EFFECT OF *AZOTOBACTER* AND *AZOSPIRILLUM* ON GROWTH AND YIELD OF RICE GROWN ON TIDAL SWAMP RICE FIELD IN SOUTH KALIMANTAN

Fakhrur Razie[1] and Iswandi Anas[2]

[1]Department of Soil Science, Faculty of Agriculture, Lambung Mangkurat University
Jl. A. Yani KM.36 Banjarbaru 70714, South Kalimantan
email: fakhrurrazie@yahoo.com
[2]Laboratory of Soil Biotechnology, Faculty of Agriculture, Bogor Agricultural University (IPB).
Kampus IPB Darmaga, 16680 Bogor, West Java

ABSTRACT

Tidal swamp land is a potential area for rice cultivation. However, tidal swamp is fragile ecosystems, so that when this area is used for rice cultivation, it has to be done carefully. To reduce a risk of environmental pollution in the tidal swamp area due to rice cultivation, the area should be managed properly and wisely especially when using agrochemicals such as fertilizers and pesticides. In relation to this, the use of bio-fertilizer such as Azotobacter or/and Azospirillum, an atmospheric nitrogen fixing bacterium, might be an important thing for this area. The objectives of this study were to evaluate the number of NFB (i.e. Azotobacter and Azospirillum spp.) found in tidalswamp rice fields of South Kalimantan, and their ability in fixing atmospheric nitrogen and supplying this fixed nitrogen to rice, and in increasing rice yields. There were three research stages performed in this study. The first, collecting, isolating and purifying the NFB on the selective media of nitrogen free media. The second, a hydroponic experiment in the greenhouse using Yoshida nutrition solution (Yoshida et al., 1976 in IRRI, 2003) as culture media and selected Azotobacter and Azospirillum. The third experiment was to study the effect of selected Azotobacter isolates to increase growth and yield of IR64 and Margasari rice cultivars, and two Azotobacter isolates were combined with six inoculation methods for Siam Unus rice cultivar as treatments. The result showed that the number of NFB strains found in rhizospere rice cultivars were varied widely. Then, Azotobacter and Azospirillum spp selected from rice fields in the tidal land of South Kalimantan when associated with IR64 and Siam Unus rice cultivars have ability in fixing atmospheric N2 and in supplying N on the initial rice growth. Azotobacter T.B.PDST.2b and T.HM.BPMT.2b were significantly supplying N for early growth of IR64 rice cultivars (2.34 and 2.13 %N). The ability of these isolates to fix atmospheric N_2 was similar to N fertilizer (urea) in supplying N (2.2% N). Only Azotobacter T.B.PDST.2b was relatively significant to supply N (1.36% N) for Siam Unus rice cultivar early growth, even though this strain has less ability than N fertilizer (1.94% N) in supplying N. Azotobacter isolates grow in media containing no urea were higher IAA produced than in media containing Urea. Furthermore, yield of IR64, Margasari and Siam Unus inoculated with Azotobacter isolates were 3.87–4.93; 4.63-5.36 and 5.44-6.42 ton/ha respectively. In conclusion, the utilization of Azotobacter and Azorpirillum spp to substitute N fertilizer would be able to increase effeciency of N nutritions and to avoid environment pollutions risks from agrochemical N fertilizer other than to increase rice yields on tidal land of South Kalimantan.

Keywords: Azotobacter, Azospirillum, rice cultivars, tidal lands and rice yields.

INTRODUCTION

In South Kalimantan, tidal swamp area is used for rice cultivation. This swampy area is a very fragile ecosystem. Therefore, application of agrochemicals such as synthetic fertilizer i.e. Urea, SP-36 and KCl as well as pesticide should be done carefully. So far, the use of Urea fertilizer with very high dosage has caused environmental pollution. Up-take efficiency of nitrogen fertilizer in this area is low. According to Biswas *et al.* (2000), up-take efficiency of Urea in tidal swamp area was reported to be much less than 50% (Biswas *et al*., 2000). On the other hand, recently there were tremendous increase in price of synthetic fertilizer such including Urea, SP-36 and KCl. Application of bio-fertilizer, organic fertilizer, or combination of both (bio-organic fertilizer) might reduce the dosage of synthetic fertilizer applied, hence reduce the cost of production (input) as well as reduce the environmental problems. Many reports have been published on the potential of *Azotobacter* and *Azospirillum* as bio-fertilizer in relation to their ability to fix atmospheric N2 and to produce plant growth promoting substances (i.e. *indole acetic acids* - IAA). Both nitrogen fixing bacteria supply nitrogen to the pant and stimulate the growth of rice roots system and increase rice yield

