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# The role of endophytic bacteria in increasing nitrogen concentration, root length, and dry weight of rice plants (*Oryza sativa* L.) in saline soil

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## Abstract

Soil salinity can interfere with the absorption of water and plant nutrients, causing a decrease in growth and yield of rice plants in saline soil. This negative effect can be reduced by the application of endophytic bacteria biofertilizer. Endophytic bacteria live in plant tissues and play a role in increasing plant growth through nitrogen fixation or growth hormone production. The purpose of this study was to determine the effect of endophytic bacterial biofertilizer application on N concentration, root length, and dry weight of rice seedlings (*Oryza sativa* L.) in soils with different salinities. This research was conducted in the greenhouse of the Faculty of Agriculture, Universitas Padjadjaran. The experimental design used was a Randomized Block Design (RBD) with 8 combinations. The treatments were with and without endophytic bacterial biofertilizers, and soils with different levels of salinity (non-saline soil and saline soil with salinities of 2, 4, and 6 mmhos $cm^{-1}$) and repeated 4 times. The experimental results showed that the combination treatment of endophytic bacterial biofertilizer in saline soil of 2 mmhos $cm^{-1}$ was able to increase the N concentration, root length, and dry weight of rice plants which were higher than the treatment of endophytic bacterial biofertilizer in soil with salinity of 4 and 6 mmhos $cm^{-1}$. The application of endophytic bacteria biofertilizer has higher dry weight of rice plants than without endophytic bacteria at each different salinity level.

**Keywords:** Biofertilizer; Endophytic bacteria; Plant N; Rice plant; Saline soil

## 1 Introduction

Rice is the most important cereal crop in the world, feeding more than 50% of the world's population [1]. In Asia, rice important as the main staple food and to those who depend on rice farming for their livelihoods. Approximately 67% of agricultural area is temporarily associated with salinity [2]. Salinization is the accumulation of water-soluble salt in soil solum to a level which endangers agricultural production, environmental health, and welfare.

The rice harvested area in Indonesia in 2021 will reach around 10.41 million hectares, a decrease of 245.47 thousand hectares or 2.30 percent compared to the rice harvested area in 2020 of 10.66 million hectares. Rice production in Indonesia in 2021 for the population's food consumption will reach 31.3 million tons. Rice production decreased by 140.73 thousand tons or 0.45 percent compared to rice production in 2020 which was 31.50 million tons [3]. Efforts to overcome this are through agricultural intensification and extensification. Agricultural intensification is an increase in agricultural production per unit area of land through the use of better production technology, while agricultural extensification is an increase in agricultural production that is sought through the expansion of planting areas [4].

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Agricultural intensification and extensification in rice cultivation can be pursued in saline land. Saline land is land that has an excess dissolved salt content in the soil solution [5]. The total area of saline land in Indonesia is 0.44 million ha [6]. These saline lands are often found in coastal areas due to seawater intrusion. Saline land on the north coast of Java island is widely used as land for rice cultivation, which is a staple food source in Indonesia.

There are several things that need to be considered in the use of saline land for intensification and extensification. Massoud et al. [7] reported that rice is moderately sensitive to salinity stress. Putri [8] stated that the biggest problem in using saline land is its effect on decreasing plant growth and productivity and even plant death due to disturbances in water and nutrient absorption. The high salt content in the soil will reduce the osmotic potential so that plants have difficulty absorbing water. The difficulty of plants in taking water from the soil also has an impact on the disruption of the process of absorption of nutrients in the form of ions dissolved in water. Special attention is needed in the use of saline land for agriculture, especially in overcoming obstacles to nutrient absorption by plants. One of the efforts that can be done to increase nutrient absorption by plants is by utilizing nitrogen-fixing endophytic bacteria in rice plants that grow on saline soils.

The biggest problem in using saline soil is its effect on decreasing plant growth and productivity and even plant death due to disturbances in water and nutrient absorption. The high salt content in the soil will make it difficult for plants to absorb water. The difficulty of plants in taking water from the soil also has an impact on the disruption of the process of absorption of nutrients in the form of dissolved ions in water. Special attention is needed in the use of saline land for agriculture, especially in overcoming obstacles to nutrient absorption by plants. One of the efforts that can be done to increase nutrient absorption by plants is by utilizing nitrogen-fixing endophytic bacteria biofertilizer in rice plants that grow in saline soil.

