Net Assimilation Rate, Growth and Yield of Rice (Oryza sativa L cv Inpago Unsoed 1) with PGPR Application in Different Rate of Nitrogen

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Abstract. Dependence on chemical fertilizers, especially nitrogen has caused nutrient imbalance, but nitrogen as macro nutrient is needed in all plant growth phase. An ecological approach to restoring soil fertility application of biological fertilizers in the form of a consortium. Therefore, the PGPR consortium application is expected to cut the need for chemical fertilizers and increase plant growth. This research was aimed to study the net assilation rate, growth and yield of rice with PGPR application in different rate of nitrogen. The pots experimental was conducted in Laboratorium of Agronomy and Horticulture, and experimental farm of Agriculture Faculty of UNSOED Purwokerto from July until November 2020. The research was arranged by Randomized Completely Block Design with three replication. The first factor was the PGPR consortium, and the second factor was the dosage of nitrogen fertilizer application. The observed variables were number of tiller per hill, plant height, plant biomassa, leaf greenness, plant biomassa, net assimilation rate, relative growth rate and yield. The data were analyzed by anova and if significant different was continued by DMRT 95%. The results showed that the interaction of the PGPR consortium and N fertilization can increase the net assimilation rate of rice plants and the relative growth rate of rice plants and plant biomass. however, the PGPR consortium has not been able to increase plant height growth, number of tillers, root shoot ratio, width of openings and stomata density of rice leaves, and yield. The highes yield was achieved by application of nitrogen at 200 kg ha-1 reached 36.17 g plant^{-1}

Keywords: PGPR, nitrogen, net assimilation rate, growth, yield

1. Introduction

Nitrogen is macro nutrient for the growth of rice plants, so that without the application of nitrogen, the production of rice plants will be disrupted. Among rice farmers, nitrogen source sourced from urea is the most widely used type of fertilizer since the launch of agricultural intensification and has always received a subsidy allocation from the Government. In 2020 the Government will provide fertilizer subsidies for both urea, SP-36 and NPK compounds reaching 7.9 million tons [1]. The availability and ease of obtaining urea fertilizer among farmers causes the fertilization practice done by most farmers to exceed the recommended dosage. [2] stated that the practice of urea fertilization among farmers has exceeded the recommended dosage, reaching a dosage of 300-500 kg ha^{-1}. Excessive doses of nitrogen cause low fertilization efficiency, and cause environmental damage due to NO_2 emissions resulting from ammonification, nitrification and denitrification processes [3]. [4] stated that the loss of N from plant and soil are estimated to be between 50-70% of the fertilizer dose application.

The application of high nitrogen dosage causes nutrient imbalance in paddy soil. Long-term nitrogen application without the application of organic fertilizers has an effect on reducing soil organic matter content and affects soil biological diversity [5]. [6] reported that application of urea fertilizer that were a NH_4^- producing compound and its inhibitory effect on methanotroph bacteria in paddy soil. On the other hand, [7] reported that the application of nitrogen fertilizers for a long period in the mulberry field soil had an effect on reducing the levels of organic matter content and soil pH, thereby suppressing the population of Acidobacteria. [8] reported that application of nitrogen fertilizers at 50 kg ha^{-1} increased the diazotrophic bacteria population in the rhizosphere of rice plants due to low NH_4 concentrations in the soil, but increasing the N dose actually decreased the diazotropic bacteria population.

