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The Role of Endophyte Bacteria in The Growth and Yield of Various Rice Varieties in Rainfed Rice Lands

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Abstract. Rainfed land typically lacks essential nutrients, including nitrogen. An endophytic bacterial consortium can fix nitrogen from the air, potentially enhancing the growth and yield of lowland rice varieties. The aim of this research was to assess the growth and yield of various lowland rice varieties when treated with a consortium of diazotrophic endophytic bacteria in rainfed land. This study was conducted in rainfed rice fields in Demangan, Sambi, Boyolali, Central Java, Indonesia, to evaluate the growth of rice varieties at different doses of the endophytic bacterial consortium. A completely randomized block design was employed, featuring two factors and three replications. The first factor was the endophytic bacterial consortium, applied at doses of 0, 20, 30, and 40 L/ha/application. The second factor consisted of three rice varieties: Situbagendit, Ciherang, and Mekongga. The results indicated that (1) the dose of endophytic bacteria had a very significant effect on dry shoot weight, dry plant weight, 1,000 grain weight, and panicle length; (2) rice varieties had a significant effect only on fresh shoot weight; and (3) the interaction between the dose of endophytic bacteria and rice varieties did not significantly affect any of the observed parameters. This research suggests that to enhance rice growth and yield in rainfed rice fields, a dose of 40 L/ha/application of the diazotrophic endophytic bacterial consortium is recommended, along with the use of the Situbagendit, Ciherang, or Mekongga varieties.

Keywords: Endophytic bacteria; nitrogen; phosphorus; rainfed rice fields; rice varieties

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INTRODUCTION

In Indonesia, rainfed land covers 3,292,578 hectares, with 24% designated for rice cultivation (Siek et al., 2024; Asfaw et al., 2024).This type of land relies on rainwater for irrigation, distinguishing it from irrigated rice fields. Rainfed rice fields tend to have low phosphorus availability due to groundwater leaching (Mote et al., 2020; Rajapaksha et al., 2024). Overall, inadequate agricultural management, prolonged use of chemical fertilizers, and inefficient fertilizer application contribute to reduced productivity in these rice fields.

Drought stress is one of the most detrimental abiotic factors affecting plant growth and development. It disrupts physiological processes, triggers biochemical changes, and alters secondary metabolite formation. Additionally, drought stress leads to a significant accumulation of endogenous reactive oxygen species (ROS) and increases toxin levels (Sultana et al., 2024).

Drought stress has a significant negative impact on both grain yield and vegetative growth. (Hou et al., 2024). Water deficit conditions typically lead to reductions in grain size, weight, and seed set rates. (Mohan et al., 2024). Additionally, drought stress during critical phases such as budding, flowering, and the terminal stages can disrupt bud initiation, induce grain sterility, decrease grain weight, and ultimately lower grain yields (Missa et al., 2024). The extent of yield loss is influenced by the duration of water scarcity, the growth stage of the plant, and the intensity of the stress (Shantharaja et al., 2024).

One approach to mitigating drought stress is through the use of microbial-based technology, such as endophytic bacterial consortia. Endophytic bacteria reside within their host plants (Medison et al., 2022), forming complex relationships that promote plant growth. According to (Saeed et al., 2021), these plant bacteria play a significant role in enhancing crop production and soil fertility. Microbial components found in the endosphere and rhizosphere create beneficial associations with plants, contributing to increased productivity (Ali et al., 2021). These bacteria enhance plant resistance to various abiotic and biotic factors that can hinder growth and production (Kumar & Verma, 2018). Additionally, these microbes can exist both internally and externally within host plant tissues; for instance, rhizosphere bacteria colonize the roots in the soil, while epiphytic bacteria live on the surfaces of plant leaves.

Rhizobacteria are plant growth-promoting bacteria found in the rhizosphere, a narrow zone of soil surrounding plant roots where microbial activity is at its peak (Verma et al., 2019). This zone serves as an ecological niche, providing a rich source of nutrients and energy essential for plant growth. While rhizobacteria are abundant partners in the rhizosphere, they fulfill various roles in supporting plant development. Numerous interactions take place between plants and rhizobacteria in this environment. These interactions, which involve signaling between rhizobacteria and plant roots, are crucial for regulating biochemical activities (Ameen et al., 2024). Rhizobacteria play a vital role in nutrient cycling, carbon sequestration, and ecosystem functions that enhance plant growth, yield, and nutritional quality. Several bacterial genera have been identified as plant growth-promoting rhizobacteria (PGPR), including Burkholderia, Pseudomonas, Arthrobacter, Bacillus, Serratia, Micrococcus, Chromobacterium, Erwinia, Azospirillum, Caulobacter, Agrobacterium, and Azotobacter (Verma et al., 2019).

