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Contribution of the endophyte bacterial consortium on rooting and rice yield in suboptimal rainfed lands, under different nitrogen and phosphorus doses

ABSTRACT

Rice (*Oryza sativa* L.) is a vital food crop in Indonesia's economy. It is grown in both irrigated and rainfed rice fields. However, rainfed rice fields have low nutrient content, requiring significant fertilization. To reduce reliance on chemical fertilizers, agricultural technological advancements are necessary, including the use of endophytic bacteria. The research aimed to assess the impact of an endophytic bacterial consortium on rice rooting and yield under various nitrogen and phosphorus doses in suboptimal rainfed land. The study was conducted in Demangan Village, Sambi District, Boyolali Regency, from March 2023 to June 2023, using a factorial Complete Group Design. The research consisted of two factors , each repeated three times. The first factor was nitrogen fertilizer, with four levels: no urea, 100 kg/ha urea, 200 kg/ha urea, and 300 kg/ha urea. The second factor iwas phosphorus fertilizer, with four levels,: no phosphorus, 80 kg/ha phosphorus, 160 kg/ha phosphorus, and 240 kg/ha phosphorus. The observed parameters included fresh root weight, dry root weight, root volume, root length, root diameter, root surface area, weight of 1000 grains, grain weight per plant, and grain weight per plot. The results revealed no significant effects of nitrogen, doses, phosphorus doses, or interactions between nitrogen and phosphorus doses on any of the observed parameters. In conclusion, the application of an endophytic bacterial consortium can reduce the need for nitrogen and phosphorus fertilizers in rainfed rice fields.

Keywords: endophytic bacterial consortium, nitrogen, phosphorus, root, yield,

INTRODUCTION

Rice is a crucial food crop for the Indonesian economy due to its high rice production. It serves as a staple food that is not easily replace able by other food sources. Rice plants require various nutrients, both macro and micro, for their growth. Macro nutrients, such as nitrogen (N), phosphorus (P), and sulfur (S), play a vital role in plant protein production. Plants obtain the energy needed to absorb these nutrients through respiration, which is fueled by carbohydrates produced through photosynthesis. Hence, factors influencing photosynthesis rate also impact nutrient absorption in plants (Messig & Groß, 2018).

The availability of nitrogen in the soil plays a crucial role in supporting rice growth and development. Although approximately 78% of the air is composed of nitrogen in the form of N2 gas, plants cannot directly utilize it as it remains inactive. This is why nitrogen fertilizer is consistently added in crop production (Hindersah et al., 2021).

Phosphorus is extremely important for the growth and production of plants. In rice plants, specifically, phosphorus (P) promotes root growth, triggers flowering and fruit ripening, and enhances the formation of tillers, which aids in stress recovery and adaption (Shamuyarira et al., 2022).

Many plants are grown in rainfed rice fields, despite the fact that these fields have low nutrient content, particularly nitrogen and phosphorus. To address these nutritional deficiencies, especially N and P, agricultural technology involving the use of endophytic bacteria is necessary. There are groups of soil bacteria, both symbiotic and free-living, that have the ability to fix nitrogen from the air. One option for biologically supplying nitrogen is to utilize nitrogen-fixing bacteria such as Azotobacter and Azospirillum (Desriani et al., 2013; Yanti et al., 2015).

Endophytic bacteria have a significant influence on the production of infected rice plants because they can provide the necessary factors for plant growth when infection occurs. This enables the plants to thrive. According to Bustami et al. (2012), rice production reaches its maximum level when the factors supporting plant growth are optimal and the necessary nutrients are available. Backman & Sikora (2008)reported that endophytic bacteria found in plant tissue can enhance plant growth, produce growth -promoting substances, fix nitrogen, mobilize phosphate, and contribute to plant health (Mbai et al., 2013). Khan et al. (2020), also stated that endophytic bacteria play a crucial role in plant adaptation and improving soil quality.

Plant growth-promoting bacteria possess specific mechanisms that significantly contribute to growth promotion, plant enhancement, and stress tolerance. These bacteria stimulate plants to produce growth hormones and regulators that aid in plant growth and yield (Numan et al., 2018). Endophytic bacteria play a vital role in plant health and development by enhancing nutrient uptake, regulating growth, and enhancing stress tolerance. Additionally, they can protect plants from various stresses, such as drought, low temperatures, and salinity (Afzal et al., 2019).