Increasing rice production needs to be rationalized without having a negative impact from excessive N fertilizer doses on the soil and agricultural environment. Increasing soil biological fertility can be done by applying rhizobacteria indigenous paddy soil. The application of biofertilizer in rice plants aims to increase the yield and quality of grain yields [9]. Rhizobacteria indigenous wetland soils that have been isolated include Rhizobium sp. LM-5 as N_2 fixer bacteria, R08 isolate and R11 isolate as rhizobacateria producing IAA [10][11]. According to [9] that biofertilizer cannot replace inorganic fertilizers, but as a key to increasing nutrient availability, so that the application of biofertilizer must be precise with the dosage of inorganic fertilizers, especially nitrogen. [12] reported that application of biofertilizer 300-400 kg ha^{-1} combined with inorganic fertilizers at 75% of the recommended dose increased NPK nutrient uptake and grain yield.[8] reported that inoculation of *Stenotrophomonas maltophila* at a nitrogen level of 50 kg ha^{-1} increased photosynthesis and biomass of rice plants, but an increase in nitrogen doses to 300 kg ha^{-1} actually had a negative impact on plant photosynthetic activity. Furthermore [13] reported that the inoculation of *Pseudomonas putida*-1 at a nitrogen fertilizer dose of 200 mg kg^{-1} of soil increased leaf chlorophyll content, and the application of *Pseudomonas putida*-1 + *Azotobacter* in 200 mg nitrogen fertilizer mg kg^{-1} soil increases grain yield. This research was aimed to study the effect of PGPR consortium inoculation to increase net assimilation rate and yield of rice at different rate of nitrogen fertilizer.

2. Material and Methods

This research was conducted in Laboratorium of Agronomy and Hortoculture, and Experimental Farm faculty of Agriculture, Jenderal Soedirman Purwokerto, Central Java, Indonesia from May until October 2019. The experimental was conducted on Inpago Unsoed 1 aromatic and high yielding rice variety. The pot experiment was carried out in Randomized Completely Block Design (RCBD) with three replications. The treatments consisted of two factors i.e. consortium of PGPR, and nitrogen dosage. The first factors consisted of K_0: control (without PGPR application), K_1: R08 isolate+R11 isolate, K_2: R08 isolate+Rhizobium sp. LM-5, K3: R08 isolate+R11 isolate+ Rhizobium sp. LM-5. The second factor consisted of N_0: without nitrogen fertilizer application, N_1: 100 kg ha^{-1} nitrogen fertilizer (equivalent to 1.36 g urea pot^{-1}), N_2: 200 kg ha^{-1} nitrogen fertilizer (equivalent to 2.72 g urea pot^{-1}).

Rice seeds were sown on wet paper in petridish. Before sowing, rice seeds were sterilized using $HgCl_2$ 0.1% for a minute, then rinsed with sterile water. The sterile seeds were germinated on moist paper in the petridish for 2 weeks. Inoculant PGPR was prepared by inoculating a ose of bacterial isolate into 500 ml of NB (Himedia) media, then culturing it in a shaker for 24 h at a speed of 120 rpm. Bacterial culture was ready for use once it reaches a population density above 10^7 cfu ml^{-1}.

Inceptisol soil was prepare as growth medium of rice, it was sieved and powdered with a 2 mm sieve. The pots were filled with 12 kg of Inceptisol soils. The phosphate and potassium fertilizer was applied before planting with 0.94 g of SP-36 and KCl per pot, respectively. The pot containing the soil was watering until it stagnates before planting. The PGPR treatment was carried out by immersing the roots of rice seedlings in bacterial culture for 15 minutes, then planting one seedling into per pot. After planting, the soil is kept moist by providing adequate irrigation. After the plants are 2 weeks old, 2 to 5 cm of water was maintained on the soil surface of each pot. Nitrogen fertilizer application was carried out at the age of 7 weeks after planting and 35 days after planting each half the dose of treatment.

Observations were made during vegetative growth until harvets, and harvesting was carried out after physiologically maturity of rice grains. The observed variables were root length (measured by intersection method, [14]), leaf greenness (Chlorophyl meter SPAD-502Plus Minolta), leaf area, plant biomassa, net assimilation rate [15], and grains yield. The data was analysed by ANOVA, and if significant different was continued by DMRT 5%.

3. Results and Discussion

Based on the analysis of variance, it shows that the application of the PGPR consortium has an effect on the total root length of rice plants, but has not had an effect on leaf greenness, stomata density, and stomata opening width. On the other hand, an increase in the level of nitrogen fertilization had an effect on total root length and leaf greenness, but had not yet had an effect on stomata density and stomatal opening width.

