Physiological Traits and Rainfed Rice Grain Yield Treated with Cow Manure and Plant Growth Promoting Rhizobacteria Consortium

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Abstract. Agricultural intensification in the long term has had the impact of decreasing soil organic matter levels so that rice production tends to stagnate. The return of organic matter and the application of rhizobacteria is an action in the effort to the reclamation of paddy soil to be productive again. This research was conducted to examine the effect of the dosage of organic manure combined with the application of the PGPR consortium on the agronomic, physiological traits, and yield of aromatic rice plants to achieve sustainable rice production. The experiment was carried out using a Randomized Block Design 3 x 3 factorial and provide with replication. The first factor was the selected PGPR consortium application (P0 = control: P1+ PGPR consortium isolate R08 + R11, and P2 = PGPR consortium isolate R08 + Rhizobium sp. LM-5) and the second factor was cow manure application (0, 15 and 30tons ha^{-1}). Application of organic fertilizer and the PGPR consortium was able to increase vegetative growth, nutrient uptake (N, P, and K), as well as the physiological character of rice plants. The interaction between the dose of organic fertilizer and the PGPR consortium affects the net assimilation rate, crop growth rate, and yield of rice plants. The highest yield was achieved at a dose of organic fertilizer of 30 tons ha^{-1} and the PGPR consortium isolate R08+Rhizobium sp. LM-5 of 11.68 tons ha^{-1}.

1. Introduction

The intensification of rice production in the last 40 years has led to a condition where the carrying capacity of the land has decreased with intensive agricultural exploitation and very high agrochemical inputs. The massive use of agrochemicals has caused a decrease in soil fertility through decreased physical, chemical and biological fertility [1]. One of the indicators of decreasing land quality is the removal of soil organic matter and decreasing soil

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biological biodiversity [2]. Soil organic C content and C/N ratio are indicators of soil quality and the sustainability of crop production [3] The loss of soil organic matter will reach 30-60% due to agricultural production resulting in decreased soil fertility and will require a higher fertilizer dose in crop cultivation [4].

Returning organic matter to the soil can be done by applying organic fertilizers. Sources of organic fertilizer are very diverse, both from plant litter and also from chicken manure, and cow manure. The application of organic materials is one of the efforts to increase the fertility of rice field soil in Indonesia, which generally has a low C-organic content [5]. The level of organic matter in paddy soil is generally low below 2 percent so to increase the organic matter content by 1 percent, at least 20 tons ha^{-1} of organic fertilizer is needed [6]. Previous research reported that the application of goat manure, green manure, and Azolla was able to increase soil organic C content of paddy soil from 0.82% to more than 2% [7]. Furthermore, the application of organic fertilizers up to 17.5 tons ha^{-1} in Cichorium endivia plant cultivation increased fresh matter weight, the number of leaves and plant height to 280, 58, and 101%, and linearly increased nutrient uptake [8].

Increasing the level of soil organic matter not only increases physical, and chemical fertility, but also soil biological fertility. Soil organic matter acts as a source of C for the metabolism of soil microorganisms. The activity and abundance of soil bacteria were twice increased with the application of straw with the addition of N and this indicated that soil organic matter was central to soil bacterial activity [9,10]. Returning rice straw as an organic material can stimulate the growth of soil microbes where dissolved organic matter has higher biodegradability so that dissolved organic matter can be easily used by microbes as subtract C [10].

The abundance of microorganisms in the plant rhizosphere will have a positive impact and stimulate plant growth. Microorganisms that live in plant rhizosphere can have a positive effect on plant growth known as plant growth promoter rhizobacteria (PGPR). PGPR affects plant growth through various mechanisms including its ability to produce hormones, increase the availability of nutrients both nitrogen, phosphate, potassium, and various essential nutrients [11]. The application of indigenous bacteria will have a faster impact on plants because they can dominate the rhizosphere more quickly [12]. Other researchers reported that inoculation of *Pseudomonas aeruginosa* was able to significantly improve rice yields [13]. Furthermore, the indigenous rhizobacteria that isolated from paddy fields have the ability to produce high concentrations of IAA [14]. Application of indigenous rhizobacteria can reduce nitrogen doses by up to 25 percent and increase rice productivity [15]. Selected rhizobacteria applications can be applied in the form of a bio-fertilizer. The application of multi strains in the form of a consortium will have a positive impact through the activity of rhizobacteria producing IAA, fixing of N, solubilizing of P, production of siderophore, and antibiotics [16–18]. The application of poultry manure (12 tons ha^{-1}) enriched with PGPR significantly improved the root attributes of okra plants, the root and shots dry weight of okra plants [18]. Other researcher reported that the application of organic manure combined with a bio-fertilizer has a positive effect on the roots of cotton plants so that it can be used to improve root health in sustainable cotton cultivation. In mustard crop plants, applications of chemical fertilizers, vermicompost, and bio-fertilizer can increase chlorophyll content, plant biomass, leaf area, LAI, net assimilation rate, and plant growth rate [19].

The application of multi-strain rhizobacteria combined with the ability of the bacteria to both fix nitrogen and produce IAA will further increase its effectiveness. This will be supported by the application of organic fertilizers as a carbon source for bacteria so that the abundance of microbes in the rhizosphere of rice plants will increase. This research was conducted to examine the effect of the dosage of organic manure combined with the application of the PGPR consortium on the agronomic, physiological traits, and yield of aromatic rice plants to achieve sustainable rice production.