Endophytic bacteria are crucial for agriculture and environmental sustainability. These bacteria reside in plant tissue without causing disease and provide various benefits,

including promoting plant growth. They are capable of producing phytohormones such as gibberellins, which enhance plant growth (Sowjanya et al., 2024).

Endophytes, which are bacteria that inhabit different parts of plants, have the potential to help plants withstand both biotic and abiotic stress, resulting in reduced crop yield losses (Mengistu, 2020). These endophytes colonize plants in a systematic manner and provide benefits throughout various stages of plant growth. One of their roles is to activate plant defense systems, and they can also produce secondary metabolites that act as antibiotics against pathogens (Pndey et al., 2017). Additionally, endophytes have the potential to serve as a source of new and environmentally friendly natural products, which can be used for medical, agricultural, and industrial purposes (Sahoo et al., 2018).

The objective of this study was to investigate the impact of a consortium of endophytic bacteria on the root development and yield of rice under different nitrogen and phosphorus levels in suboptimal rainfed land.

RESEARCH METHODS

This research utilized a factorial Complete Randomized Block Design (RCBD) with 2 factors, each repeated 3 times. The first factor was the N fertilizer, with 4 levels: without urea (N0), 100 kg/ha urea (N1), 200 kg/ha urea (N2), and 300 kg/ha urea (N3). The second factor was the P fertilizer with 4 levels: without phosphorus (P0), 80 kg/ha phosphorus (P1), 160 kg/ha phosphorus (P2), and 240 kg/ha phosphorus (P3). The research was conducted in Demangan Village, Sambi District, Boyolali Regency, at an altitude of approximately 130 meters above sea level on Regosol soil from March 2023 to June 2023.

The materials used included Mekongga variety rice seeds, water, raffia rope, labels, nitrogen fertilizer, and phosphorus fertilizer. The tools used included a measuring tape, bamboo spacing, scissors, writing tools, rulers, hoes, buckets, basins, scales, treatment nameplates, and bamboo.

The basic fertilizer applied was 10 tonnes/ha of cow manure, followed by tilling the soil with a tractor. Prior to sowing, the seeds were soaked in water mixed with a consortium of endophytic bacteria. After 3 weeks of sowing, the seeds were transplanted to the field. The endophytic bacterial consortium was used as a seed treatment at sowing, with a ratio of

20:1000. Soil treatment was performed by shaking at 1, 2, 3, 4, and 5 weeks after planting (WAP), at a dose of 40 ml/ha. Fertilization was carried out twice, at 2 WAP and 5 WAP, according to the specified dosage. The observations included rooting and yield components.

The rooting parameters observed included fresh root weight, root dry weight, root volume, root length, root diameter, and root surface area. The yield component parameters observed included the weight of 1000 grains, the weight of grain per plant, and the weight of grain per plot.

Root length measurement was conducted using an Areameter (Figure 1) following these steps: connect the power cable to the power source, press the ON button on the voltage regulator, press the ON button on the ΔT Areameter, press the ON button to the left until the light turns on, press the ON button on the tool to focus on the object, adjust the lens if necessary, press the ON button to turn on the screen, adjust the brightness by turning the Bright knob clockwise, adjust the contrast level by turning the contrast knob, place the sample on the lighting table, calibrate the instrument according to the objective of measuring root length, place the sample to be observed on the instrument until it is visible on the screen, select the parameters to be measured (AREA for leaf area and LENGTH for length), record the numbers displayed on the screen as the observation results.

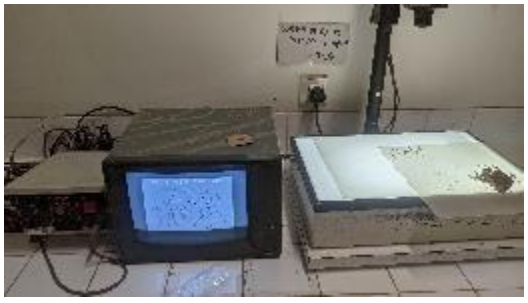

Figure 1. Areameter. is a tool for measuring root length, root diameter and root surface area

Connect the power cord to the electricity source, then press the ON button on the Voltage Regulator. Next, press the ON button on the Conveyor Control , followed by the ON button on the T area meter. Press the ON button to the left until the light comes on, and finally, press the ON button on the tool to view the object. If the object is not in focus, adjust the lens.