Rhizobacteria produce phytohormones that regulate plant growth, including ethylene, gibberellins, and auxins. They also generate important metabolites such as siderophores, enzymes, organic acids, antibiotics, biosurfactants, nitric oxide, and osmolytes. These metabolites enhance nutrient uptake, improve tolerance to abiotic stresses, facilitate nitrogen fixation, and suppress pathogenic organisms (Khan et al., 2020).

Moreover, this beneficial trait is heritable and can be transmitted through seeds, making it particularly effective in promoting plant growth (Verma et al., 2019). The heritability of these traits is crucial for selecting adaptive and effective endophytes associated with specific crops, which is vital for agriculture, especially in plant breeding and tackling challenges related to climate change. The ability of endophytes to enhance tolerance and induce resistance to biotic and abiotic stresses can help address the edaphic and pathogenic challenges faced by the crop production sector. According to Afzal et al. (2019), the benefits associated with endophytes are often more pronounced when plants are subjected to environmental stress. This habitat-induced stress triggers plant-microbe signals, leading to complex communication between them.

Endophytic bacteria positively influence the development of host plants without causing significant harm, while also suppressing potential pathogens (Zhang et al., 2020). In return, these endophytic microbes benefit from the plant endosphere, which serves as a unique and safe haven, shielded from harsh climatic conditions that could otherwise disrupt their function (Mengistu, 2020). Additionally, many endophytic bacteria exhibit a biphasic life cycle, alternating between soil and plant environments to survive across seasons Afzal et al. (2019). Some bacteria form symbiotic structures, such as nodules in beans, which host various types of bacteria. While the rhizobia responsible for nitrogen fixation are well-documented, other endophytic bacteria remain less studied (Afzal et al., 2019).

The aim of this research was to assess the growth and yield of various lowland rice varieties when treated with a consortium of diazotrophic endophytic bacteria in rainfed land. The expected outcome is to enhance our understanding of how endophytic bacteria contribute to improving the productivity of rainfed land, ultimately increasing its potential as a food source.

METHODS

The study was conducted in Demangan, Sambi, Boyolali, Central Java, Indonesia, from June to September 2022, using alfisol soil. The geographical coordinates of the location are between 110 °22'- and 110 °50' East Longitude and between 7 °7' and 7°36' South Latitude, at an altitude of 184 meters above sea level (asl). The average monthly rainfall is 139 mm, and the temperature is 139 mm per month and 26-32^{o} C, respectively ranging from 26 to 32 °C.

Experimental design

This study utilized a randomized completely block design with two factors and three replications. The first factor was the dose of an endophytic bacterial consortium, which included four levels: 0 L/ha/application, 20 L/ha/application, 30 L/ha/application, and 40 L/ha/application. The second factor consisted of three rice field varieties: Situbagendit, Ciherang, and Mekongga. In total, there were 12 treatment combinations, each replicated three times, with each replication comprising five plant samples.

Research procedures

Before conducting the research, a chemical analysis was performed on the soil used as the research substrate. The results indicated a pH of 6.52 (slightly acidic), a carbon concentration of 1.34% (low), an organic matter concentration of 2.28% (low), a total nitrogen concentration of 0.22% (low), an available phosphorus level of 9.49 ppm (very high), and an available potassium level of 0.28 me/100 g (high).

The media used for the experiment was alfisol soil. The experimental plot measured 500 cm in length and 200 cm in width. The water level was maintained at 5 cm, and the plants were spaced 20 cm apart in a grid of 20 cm x 20 cm. Weeding was conducted at 2 and 4 weeks after planting, and pest and disease control was managed using organic pesticides. Fertilizers, including urea, NPK Phonska, and SP-36, were applied at doses of 200 kg/ha, 100 kg/ha, and 75 kg/ha, respectively, at the time of planting and again five weeks after planting. The criteria for harvest were that the seed coat above the panicle was clean and hard.

Measurement

The observed growth parameters included shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, plant fresh weight, and plant dry weight. Yield parameters consisted of the number of panicles, panicle length, grain weight per hill, grain weight per plot, weight of 1000 grains, and harvest index.

Statistical analysis

Observation data were analyzed using analysis of variance (ANOVA) with the SAS 9.1 program. If the treatment showed a significant effect, Duncan's New Multiple Range Test (DMRT) was employed to determine the differences between treatments, using a significance level of 5%.

RESULTS AND DISCUSSION

Rice plant growth

The analysis of variance presented in Table 1 indicates that the dose of the endophytic bacterial consortium significantly affected both the dry weight of the shoot and the dry weight of the plant. In contrast, the rice variety had a significant influence only on the fresh weight of the shoot. Additionally, there was no interaction observed between the dose of the endophytic bacterial consortium and the rice variety for any of the parameters measured.