2. Materials and Methods

2.1 Experimental Site

Field trials were carried out at Experimental Farm, Faculty of Agriculture, General Sudirman University, Purwokerto, Central Java, Indonesia. The research was carried out in the wet season 2020 (February–May) with the aromatic type of Inpago Unsoed 1 rice variety. The research location has Inceptisol soil type. The soil at the study site was a dusty clay loam texture with a pH of 6.5, a cation exchange capacity of 19.96 cmol kg^{-1}, a C/N ratio of 12, and a C-organic content of 2.47%. Besides the nutrient content of the soil, each contains 12 ppm, 70 ppm, and 484 ppm N, P_2O_5, and K_2O respectively.

2.2 Treatments and Experimental Design

The experiment was carried out using a randomized block design 3 x 3 factorial. The first factor was the selected PGPR consortium application consisting of P0: without PGPR application, P1: PGPR consortium isolate R08 + R11, and P2 PGPR consortium isolate R08 + Rhizobium sp. LM-5. The second factor was the provision of soil amendment material in the form of cow manure with a dose of B0: without manure, B1: cow manure 15 tons ha^{-1}, and B2: cow manure 30 tons ha^{-1}. The combination of treatments obtained was 9 treatments and repeated 3 times so that the total research units were 27 research units. The rice plant variety used was Inpago Unsoed 1. The cow manure compost contained 38.28%, 16, 2.43%, 1.60%, and 0.63 C-organic matter, C/N ratio, N, P_2O_5, and K_2O, respectively.

2.3 Preparation of PGPR Isolates and Application of Rice Plants

The PGPR isolates used in this study were collected from the Agronomy and Horticulture Laboratory of the Faculty of Agriculture, UNSOED Purwokerto. PGPR isolate used in this study was the result of previous studies where Rhizobium sp. LM-5 has the ability to fix N2 and produce IAA. Isolates R08 and R11 were rhizobacteria isolates that have the ability to produce IAA. Inoculant propagation was carried out using Nutrient Broth (HIMEDIA) media. One ose was inoculated into 250 mL of Nutrient Broth media, then shaken at 120 rpm for 48 hours with a population density above 107 CFU mL^{-1}. PGPR application in the field is carried out using a liquid carrier material, namely Molasses with a concentration of 5%. Molasses nutrition consists of water content 23%, dry matter 77%, crude protein 4.2%, 0.2% crude fat, 7.7% crude fiber, Ca 0.84%, P 0.09%, BETN 57.1%, and ash 0.2% [20]. Preparation of the molasses formulation was carried out by making a 5% molasses solution, whereas much as 50 mL of molasses was dissolved in 950 ml of distilled water then sterilized using an autoclave for 15 minutes with a pressure of 15 psi. The molasses 5% solution was then cooled before being inoculated with the PGPR inoculant. PGPR isolates inoculation was carried out aseptically, as much as 1 mL of PGPR inoculant was inoculated into molasses 5% solution and shaken at 120 rpm for 48 hours and the population density had reached above 107 CFU mL^{-1}. The PGPR solution was applied with 5% concentration and volume by 600 L ha^{-1} by spraying at 15, 30, and 45 days after planting (DAP)

The cow manure comes from a farm in Purwokerto and has undergone a thorough composting process. The treatment of cow manure was given at doses of 0 tons ha^{-1}, 15 tons ha^{-1}, and 30 tons ha^{-1}. After the land was plowed and harrowed, a 4 x 4 m research plot was then made. The distance between blocks was 50 cm as irrigation channels, and water flow from the plot did not enter the other plots. The application was carried out by spreading it evenly over the land that had been harrowed before planting rice. Rice planting was carried out using 21 days old seedlings after sowing, and the spacing used was 25 x 25 cm. Chemical fertilization was given in the form of nitrogen of 100 kg ha^{-1} which was given twice at the age of 7 days after planting and 28 days after planting. 90 kg ha^{-1} of K_2O and 54 kg ha^{-1} of P_2O_5 were given before planting as basal fertilizers.

2.4 Growth and yield parameters

Observation of the growth and yield of rice plants was carried out based on the Standard Evaluation System (SES) for rice [21]. The variables observed included plant height (cm), number of tillers, plant biomass (g), root biomass (g), total root length measured by intersection method, leaf area (cm^2), leaf area index (LAI), number of productive tillers, panicle length (cm), number of grain per panicle, 1000 grains weight (g), grains weight per clumps (g), and grains yield (tons ha^{-1}).

2.5 Nutrient contents

Plant samples were taken by pulling out a clump of rice plants and then cleaning and drying the plant canopy. The dry sample was then in an oven at 80 0C for 48 hours. Dried plant samples were pulverized in a blender to analyze the N, P, and K content. The N content of plant tissue was measured by the micro-Kjeldahl method. A total of 200 mg of the ground dry sample that had been mashed was added to a 100-ml Kjeldahl flask, then added with salt mixture and 3 ml of concentrated H_2SO_4. Place the Kjeldahl flask in an empty tin can of a suitable size and heat over a flame to digest the sample. When the sample was clear, cool it and then add 10 mL of distilled water. Mix thoroughly and allow the sample to cool again. Phosphorus levels were measured using a colorimetric method, and potassium levels were measured using a Flame photometer.