To determine the root diameter, first measure the root surface area. Then, read the root projection area at the position of the area button. Assuming the root is cylindrical, the root projection areas calculated as 2RP, where R is the radius, and P is the area of the root. From this calculation, the value of R is obtained. The diameter of the root is the area of the cylindrical skin without a cover at both ends of the root. It is equal to 32 times the circumference multiplied by the length of the root, which can be expressed as 12πRP. By observing this, the average root diameter value, 2R, is also obtained

The data was analyzed using a variance of 5% and 1%. If there were significant differences between treatments, further tests were conducted using the Duncan Multiple Range Test (DMRT) with a significance level of 5%.

RESULTS AND DISCUSSION

Prior to conducting research, on the land, soil testing is performed to assess various factors including organic C content, organic matter, total N, available P, exchangeable K, and soil pH. The laboratory tests revealed the following results: organic C content 1.34%, organic matter 2.28%, total N 0.22%, available P (as P2O5) - 9.49 ppm, exchangeable K 0.28 me%, and pH 6.52 (Table 1.). It should be noted that all the aforementioned factors - organic C, organic matter, total N, P2O5, and exchangeable K fall within the low category.

Table 1 . Analysis of soil before experiment

Types of Analysis	Method	Test			Average
		1	2	3	
C Organic (%)	Walkey & Black	1.2 9	1.41	1.32	1.34
Material Organic (%)	Walkey & Bla c k	2.23	2.44	2.17	2.28
N Total (%)	Kjeldhal	0.20	0.25	0.22	0.22
P_2O_5 Available (ppm)	Bray II	8.25	10.47	9.75	9.49
K swapped (me%)	Extraction Am. Ace t at	0.26	0.31	0.28	0.28
pH	Electrodes glass	6.40	6.60	6.57	6.52

Source : Laboratory Chemistry And Fertility Land , Faculty Agriculture Sebelas Maret University, Surakarta.

Based on the analysis of diversity (Table 2), it is evident that differences in nitrogen dose, phosphorus dose, or the interaction between nitrogen dose and phosphorus dose did not have a significant impact on the observed parameters. This lack of difference is believed to be attributed to the presence of the endophytic bacterial consortium, which was application did during seed treatment and soil treatment. This consortium consists of beneficial soil microorganisms that enhance soil fertility through biochemical processes. The combination of microbacteria with chemical fertilizers, manure, or compost is highly advantageous in improving land productivity and increasing the quality and quantity of crops. The bacterial consortium itself comprissa variety of bacteria that positively contribute to the plant growth process, including Azotobacter sp ., Azospirillum sp ., Bacillus sp ., Pseudomonas sp., and Cytophaga sp. (Numan et al., 2018).

Table 2. Analysis of variance of root component parameters and yield components

No.	Treatment	Treatment		
		Nirogen (N)	Phosphorus (P)	N x P
	Rooting components:			
1.	Fresh weight of roots	1.30 ns	0.63 ns	0.59 ns
2.	Root dry weight	1.52 ns	4.21 ns	5.54 ns
3.	Root volume	6.64 ns	17.39 ns	0.79 ns
4.	Root length	0.73 ns	0.36 ns	0.65 ns
5.	Root diameter	0.87 ns	3.40 ns	0.49 ns
6.	Root surface area	0.43 ns	0.15 ns	0.72 ns
	Yielding Components:			
1.	Weight of 1000 grains	1.39 ns	0.35 ns	0.72 ns
2.	Grain weight per plant	0.22 ns	0.89 ns	1.22 ns
3.	Grain weight per plot	0.62 ns	0.85 ns	0.20 ns

Note : ns = non significant

Rooting Parameters of Rice Plants

According to Table 3, there are no variations in fresh root weight, root dry weight, and root volume across different nitrogen doses, phosphorus fertilizer doses, or the interaction between nitrogen and phosphorus doses. This lack of difference in the three parameters is due to the relationship between fresh root weight, root dry weight, and root

volume. When the fresh weight of the roots increases, the dry weight of the roots and root volume also increase, provided that other factors are optimal.

Root dry weight plays a crucial role in the growth of plant organs below the soil surface, which in turn affects the development of above-ground organs. A good rooting system is characterized by a favorable dry root weight/crown ratio. Additionally, root dry weight represents the distribution of assimilation to the rooting system. Transport tissues distribute assimilation products produced by the plant crown throughout the entire plant body.

Root volume significantly impacts the process of water and nutrient absorption by the roots, essential for the survival of plants. Genotype and irrigation systems influence root volume (Munarso, 2011). Plants with larger root volumes have an increased capacity to absorb water, enabling them to withstand water shortage conditions (Palupi & Dedywiryanto, 2008).