Endophytic bacteria are bacteria that are able to live in a plant tissue that can perform endosymbiosis without harming the host plant [9]. Endophytic bacteria can help the absorption of plant nutrients by means of nitrogen fixation. The potential for the development of endophytic bacteria is greater than that of non-endophytic bacteria because the N that has been fixed is not lost due to leaching so that the efficiency of N supply to plants becomes more optimal [10].

The use of endophytic bacterial biofertilizers in rice cultivation in saline soils aims to increase the uptake of N by plants so that the N needs of plants can be more fulfilled which leads to more optimal plant growth and productivity.

## 2 Material and methods

The research was conducted at the Greenhouse Faculty of Agriculture, Universitas Padjadjaran. The experimental design used was a randomized block design (RBD) with 8 combination treatments of endophytic bacterial biofertilizer application (with and without endophytic bacterial biofertilizer) and Inceptisol paddy soil with different salinity levels (non-saline soil and saline soil with EC 2, 4, and 6 mmhos $cm^{-1}$) which was repeated 4 times. Inceptisol paddy soil samples were given 2.9 g; 24.6 g; and 49.9 g of NaCl for treatments 2, 4, and 6 mmhos $cm^{-1}$, respectively. Rice field soil with a weight of 1 kg of air dry is then put into a pot, given water, and stirred evenly to create a stagnant soil. The soil was incubated for 7 days [11].

Gram-negative endophytic bacteria were isolated from samples of rice plants growing in saline rice fields in Karawang Regency, the North coast of West Java, selected, then formulated into biofertilizers with a carrier material of 2% molasses solution. Inpari 34 rice seeds were soaked (seed priming) in a suspension of biofertilizers for 12 hours and then sown. Rice seedlings aged 14 DAS were transferred to the media in pots carefully. Inoculation of 10 mL of endophytic bacterial biofertilizer with a density of $10^7$ CFU $mL^{-1}$, urea fertilizer (1.5 g), SP-36 fertilizer (0.5 g), and KCl fertilizer (0.5 g) were applied when transplanting rice into the pot. Sampling of rice plants was carried out at 14 days after planting (DAP) to observe endophytic bacterial population, nitrogen concentration of plants, root length, and dry weight of rice plants. Rice plants only survived up to 14 days after planting and then died, especially those treated with a salinity of 4 and 6 mmhos $cm^{-1}$. The data obtained was analyzed using Microsoft Office Excel 2019 software and analyzed for variance (ANOVA) using SPSS version 16.0. If there is a significant difference, then the test is continued with Duncan's multiple range test (DMRT) at 95% confidence level [12].

# 3 Results and discussion

## 3.1 Rice plant height

Plant growth is vegetative growth which is closely related to the availability of nitrogen elements [13] suggested that nitrogen plays an important role in plant vegetative growth such as root elongation and increase in plant height. Observation of rice plant height was carried out at 0, 3, 5, and 7 days after plant (DAP). This observation was carried out by measuring the height of the rice plant from its base at the soil surface to the tip of the leaf.

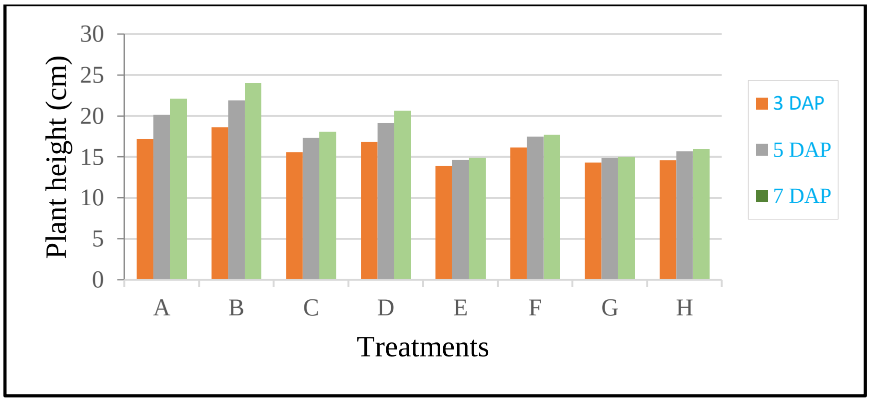


**Figure 1** Rice plant height 0 - 7 DAP at various treatments with different salinity values

Observations showed that the height of the rice plants decreased with increasing salinity. Plant height in saline treatment (Treatment C - H) was lower than in non-saline treatment (Treatment A and B). This indicates that the greater the osmotic stress on plants, the more stunted plant growth becomes (Figure 1).

