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The Effect of Inoculating Nitrogen Fixing Bacteria on Production of Rice

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Abstract: The combination use of biofertilizer and inorganic nitrogen (N) fertilizer is required to increase rice productivity and reduce land degradation. This study aims to determine the responses of four rice varieties in various fertilization packages that can save inorganic N by using nitrogen fixing bacteria as bio-fertilizer. The research was conducted in the Experimental Farm Faculty of Agriculture, Hasanuddin University. A randomized block design (RBD) with two-factors between varieties and fertilizer package was selected. The results revealed that Pandan Putri variety had the best result based on the 1,000 grains (28.42 g) weight while Inpari Sidenuk variety was excellent based on per clump weight of rice grain of 71.64 g. Ciliwung variety showed the best quantity of productive tiller (16.44 tiller). The fertilization package of half-dose urea in combination with 2.5L of *Azotobacter* demonstrated the best number of productive tillers (17.03 tiller) and grain weight (73.10 g) with a two times (1.25-0-1.25) application. It was concluded that the combination of half-dose urea and biofertilizer of *Azotobacter* increased the growth and production of rice plant.

Key words: Rice · Varieties · Inorganic N · Fertilizer · Nitrogen fixing bacteria

INTRODUCTION

Indonesia has a high demand for rice as a staple food. Until now and for the next few years rice remains as the main source of nutrition and energy for more than 90% of Indonesia's population. BPS data showed that in 2011, the level consumption of rice in Indonesia reached 139 kg per capita, which is higher than Malaysia and Thailand with a 65kg - 70 kg per capita per year. Since 1950, rice is the main staple food of Indonesian people. Even though there is a program to increase other source of food and nutrition through diversification but demand for rice is still increasing in line with the population growth. However, rice production in Indonesia is still lower than the target. The paddy production in Indonesia in 2011 only reached 65.39 million tons in comparison with the targets of 70.06 million tons of rice paddies [1].

Sustainability of rice farming production relies heavily on intensive fertilization. Nevertheless, the use of chemical fertilizers continuously can upset the balance of nutrients, depletion of micro elements such as Zn, Fe, Cu, Mn and Mo in the soil, affecting the activity of soil organisms, as well as lower productivity of rice farming in the long term. In addition, the use of highly chemical fertilizers is quite expensive and driving up the cost of rice production. If this situation is not properly and timely addressed these rice paddy lands will be no longer sustainable. In an effort to increase rice production, farmers usually provide fertilizers, especially urea and ZA with a high enough dose of up to 300 kg urea and ZA 50-100 kg ha^{-1}. Even in some areas, the proportion reaches 400-500 kg urea, equivalent to 184-230 kg N ha^{-1} despite a recommendation of 90-120 kg N ha^{-1}, equivalent to 200-260 kg urea ha^{-1} proposed [2]. Excessive use of inorganic

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fertilizers will generally lead to a 1% reduction in the organic matter of agricultural land. Thus, the use of alternative fertilizer need to be carefully considered [3]. Alternative fertilizer is expected to reduce the use of chemical fertilizers particularly urea which is most widely used in rice cultivation. Alternative fertilizer that has potential to reduce the dose of N is soil bacteria, which have ability in nitrogen fixation. The addition of alternative fertilizer can improve the growth and production of rice. It is also expected to improve the chemical and biological properties of the soil and does not pollute the environment. *Rhizosphere* bacteria such as *Azospirillum* and *Azotobacter* can be used as the alternative fertilizer. *Azospirillum* and *Azotobacter* live freely in the root zone of plants and have ability to fix nitrogen.

The efficient use of nitrogen on rice planting increases with the use of *Azotobacter* and *Azospirillum* as bio fertilizer. *Azotobacter* has some metabolic capabilities, including the fixation of free nitrogen and conversion to ammonia [4]. *Azotobacter* has capability to fix nitrogen freely in the rhizopore as non-biological fixation. In addition to nitrogen fixation, *Azotobacter* also produces thiamin, riboflavin, nicotine, indole acetic acid and gibberellins. When *Azotobacter* was applied to the seed, the seed germination rate is improved. *Azotobacter* also has play a role in the control of plant diseases through the substances that produced by *Azotobacter*. The application of isolate *Azotobacter* sp in rice, both with the addition of urea fertilizer and without fertilizer urea [5], obtained high yield crops, the number of tillers and weight of grain more than the rice plants that were only given urea alone.