Plant biomass, including roots, is influenced by nutrient availability, drought stress, and biomass allocation (Shamuyarira et al., 2022; Yan et al., 2023). Under drought stress, plants allocate photosynthesis products to enhance water uptake from deeper soil layers, making root biomass closely related to overall biomass weight.

Table 3. Effect of nitrogen and phosphorus doses on fresh root weight, root dry weight and root volume of rice applied by the endophytic bacterial consortium in rainfed rice fields.

	Rooting Parameters		
	Fresh weight of roots (g)	Root dry weight (g)	Root volume (cm3)
Fertilizer (N)			
N0	24.801	1.1175	18.0550
N1	28.359	1.5175	18.8600
N2	27.713	1.2758	19.9717
N3	28.612	1.9108	20.4725
Fertilizer Phosphorus (P)			
P0	27.188	1.1600	18.0550
P1	25.718	1.2617	18.8600
P2	28.297	1.6783	19.9717
P3	28.283	1.0175	20.4725
Interaction Nitrogen and Phosphorus Fertilizer (N x P)			

N0P0	26.493	1.4167	14.777
N0P1	24.443	0.8767	17.223
N0P2	25.057	1.4000	19.777
N0P3	23.210	0.7767	20.443
N1P0	27.537	0.7200	16.667
N1P1	22.580	0.9633	18.330
N1P2	31.547	3.2333	19.557
N1P3	31.773	1.1533	20.887
N2P0	27.203	1.3333	17.777
N2P1	28.410	1.6167	19.663
N2P2	27.130	1.1267	20.890
N2P3	28.107	1.0267	21.557
N3P0	27.517	1.1700	19.110
N3P1	27.437	1.5900	20.667
N3P2	29.453	0.9533	20.113
N3P3	30.040	1.1133	22.000

Notes :
No = without urea; N1=100 kg/ha urea, N2= 200 kg/ha urea, N3= 300 kg/ha urea
P0=without Phosphorus; P1= 80 kg/ha Phosphorus; P2=160 kg/ha Phosphorus; P3= 240 kg/ha Phosphorus

Based on Table 4, there are no differences observed in root length, root diameter, and root surface area when considering nitrogen dosage, phosphorus dosage, or the interaction between nitrogen and phosphorus dosage. The absence of a difference in root length, can be attributed to the fact that, during the growth cycle, roots tend to elongate when they receive potassium nutrients. It is known that long and lender roots have a greater capacity to freely reach and penetrate pores, allowing for enhanced nutrient absorption (Costa et al., 2002). The root surface area indicates the roots' ability to absorb water and nutrients from the soil. A wider root area, corresponds to a larger surface available for water and nutrient absorption (Aziez et al., 2023).

Although nitrogen and phosphorus levels do influence root growth and nutrient uptake efficiency, it appears that root diameter is not affected by the dosage of nitrogen or phosphorus (Li et al., 2022). Various plant species that have undergone different nutrient treatments have shown that long and slender roots are effective in exploring soil pores, thereby increasing their ability to absorb nutrients. This adaptive root morphology allows for efficient nutrient uptake without being significantly impacted by specific

nitrogen or phosphorus dosages, highlighting the crucial role of root architecture in nutrient acquisition strategies (G. Li et al., 2016).

Table 4. Effect of Nitrogen and Phosphorus Doses on root length, root diameter and root surface area of rice applied by the Endophytic Bacteria Consortium in Rainfed Rice Fields.

	Rooting Parameters		
	Root length (cm)	Root diameter (x 10^{-4} cm)	Root surface area (cm^2)
Fertilizer (N)			
N0	1131.5	767	275.77
N1	1109.8	783	269.64
N2	988.3	750	228.60
N3	1295.4	7167	282.88
Fertilizer Phosphorus (P)			
P0	1156.2	6917	254.58
P1	1174.5	7833	282.20
P2	1193.9	725	268.92
P3	1000.4	8167	251.19
Interaction Nitrogen and Phosphorus Fertilizer (N x P)			
N0P0	1035.9	7000	245.1
N0P1	1435.0	8333	367.2
N0P2	1380.3	7000	313.8
N0P3	674.8	8333	176.9
N1P0	1340.3	7333	306.6
N1P1	953.2	8000	236.8
N1P2	1248.1	8000	314.4
N1P3	897.7	8000	220.7
N2P0	113.3	6333	228.1
N2P1	987.4	8000	246.9
N2P2	866.8	7333	194.5
N2P3	966.5	8333	245.0
N3P0	1116.3	7000	238.5
N3P1	1322.5	7000	277.9
N3P2	1280.3	6667	253.1
N3P3	1462.5	8000	362.1