2.6 Physiological traits

Plant samples in the form of plant dry weight and leaf area were taken by destroying the sample plants at the age of 3, 5, 7, and 9 weeks after planting to calculate the effect of cow manure and the PGPR consortium on the physiological characters of rice plants including leaf area index (LAI), net assimilation rate (NAR), crop growth rate (CGR), and leaf area duration (LAD). Leaf area was measured using Yoshida methods [22], where each leaf sample was measured for the length and the maximum width and then the leaf area was calculated using the following formula

$$Leaf\ area\ (cm^2) = Length\ x\ width\ x\ K \quad (1)$$

Where K is the adjustment factor. The value of 0.75 can be used for all stages of growth.
Leaf area index (LAI) was measured by the following formula [24]:

$$LAI = \frac{total\ leaf\ area\ per\ hill\ (cm2)}{area\ of\ land\ covered\ by\ a\ hill\ (cm2)} \quad (2)$$

Leaf area duration (LAD) was measured by the formula below [13]:

$$LAD = \frac{(LAI1+LAI2)x\ (t2-t1)}{2} \quad (3)$$

Where: LAI1 and LA2 are the leaf area index at the time of observation t2 and t1
Net assimilation rate (NAR) was measured by the following formula :

$$NAR = \frac{W2-W1}{T2-T1} \text{ x } \frac{ln ln\ La2 - lnLa1}{La2-La1} \text{ (g dm}^{-2}\text{ week}^{-1}) \quad (4)$$

Where: where W2 and W1 are plant dry weights at times t1 and t2, ln La1 and ln La2 are the natural logs of leaf areas A1 and A2 at times t1 and t2
Crops growth rate (CGR) was measured by the following formula:

$$CGR = \frac{W2-W1}{t2-t1} \text{ (g dm}^{-2}\text{ week}^{-1}) \quad (5)$$

Where: where W2 and W1 are plant dry weights at times t2 and t1

2.7 Data analysis

The collected and tabulated research data were analyzed using analysis of variance (ANOVA) using SAS. Version 9.1. If the ANOVA results showed significantly different results, then continued with DMRT 5%.

3. Results and Discussion

3.1 Root Growth and Nutrient Uptake

The application of the PGPR consortium and organic fertilizer affected on the root length and root length density of rice plants. However, there was no interaction between the PGPR consortium and organic fertilizer on root length and root length density. The application of the PGPR consortium significantly increased the root length and root length density compared to the treatment without the PGPR consortium treatment. The longest root length was achieved in the treatment of PGPR R08 isolate+Rhizobium sp. LM-5, although not different from the treatment of PGPR R08 isolate+R11 isolate were 3875.40 and 3849.00 cm, respectively. The average PGPR consortium application was able to increase root length by 39.39 percent. Increasing the dose of organic fertilizer significantly increases the root length growth of rice plants. The longest root length was achieved at a dose of 30 tons ha^{-1} of manure of 4312.20 cm.

In the variable root length density, it can be seen that the application of the PGPR consortium and organic fertilizer significantly increased the root length density, but there was no interaction. The greatest root length density was achieved in the treatment of PGPR R08 isolate+Rhizobium sp. LM-5, followed by PGPR R08 isolate+R11 isolate treatment of 5.64 and 6.22 cm/cm^3, respectively. The lowest root length density was achieved by the control treatment, which was 2.88 cm/cm^3 (Table 1). Increasing the dose of organic fertilizer significantly increased root length density, and the highest root length density was achieved at a dose of 30 tons/ha reaching 6.14 cm/cm^3 (Table 1).

Table 1. The effect of PGPR consortium and cow manure on total root length of rice plant

Treatments	Total root lenght (cm)	Root Length Density (cm.cm^{-3})
PGPR Consortium		
Control (without PGPR)	2770.80 b	2.88 c
PGPR isolates R08+isolate R11	3849.00 a	5.64 b
PGPR isolates R08+ Rhizobium sp. LM-5	3875.40 a	6.22 a
Organic manure		
0 tons ha^{-1}	2755.20 c	4.12 b
15 tons ha^{-1}	3427.70 b	4.48 b
30 tons ha^{-1}	4312.20 a	6.14 a

Remark: The number is followed by the same letter in the same column is not significantly different according to DMRT 5%.

Plant roots are plant organs that are vital for plant growth and development. Roots interact directly with the soil and soil colloids to absorb nutrients and water for plant metabolic processes. The interaction between plants and microorganisms creates a positive synergism, in which plants release root exudates which are utilized by soil microorganisms as a carbon source. On the other hand, soil microbes have a positive effect through the synthesis of growth regulators that have an impact on plant root growth. Beneficial microorganisms that live to colonize roots and provide positive effects on plants are often referred to as plant growth promotion rhizobacteria (PGPR). PGPR affects plant growth through the production of regulatory substances such as IAA, as well as through its ability to fix N_2 and solubilize phosphate [23]. The Inocultaion of rhizobacteria genus Bacillus, Paenibacillus and Comamonas can increase root growth in kiwifruit plants through the ability to produce IAA [24].

