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THE LEAF GREENNESS AND YIELD COMPONENTS OF PADDY PLANTS INFLUENCED BY BIOLOGICAL FERTILIZER WITH AND WITHOUT COMBINATION OF INORGANIC FERTILIZERS

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Abstract—The use of biological fertilizer seems to be most important in creating environments for sustainable agriculture. This fertilizer is made up of living bacteria cells which depending on the soil conditions, are able to reproduce. In soil these bacteria have the greatest benefits for agricultural crops. They can increase the level of nitrogen in soil after converting the atmospheric nitrogen. Other species of these bacteria increase the availability of essential nutrient, Phosphorous. However, the availability of these two essential nutrients (nitrogen and phosphorous) in the soil is one factor in plant growth and yields. The second important factor is the ability of the roots to absorb these nutrients. In this aspect, the volume and type of roots of the respective plant also contribute significantly to plants growth as well as provision of expected yields. This study was carried on from September to December, 2018 at Sawah Baru, Bogor Agricultural University. The objectives were to observe the influence of biological fertilizer with and without combination of NPK and Urea fertilizers to enable rice plants to attain leaf green level which could produce expected paddy yields. The interaction effect between fertilizer treatments and the paddy varieties was also observed. Experiment design was two factor treatments arranged in a split plot. Fertilizer as main plot and paddy varieties as sub-plot laid in a Completely Randomized Block Design in 3 replications. The results showed significant differences in leaf green conditions and 1,000 grains weight of paddy.

Keywords— Nitrogen fixation; rhizosphere; root exudates; free-living bacteria.

I. INTRODUCTION

Nitrogen is an essential constituent of protein and chlorophyll (photosynthetic pigment). Furthermore, the nutrient N plays a fundamental role in agriculture by increasing crop yields [1]. Among of three essential nutrients for plants, it's the Nitrogen always insufficient in soils and is needed by high amounts. [2]

have also stated most of paddy soils of the world are N-deficient. Free-living bacteria (including saprophytes-bacteria living on plant residues, in close association with the rhizosphere and which live entirely within plants i.e., endophytes) e.g., *Bacillus* species may fix significant amounts of nitrogen 0-60 kg N ha⁻¹ per year [3]. The process is dependent on high energy, 16 ATP, thus the microbes must find the source of energy either from crop residues or root exudates. Free-living nitrogen fixers that live only in aerobic conditions have evolved a specialized biochemical pathway for nitrogen fixation. According to [4], *Bacillus* species are among of these free-living nitrogen fixers. The bacteria are also well known to promote the release of Plant growth hormones, from their microbial activities [5]. They enhance biosynthesis of indole-3-acetic acid (IAA) wherein have a closely relationship with the availability of nutrients to plants [6]. The IAA produced by rhizobacteria also induce root morphogenesis and consequently enhance its (1) size and weight, (2) branch numbers and patterns and (3) the surface area of roots as reported in non-legumes [7]. Indole-3-acetic-acid also essentially role in the origination and formation of adventitious roots [8-9], as well as in the enhancement of shoot development by the influence in cell division and differentiation. In this research the objectives were to observe the potential of biological fertilizer only or in combination with other chemical fertilizers to enhance leaf green color of rice plants which could facilitate the Rice plants to enable photosynthesis and produce expected yields. The second one was to observe the interaction effect of the fertilizer treatments with the paddy varieties. Two paddy varieties (Mekongga and IPB 3S) were used. Mekongga variety includes a new modern rice variety recognized by IRRI varieties and IPB 3S rice variety is a rice variety resulted from crossing of modern and local varieties. The latter was created by a research team at Bogor Agricultural University at the end of 2014.



II. MATERIALS AND METHODOLOGY

Materials

One Indonesian Paddy variety (IPB 3S) and one Improved variety (Mekongga) were used during this research. The fertilizers were of three types, NPK; Urea; and Biological Fertilizer (BF)-contained ten strains of *Bacillus* Species (*B. catenulatus*, *B. cereus*, *B. drentensis*, *B. firmus*, *B. flexus*, *B. megaterium*, *B. niacin*, *B. subtilis*, *B. tequilensis* and *B. thuringiensis*).