However, the use of bio-fertilizers alone cannot simply increase crop productivity. There is a need to encourage the integrated nutrient management system that combines biological fertilizer/organic and inorganic fertilizers in order to increase land productivity and environmental sustainability. A farming system called LEISA (low external input and sustainable agriculture) using a combination of biological fertilizer /organic and inorganic based on the concept of good agricultural practices need to be implemented in order to preserve the environment and reduce land degradation [6]. Therefore the effectiveness and efficiency of bio-fertilizers, especially in the use of various doses in combinations with inorganic N, as well as the appropriate timing of application and choosing the right variety need to be further studied. The objective of this paper is to determine the response of four different rice varieties to different application of fertilizer package that can save inorganic N through the use of bacteria as biological nitrogen fixation.

MATERIALS AND METHODS

Media Preparation: The experiment was conducted at the Experimental Farm, Faculty of Agriculture, Hasanuddin University in Makassar, South Sulawesi, Indonesia. Rice soils that have dried for seven days were stored in plastic pots and then saturated with water for two days. Rice seed were grown in plastic containers and after 20 days, matured seedlings were transferred to plastic pots. The application of fertilizer package was in accordance with the treatment. Application of bio-fertilizer was given by way of close planting medium dripped into the roots of the plants. Plant growth was done according to the guidelines of rice cultivation until the next harvest.

Treatment: This experiment tested varying application of microbial N fertilization on four rice varieties. Inorganic N fertilizer was based on the recommendation dose of 250 kg ha^{-1} and half of the urea dose recommendation. Thus, a urea dose of 125 kg ha^{-1} was applied. Biofertilizers were used in the form of microbes *Azospirillum* and *Azotobacter* at three different times, namely given one time in the early planting (0 day after planting-DAP), given two times (0 DAP and 45 DAP) and given 3 times (0 DAP, 21 DAP and 45 0 DAP). A Factorial Randomized Block Design (RBD) was used. The first factor is the different of variety of rice (v), which is composed of four types, namely: v1 = Pandan Putri, v2 = Inpari Sidenuk, v3 = Ciliwung dan v4 = Ciherang. The second factor is 15 Fertilizer Packagas that can save using inorganic nitrogen fertilizer (p), namely: p1 = 5.0 L *Azospirillum* 1 times (5-0-0), p2 = 5.0 L *Azospirillum* 2 times (2.5-0-2.5), p3 = 5.0 L *Azospirillum* 3 times (1.7 -1.7-1.7) p4 = half-dose of urea + 2.5 L *Azotobacter* 1 times (2.5-0-0), p5 = half-dose of urea + 2.5 L *Azotobacter* 2 times (1.25-0-1.25), p6 = half- dose of urea + 2.5 L *Azotobacter* 3 times (0.83-0.83-0.83), p7 = half-dose of urea + 5.0 L *Azotobacter* 1 times (5-0-0), p8 = ½ dose of urea + 5.0 L *Azotobacter* 2 times (2.5-0-2,5), p9 = half-urea + 5.0 L *Azotobacter* 3 times (1.7-1.7-1.7), p10 = 5.0 L *Azotobacter* 1 time (5-0-0), p11 = 5.0 L *Azotobacter* 2 times (2.5-0-2,5), p12 = 5.0 L *Azotobacter* 3 times (1.7-1.7-1.7), p13 = half-dose of urea + 2.5 L *Azospirillum* 1 times (2,5-0-0) + 2.5 L *Azotobacter* 1 time (2.5-0-0), p14 = half-dose of urea + 2.5 L *Azospirillum* 2 times (1.25-0-1.25) +

2.5 *Azotobacter* 2 times (1.25 – 0-1..25), p15 = half-dose of urea + 2.5 L *Azospirillum* 3 times (0.83-0.83-0.83) + 2.5 + *Azotobacter* 3 times (0.83-0.83-0.83). Of those two factors there are 60 (4 x 15) combination treatments and each combination treatment was repeated three times, which consisted of three units pots with an overall 540 units of pot experiments.

RESULTS AND DISCUSSION

Number of Productive Tiller: Analysis of variance showed that the treatment of varieties and fertilizer package was highly significant on the number of productive tillers, but the interactions of both treatments were not significant. Table 1 show that the variety Ciliwung (v3) produces the highest average number of productive tillers (16.44 tillers) and significantly different compared with other varieties. While the package fertilizer of half- dosage of urea + 2.5 L *Azotobacter* in two times application (2.5-0-2.5) (p5), produces the highest average number of productive tillers (17.03 tillers) and significantly different compared to treatment package p1, p3, p5, p10, p11 and p12, but did not differ significantly compared to the treatment package p4, p6, p7, p8, p9, p13, p14 and p15.