The average increase in plant height at 0 - 3 DAP was better than that at 5 - 7 DAP at 2, 4, and 6 mmhos $cm^{-1}$ salinity treatments (C - H treatment). This is thought to be due to excessive accumulation of salt ions in plants which is characterized by a change in leaf color from green to yellow then dry and die. The results also showed that the application of endophytic bacterial biofertilizers on non-saline soils (Treatments A and B) and saline (Treatments C - H) showed better plant height compared to treatments without biofertilizers. This proves that the application of endophytic bacterial biofertilizers can increase the height of rice plants.

## 3.2 Endophytic bacterial population

Table 1 shows the population of endophytic bacteria in root rice plant increase compared to without endophytic bacteria application treatments at each salinity level.

**Table 1** Effect of endophytic bacterial biofertilizer on endophytic bacterial population in root rice plants on saline soil

| **Treatments** | **Endophytic Bacterial Population ($x10^3$ CFU $g^{-1}$)** |
|---|---|
| A (No endophytic bacteria biofertilizer + Non saline soil) | 6,4 b |
| B (Endophytic bacteria biofertilizer + Non saline soil) | 11,5 c |
| C (Without biofertilizers endophytic bacteria + saline soil 2 mmhos $cm^{-1}$) | 4,9 ab |
| D (Endophytic bacteria biofertilizer + saline soil 2 mmhos $cm^{-1}$) | 6,7 b |
| E (Without biofertilizers endophytic bacteria + saline soil 4 mmhos $cm^{-1}$) | 3,2 ab |
| F (Endophytic bacteria biofertilizer + saline soil 4 mmhos $cm^{-1}$) | 5,4 ab |
| G (Without biofertilizers endophytic bacteria + saline soil 6 mmhos $cm^{-1}$) | 2,6 a |
| H (Endophytic bacteria biofertilizer + saline soil 6 mmhos $cm^{-1}$) | 5,1 ab |

Note: The average value in the same column with the same letter is not significantly different according to Duncan's Test at a significance level of 5%.

In non-saline soils, rice plants were naturally colonized by endophytic bacteria and the number was higher than the population in rice plants growing on saline soil of 6 mmhos $cm^{-1}$. Several factors affect the survival of endophytic bacteria living in plant tissues, both abiotic and biotic factors. Biotic factors are the ability of endophytic bacteria to live in plant tissues, while abiotic factors are the environmental carrying capacity that ensures the survival of endophytic bacteria. Abiotic factors, namely salinity levels, are the cause of the inhibition of the endophytic bacterial population living in plant tissues. Endophytic bacteria colonize the most in the root area due to the response of root exudates which results in a chemotaxis reaction, which is then transmitted to plant tissues [14]. Only saline-resistant endophytic bacteria, which are fewer than non-saline, survive in high saline environments and can infect rice roots. According to Rolfe and Weinman [15] bacterial colonization in the crevices of the epidermal cells of the root caused the emergence of lateral roots which were detected three days after inoculation. Epidermal cell growth will extend longitudinally so that endophytic bacteria can invade other tissues.

Endophytes inside a plant may either become localized at the point of entry or spread throughout the plant [16]. These microorganisms can reside within cells, in the intercellular spaces, or in the vascular system [17]. Significant variations in the populations of both indigenous and introduced endophytes have been reported. These variations are attributed to plant source, plant age, tissue type, time of sampling, and environment. Generally, bacterial populations are larger in roots and decrease in the stems and leaves. Natural endophyte concentrations can vary between $10^2$ – $10^6$ CFU $g^{-1}$ plant tissue. Similar results were obtained for endophytic bacteria inoculated by root or seed drenching, with the population levels reaching between $10^3$ – $10^5$ CFU $g^{-1}$ of plant tissue for tomato and potato [18].

### 3.3 Nitrogen concentration

The highest N concentration of 4.44% was found in the non-saline treatment with the addition of endophytic bacterial biofertilizer. In the treatment of 6 mmhos $cm^{-1}$ saline soil that was not applied with endophytic bacterial biofertilizers, the lowest N concentration was 3.04%. Endophytic bacterial biofertilizer was able to increase plant N concentration by 3.76% – 15.96% compared without endophytic biofertilizer in the same salinity.