Based on Table 2, the dry weight of the crown and the highest dry weight of the plant at the highest dose (40 L/ha/application) were significantly different from the lower dose, including the absence of endophytic bacteria. Similarly, while not significantly different, the parameters for fresh weight of the shoot, fresh weight of the plant, and dry weight of the plant showed trends that support this finding. This highlights the role of endophytic bacteria in fixing nitrogen and providing phosphorus that is retained by soil colloids (Medison et al., 2022; Ramírez et al., 2024). Endophytic bacteria are known to enhance host plant growth and resistance through the plant growth-promoting rhizobacteria (PGPR) mechanism (Purwanto & Suharti, 2021). Plant growth-promoting bacteria (PGPB), including rhizobacteria, are recognized for their ability to convert macronutrients (potassium, phosphorus, and zinc) from the soil and atmospheric nitrogen into forms that plants can absorb. Many PGPBs also synthesize phytohormones to regulate plant growth. (Badrudin et al., 2024).

The fresh weight of the Mekongga variety shoot was significantly different from that of the Situbagendit variety, but did not differ from the Ciherang variety; conversely, the Ciherang variety showed no difference from the Mekongga variety (Table 2). Differences in plant growth are heavily influenced by the genetic characteristics of each variety, which aligns with previous research (Basith et al., 2021; Kartahadimaja et al., 2021)

Table 1. Investigation of the role of endophytic bacteria in the growth of various rice varieties (*Oryza Sativa* L) in rainfed rice fields.

Parameter	Source of diversity (SV)		
	D	V	DV
1. Fresh weight of the crown	NS	*	NS
2. Dry weight of the crown	**	NS	NS
3. Fresh weight of roots	NS	NS	NS
4. Dry weight of roots	NS	NS	NS
5. Fresh weight of plants	NS	NS	NS
6. Dry weight of plants	**	NS	NS

Description = NS = non significant, * = significant, ** = very significant

Table 2. Effect of endophytic bacterial doses on various varieties of lowland rice on plant growth characteristics

Treatment	Crown weight		Root weight		Plant weight	
	Fresh weight of the crown (g)	Dry weight of the crown (g)	Fresh weight of roots (g)	Root dry weight (g)	Fresh weight of plants (g)	Dry weight of plants (g)
Dose of endophytic bacteria (D)						
D_0	74.18	18.15 b	24.67	17.95	98.85	36.11 b
D_1	72.15	24.54 b	23.19	16.17	95.35	40.72 b
D_2	77.86	25.15 b	21.16	17.12	99.02	41.04 b
D_3	79.75	34.96 a	22.47	14.15	102.22	49.12 a
Rice varieties (V)						
V_1	65.40 b	24.13	21.84	15.91	87.25	40.05
V_2	78.78 ab	24.72	25.09	17.90	103.88	42.62
V_3	83.77 a	28.26	21.68	15.56	104.45	43.88
Interaction of endophytic bacterial doses with rice varieties (D x V)						
D_0V_1	64.74	16.12	28.53	20.36	93.27	36.48
D_0V_2	80.91	19.51	27.54	18.12	108.45	37.63
D_0V_3	76.90	18.82	17.92	15.39	94.82	34.22
D_1V_1	57.78	24.17	21.61	15.48	79.39	39.65
D_1V_2	73.22	22.17	24.52	17.50	97.74	39.67
D_1V_3	85.48	26.50	23.44	15.52	108.92	42.07
D_2V_1	69.08	26.92	16.98	12.88	86.06	39.81
D_2V_2	82.08	20.69	24.34	22.03	106.43	42.72
D_2V_3	82.42	27.85	22.16	16.45	104.68	44.31
D_3V_1	70.03	29.31	20.24	14.94	90.27	44.25
D_3V_2	78.94	35.71	23.97	13.95	102.91	49.66
D_3V_3	90.29	39.97	23.21	14.94	113.5	54.91

Description: Treatments in the same column followed by the same letter show no significant difference according to the 5% DMRT test.

Table 3. Analysis of the role of endophytic biofertilizer doses on the yield of various rice varieties (*Oryza Sativa* L) in rainfed rice fields.

Parameter	Source of diversity (SV)		
	D	V	DV
1. Number of panicles	NS	NS	NS
2. Length of panicle	*	NS	NS
3. Weight of grain per clump	NS	NS	NS
4. Weight of grain per plot	NS	NS	NS
5. Weight of 1000 grains of rice	**	NS	NS
6. Harvest index	NS	NS	NS

Rice plant yields

The analysis of variance presented in Table 3 indicates that the dose of endophytic bacteria significantly influenced panicle length and the weight of 1000 grains. However, it did not affect other parameters. Additionally, there were no significant differences observed in relation to the type of variety or the interaction between the variety type and the dose of bacteria.