The objectives of this study were (1) to determine the number of atmospheric nitrogen (N2) fixing bacteria (NFB) i.e. *Azotobacter* and *Azospirillum* spp. in the tidal land rice fields of South Kalimantan; (2) to isolate and selected these N2 fixing bacteria, and (3) to determine their ability to supply nitrogen to the rice plant, (4) to evaluate the effect of *Azospirillum* and *Azotobacter* on growth and yield of rice grown on tidal land area rice field area in South Kalimantan.

MATERIALS AND METHODS

Three stages of research were performed in studying the NFB on tidal land rice fields of South Kalimantan. The first study was conducted to evaluate the number of NFB on tidal lands rice field of South Kalimantan and to determine effectiveness of selected NFB in fixing atmospheric N_2 in culture medium. Soil and rhizosphere of rice plant samples were collected from several sites where three local rice cultivars (Bayar Pahit, Siam Pandak and Siam Unus), one high yield local rice cultivar (Margasari) and one national high yield rice cultivar (IR64) were grown. Isolating and purification of the NFB was carried out at the Soil Laboratories, Faculty of Agriculture, Lambung Mangkurat University. These bacteria were isolated using the selective media of Nitrogen Free Media (NFM) for *Azotobacter* and Nitrogen Free Bromthymol Blue (NFB) for *Azospirillum* (Atlas, 1997). The effectiveness of these bacteria to fix atmospheric N2 was determined in culture medium according to *nitrogenase* activities (Zuberer, 1998). Total nitrogen in the culture media was measured by using the Micro Kjeldahl methods and the *nitrogenase* activities was measured according to Acethylene Reduction Assay (ARA) value using Gas Chromatographic (Anas, 1999).

The second study was a hydroponic experiments. The purpose of this study was to evaluate the ability of the selected BNF to fix atmospheric N_2 and to supply this fixed nitrogen to rice seedling. This experiment was conducted at the greenhouse of the Faculty of Agriculture IPB Dramaga Bogor. Nine isolates of *Azotobacter* and *Azospirillum* were tested. As a control, two treatments were performed i.e. Urea fertilizer and without urea or isolates) The treatments had three replications. Two rice cultivars (IR64 and Siam Unus) were used in this experiment. The hydroponic medium was Yoshida nutrition solution without N nutrient (Yoshida *et al.*, 1976 *in* IRRI, 2003). The nutrient solution consisted of $NaH_2PO_4 \cdot 2H_2O$ (0.37 mM), K_2SO_4 (0.5 mM), $CaCl_2$ (1.00 mM), and $MgSO_4 \cdot 7H_2O$ (1.6 mM). The ability of isolates in fixing atmospheric N_2 measured based on the difference in total Nitrogen content (total of plant N + N in solution) between the inoculated treatments and uninoculated treatment.

The third experiment was the field experiments. The aim of this study was to evaluate the ability of selected *Azotobacter* isolate to increase the plant growth and to increase rice yield. The experiments was conducted at the Rice Research Stations of the Faculty of Agriculture, University of Lambung Mangkurat, Sungai Rangas-Banjar Regencies, South Kalimantan. A completely randomized block design experiments was performed. Three rice cultivars used in this experiment were IR64, Margasari and Siam Unus. Two selected *Azotobacter* were tested using IR64 cultivar and two selected Azotobacter were also tested using Margasari rice cultivar. The treatments were arranged in 30 plots (5x5 m^2) with three replication for IR64 and 36 plots (5x5 m^2) with three replication for Siam Unus rice cultivar.

RESULTS AND DISCUSSION

The presence of *Azotobacter* and *Azosprillum* in the soil and rhizophere of ricegrown on tidal swamp rice field of South Kalimantan

The numbers of *Azotobacter* and *Azospirillum* isolated from A type of tidal land (on Balandean fields) was seven *Azotobacter* and 16 *Azospirillum* , whereas in B and C types (Handil Manarap and Handil Malintang fields) as much as 12 and 14 *Azotobacter*; 23 and 22 *Azosprillum* isolates were found respectively. The numbers of *Azotobacter*s isolates from Siam Pandak, Bayar Pahit, Sian Unus, Margasari IR64 rice cultivars were. 13, 12, 8, 5 and 4 *Azotobacter* isolates respectively. On the other hand, the number of *Azospirillum* isolated from Bayar Pahit, Siam Unus, Siam Pandak, IR64, Margasari rice cultivars were 27, 22, 20, 13 and 7 *Azospirillum* isolates respectively. This means that the number of *Azotobacter* and *Azospirillum* depent on the type of tidal swamp area (Type A, B or C) and rice cultivars.