**Table 2** Effect of endophytic bacterial biofertilizer and soil salinity on n concentration of rice plants

| **Treatments** | **N-Concentration (%)** |
|---|---|
| A (No endophytic bacteria biofertilizer + Non saline soil) | 3.83 d |
| B (Endophytic bacteria biofertilizer + Non saline soil) | 4.44 e |
| C (Without biofertilizers endophytic bacteria + saline soil 2 mmhos $cm^{-1}$) | 3.36 c |
| D (Endophytic bacteria biofertilizer + saline soil 2 mmhos $cm^{-1}$) | 3.76 d |
| E (Without biofertilizers endophytic bacteria + saline soil 4 mmhos $cm^{-1}$) | 3.22 b |
| F (Endophytic bacteria biofertilizer + saline soil 4 mmhos $cm^{-1}$) | 3.32 c |
| G (Without biofertilizers endophytic bacteria + saline soil 6 mmhos $cm^{-1}$) | 3.04 a |
| H (Endophytic bacteria biofertilizer + saline soil 6 mmhos $cm^{-1}$) | 3.15 b |

Note: The average value in the same column with the same letter is not significantly different according to Duncan's Test at a significance level of 5%.

The treatment of endophytic bacterial biofertilizer application on saline 2 mmhos $cm^{-1}$ had a higher N concentration value than the treatment of endophytic bacterial biofertilizer application on saline 4 and 6 mmhos $cm^{-1}$ and was equivalent to the treatment without endophytic bacterial biofertilizer application on non-saline soil. This is in line with the report of [19] that inoculation of *Bacillus pumilus* in soil given 1.5% NaCl had a better N concentration of rice plants than in soil given 2.0% and 2.5% NaCl and equivalent to treatment without inoculation of bacteria in the soil without the addition of NaCl.

The application of endophytic bacterial biofertilizer on non-saline and saline soils showed a higher concentration of N than without the application of endophytic bacterial biofertilizer. This is the effect of the ability of endophytic bacteria to increase plant N concentration through the nitrogen fixation process. A similar thing was reported by Carvalho et al. [20] that nitrogen-fixing endophytic bacteria *Herbaspirillum* sp. and *Burkholderia* sp. which was inoculated on rice plants was able to increase the plant N concentration up to 31%.

Observations also showed that the N concentration of rice plants decreased with increasing salinity in all treatments. Chookietwattana [21] reported that high soil salinity can increase osmotic stress for plants so that plants are not able to absorb nitrogen optimally. On the other hand, the nitrogenase enzyme produced by bacteria is thought to be inactive under certain saline conditions. This is supported by Tripathi et al. [22] who reported that the activity of the nitrogenase enzyme *Azospirillum brasilense* decreased at NaCl concentration of 200-400 mM so that the nitrogen fixation process was inhibited.

### 3.4 Root length of rice plant

The highest root length of 9.8 cm was found in non-saline soil treatment with the addition of bacterial biofertilizer. In the treatment of 6 mmhos $cm^{-1}$ saline soil which was not applied with endophytic bacterial biofertilizer, the lowest root length was 2.1 cm. The best treatment was found in the application of endophytic bacterial biofertilizer on saline 2 mmhos $cm^{-1}$ which had a higher root length than the treatment of endophytic bacterial biofertilizer application on 4 and 6 mmhos $cm^{-1}$ salinity and was equivalent to the treatment without endophytic bacteria biofertilizer application in non-saline soil.

The application of endophytic bacterial biofertilizer on non-saline and saline soils showed a higher root length compared without the application of endophytic bacterial biofertilizer. This is in line with the research of Sen and Chandrasekhar [23] who reported that inoculation of *Pseudomonas* sp. strain TDK1 in rice plants grown in Yoshida liquid media with 100 mM NaCl concentration was 22% higher in a root length compared to the control. Mattos et al. [24] said that the nitrogen-fixing endophytic bacteria *Burkholderia kururiensis* produces auxin (IAA) which can increase root growth in rice plants.

**Table 3** Effect of endophytic bacteria biofertilizer and soil salinity on root length of rice plants

| Treatments | Root Length of Rice Plants (cm) |
|---|---|
| A (No endophytic bacteria biofertilizer + Non saline soil) | 6.5 c |
| B (Endophytic bacteria biofertilizer + Non saline soil) | 9.8 d |
| C (Without biofertilizers endophytic bacteria + saline soil 2 mmhos $cm^{-1}$) | 3.9 b |
| D (Endophytic bacteria biofertilizer + saline soil 2 mmhos $cm^{-1}$) | 5.7 c |
| E (Without biofertilizers endophytic bacteria + saline soil 4 mmhos $cm^{-1}$) | 3.1 ab |
| F (Endophytic bacteria biofertilizer + saline soil 4 mmhos $cm^{-1}$) | 3.8 b |
| G (Without biofertilizers endophytic bacteria + saline soil 6 mmhos $cm^{-1}$) | 2.1 a |
| H (Endophytic bacteria biofertilizer + saline soil 6 mmhos $cm^{-1}$) | 3.6 b |

Note: The average value in the same column with the same letter is not significantly different according to Duncan's Test at a significance level of 5%.