The application of the PGPR consortium was able to increase the root length density (RLD) of rice plants. The results of this study showed that the PGPR isolates consortium R08+Rhizobium sp. LM-5 had the highest RLD value compared to the PGPR isolate R08+isolate R11 consortium and control. The ability of PGPR to secrete growth hormones such as IAA will improve root architecture where PGPR will decrease main root growth, but increase the number and length of lateral roots and stimulate elongation of root hairs so that root length per soil volume will increase [25,26]. The consortium of PGPR isolate R08+ Rhizobium sp. LM-5 can provide nutrients in the form of nitrogen through the ability of Rhizobium sp. LM-5 binds N2 so that the availability of N for plants increases. Nitrogen fertilization with moderate doses can increase the RLD of cotton plants, and is much greater than without nitrogen fertilization [25].

Increasing the dose to 30 tons ha^{-1} resulted in the highest root length and RLD compared to doses of 15 tons ha^{-1} and doses of 0 tons ha^{-1}. Organic fertilizers play a role in increasing physical, chemical and biological fertility. Organic matter is one of the soil components that are very important for soil physicochemistry, namely as a buffer and increasing soil biological activity [27]. The increase in root length and RLD in the application of organic fertilizers is related to the effect of organic fertilizers on the physical properties of the soil and the availability of nutrients in the soil. Previous researcher reported that the RLD of maize increased significantly due to the application of manure, where the effect of manure on increasing root proliferation in the topsoil was in line with improving soil physical properties and increasing soil nutrient levels [28]. The advantages of using organic fertilizers include that organic fertilizers are a source of macro and micronutrients so that they can increase their availability for plants, and improve soil physical properties [29].

Table 2. The effect of PGPR consortium and cow manure on the nutrient content of rice plant

Treatments	N (g $plant^{-1}$)	P (g $plant^{-1}$)	K (g $plant^{-1}$)
PGPR Consortium			
Control (without PGPR)	0.47 a	0.069 b	0.79 a
PGPR isolates R08+isolate R11	0.53 a	0.081 a	0.80 a
PGPR isolates R08+ Rhizobium sp. LM-5	0.49 a	0.071 a	0.77 a
Organic manure			
0 tons ha^{-1}	0.43 b	0.069 b	0.70 b
15 tons ha^{-1}	0.53 a	0.081 a	0.78 ab
30 tons ha^{-1}	0.54 a	0.072 ab	0.89 a

Remark: The number is followed by the same letter in the same column is not significantly different according to DMRT 5%.

The application of the PGPR consortium only affected the uptake of K nutrients, while the uptake of N and P nutrients was not affected by the application of the PGPR consortium. Based on the results of the study, it was seen that there was a tendency that the application of the PGPR consortium R08 isolate+R11 isolates to result in higher nutrient uptake in both N, P, and K uptake (Table 2). The nutrient uptake of rice plants is strongly influenced by the availability of nutrients and nutrient status in the paddy fields. Based on the results of soil analysis, it was found that the soil nitrogen content was in low status, while P and K were in high nutrient status. Nitrogen in flooded soil conditions, nitrification and nitrification processes occur so that its levels in the soil are always low [30]. The increase in P nutrient uptake in rice plants that were inoculated by the PGPR consortium was strongly influenced by root growth factors, both root length, and RLD. Increasing root length and RLD value will increase the root surface area that interacts with soil colloids.

Nutrient P in lowland soils is generally high as a residue from fertilization, but its availability is low. The P content of paddy soil varies greatly depending on the location, and in general the total P content of paddy soil is in high concentration and P is fixed by Fe and Al oxides [31]. The increase in nutrient uptake by plants indicates that the amount of P is available and can be absorbed by plants in sufficient quantities. Absorption of nutrients from the rhizosphere is the first stage of the accumulation process in plants, and PGPR has an important role in the solubilizing of nutrients from the soil and increases their availability to plants [32]. The application of the Klebsiella Klebsiella variicola, Pseudomonas furukawaii, Klebsiella pneumonia, and Bacillus niacin significantly increased the availability of N, P, and K in the soil and effectively increased the uptake of N, P, and K nutrients, as well as increasing the growth of wheat plants [33].

Organic manure application has the effect of increasing nutrient uptake both N, P, and K uptake compared to organic manure application (0 tons ha^{-1}). Nutrient uptake of N and K showed that the dose of 30 tons ha^{-1} showed the highest nutrient uptake although it was not different from the dose of 15 tons ha^{-1} (Table 2). The highest P nutrient uptake was achieved at a dose of 15 tons ha^{-1}, although it was not significantly different from nutrient uptake at a dose of 30 tons ha^{-1}. Organic manure is a complete source of macro and micronutrients for plants. Mineral nutrients will be released from organic matter through the mineralization process so that it will increase nutrients in the soil. The increase in nutrient uptake of N, P, and K in the treatment dose of organic fertilizer was related to the increase in root growth of rice plants. It can be seen that in the treatment of variable doses of organic fertilizer, root length and RLD were increased so that root occupancy in the soil was wider so that nutrient absorption increased. The application of soil amendments was able to increase the stability of soil aggregates, and increase root growth [34]. Furthermore, the application of organic fertilizers combined with inorganic fertilizers increased N, P, and K nutrient uptake in sweet corn plants [35]. The application of organic fertilizers directly affects the increase in inorganic N content from the process of mineralization of organic N from organic fertilizers by microorganisms to create good soil conditions for N absorption, besides that the application of organic fertilizers helps reduce P fixation and increases the availability of P in the soil [36].