The influence of sea water on soil properties is shown by electrical onductivity (EC) of soils. The EC value of Balandean soils (i.e. 0.20 µS/cm) was higher than Handil Manarap and Handil Malintang soil (i.e. 0.15 and 0.12 µS/cm respectively). Application of composted rice straws in Handil Malintang seems to stimulate the number of both atmospheric N2 fixing bacteria in this area. Roper and Ladha (1995) showed that the asymbiotic diazotrophic bacteria such as *Azospirillum lipoferrum* and *A. brasilense* was using a high mole weight carbohydrates such as *xylan* (mainly component of *hemisellulose*) from rice straws as energy and carbon sources. In addition, differences of rice cultivars in releasing organic acid substances that can also influence presence of these bacteria. Nursyamsi (2000) reported that difference of rice cultivars produce difference of organic acid substances. The production of malic acid (2.532±167 nmol/g dry soil) by IR 66 cultivar was higher than Cisadane rice cultivar (1.793±153 nmol/g dry soil) and succinate acids produced by IR66 was 535±153 nmol/g dry soil, but this acids was not produced by Cisadani rice cultivar.

The effectiveness of *Azotobacter* and *Azorpirillum* spp in fixing N_2 atmosphere is presented in Table 1. All selected BNF isolates were able to fix atmospheric N2. Zuberer (1998) suggested that the effectiveness ranges of bacterias to fix of atmospheric N_2 in their environments were from 0.04 to 0.67 nmole N/ nmol C_2H_4. The *Azotobacter* isolates (i.e. 1.62-7.57 nmole N/nmole C_2H_4) were more effective than *Azorpirillum* isolates (i.e. 0.42-0.89 nmole N/nmole C_2H_4). The effectivity of isolates T.B.BPMT.1 and T.B.PDST.2b in fixing atmospheric N2 (4.07 and 2.92 nmole N/nmole C_2H_4) was less than *Azotobacter* 07.1/TNH/II, but these effectivity was more than *Azotobacter*s. Moreover, the *Azotobacter* 07.1/TNH/II (i.e. 7.56 nmole N/nmole C_2H_4) was the most effective isolate in fixing atmospheric N_2.

Table 1. Effectiveness of *Azotobacter* Isolates and *Azospirillum* in Fixing Atmospheric N_2

Genera	Isolates	Effectiveness (nmole N/ nmol C_2H_4)
Azorpirillum	S.0.4/TNM *	0.89
	S.HM.MGSR.3b	0.42
	S.TSB.BPMT.1c	0.81
Azotobacter	T.07.1/TNH/II *	7.56
	T.B.BPMT.1	4.07
	T.B.MGSR.1	1.78
	T.B.PDST.2b	2.92
	T.HM.BPMT.2b	1.95
	T.M.UNST.3	1.62

Effectiveness = sum of N fixed /ARA values (Zuberer, 1998)

*) Standard comparison strains from the laboratory of Soil Biotechnology, IPB

Role of *Azotobacter* and *Azospirillum* spp. in fixing atmospheric N2 and supplying nitrogen to rice seedling

The capability of *Azotobacter* and *Azospirillum* isolates in fixing atmospheric N_2 and in supplying nitrogen to the rice seedling is shown in Figure 1. All NFB isolates when associated on IR64 rice cultivar were significantly to fix N_2 atmosphere, but difference of the strains were not significantly different. We can see that its difference of N_2 atmosphere amounts were fixed by NFB strains when these strains were inoculated to Siam Unus rice cultivars. *Azotobacter* 07,1/TNH/II; T.B.BPMT.l; T.B.PDST.2b; THM.BPMT.2b and T.M.UNST.3 and *Azospirillum* 04/TNM to fix of N_2 atmosphere were higher than other strains i.e. 7.77 to 9.85 mg N/pot. Malarvizhi and Ladha (1999) suggested that the amount of atmospheric N2 fixed by isolates depend on plant needs and available of nitrogen in the soil. The increase of N-content in the leaf tissue was also caused by different in genotype of the rice to absorb specific nutrients. Root metabolism might modify the BNF in the rhizosphere of the plant.

