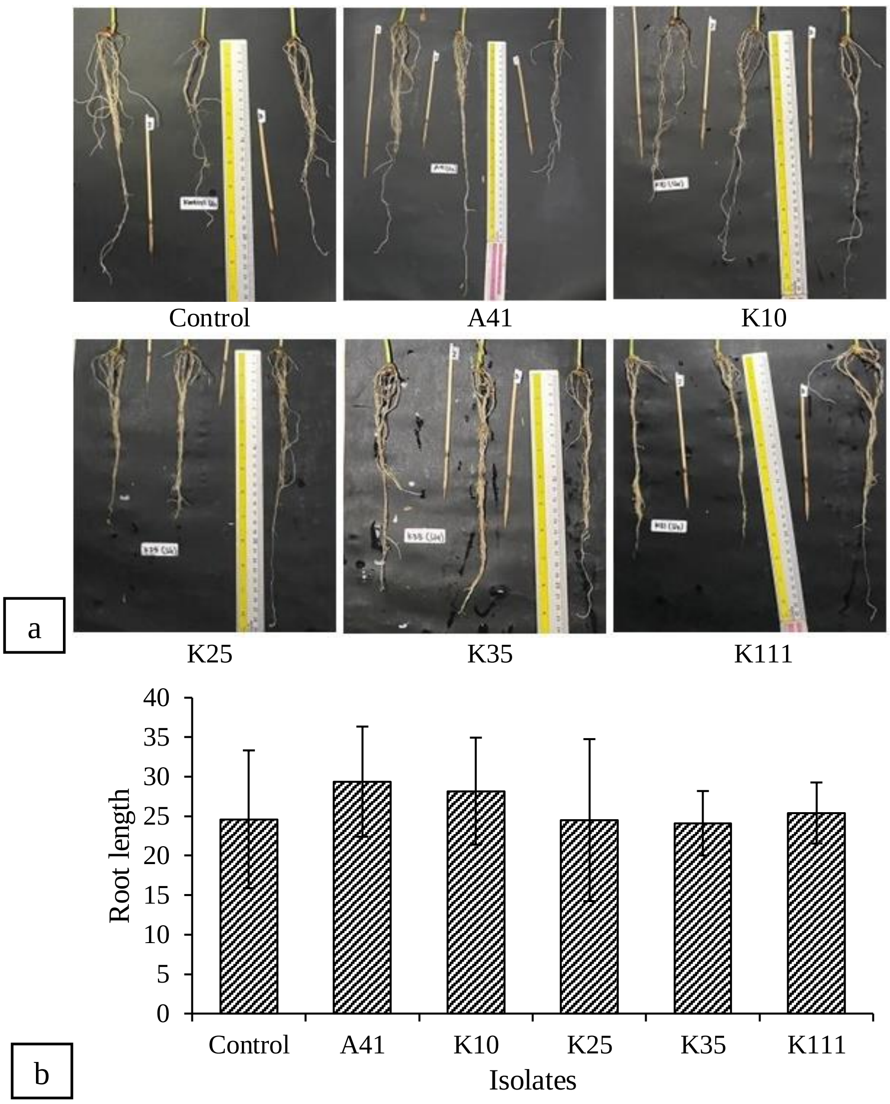
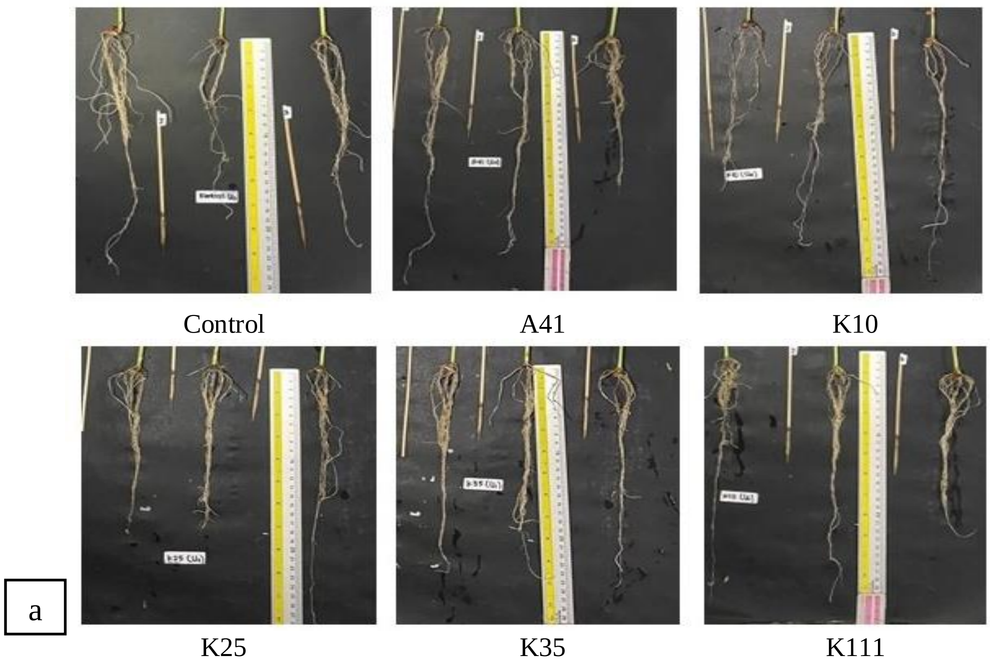