Weight of Grain per Clump: Analysis of variance showed that the varieties and fertilizer treatment package was highly significant on the grain weight per hill of rice plants. The interactions from both treatments have no significant effect. Table 2 shows that the variety Inpari Sidenuk (v2) produces the highest average weight of grain per clump (71.64 g) and significantly different if compare with other varieties. While the fertilizer treatment of ½ dosage of urea + 2.5 L *Azotobacter* with two times application (2.5-0-2.5) (p5) produces the highest average number of productive tillers (17.03 tillers) and has significantly different compared with treatment package p1, p3, p5, p10, p11 and p12, but did not differ significantly compared to treatment package p4, p6, p7, p8, p9, p13, p14 and p15.

Weight of 1,000 Grains: Analysis of variance showed that the different varieties have significant effect on the grain weight while there were no significant effects on the interactions between the different fertilizer package and rice varieties. Table 3 shows that the Pandan Putri variety (v1) produces the highest significant mean weight of 1000 grains (28.42 g) compared with other varieties. The result also showed that there is variability in the response of rice plants at various fertilizer package because each variety

Table 1: The average number of productive tillers in various varieties and package of fertilization

Fertilizer Package (P)	Variety (V) v_1	v_2	v_3	v_4	Average	DMRT $_{á=0,01}$
p_1	9,22	13,78	14,22	13,33	12,64 cde	2,26
p_2	9,44	14,11	16,44	14,78	13,69 b-e	2,35
p_3	8,67	11,22	14,67	11,67	11,56 e	2,42
p_4	11,67	15,89	18,11	16,67	15,58 ab	2,47
p_5	12,33	17,78	20,44	17,56	17,03 a	2,50
p_6	13,78	17,11	15,44	18,33	16,17 ab	2,54
p_7	10,33	16,22	18,56	16,89	15,50 ab	2,56
p_8	11,78	16,56	19,33	16,67	16,08 ab	2,59
p_9	12,89	14,11	15,78	15,44	14,56 a-d	2,61
p_{10}	9,67	12,33	13,67	14,00	12,42 cde	2,65
p_{11}	9,00	13,22	13,67	12,78	12,17 de	
p_{12}	10,11	12,22	12,78	13,89	12,25 cde	
p_{13}	11,11	16,78	15,44	15,67	14,75 abc	
p_{14}	10,56	18,22	18,67	16,44	15,97 ab	
p_{15}	13,00	15,67	19,33	16,67	16,17 ab	
Average	10,90 x	15,01 w	16,44 v	15,39 w		
NP JBD$_{á=0,01}$	1,167	1,214	1,252			

Note: Mean in the same column suffixed with different lower case letters or mean in the same row suffixed with different capital letters are different at 5% levels of significance according to DMRT.

Table 2: The average weight of grain per hill (g) in various varieties and different package fertilization

Fertilizer Package (P)	Variety (V) v_1	v_2	v_3	v_4	Average	DMRT $_{á=0,01}$
p_1	55,04	71,88	58,53	56,36	60,45 [bc]	7,48
p_2	54,66	65,58	59,03	61,85	60,28 [bc]	7,78
p_3	55,59	57,62	60,77	53,43	56,85 [c]	8,02
p_4	63,63	73,14	76,02	67,42	70,05 [a]	8,18
p_5	69,49	74,54	79,45	68,92	73,10 [a]	8,28
p_6	69,67	78,99	60,17	76,92	71,44 [a]	8,40
p_7	64,31	78,10	64,27	69,54	69,05 [a]	8,48
p_8	64,99	78,03	70,01	69,65	70,67 [a]	8,57
p_9	64,77	69,25	66,15	67,14	66,83 [ab]	8,65
p_{10}	54,35	67,37	57,36	61,54	60,16 [bc]	8,77
p_{11}	51,80	66,53	58,62	61,18	59,53 [bc]	
p_{12}	55,30	56,45	54,29	60,32	56,59 [c]	
p_{13}	63,91	75,80	67,35	66,42	68,37 [a]	
p_{14}	59,51	86,41	72,13	68,64	71,67 [a]	
p_{15}	67,83	74,91	74,66	70,31	71,93 [a]	
Average	60,99 [x]	71,64 [v]	65,25 [w]	65,31 [w]		
NP JBD$_{á=0,01}$	3,861	4,017	4,142			

Note : Mean in the same column suffixed with different lower case letters or mean in the same row suffixed with different capital letters are different at 5% levels of significance according to DMRT.