Table 1. The effect of PGPR consortium and dosage of nitrogen fertilizer on total root length and leaf greennes

Treatments	Total Root Length (cm)	Leaf Greenness (SPAD unit)
PGPR Consortium		
Control	2448.80 b	29.47 a
R08 isolate + R11 isolate	4396.50 a	27.82 a
R08 + *Rhizobium* sp. LM-5	3231.50 ab	29.55 a
R08 isolate + R11 isolate + *Rhizobium* sp. LM-5	3154.20 ab	29.30 a
Dosage of Nitrogen Fertilizer (urea)		
0 g plant^{-1}	2396.40 b	22.87 c
1.36 g plant^{-1}	4045.80 a	29.72 b
2.72 plant^{-1}	3481.00 ab	34.49 a

Remark : The number following by same letter in the same coloum and treatments were not significant different according DMRT 5%.

Table 2. The effect of PGPR consortium and dosage of nitrogen fertilizer on stomata density stomata opening width

Treatments	Stomata Density (unit/mm^2)	Stomata Opening Widht (μm)
PGPR Consortium		
Control	2085.7 a	3.44 a
R08 isolate + R11 isolate	2271.2 a	3.61 a
R08 + *Rhizobium* sp. LM-5	2670.4 a	3.61 a
R08 isolate + R11 isolate + *Rhizobium* sp. LM-5	2338.7 a	3.67 a
Dosage of Nitrogen Fertilizer (urea)		
0 g plant^{-1}	2255.8 a	3.63 a
1.36 g plant^{-1}	2390.7 a	3.67 a
2.72 plant^{-1}	2378.1 a	3.46 a

Remark : The number following by same letter in the same coloum and treatments were not significant different according DMRT 5%.

The PGPR consortium application showed that it affects the total root length, where the consortium isolate R08 + isolate R11 gives the longest root length of 4396.50 cm, greater than the consortium R08 + *Rhiszobium* sp. LM-5, R08 + isolate R11 + *Rhiszobium* sp. LM-5, and control, respectively 3231.50 cm, 3154.20 cm and 2448.80 cm (Table 1). The application of the consortium isolate R08 + isolate R11 increased the root length of rice plants by 79.54 percent for plants without PGPR application. This shows that the application of the PGPR consortium increased the total root length compared to the control, even though the application of the consortium R08 + *Rhiszobium* sp. LM-5, R08 + isolate R11 + *Rhiszobium* sp. LM-5 did not show a significant different total root length compared to the treatment without the PGPR consortium application. [11] reported that the PGPR isolates R08 and R11 were isolates of indigenous paddy soil that were able to produce the auxin group phytohormones, namely IAA and were able to increase the roots of rice seedlings. IAA is one of the phytohormones that are classified as important natural hormones and can be produced by rhizobacteria so that inoculation with bacteria capable of producing IAA will induce the proliferation of lateral roots and root hairs [16]. [17] reported that the inoculation of *Bacillus paenibacillus* and *Comamonas* on kiwifruit cuttings was able to stimulate root formation, this correlated with the ability of bacteria to produce indole-3-acetic acid.

Nitrogen is a macro nutrient that is really needed by plants in the vegetative phase, and is a nutrient that plays an important role in photosynthetic organelles, especially chlorophyll. The results showed that the application of nitrogen derived from urea affected the total root length and leaf greenness of rice plants. Application of urea 1.26 g plant^{-1} gave a total root length of 4045.80 cm, however, increasing the dose of urea to 2.72 g plant^{-1} actually decreased the total root length and was not significantly different from that without urea fertilization of 3481.00 cm and 2396.40 cm, respectively. The results of this study were in line with [18] where nitrogen fertilization significantly increases root length than without nitrogen fertilization, and nitrogen dosage of 240 kg ha^{-1} was a moderate dose giving longer root length, and increasing the dose actually decreases root length.