Notes :
No = without urea; N1=100 kg/ha urea, N2= 200 kg/ha urea, N3= 300 kg/ha urea
P0=without Phosphorus; P1= 80 kg/ha Phosphorus; P2=160 kg/ha Phosphorus; P3= 240 kg/ha Phosphorus

Rice Crop Yield Parameters

The observed components of rice yield included the weight of 1000 grains, the weight of grain per plant, and the weight of grain per plot. No differences were found in these components based on nitrogen dose, phosphorus dose, or the interaction between nitrogen dose and phosphorus dose. (Hamid et al., 2015)stated that the weight of 1000 grains is more influenced by genetic factors of the variety than environmental factors such as nitrogen and phosphorus fertilizer doses. Grain ripeness is influenced by photosynthates from two sources, : assimilates before fertilization, which are stored in the stem tissue and later converted into sugars and transported to the seeds, and assimilates produced during ripening (Mungara & Rogomulyo, 2013). The size of the husk on the seed determines its weight, with larger husks resulting in heavier seeds. Lagasca et al., (2024)Click or tap here to enter text. mention that the size of the grain is controlled by the size of the husk.

According to (Park et al., 2023), grain yield in rice plants is mainly determined by factors such as the number of panicles per plant, the number of grains per panicle, and the weight of 1000 grains, all of which contribute significantly to the overall yield . Additionally, the average seed weight affects the shape and size of the seeds, highlighting its importance in determining the final yield (Zhang et al., 2023). Genetic factors also play a crucial role in ensuring uniform grain size when there are no individual differences in grain size (Gunasekaran et al., 2023). Furthermore, competition among tillers for nutrients can affect the size of rice grains, demonstrating the influence of the environment on grain characteristics (Gudepu et al., 2022). Increasing grain weight per plant can also lead to an increase in grain weight per plot, underscoring the significance of individual grain weight in overall yield improvement (Yadav & Pitha, 2022).

Table 5. Effect of Nitrogen and Phosphorus Doses on Rice (*Oryza Sativa* L*)* Yield which was applied by the Endophytic Bacteria Consortium in Rainfed Rice Fields.

	Result Parameters		
	Weight of 1000 grains (g)	Grain weight per plant(g)	Grain weight per plot (kg)
Fertilizer (N)			
N0	48.075	38.418	3.7617

N1	45.721	40.051	3.9350
N2	45.296	39.980	3.9242
N3	54.375	40.348	3.9750
Fertilizer Phosphorus (P)			
P0	45.433	37.508	3.9125
P1	48.758	40.454	4.0292
P2	48.804	39.263	3.7592
P3	50.471	41.571	3.8950
Interaction Nitrogen and Phosphorus Fertilizer (N x P)			
N0P0	46.37	34.603	3.7167
N0P1	48.23	35.653	3.8667
N0P2	51.03	39.723	3.7167
N0P3	46.67	43.690	3.7467
N1P0	39.43	37.040	3.9167
N1P1	42.08	41.117	4.1667
N1P2	50.03	44.133	3.7567
N1P3	51.33	37.913	3.9000
N2P0	39.68	40.353	4.0000
N2P1	43.17	38.723	3.9500
N2P2	42.38	35.353	3.6633
N2P3	55.95	45.490	4.0833
N3P0	56.25	38.033	4.0167
N3P1	61.55	46.323	4.1333
N3P2	51.77	37.843	3.9000
N3P3	47.93	9.190	3.8500

Notes :
No = without urea; N1=100 kg/ha urea, N2= 200 kg/ha urea, N3= 300 kg/ha urea
P0=without Phosphorus; P1= 80 kg/ha Phosphorus; P2=160 kg/ha Phosphorus; P3= 240 kg/ha Phosphorus

CONCLUSION

The research results indicate that the root fresh weight, root dry weight, root volume, root length, root diameter, root surface area, as well as yield component parameters such as the weight of 1000 grains, grain weight per plant, and grain weight per plot were not affected by the nitrogen dose, phosphorus dose, or the interaction between nitrogen and phosphorus doses. However, it is important to note that endophytic bacterial consortia have a significant impact on soil fertility, particularly in regards to nitrogen and phosphorus content.

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