Table 3: The average weight of 1,000 grains (g) on different varieties

Fertilizer Package (P)	Variety (V) v_1	v_2	v_3	v_4
p_1	28,40	24,15	21,83	23,67
p_2	28,60	25,38	23,75	25,50
p_3	28,62	24,63	22,80	23,33
p_4	28,23	26,00	23,28	24,20
p_5	27,95	25,42	21,88	23,12
p_6	27,75	24,00	22,57	22,70
p_7	28,93	24,30	21,70	23,38
p_8	27,75	24,73	26,77	22,38
p_9	28,70	26,02	24,35	24,25
p_{10}	26,78	25,68	23,45	23,67
p_{11}	34,37	22,45	22,57	23,07
p_{12}	27,32	24,67	23,12	23,43
p_{13}	28,53	25,05	22,97	23,55
p_{14}	26,35	24,70	23,60	24,25
p_{15}	27,98	23,12	22,50	23,25
Average	28,42 [v]	24,69 [w]	23,14 [x]	23,58 [wx]
DMRT $_{=0,01}$	1,260	1,311	1,352	

Note: Mean in the same column suffixed with different lower case letters or mean in the same row suffixed with different capital letters are different at 5% levels of significance according to DMRT.

has a different genetic potential to grow. In addition, each variety has a different adaptation capability to the environment [7].

Based on 1000 grain weight, Pandan Putri variety showed the highest yield but on the productive tiller number and grain weight per clump, Pandan Putri variety had the lowest yield compared to other varieties. The differences character of variety arises due to the genetic differences of variety that regulate the yield characteristics. The genes that varied from each variety were visualized in diverse characters. Each gene has its own work to develop and manage various types of characters in a living body [8]. Plants have the ability to change the appearance of the character as a response to fluctuations due to environmental influences. The variability due to environmental factors and genetic diversity generally interact with each other to affect the appearance of the plant phenotype [9]. Genetic factors will not disclose the nature of plant yield except with certain environmental factors. Instead, the manipulation and improvement of the environmental factors will not lead to the development of a trait, unless there is a genetic factor that is required on the individual concerned.

Statistical analysis showed that the half dose of urea fertilizer package plus 2.5 L *Azotobacter* with two time's application (2.5-0-2.5) showed the best yield of productive tillers and grain weight per hill. The result showed that good growth on rice plants if treated with combined biological fertilizer and urea as a source of N because it can be directly available to the plant in accordance with the requirements of the plant. The application of urea caused nitrogen content in the soil increased. Nitrogen causes the plant chlorophyll content to be higher and the subsequent rate of photosynthesis also increases. The increase rate of photosynthesis caused the synthesis of carbohydrates and the synthesis of other organic compounds also increased. Nitrogen is a constituent element of amino acids. While the amino acids make up the protein, the protein absorbs various enzymes for catalyzing biochemical reactions and new cell structure which further contribute to the growth of the vegetative growth of plants including plant height [10]. Nitrogen is the primary nutrient elements which constitute the main component of various compounds in the plant body. Plants that grow must contain N in forming new cells. Photosynthesis produces carbohydrates, O_2 and H_2O, but the process can not take place to produce proteins and nucleic acids, when N is not available. Nitrogen available to plants can affect the formation of proteins and in addition it is also an integral part of chlorophyll [11].

Statistical analysis also showed that the treatment of 5.0 L fertilization treatment package *Azotobacter* with three times application (1.7-1.7-1.7) without additional urea dosage resulted in the lowest number of productive tillers and grain weight per clump, although the results were not significantly different to fertilizer package of 5.0 L *Azospirillum* three times (1.7-1.7-1.7) applications. It shows that in both of these treatments, rice plants need the available N especially in the early of growth that has not adequately met from soil bacteria fixation process. It also showed that the nitrogen fixation by bacteria is not sufficient to meet the N requirements of rice plants. The inorganic nitrogen compounds (urea) in a small amount is required to address the needs of the early growth before the plants can rely on nitrogen requirement of N_2 fixation by bacteria [12]. Besides that the bacteria also need the nitrogen to grow and thrive indirectly influenced by the availability of nitrogen in the early growth. The growth of bacteria is also influenced by the availability of nutrients in the root environment and will certainly affect on the N_2 fixation [13].