3.2 Physiological traits of rice

The LAI value of rice plants showed that until the age of 9 weeks after planting (WAP) it was seen that the application of PGPR isolate and organic fertilizer affected on LAI, however, there was no interaction between the PGPR consortium and organic fertilizer. LAI on the application of the PGPR consortium isolate R08+isolate R11 and isolate R08+ Rhizobium sp. LM-5 showed a higher value than control respectively 3.95 and 3.57, while control showed an LAI value of 3.07. The LAI value for the application of organic fertilizer at a dose of 15 tons ha^{-1} and 30 tons ha^{-1} was higher than the control at 3.61 and 4.05, respectively, while without the application of organic fertilizer the LAI value was 2.94 (Table 3).

The LAI value of rice plants gradually increased according to the stage of plant growth, and as the number of leaves and leaf area of the plant increased. Until the age of the plant 9 weeks after planting, it was seen that the PGPR consortium and the dose of organic fertilizer had an effect on increasing the LAI value. This is related to the availability of nutrients for plants in the PGPR consortium treatment and organic fertilizers, and increasing availability so that nutrient uptake increases, ensuring adequate vegetative growth (LAI) is achieved [37]. The availability of nutrients N, P, and K have a big effect on the increase in LAI and the LAI value and leaf biomass of rice plants increased significantly with the input of N and K nutrients [38].

LAI describes plant properties related to plant interactions with the atmosphere, where the LAI value is highly correlated with the number of leaves and leaf area of plants. Increasing the LAI value will guarantee the greater absorption of solar energy for the photosynthesis process of plants [38]. Therefore, plant productivity is strongly influenced by the ability of leaves to produce assimilate which is largely determined by the productivity per unit leaf area and the total leaf area of the plant [39].

Leaves as a source and center of photosynthetic activity, and productivity are described as the dry biomass yield of plants as a net result of the assimilation process per leaf area per time. This is described as the net assimilation rate of rice plants. The results showed that the application of the PGPR consortium and organic fertilizer has not had an effect on the Net assimilation rate and crops growth rate at the age of 3-5 WAP, and 5-7 WAP (Table 3). There was an interaction effect between the application of the PGPR consortium and organic fertilizer on the net assimilation rate and plant growth rate at the age of 7-9 WAP (Tables 4 & 5).

The interaction between the PGPR consortium and organic fertilizer resulted in the net assimilation rate and the growth rate of rice plants increasing significantly. The highest net assimilation rate and crops growth rate occurred in the combination treatment of the PGPR isolate consortium R08+ Rhizobium sp. LM-5 and 30 tons/ha organic fertilizer application doses were 1.47 and 4.93 g/dm^2/week, respectively (Tables 4 & 5). The application of organic fertilizer to paddy fields will increase the nutrient and organic carbon content which will increase the biological activity of the soil. Soil organic carbon is a carbon source for rhizobacteria. This is thought to stimulate soil biological activity and increase the abundance of useful microorganisms in the rhizosphere of rice plants. The consortium of PGPR isolates R08+ Rhizobium sp. LM-5 is a combination of PGPR isolates that have the dual ability to produce IAA and fix N_2. This results in improving plant roots and providing nitrogen nutrients to plants. It can be seen that the root variable increased both length and RLD both in the treatment of the PGPR consortium and the dose of organic fertilizer. This condition causes the absorption of nutrients and water as one of the ingredients in plant metabolism, especially photosynthesis and increases vegetative growth both in the shot and roots of plants. The increase in the shot growth of rice plants from the results of this study was seen from the increase in the LAI of plants to the maximum vegetative phase (9 weeks after planting) both in the treatment of the PGPR consortium and the dose of organic fertilizer. LAI acts as an interface between the plant canopy and the atmosphere where gas and water exchange occur, and changes in LAI will cause changes in plant productivity, besides the LAI plant growth rate depends on the LAI value of the plant [40].

Tabel 3. Physiological traits of rice plants under treatments of PGPR consortium and cow manure

Treatments	Leaf Area Index				Net Assimilation Rate (g dm^{-2} week^{-1})		Crop Growth Rate (g dm^{-2} week^{-1})		Leaf Area Duration (days)
	week after planting				week after planting		week after planting		
	3	5	7	9	3–5	5–7	3–5	5–7	

PGPR Consortium									
Control (without PGPR)	0.38 b	0.65 c	2.66 a	3.07 b	0.49 a	0.43 a	0.23 a	0.58 a	64.86 b
PGPR isolates R08+isolate R11	0.49 a	0.78 b	2.65 a	3.95 a	0.54 a	0.53 a	0.34 a	0.71 a	83.22 a
PGPR isolates R08+ Rhizobium sp. LM-5	0.48 a	0.89 a	2.53 a	3.57 ab	0.43 a	0.51 a	0.30 a	0.78 a	75.93 ab
Organic manure									
0 tons ha^{-1}	0.36 b	0.63 c	2.09 b	2.94 b	0.51 a	0.64 a	0.24 a	0.73 a	61.89 b
15 tons ha^{-1}	0.47 a	0.80 b	2.69 a	3.61 a	0.58 a	0.39 a	0.37 a	0.60 a	76.39 a
30 tons ha^{-1}	0.52 a	0.88 a	3.08 a	4.06 a	0.38 a	0.43 a	0.26 a	0.74 a	85.72 a

Remark: The number is followed by the same letter in the same column is not significantly different according to DMRT 5%.