Table 4. Effect of bacterial dose, variety type, and their interaction on rice yield components in rainfed rice fields

Treatment	Number of panicles (cm)	Panicle length (cm)	Weight of grain per clump (g)	Weight of grain per plot (kg)	Weight of 1000 grains (g)	Harvest index
Dose of endophytic bacteria (D)						
D_0	22.86	20.91 b	22.14	1.91	24.40 ab	0.31
D_1	19.22	20.67 b	20.12	1.52	22.55 b	0.29
D_2	20.63	20.48 b	20.31	1.92	22.68 b	0.27
D_3	23.27	22.69 a	27.13	2.53	26.19 a	0.65
Rice varieties (V)						
V_1	21.72	20.87	20.88	1.84	24.32	0.38
V_2	21.00	21.25	24.00	2.11	24.32	0.47
V_3	21.77	21.43	22.35	1.84	23.24	0.29
Interaction of endophytic bacterial doses with rice varieties (D x V)						
D_0V_1	21.83	19.99	18.03	1.86	23.47	0.39
D_0V_2	22.25	19.99	25.87	1.80	24.99	0.34
D_0V_3	24.50	22.75	22.46	2.06	24.75	0.22
D_1V_1	21.00	21.06	22.20	1.48	21.51	0.28
D_1V_2	19.33	20.93	18.94	1.63	22.78	0.28
D_1V_3	17.33	20.01	19.22	1.46	23.37	0.30
D_2V_1	22.00	19.49	18.73	1.90	22.18	0.31
D_2V_2	19.16	20.92	22.08	2.16	23.50	0.26
D_2V_3	20.75	21.03	20.11	1.70	22.37	0.23
D_3V_1	22.08	22.94	24.49	2.13	25.79	0.53
D_3V_2	23.25	23.18	29.27	2.86	26.02	1.01
D_3V_3	24.50	21.95	27.62	2.60	26.78	0.42

Description: Treatments in the same column followed by the same letter show no significant difference according to the 5% DMRT test.

Figure 1. The best performance of research results in the Ciherang variety with an endophytic bacterial consortium dose of 40 l/ha/application

According to Table 4, the longest panicle length and the heaviest 1000-grain weight were obtained with an application of endophytic bacteria at a dose of 40 L/ha. This dose significantly differed from both lower doses and the control. Other parameters, including the number of panicles, grain weight per clump, grain weight per plot, 1000 grain weight, and harvest index, exhibited similar results. This indicates that the minimum effective dose for increasing panicle length and 1000-grain weight is 40 L/ha/application. Endophytic bacteria play a crucial role in plant growth, exerting both direct and indirect effects. As nonpathogenic microorganisms, they are essential components of the plant microbiome, colonizing all accessible host plant tissues (Faria et al., 2021). Endophytic bacteria have garnered significant attention due to their beneficial impacts on plant development and health (Afzal et al., 2019; White et al., 2019).

Most endophytic bacteria exhibit a broad host range. When isolated and introduced into both host and non-host plants, many have demonstrated the ability to enhance plant health, boost productivity, and improve stress responses (Faria et al., 2021; Zhang et al., 2023;Wang & Zhuang, 2019; Wang et al., 2020;Wei et al., 2024). Because of their environmentally friendly and sustainable characteristics, endophytic bacteria can be used as biofertilizers, biocontrol agents, and bioremediation efforts, contributing to better agricultural management and adding value to agricultural markets. The various rice varieties SituBagendit, Mekongga, and Ciherang do not show differences in all yield parameters. This is attributed to the distinct genetic factors of each variety. Planting different rice varieties on the same land results in varying levels of productivity (Devi et al., 2019; Chen et al., 2021; Kartahadimaja et al., 2021).

The novelty of this research lies in the application of a consortium of endophytic bacteria in rainfed rice fields, utilizing varieties that are well-suited for the land. This research contributes to enhancing rice growth and yield in rainfed fields by recommending the use of a diazotrophic endophytic bacteria consortium at a dosage of 40 L/ha/application, in combination with the Situbagendit, Ciherang, or Mekongga rice varieties.

CONCLUSION

This study concludes that the application of endophytic bacteria in rice plants (*Oryza sativa* L.) grown in rainfed areas can enhance the dry weight of the canopy, dry weight of the plant, panicle length, and weight of 1,000 grains. Future research should focus on determining the organic matter content requirements of the soil prior to the land being used to carry out research and conducting studies. Additionally, using optimal organic fertilizers will ensure suitable growing media for the growth, development, and activity of endophytic bacteria.

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