The results also showed that there was a decrease in root length along with an increase in salinity in all treatments. A similar thing was reported by Balkan et al. [25] that increasing salinity from 4 mmhos $cm^{-1}$ to 16 mmhos $cm^{-1}$ decreased the root length of rice seedlings by 16%. The decrease in root length is due to disruption of the root elongation process. This is due to the accumulation of reactive oxygen species (ROS) in root meristems because of plant oxidative stress mechanisms induced by salinity stress [26].

### 3.5 Rice Plant Dry Weight

The highest plant dry weight of 0.22 g was found in non-saline soil treatment with the addition of endophytic bacterial biofertilizer. In the treatment of 6 mmhos $cm^{-1}$ saline soil which was not applied with endophytic bacterial biofertilizer have the lowest plant dry weight it was 0.10 g. The best treatment was found in the application of endophytic bacterial biofertilizer on saline soil 2 mmhos $cm^{-1}$ because it had a higher plant dry weight value than the treatment with endophytic bacterial biofertilizer application on saline soil 4 and 6 mmhos $cm^{-1}$ and was higher than without application endophytic bacteria biofertilizer in non-saline soil.

**Table 4** Effect of endophytic bacterial biofertilizer and soil salinity on dry weight of rice plants

| Treatments | Dry Weight of Rice Plants (g) |
|---|---|
| A (No endophytic bacteria biofertilizer + Non saline soil) | 0.17 d |
| B (Endophytic bacteria biofertilizer + Non saline soil) | 0.22 e |
| C (Without biofertilizers endophytic bacteria + saline soil 2 mmhos $cm^{-1}$) | 0.13 c |
| D (Endophytic bacteria biofertilizer + saline soil 2 mmhos $cm^{-1}$) | 0.15 d |
| E (Without biofertilizers endophytic bacteria + saline soil 4 mmhos $cm^{-1}$) | 0.12 ab |
| F (Endophytic bacteria biofertilizer + saline soil 4 mmhos $cm^{-1}$) | 0.13 c |
| G (Without biofertilizers endophytic bacteria + saline soil 6 mmhos $cm^{-1}$) | 0.10 a |
| H (Endophytic bacteria biofertilizer + saline soil 6 mmhos $cm^{-1}$) | 0.11 ab |

Note: The average value in the same column with the same letter is not significantly different according to Duncan's Test at a significance level of 5%.

The results showed that there was a decrease in plant dry weight along with an increase in salinity in all treatments. This is thought to be due to chloroplast damage caused by ROS. Suo et al. [27] suggested that high salinity stress increases the production of large amounts of ROS in plant cells causing damage to chloroplasts. Chloroplasts play a role in the process of photosynthesis so that the destruction of chloroplasts will reduce the rate of photosynthesis [28]. Qurashi and Sabri [29] proved that there was a decrease in the amount of chlorophyll a, b, and carotenoids as well as the dry weight of *Lens esculenta* grown on soil treated with 200 mM NaCl concentration.

The application of endophytic bacterial biofertilizer showed higher plant dry weight than without the application of endophytic bacterial biofertilizer at salinity treatments of 0, 2, and 4 mmhos [15].

Endophytic bacteria are thought to be able to produce antioxidant enzymes that can reduce damage to chloroplasts so that the rate of photosynthesis can run more optimally and produce better plant dry weight. Jha and Subramanian [19] reported that rice plants inoculated with antioxidant enzyme-producing bacteria showed a higher dry weight than the control in soil with a concentration of 0-2.5% NaCl.

## 4 Conclusion

Endophytic bacteria biofertilizer and soil salinity affect the N concentration, root length, and dry weight of rice plants. The application of endophytic bacterial biofertilizer on 2 mmhos $cm^{-1}$ salinity was able to increase the N concentration, root length, and dry weight of rice plants. This treatment had higher dry weight of rice plants compare the application of endophytic bacterial biofertilizer on 4 and 6 mmhos $cm^{-1}$ salinity. The endophytic biofertilizer on 2 mmhos $cm^{-1}$ salinity produced higher dry weight of rice compared without endophytic bacterial biofertilizer application on non-saline soil. The application of endophytic bacteria biofertilizer has higher dry weight of rice plants than without endophytic bacteria at each different salinity level.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The author declares there is no conflict of interest.

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