Table 4. Interaction effect between PGPR consortium and cow manure to NAR at 7-9 weeks

Treatments	Cow Manure			Average
	0 tons ha^{-1}	15 tons ha^{-1}	30 tons ha^{-1}	
PGPR Consortium				
Control (without PGPR)	1.14 a A	1.12 a A	0.71 b B	0.99
PGPR isolates R08+isolate R11	1.03 a A	0.98 a A	0.97 a AB	0.99
PGPR isolates R08+ Rhizobium sp. LM-5	1.20 b A	0.90 c A	1.47 a A	1.19
Average	1.13	1.00	1.05	+

Remark: The number followed by the same small letter in the same line, and the number followed by the same capital letter in same column is not significantly different according to DMRT 5%.

Table 5. Interaction effect between PGPR consortium and cow manure to CGR at 7-9 weeks

Treatments	Cow Manure			Average
	0 tons ha^{-1}	15 tons ha^{-1}	30 tons ha^{-1}	
PGPR Consortium				
Control (without PGPR)	2.53 a A	3.14 a A	2.64 a B	2.77
PGPR isolates R08+isolate R11	2.90 a A	3.20 a A	3.48 a B	3.19
PGPR isolates R08+ Rhizobium sp. LM-5	2.94 b A	2.94 b A	4.93 a A	3.60
Average	2.79	3.09	3.69	+

Remark: The number followed by the same small letter in the same line, and the number followed by the same capital letter in same column is not significantly different according to DMRT 5%.

The application of the PGPR consortium and organic fertilizer showed the effect of increasing the leaf area duration (LAD) of rice plants. The application of the PGPR isolate R08+isolate R11 consortium showed the highest LAD value of 83.22 days, although the PGPR isolates R08+ Rhizobium sp. LM-5 consortium did not differ, which reached 75.93 days. The LAD value without PGPR application showed the lowest LAD value of 64.86 days. The application of 15 tons ha^{-1} and 30 tons ha^{-1} organic fertilizers showed higher LAD values than the 0 tons ha^{-1} doses of 76.39 and 85.72 days, respectively (Table 3).

3.3 Rice grain yield

The pplication of the PGPR consortium gave a nonsignificant effect on the yield components (number of productive tillers, and the weight of 1000 seeds), while panicle length and the number of spikes per panicle were affected significantly. The panicle length in the consortium treatment was seen to increase compared to without the PGPR consortium application. The same thing was seen in the variable number of grains per panicle. The application of organic fertilizer up to 30 tons ha^{-1} has not shown any effect on the variables of the number of productive tillers, panicle length, number of spikes per panicle, and weight of 1000 seeds (Table 6). There was an interaction between the PGPR consortium and the dose of organic fertilizer on the yield of rice plants. The results showed that the combination of the PGPR isolates R08+ Rhizobium sp. LM-5 and a dose of organic fertilizer of 30 tons ha^{-1} gave the highest yield reaching 11.68 tons ha^{-1} (Table 7).

The application of the PGPR consortium was able to increase the average panicle length by 8.58 percent against the control, and the longest panicle length was achieved in the treatment of the PGPR consortium PGPR isolates R08+ Rhizobium sp. LM-5 was 22.05 cm, although it was not different from the treatment of PGPR isolate R08+isolate R11 with panicle length reaching 21.75 cm, and in the control treatment of 20.17 cm (Table 6). On the other hand, the PGPR consortium treatment was also able to increase the number of grains per panicle whereas the PGPR consortium treatment on average was able to increase the number of grain per panicle by 8.67 percent against the control. The consortium PGPR isolates R08+isolate R11 and the consortium PGPR isolate R08+ Rhizobium sp. LM-5 based on the results of the study showed that there was no difference in the average number of grains per panicle, respectively 71.01 and 71.98 grains per panicle, and this result was greater than without the PGPR consortium inoculation which only produced 65.83 grains per panicle.

Table 6. Yield component of rice under treatments of PGPR consortium and cow manure

Treatments	Number of productive tillers	Panicle length (cm)	Number of grains per panicle	1000 seeds weight (g)
PGPR Consortium				
Control (without PGPR)	14.60 a	20.17 b	65.83 b	25.02 a
PGPR isolate R08+isolate R11	14.29 a	21.75 a	71.01 a	25.56 a
PGPR isolate R08+ Rhizobium sp. LM-5	14.68 a	22.05 a	71.98 a	25.55 a
Organic manure				
0 tons ha^{-1}	13.46 a	20.96 a	68.42 a	24.46 a
15 tons ha^{-1}	14.29 a	21.08 a	68.82 a	25.57 a
30 tons ha^{-1}	15.83 a	21.93 a	71.57 a	26.17 a

Remark: The number is followed by the same letter in the same column is not significantly different according to DMRT 5%.