The ability of two bacterias genera in supplying N for IR64 and Siam Pandak rice cultivars at initial growth can be seen from the N content of the leaves (Figure 2). At the initial growth of IR64 rice cultivars, the total of N content was significantly increased by *Azotobacter* T.B.PDST.2b and THM.BPMT.2b (2.34 and 2.13 %N), and these values were similar to treatment with N fertilizer (2.2% N). At the initial growth of Siam Unus rice cultivar, only *Azotobacter* T.B.PDST.2b has ability to increase nitrogen content of the leaf (1.36% N), eventhough the isolate has less ability in supplying nitrogen than N fertilizer (1.94% N). Dobermann and Fairhurst (2000) suggested that the optimum range of N-tissue in rice during vegetatif growth was 2.9 – 4.2% N, so Shrestha and Ladha (1996) found that the range N-tissue contents in rice from 25 rice cultivars from short until long-term aged were 1.84 – 2.35% ^{15}N.

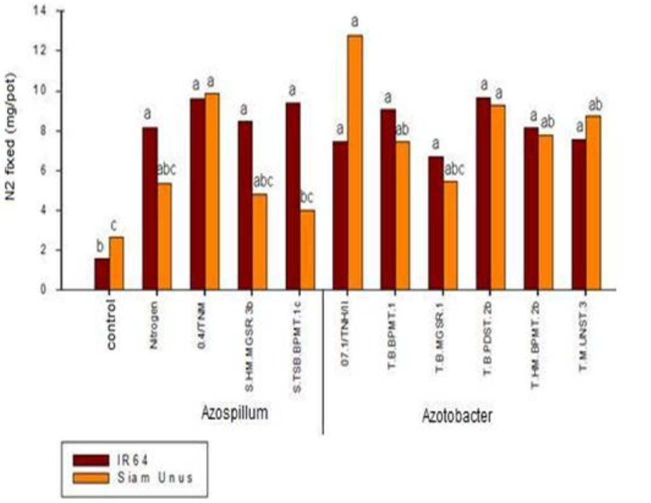


Figure 1. The Amounts of Atmospheric N2 Fixed by NFB on The Early Growth of IR64 and Siam Unus Rice Cultivars

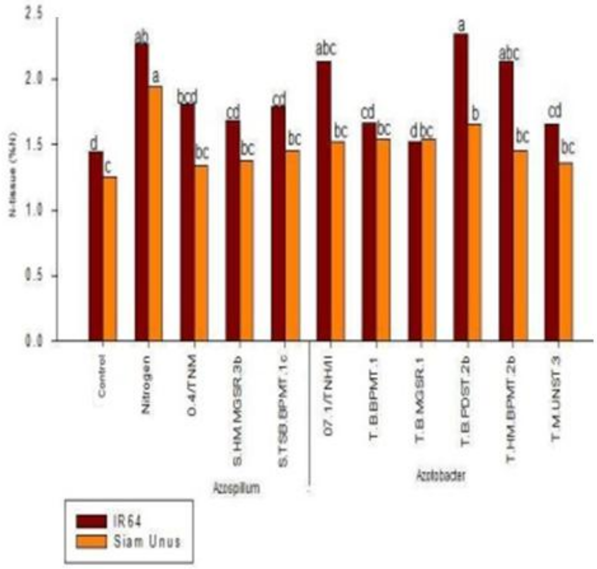
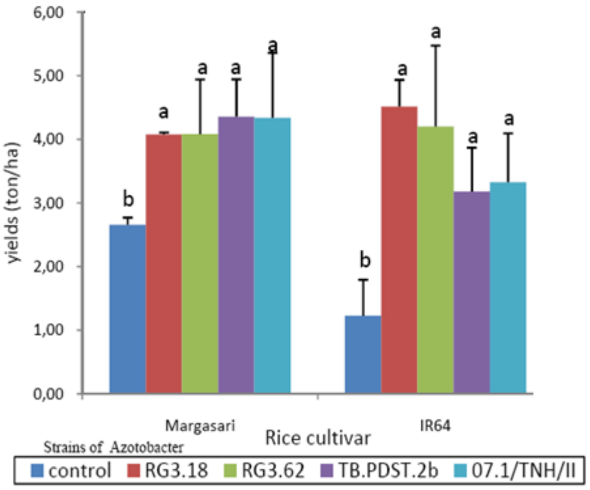


Figure 2. Nitrogen Content of The Leaf of IR64 and Siam Unus Rice Cultivars.