The high number of productive tillers was obtained when a fertilizer package at half-dose urea plus a twice (2.5-0– 2.5) 2.5 L *Azotobacter* was applied. This is due to the optimum bacterial population to perform the required N fixation in the early growth of rice plants and the period of development of productive tillers. In the rice plants, nitrogen is needed in large quantities in the early and middle phases of rice growth to maximize the number of panicles [14]. The half-dose package of urea plus 2.5 L *Azotobacter* is sufficient enough to supply nitrogen that is required in the whole growth of rice plant. When nitrogen availability is present in a sufficient amount, it can encourage rapid division, elongation and enlargement of cells in the apical meristematic region that leads to a taller plant growth. Nitrogen crop requirement can be fulfilled through the application of urea fertilizer and through the use of non-symbiotic nitrogen-fixing bacteria [15].

CONCLUSION

Pandan Putri rice variety produces the best weight per 1,000 grain while Inpari Sidenuk on the grain weight per hill and Ciliwung on the number of productive tillers. The fertilizer package with half dose urea fertilizer combined with a twice 2.5L *Azotobacter* application (2.5 - 0 - 2.5) demonstrated the maximum productive tillers and grain weight per hill. In the efforts to increase rice productivity, it is recommended that rice paddy farmers use biological fertilizers combined with inorganic N fertilizer to increase their rice paddies production.

REFERENCES

1. Badan Pusat Statistik (BPS)., 2011. Data Luas Panen, Produktivitas dan Produksi Tanaman Padi Seluruh Provinsi Indonesia. Badan Pusat Statistik. Jakarta (*In Indonesian*).
2. Wahid, A.S., 2003. Peningkatan efisiensi pupuk nitrogen pada padi sawah dengan metode bagan warna daun. Jurnal Litbang Pertanian 22(4): 156-161 (*In Indonesian*).

3. Pusat Penelitian Tanah dan Agroklimat (PPTA), 2004. Sumberdaya lahan Indonesia dan pengelolaannya. Badan Penelitian dan Pengembangan Pertanian (BPPP), Bogor (*In Indonesian*).
4. Hindersah, R. dan and T. Simarmata, 2004. Potensi rhizobakteri *Azotobacter* dalam meningkatkan kesehatan tanah. Artikel ulas balik. Jurnal Natur Indonesia, 5(2): 127-133 (*In Indonesian*).
5. Syam'un, E., A. Dachlan, I.N.P. Aryantha and dan G. Suantika, 2006. Respon dua varietas padi terhadap isolat bakteri Azotobacter sp. Jurnal Agrivigor., 6(1): 57-56 (*In Indonesian*).
6. Simanungkalit, R.D.M., 2001. Aplikasi pupuk hayati dan pupuk kimia: suatu pendekatan terpadu. Buletin AgroBio., 4(2): 56-61 (*In Indonesian*).
7. Ghosh, P. and A.K. Kashyap, 2003. Effect of rice cultivars on rate of N mineralization nitrification and nitrifier population size in an irrigated rice ecosystem. Applied Soil Ecology, (24): 27-41.
8. Yatirn, W., 1991. Genetika. Penerbit Tarsito, Bandung (*In Indonesian*).
9. Bari, A., M. Sjarkani and dan S. Endang, 1974. Pengantar pemuliaan tanaman. Departemen Agronomi, Fakultas Pertanian Institut Pertanian Bogor, Bogor (*In Indonesian*).
10. Sugiyanta, F. Rumawas, M.A. Chozin, W.Q. Mugnisyah and dan M. Ghulamahdi, 2008. Studi serapan hara N, P, K dan potensi hasil lima varietas padi sawah (Oryza sativa L.) pada pemupukan anorganik dan organik. Bul. Agron., 3(36): 196-203(*In Indonesian*).
11. Nyakpa, M.Y., A.M. Lubis, M.A. Diha, A.G. Amrah, A. Munawar, G.B. Hong and N. Hakim, 1988. *Kesuburan Tanah*. Penerbit Universitas Lampung (*In Indonesian*).
12. Budiyanto dan H.M.A. Krisno, 2004. Mikrobiologi Terapan. Universitas Muhammadiyah Malang (*In Indonesian*).
13. Sutanto, R., 2002. Penerapan pertanian organik. Kanisius, Yogyakarta (*In Indonesian*).
14. Suyamto, 2010. Peranan unsur hara N, P, K dalam proses metabolisme tanaman padi. Balai Besar Litbang Sumberdaya Lahan Pertanian, Badan Penelitian dan Pengembangan Pertanian. Bogor (*In Indonesian*).
15. Dachlan, A., E. Syam'un and dan A.U. Singkerru, 2008. Pertumbuhan dan produksi tiga varietas padi pada berbagai paket pemupukan N sintetik-bakteri Azotobacter. J. Agrivigor, 7(3): 230-241 (*In Indonesian*).