Table 7. Interaction effect between PGPR consortium and cow manure on rice grain yield (tons ha^{-1})

Treatments	Cow Manure			Average
	0 tons ha^{-1}	15 tons ha^{-1}	30 tons ha^{-1}	
PGPR Consortium				
Control (without PGPR)	4.42 b C	5.04 b C	5.90 a C	5.12
PGPR isolates R08+isolate R11	6.84 c A	8.96 b A	10.76 a B	8.85
PGPR isolates R08+ Rhizobium sp. LM-5	5.46 c B	8.11 b B	11.68 a A	8.42
Average	5.57	7.37	9.45	+

Remark: The number followed by the lower letter in the same line and the number followed by the capital letter in same column are not significantly different according to DMRT 5%.

The results showed that the interaction between the PGPR consortium and the compost dosage affected the grain yield per ha. Increasing the dose of organic fertilizer to 30 tons per ha can increase the grains yield per ha. Increasing the dose of organic fertilizer to 30 tons per ha in the control, consortium PGPR isolates R08+isolate R11, PGPR isolates R08+ Rhizobium sp. LM-5 respectively showed the highest yields with values reaching 5.90, 10.76, and 11.68tons ha^{-1}. The application of the PGPR consortium without the application of organic fertilizer (0 tons ha^{-1}) was able to significantly increase the yield by 39.14%, while at a dose of 15 tons ha^{-1} of organic fertilizer the application of the PGPR consortium was able to increase the average yield by 69.34%, and at a dose of 30 tons ha^{-1} was able to increase the average yield by 90.17%. The PGPR consortium isolates R08+isolate R11 showed that there was an increase in yield at various doses of cow manure. Application doses of 15 tons ha^{-1} and 30 tons ha^{-1} significantly increased grain yields by 31.57 and 58%, respectively, compared to no cow manure. The PGPR consortium isolates R08+ Rhizobium sp. LM-5 at cow manure doses of 15 and 30tons ha^{-1} was able to increase by 48.53 and 113.92 percent respectively compared to without the application of cow manure compost. The application of organic fertilizers in general can increase crop yields. Organic fertilizer amendments can support the growth of soil bacteria, and increase soil aggregates, water holding capacity, and soil pH [41]. The combination of PGPR and organic fertilizers in increasing rice yields was also reported by several previous researchers. The application of PGPR combined with FYM was able to increase rice yields between 3.86 to 5.0 tons ha^{-1} [42].

4. Conclusions

The application of cow fertilizer and the PGPR consortium were able to increase vegetative growth, nutrient uptake (N, P, and K), as well as the physiological character of rice plants. The interaction between the dose of organic fertilizer and the PGPR consortium affects the net assimilation rate, crop growth rate, and yield of rice plants. The highest yield was achieved at a dose of organic fertilizer of 30 tons ha^{-1} and the PGPR consortium isolate R08+Rhizobium sp. LM-5 of 11.68 tons ha^{-1}. Based on the research results, the consortium isolate R08+Rhizobium sp. LM-5 can be recommended as a biofertilizer formulation and application in combination with organic fertilizers.