The leaf greenness variable showed that an increase in the dose reached 2.72 g plant^{-1} gave the highest leaf greenness value of 34.49, significantly different from the 1.72 g plant^{-1} dose and without urea fertilization of 29.72 and 22.87, respectively (Table 1). Leaf greenness values correlate with chlorophyll content in rice leaves [19]. Nitrogen is one of the important nutrients in chlorophyll biosynthesis, where the synthesis of chlorophyll depends on mineral nutrients so that nitrogen availability will play a role in cell division and the formation of photosynthetic active pigments [20]. [21] reported that chlorophyll a and total chlorophyll contents of sunflower plants increased along with the increase in nitrogen availability, but the chlorophyll b levels decreased.

Table 3. The effect of PGPR consortium and dosage of nitrogen fertilizer on leaf area (cm^2)

Treatments	**3 WAP**	**5 WAP**	**7 WAP**
PGPR Consortium			
Control	61.81 a	527.80 a	849.40 a
R08 isolate + R11 isolate	38.89 b	673.30 a	1312.00 a
R08 + *Rhizobium* sp. LM-5	35.87 b	594.30 a	1201.00 a
R08 isolate + R11 isolate + *Rhizobium* sp. LM-5	48.19 ab	513.00 a	1162.50 a
Dosage of Nitrogen Fertilizer (urea)			
0 g plant^{-1}	42.33 a	421.46 b	885.20 a
1.36 g plant^{-1}	46.39 a	708.03 a	1188.70 a
2.72 plant^{-1}	49.84 a	601.80 ab	1320.00 a

Remark : The number following by same letter in the same coloum and treatments were not significant different according DMRT 5%. WAP : week after planting.

Table 4. The effect of PGPR consortium and dosage of nitrogen fertilizer on plant biomassa (g)

Treatments	3 WAP	5 WAP	7 WAP
PGPR Consortium			
Control	0.32 a	6.82 a	20.27 b
R08 isolate + R11 isolate	0.23 a	7.19 a	46.22 a
R08 + *Rhizobium* sp. LM-5	0.23 a	7.13 a	26.96 a
R08 isolate + R11 isolate + *Rhizobium* sp. LM-5	0.28 a	6.40 a	23.27 a
Dosage of Nitrogen Fertilizer (urea)			
0 g plant^{-1}	0.24 a	5.31 a	20.09 b
1.36 g plant^{-1}	0.21 a	7.42 a	37.66 a
2.72 plant^{-1}	0.34 a	7.93 a	29.85 ab

Remark : The number following by same letter in the same coloum and treatments were not significant different according DMRT 5%. WAP : week after planting

The results showed that the plant height at 7 weeks after planting (WAP) did not show any difference in plant height in both the PGPR consortium and nitrogen fertilizer. However, at the age of 7 WAP, it was seen that the total leaf area increased in the PGPR consortium isolate R08 + R11, isolate R08 + *Rhizobium* sp. LM-5, as well as isolates R08 + R11 + *Rhizobium* sp. LM-5 when compared without inoculation were 1321.00 cm^2, 1201.00 cm^2, 1162.00 cm^2 and 849.40 cm^2, respectively. Nitrogen fertilization tends to increase the leaf area of rice plants, it can be seen that the widest leaf area was reached at a dose of 2.72 g $plant^{-1}$ of 1320.00 cm^2, which is greater than that at a dose of 1.26 g $plant^{-1}$ and without nitrogen fertilization were 1188.70 cm^2 and 885.20 cm^2 respectively (Table 3).

PGPR consortium inoculation and nitrogen fertilization until the age of 7 WAP gaved a significant different among the treatments on plant dry biomass. PGPR consortium inoculation has an effect on increasing plant biomass compared to without inoculation. Without PGPR inoculation, plant biomass was only 20.27 g $plant^{-1}$, lower than the consortium R08 isolate + R11 isolate, R08 + *Rhizobium* sp. LM-5, and R08 isolate + R11 isolate + *Rhizobium* sp. The LM-5 was 46.22 g $plant^{-1}$, 26.96 g $plant^{-1}$, and 23.27 g $plant^{-1}$, respectively (Table 4). These results indicate that the PGPR consortium inoculation was able to increase plant biomass by an average of 58.61 percent against the control, and the inoculation of R08 isolate + R11 isolate was able to provide the highest plant biomassa, namely an increase of 128.02 percent. This is related to the ability of the consortium R08 isolate + R11 isolate which stimulates plant roots so that the ability to absorb nutrients and water is better so that the supply of nutrients to the leaves and increases so that plant photosynthesis is more optimal.