Figure 8. The root length of the maize plant at 14 days after inoculation treatment of endophytic bacterial isolates, representative photographs of all treatments (a), and the measurement results of the t-student test showed that the treatment with isolates A41, K10, K25, K35, and K111 did not show significant differences compared to control (b)



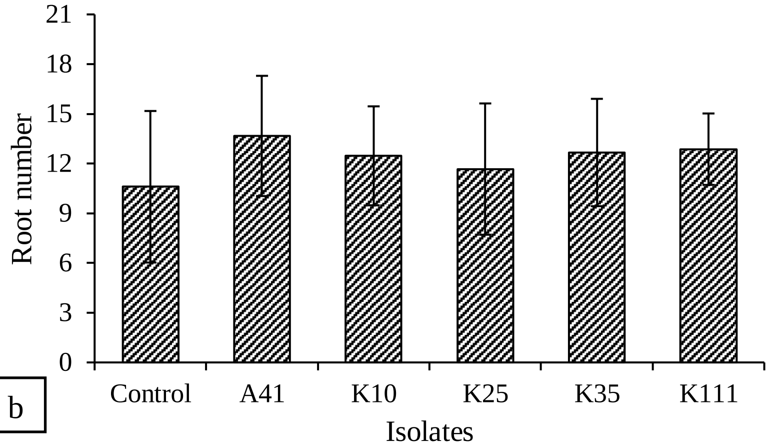


Figure 9. The number of roots of maize plants on day 14 with inoculation treatment of endophytic bacterial isolates, representative photographs of all treatments (a), and the measurement results of the t-student test showed that the treatment with isolates A41, K10, K25, K35, and K111 did not show significant differences in control (b)

DISCUSSION

In this study, bacterial growths were measured to ensure a relatively equal amount of inoculated bacteria in the plant's root. Considering the bacterial density and their multiplication are key factors in successful inoculation (Lopes et al., 2021; Renoud et al., 2022), this study utilized the exponential phase as a point for inoculant preparation. In this phase, the bacteria show rapid multiplication (Renoud et al., 2022). The result revealed a distinct growth rate of K111 isolate compared to other isolates, specifically, K111 exhibited delayed growth relative to the other four isolates (A41, K10, K25, and K35). Therefore, in this study, isolates A41, K10, K25, and K35 were used for inoculation after 2 hours of shaking, while K111 was isolated at 8 hours after shaking. Different growth rates is common among different isolates of bacteria depending on genetic factors and the nutrition of the media (Basan et al., 2020). The optimum bacterial density for inoculation is 1×10^6–10^8 cells/mL (Kumar et al., 2021; Pellegrini et al., 2021). This experiment used incubation time and OD of 0.8 to ensure equal bacterial density for inoculation into the plant's root.