Experimental Site and Duration

This experiment was done at Sawah Baru (6°33'50.4"S 106°44'09.9"E, altitude 250 meter above the sea level) – IPB University, Darmaga, Bogor, Indonesia from September to December 2018.

Experimental Design

The experiment was two-factor treatments arranged in a split plot design with three replicates. The first factor was fertilizers amounts as main plot. In this, there were seven levels of fertilizer with and without combination of other fertilizer as shown in Table 1. The second factor was paddy varieties as sub plots.

Table 1. Applied Fertilizers (NPK, Urea and BF)

Treatment code	Levels of applied fertilizers			% of applied CF
	NPK Kg/ha	Urea Kg/ha	BF L/ha	
Co (Control)	0	0	0	0
M4	75	37.50	4	25
M3	150	75	4	50
M2	225	112.50	4	75
M1	300	150	4	100
Only BF	0	0	4	0
M5	300	150	0	100

Note: CF: Chemical Fertilizers. BF: Biological Fertilizer

Experimental Procedures

The field was prepared as normal flooded paddy fields. One, 14-days-old seedling from nursery was transplanted per hill at a planting distance of 25 cm X 25 cm. The sub plot size was 5 m X 5 m. The NPK fertilizer was applied in three times at 1, 4 and 6 weeks after transplanting each time as much as 250 g per plot while Urea fertilizer applied at 1 and 4 WAT, 250 and 125 g per plot respectively as full recommended rate. The BF was applied as follows: 60 ml was used during seeds soaking and sprayed direct on soils as much as 2.5 ml per plot at 2, 4, 6 and 8 weeks after transplanting.

Data Collection and Analysis

During the growing period five plant samples were randomly selected and eye-marked in each plot for measurements and data collection. Leaf Greenness was measured at vegetative phase (4,5,6,7, 8 and 9 weeks after transplanting) by using Leaf Color Chart. Panicle length was measured by Centimeter

Ruler. Thousand grains weight of paddy was determined by choosing using fingers the filled grains until 1,000 grains per plot then weighed by using electronic balance. Data were analysed with anova using SAS 9.4. Further comparison of means was done using Duncan Multiple Range Test (DMRT) at $\alpha = 5\%$.

III. RESULTS AND DISCUSSION

Leaf Greenness

The leaf greenness is used as an indicator of plant leaf N status. Rice plants applied with 75% of CF + BF; and 100% of CF + BF at 9 WAT were observed greenish and significantly different with other five fertilizer treatments. Only unfertilized plants (Co) observed yellowish green (implies there was N deficiency) and were significantly different with rice plants treated with only BF. This might be an indication there was continuous fixation of the N-nutrient in soil by the *Bacillus*. Similarly, to Research done by [10], found strains of *B. megaterium* had ability of N₂-fixing in paddy fields. On the other side applications of only BF; 25% of CF + BF; 50% of CF + BF; and 100% of only CF were not significantly different in the observed leaf green color. In all BF treated rice plants, the level of green color observed to delay in turning into yellowing stage as the plant leaf was getting old. Comparison on paddy varieties on leaf greenness during and after the vegetative growth were also noted. The IPB 3S rice plants were the earliest to lose its green color after the vegetative stage has ended, but Mekongga variety delayed until 11 WAT, rice plants were still green in color.