Table 5. Interaction effect between PGPR consortium and dosage of nitrogen fertilizer on NAR (g dm^{-2} $week^{-1}$) of rice plant

Treatments	Dosage of N Fertilizer (urea)			
	0 g $plant^{-1}$	1.36 g $plant^{-1}$	2.72 $plant^{-1}$	
Control	1.11 b B	2.20 a B	1.44 ab B	1.58
R08 isolate + R11 isolate	2.05 b A	5.87 a A	2.12 b A	3.35
R08 + *Rhizobium* sp. LM-5	1.94 a A	1.40 a C	2.16 a A	1.84
R08 isolate + R11 isolate + *Rhizobium* sp. LM-5	2.06 a A	1.91 a B	1.40 a B	1.79
	1.79	2.85	1.78	+

Remark : The number in the same row following by lower letter and the number in the same coloum following by capital letter were not significant different according DMRT 5%.

The results of this research showed that nitrogen fertilization had an effect on rice biomass. Fertilization of nitrogen at a dose of 1.26 g $plant^{-1}$ gave the highest plant biomass of 37.66 g, greater than control which was only 20.09 g. Increasing the nitrogen dose to 2.72 g $plant^{-1}$ actually reduced plant biomass by 29.85 g. The results of this study are in line with [8] where nitrogen fertilization was able to provide the higher plant biomass, both inoculated and not inoculated by PGPR, and an increase in nitrogen doses. Nitrogen fertilization, especially urea at high doses, will reduce plant biomass due to the very high concentration of NH_4^+ [22].

Plant biomass is the net result of photosynthesis which is translocated as plant dry matter. [23] stated that photosynthesis is the main process responsible for plant growth, and photosynthesis metabolism will regulate plant growth to grow in a variety of different nitrogen sources. The increase in biomass will be measured by the change in the accumulation of plant dry matter per unit leaf area per unit time or known as the net assmilation rate. The results showed that the interaction between the PGPR consortium and nitrogen fertilization dose had an effect on NAR of rice plants. The increasing nitrogen fertilizers dosage without inoculation of PGPR showed that the highest NAR of rice was achieved at a dose of 1.26 g $plant^{-1}$

at 2.20 g dm^{-2} $week^{-1}$, greater than the control which was only 1.11 g dm^{-2} $week^{-1}$. Increasing the dose to 2.72 g $plant^{-1}$ decreased the net assimilation rate by 1.44 g dm^{-2} $week^{-1}$. Inoculation of consortium R08 isolate + R11 isolate at various doses of nitrogen fertilizer showed that the nitrogen fertilization dose of 1.26 g $plant^{-1}$ gave the highest net assimilation rate of 5.87 g dm^{-2} $week^{-1}$, higher than without nitrogen fertilization which was only 2.05 g dm^{-2} $week^{-1}$. Increasing the nitrogen dose actually decreased the net assimilation rate by 2.12 g dm^{-2} $week^{-1}$. Inoculation of the PGPR consortium R08 + *Rhizobium* sp. LM-5 and R08 isolate + R11 isolate + *Rhizobium* sp. LM-5 at various nitrogen fertilization doses had no effect on the net assimilation rate of rice (Table 5).

[8] reported that increasing dosage of nitrogen after addition of 300 kg ha^{-1} reduced photosynthetic activity of rice plant of 56 percent, and photosynthetic activity tended to be high at low nitrogen doses possibly due to the initial low requirement of N application. [24] reported that the inoculation of *Azotobacter* and *Azospirillum* showed better photosynthetic activity during the growth phase of the maize plant due to greener leaves, high accumulation of plant dry matter.