Based on the data from measuring the height of rice plants, it can be seen in Figure 2 that K25 and K35 isolates inoculated in plants have significant differences with the control treatment. Isolates K25 and K35 have a good effect on the height growth of rice plants. Some other isolates such as A41 and K10 also increased the height growth of rice plants compared to the control treatment but did not have a significant difference. Indole acetic acid which is produced by endophytic bacteria is useful in stimulating cell division to spur plant growth, regulating cell enlargement, and spurring the absorption of water and nutrients that affect plant growth (Puspita et al., 2019; Setyowati et al., 2023). The occurrence of cell division and elongation events causes high growth of a plant. Growth on the shoots of plant shoots is spurred when the process of division and elongation of plant cells takes place, increasing plant height. This is in stark contrast to the K111 isolate, where the data in Figure 2 showed that K111 isolates had no significant effect on the height growth of rice plants compared to controls. This is in line with the report of Harni et al. (2020) that the plant's fresh weight, the length of the roots, and the height of the plant are affected by the type of bacteria themselves. This is because the bacterial application method does not show a noticeable effect on the growth.

The result of measuring the number of leaves in Figure 3 was found that 5 isolates that had been inoculated in rice plants did not have a noticeable effect with control plants on the growth of leaf count. This is in line with the experiment of Saridewi et al. (2020), in which endophytic bacteria from eggplant roots on the number of leaves also did not differ markedly between the three isolates with controls, even though the control treatment was higher than that of AKa and AKb isolates. This is also the case with a report of Wulandari et al. (2023) showed that inoculation of endophytic bacteria in nail wood seedlings had no significant effect on all observed growth of nail wood seedlings (height, diameter, number of leaves, total wet weight, total dry weight, and moisture content of nail wood seedlings). The lack of availability of nutrients also affects the growth of the number of leaves. The availability of sufficient nutrients allows the optimal photosynthesis process and the resulting assimilate can be used as food reserves, large food reserves allow the formation of many leaves as well (Darise & Guniarti, 2023).

The results of measuring the root length of rice plants that had been inoculated with endophytic bacterial isolates did not provide a significant difference. The treatment of K10 and K35 in Figure 4 gives the effect of differences in plant height compared to control plants but does not have a significant difference. Root elongation in these plants is due to the IAA content in the isolate. The presence of the IAA hormone can spur plant growth by increasing the rate of root growth (Suwarni & Advinda, 2021). However, this is different from the isolates A41, K25, and K111 in the figure. All three plants inoculated by the isolate had lower root lengths compared to the control plants. Research (Suwarni & Advinda, 2021), mentioned that giving IAA-producing isolates to the length of sprouted roots has no effect, this is due to the presence of other hormones, such as ethylene in the roots produced in large quantities can inhibit root development.

Based on the data calculation of the number of roots in Figure 5, five isolates that have been inoculated in rice plants have a noticeable effect compared to control plants on the growth of the number of roots of rice plants. In rice plants that have been inoculated with K25, K35, and K111 isolates showed a significant difference in the 5% level test of the control, while plants with A41 and K10 isolates did not show a significant difference, but had a significant effect on the growth of the number of rice plant roots. The large number of roots in plants that have been inoculated with endophytic bacteria compared to control plants is caused by these endophytic bacteria. Where endophytic bacteria contain IAA in it. The Auxin hormone that plays a more important role in root formation is IAA (Yulia et al., 2020). This is following Herlina et al. (2016), that Isolates of IAA-producing endophytic bacteria affect lateral root count but do not affect germination length.