Table 2. Leaf Greenness of rice plants in seven fertilizers treatments and two paddy varieties

Combinations of Fertilizers			Leaf Greenness WAT					
NPK (%)	Urea (%)	BF (L/ha)	4	5	6	7	8	9
			LCC-Measurements					
0	0	0	2.8b	2.9d	3.2d	3.2d	3.2c	3.2c
25	25	4	3.0a	3.38bc	3.5bc	3.3c	3.6b	3.6b
50	50	4	3.0a	3.4abc	3.5bc	3.4c	3.6b	3.6b
75	75	4	3.1a	3.5a	3.7ab	3.6b	3.8a	3.7a
100	100	4	3.1a	3.5a	3.7a	3.7a	3.8a	3.7a
0	0	4	3.0a	3.2c	3.3cd	3.3c	3.6b	3.6b
100	100	0	3.1a	3.6a	3.6ab	3.7b	3.6b	3.6b
Variety								
Mekongga			3	3.3	3.4	3.5	3.6	3.6
IPB 3S			3	3.3	3.5	3.4	3.6	3.6
Interaction			ns	ns	ns	ns	ns	ns

Note: Numbers (Mean) marked by the same letter within the column shows not significantly different according to DMRT at $\alpha = 5\%$. WAT: Weeks After Transplanting. LCC: Leaf Color Chart. ns: not significantly different.

Yield Components

Number of grains per panicle varied from 197 to 203. Similar to panicle length, no significance difference was observed due to applied fertilizer treatments. The range for panicle length was 27.5 to 28.6 (Table 3). Only IPB 3S variety had a greater



Number of grains per panicle by 27.8% than the Mekongga variety and the difference was significantly. In panicle length also IPB 3S variety was the longest by 15.4% than Mekongga.

The thousand grains weight of rice plants fertilized with only BF, 75% of CF + BF; and 100% of CF + BF were heavier and significantly different than the grains weight of other four fertilizer treatments. High sink resulted from photosynthesis process might have been contributed to such heavier grains. Significant improvements on 1,000 grains weight due to application of formulations of *Bacillus* sp. on the growth of rice (*Oryza sativa* L.) was also reported by [11].

Only BF treated rice plants found significantly different in 1,000 grains weight as compared with only CF. This result, might be due to high status of initial soil organic matters (valued 4.2). So, the rice plants in BF treatments had more access to unlock essential nutrients from the organic matters as compared to only CF treated rice plants. However, strains of *Bacillus* contained in the BF have the ability to adjust soil pH [12] during the process of phosphate solubilization [13]. This process increases availability of phosphorous nutrient which is second important nutrient in plants and is the energy carrier during the photosynthesis.

Table 3. The average number of grains per panicle, panicle length and 1,000 grains weight of paddy in the combinations of fertilizers and two paddy varieties

Treatments			Parameter		
Fertilizers			Number of grains/panicle	Panicle Length (cm)	1,000 grains weight (g)
NPK (%)	Urea (%)	BF (L ha ⁻¹)			
0	0	0	199.1	27.5	24.7b
25	25	4	202.4	27.1	24.8b
50	50	4	186.5	28.3	25.3b
75	75	4	203.9	28.4	26.2a
100	100	4	191.3	28.0	26.5a
0	0	4	193.9	28.6	26.2a
100	100	0	197.8	27.5	25.2b
Varieties					
Mekongga			172.4b	25.9b	25.2b
IPB 3S			220.4a	29.9a	25.9a
Interaction			ns	ns	ns

Note: Numbers (Mean) marked by the same letter within the column shows not significantly different according to DMRT at α 5%. WAT: Weeks After Transplanting. ns: not significantly different.

IV. CONCLUSION

The use of Biological fertilizer is highly emphasized to rice plant growers. Not only can improve paddy yields but also, protect agricultural fields from the consequences of chemical fertilizers in both water sources and food produces. Based on the results of this research, reduction by 50%; 75; and 100% of CF-amounts if combined with BF was not observed significantly different on paddy leaf greenness as compared with plants treated with full recommended rates of CF. Furthermore, BF application in paddy

fields are able to increase Nitrogen amount in the soil through fixation, whereby absorbed N by roots enhances green color of Paddy leaves. This research also concluded that, the use of only BF; combination of BF with 75% and 100% of CF gave heavier 1,000 grains weight than the other four fertilizer treatments. Its therefore, has the potential of improving paddy yields. Lastly, the results found interaction between types of fertilizers and the paddy varieties used was not significantly different.

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