Increasing the dosage of nitrogen in the soil tends to suppress the soil microbial population. In addition, high nitrogen concentrations in diazotrophic bacteria will suppress the nitrogenase enzyme activity so that the N_2 fixing activity decreases. It can be seen that the consortium contains *Rhizobium* sp. LM-5 was unable to increasing the net assimilation rate even though the nitrogen dose is increased. *Rhizobium* sp. LM-5 is one of the diazotropic bacteria that are free living bacteria in the rhizosphere of rice plants [10]. The application of nitrogen fertilizers with high doses will reduce the population and number of bacteria that colonize the roots, as well as suppress the nitrogenase activity of diazotrophic bacteria in sugarcane, and *Stenotrophomonas maltophila*. in rice plants [8][25]. Furthermore, [26] stated that energy-intensive nitrogen fixation by diazotrophic bacteria is suppressed when alternative nitrogen sources are available in the rhizosphere, so that nitrogenase activity decreases at high nitrogen doses.

Based on the results of the analysis of variance, the PGPR consortium application has not shown an effect on grain yield per hill, but nitrogen fertilization up to 2.72 g $plant^{-1}$ shows an increase in grain yield per hill. The the consortium of PGPR R08 isolate + R11 isolate and R08 isolate + *Rhizobium* sp. LM-5 were the consortium of PGPR with the highest yield of 29.94 g per hill and 24.95 g per hill respectively, which was greater than without inoculation and consortium PGPR R08 isolate + R11 isolate + Rhizobium sp. LM-5 was 22.06 g per hill and 23.39 g per hill, respectively. Nitrogen fertilization increased yield with increasing nitrogen fertilizer dosage. The highest grain yield was achieved at a nitrogen fertilization dose of 2.72 g $plant^{-1}$ of 36.17 g per clump (Figure 1). Nitrogen fertilization up to a dose of 2.72 g $plant^{-1}$ can increase grain yield by 273.66 percent compared to without nitrogen fertilization. The increase in nitrogen fertilizer dosage from 1.36.- 2.72 g $plant^{-1}$ stimulated an increase in grain yield by 40.96 percent.

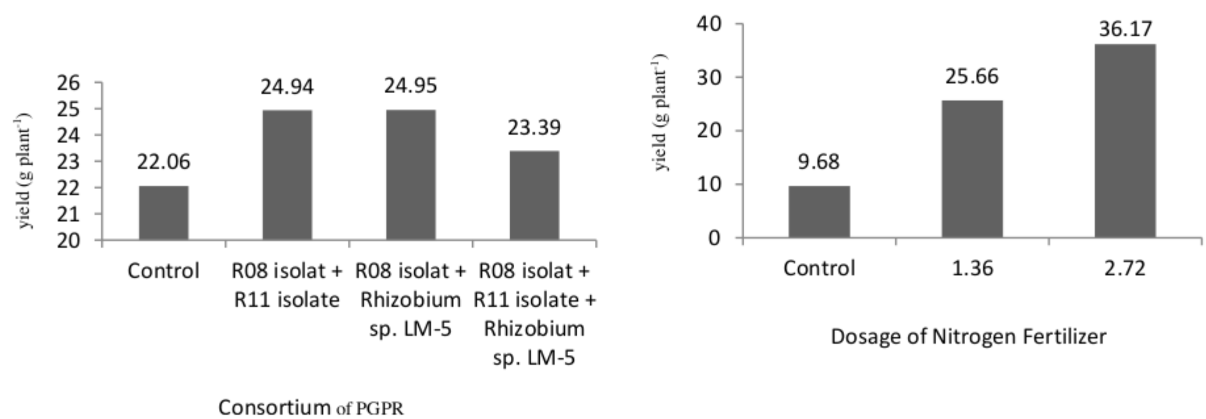

Figure 1. Rice yield under treatments of consortium PGPR and Dosage of Nitrogen fertilizer

4. Conclusion

PGPR consortium inoculation and nitrogen fertilization have an effect on root growth, leaf greenness, and plant biomass. The PGPR consortium R08 isolate + R11 isolate and nitrogen fertilization at a dose of 1.36 g $plant^{-1}$ gave the highest net assimilation rate of 5.87 g dm^{-2} $week^{-1}$. The highest grain yield was achieved at nitrogen fertilization of 2.72 g $plant^{-1}$ at 36.17 g per hill.

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