Measurement of maize plant height in Figure 6 showed results that isolates derived from endophytic bacteria were able to increase the height of maize plants. The inoculated plants with A41, K10, and K111 isolates showed significant differences from control plants, while K25 and K35 isolates are also insignificantly able to have a high effect on maize plants. This supports the report of Rahayu et al. (2021b), the isolates having the potential to support plant growth are K22, K117, K58A, and K10 isolates, and isolates capable of producing high IAA including K2, K14, K111, K25, K28, K104, K107, K35, K37, and K79 can be applied to increase plant growth. One of the mechanisms of plant growth is the production of the hormone IAA by microorganisms (Ismawanti et al., 2022). The metabolic process in the plant tissues occurs due to the utilization of IAA produced by bacteria, thus helping the growth process of height, stem diameter, number of leaves, and area of plant seeds (Puspita et al., 2019).

Measurement of the number of maize leaves Figure 7 it showed that plants that had been inoculated with endophytic bacteria with control plants did not make a noticeable difference in the 5% level test. This is in line with Yulia et al. (2020), which reported that *in Cymbidium* orchid plants, IAA treatment did not affect the number of leaves. The same results of Hazra et al. (2019), on the research of pepper plants showed that the formula of endophytic bacteria had no real effect on increasing the number of leaves. The increase in the number of leaves is also influenced by an increase in the rate of photosynthesis, where an increase in the rate of photosynthesis will be followed by the results of photosynthesis. The results of this photosynthate play a role in the formation of plant structure.

Results of maize root length data analysis Figure 8 showed that the 5 isolates did not provide a significant difference between control plants and inoculated plants. This is in line with Saridewi et al. (2020), the application of endophytic bacteria does not have a noticeable effect on the root length of eggplant plants. Allegedly because the influence of the volume of plant medium allows limited nutrients that can be absorbed by plant roots and space for movement of plants. Moreover, Apriliani (2015) since plants have a control mechanism over the administration of auxin from the outside if the synthesized hormone is enough to support the metabolic process, it affects growth. In addition, it is suspected that sufficient watering of maize plants so that water needs are fulfilled so that an increase in roots to expand the absorption field is not needed. This is following Solin et al. (2021) that mild and moderate dryness causes root growth to further increase to expand the absorption field.

Measurement results in Figure 9 number of maize plant roots found that 5 isolates of banana endophytic bacteria did not provide a significant difference with the control at the 5% level test. This is in line with Lathyfah and Dewi (2016) that variations in IAA concentration on the growth of root count, leaf length, and pseudostem length of barangan banana (*Musa acuminata* L. triploid AAA) with IAA variation of 0.6 ppm as the optimal concentration for the elongation stage did not have a significant effect. The report of Harahap and Nusyirwan (2014) also mentioned that the hormone auxin (IAA) and cytokinin interaction did not have a significant effect on the increase in the number of pineapple roots (*Ananas comosus* L.). It is understood that root formation will occur if in the media there is a higher auxin content than cytokinins. Isolates from banana plants have not been optimal to increase the growth of the number of roots in maize plants. The IAA contained in coconut water does not increase the number of roots, high concentrations of auxin inhibit root growth (Rochmah & Rahayu, 2021). The level of endogenous auxin concentration in plants affects the number of roots. Higher hormone levels can inhibit growth and even poison plants (Wuriesyliane & Sawaluddin, 2022). Overall, the findings of this study revealed the potential isolate to improve the growth of rice and maize plants.

CONCLUSION

Root inoculation using isolates K25 and K35 increases the height and number of roots. While treatment using K111 isolate significantly increased the number of roots of rice plant. In addition, root inoculation using isolates A41, K10, and K111 significantly increased the height of maize plants. These findings prove that isolates of endophytic bacteria from klutuk banana plants have the potential to improve the growths of rice and maize plants.

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