References

1. F. Liesdiana, N. P. S. T. Laksemi, R. Fachria, S. Nursaadah, Y. A. Hoesen, Z. I'lanul A.A., and Y. Yustiana, 3BIO: Journal of Biological Science, Technology and Management **1**, 22 (2019)
2. T. Gomiero, Sustainability (Switzerland) **8**, 1 (2016)
3. A. J. Franzluebbers, Soil Tillage Res **66**, 95 (2002)
4. P. M. Kopittke, N. W. Menzies, P. Wang, B. A. McKenna, and E. Lombi, Environ Int **132**, 1 (2019)
5. Iqbal, M. Achmad, and M. T. Sapsal, in *IOP Conference Series: Earth and Environmental Science 355* (Institute of Physics Publishing, 2019), pp. 1–6
6. A. Tangkesik, N. M. Wikarniti, N. N. Sooniari, and I. W. Narka, Agrotrop **2**, 101 (2012)
7. M. R. Setiawati, M. K. Prayoga, S. Stöber, K. Adinata, and T. Simarmata, Open Agric **5**, 509 (2020)
8. N. B. L. Lanna, P. N. L. Silva, L. F. Colombari, P. G. Freitas-Nakada, A. Ismael, and I. Cardoso, Hortic Bras **35**, 621 (2017)
9. J. Schnurer, M. Clarholm, and T. Rosswall, Soil Biol. Eiochem **17**, 611 (1985)
10. W. Huang, J. fu Wu, X. hua Pan, X. ming Tan, Y. jun Zeng, Q. hua Shi, T. ju Liu, and Y. hua Zeng, J Integr Agric **20**, 236 (2021)
11. M. Kumar, V. P. Giri, S. Pandey, A. Gupta, M. K. Patel, A. B. Bajpai, S. Jenkins, and K. H. M. Siddique, Int J Mol Sci **22**, 1 (2021)
12. T. Mondal, J. K. Datta, and N. K. Mondal, Journal of the Saudi Society of Agricultural Sciences **16**, 135 (2017)
13. B. Ikhajiagbe and E. Osazee Ohanmu, Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii **29**, 134 (2019)
14. S. Habibi, S. Djedidi, N. Ohkama-Ohtsu, W. A. Sarhadi, K. Kojima, R. V. Rallos, M. D. A. Ramirez, H. Yamaya, H. Sekimoto, and T. Yokoyama, Microbes Environ **34**, 347 (2019)
15. W. F. Ríos-Ruiz, E. E. Torres-Chávez, J. Torres-Delgado, J. C. Rojas-García, E. J. Bedmar, and R. A. Valdez-Nuñez, Rhizosphere **14**, 1 (2020)
16. M. Ashrafuzzaman, F. A. Hossen, M. Razi Ismail, A. Hoque, M. Z. Islam, S. M. Shahidullah, and S. Meon, Afr J Biotechnol **8**, 1247 (2009)
17. P. N. Bhattacharyya and D. K. Jha, World J Microbiol Biotechnol **28**, 1327 (2012)
18. R. E. Magana, M. Melati, H. Purnamawati, and E. Pratiwi, J Trop Crop Sci **7**, 143 (2020)
19. Z. Zhang, X. Dong, S. Wang, and X. Pu, Sci Rep **10**, 1 (2020)
20. A. Larangahen, B. Bagau, M. R. Imbar, and H. Liwe, Zootek" Journal) **37**, 156 (2017)
21. IRRI, *Standard Evaluation System for Rice* (IRRI, Los Banos, 2002)
22. S. Yoshida, D. A. Forno, J. H. Cock, and K. A. Gomez, *Laboratory Manual for Physiological Studies of Rice* (1976)
23. J. Vacheron, G. Desbrosses, M. L. Bouffaud, B. Touraine, Y. Moënne-Loccoz, D. Muller, L. Legendre, F. Wisniewski-Dyé, and C. Prigent-Combaret, Front Plant Sci **4**, 1 (2013)
24. Y. Erturk, S. Ercisli, A. Haznedar, and R. Cakmakci, Biol Res **43**, 91 (2010)
25. J. Chen, L. Liu, Z. Wang, Y. Zhang, H. Sun, S. Song, Z. Bai, Z. Lu, and C. Li, Front Plant Sci **11**, (2020)
26. M. Grover, S. Bodhankar, A. Sharma, P. Sharma, J. Singh, and L. Nain, Front Sustain Food Syst **4**, 1 (2021)
27. S. Assefa, Agric Res Technol **22**, 46 (2019)
28. M. R. Mosaddeghi, A. A. Mahboubi, and A. Safadoust, Soil Tillage Res **104**, 173 (2009)

29. M. D. Maghfoer, Koesriharti, T. Islami, and N. D. S. Kanwal, Agrivita **40**, 168 (2018)
30. N. K. Fageria, G. D. Carvalho, A. B. Santos, E. P. B. Ferreira, and A. M. Knupp, Commun Soil Sci Plant Anal **42**, 1913 (2011)
31. T. Nishigaki, Y. Tsujimoto, S. Rinasoa, T. Rakotoson, A. Andriamananjara, and T. Razafimbelo, Plant Soil **435**, 27 (2019)
32. A. Rana, B. Saharan, L. Nain, R. Prasanna, and Y. S. Shivay, Soil Sci Plant Nutr **58**, 573 (2012)
33. J. Wang, R. Li, H. Zhang, G. Wei, and Z. Li, BMC Microbiol **20**, 1 (2020)
34. M. Chen, S. Zhang, L. Liu, L. Wu, and X. Ding, Soil Tillage Res **212**, 1 (2021)
35. E. Trinurani Sofyan and D. Sapta Sara, J Trop Soils **23**, 111 (2018)
36. J. A. Dogbatse, A. Arthur, G. K. Awudzi, A. K. Quaye, S. Konlan, and A. A. Amaning, International Journal of Agronomy **2021**, 1 (2021)
37. T. Shankar, G. C. Malik, M. Banerjee, S. Dutta, S. Maitra, S. Praharaj, M. Sairam, D. S. Kumar, E. S. Dessoky, M. M. Hassan, I. A. Ismail, T. Saif, M. Skalicky, M. Brestic, and A. Hossain, Plants **10**, (2021)
38. W. Hou, M. Tränkner, J. Lu, J. Yan, S. Huang, T. Ren, R. Cong, and X. Li, Front Plant Sci **11**, 1 (2020)
39. E. D. Purbajanti and S.- Setyawati, Agrosains : Jurnal Penelitian Agronomi **22**, 83 (2020)
40. L. A. Maqueira-López, R. Morejón-Rivera, O. Roján-Herrera, and W. Torres-De-La-Noval, Agron. Mesoam **230**, 79 (2019)
41. A. Fatawi, B. Pujiasmanto, Komariah, M. K. Zaki, and K. Noda, in *IOP Conf Ser Earth Environ Sci* (IOP Publishing Ltd, 2021), pp. 1–10
42. R. Hari, P. Ram Mirjha, and P. Mahesh Kurrey, Int J Chem Stud **7**, 2406 (2019)