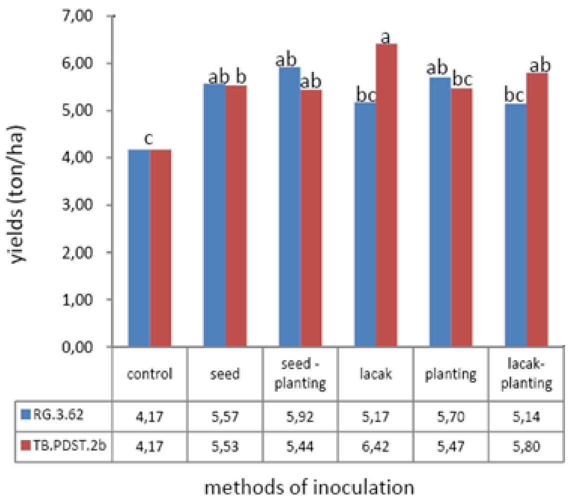
Role of *Azotobacter* spp in rice yields on tidal areas of South Kalimantan

The ability of *Azotobacter* isolates in increasing rice yields is presented in Figure 3 and 4. *Azotobacter* isolates significantly increased yield of IR64 and Margasari rice (Figure 3), but among isolates tested there were no significantly different in increasing of rice yields. Subsequently, the *Azotobacter* isolates which inoculated through seeds inoculations were able to increase IR64 rice yields from 1.26 ton/ha (Control) to 3.87 – 4.93 ton/ha and for Margasari rice cultivar yields from 2.77 ton/ha to 4.63 – 5.36 ton/ha. The yield of IR64 and Margasari inoculated with *Azotobacter* were the same as the IR64 and Margasari rice cultivars received Urea fertilizer. Urea treatment were able to improve rice yields up to 4-4.5 ton/ha. In Figure 4. It can be seen that *Azotobacter* RG.3.62 and TB.PDST.2b inoculation were significantly increasing yield of Siam Unus rice cultivars. The yield of Siam Unus rice cultivars inoculated with *Azotobacter* RG.3.62 and TB.PDST.2b were 5.17-5.92 and 5.44-6.42 ton/ha respectively.



Treatment not followed the same letter(s) are statistically different at p=0,05 (Duncan test) at each rice cultivar

Figure 3. Effect of *Azotobacter* Isolates Inoculation on Rice Yields of IR64 and Margasari Rice Cultivars.



	control	seed	seed - planting	lacak	planting	lacak-planting
RG.3.62	4,17	5,57	5,92	5,17	5,70	5,14
TB.PDST.2b	4,17	5,53	5,44	6,42	5,47	5,80

Treatments not followed the same letter(s) are statistically different at p=0,05 (Duncan test) at each Azotobacter strain. Lacak is the 3rd transplanting of multiple transplanting methods in tidal land rice fields of South Kalimantan. Control is without inoculating

Figure 4. Effect of *Azotobacter* Isolates Methods on Rice Yield of Siam Unus Rice Cultivars.

CONCLUSIONS

The number of NFB isolates found on rice fields of tidal land types of South Kalimantan were varied widely. *Azotobacter* and *Azorpirillum* isolates have a high ability in fixing atmospheric N_2 and in supplying N to rice seedlings. *Azotobacter* T.B.PDST.2b and T.HM.BPMT.2b were significantly supplying nitrogen for early growth of IR64 rice cultivar (2.34 and 2.13 %N). The ability of these isolates was similar to N fertilizer application (2.2% N). Only *Azotobacter* T.B.PDST.2b was relatively significant to supply N (1,36% N) for Siam Unus rice seedling, even though this strain has less ability than N fertilizer to supply N (1,94% N). Yield of IR64, Margasari and Siam Unus rice cultivar inoculated with *Azotobacter* sisolates were 3.87–4.93; 4.63-5.36 and 5.44-6.42 ton/ha respectively. In conclusion, the inoculation of *Azotobacter* and *Azorpirillum* spp to rice seedling were able to substitute application of synthetic N fertilizer. This will reduce the risk of environment pollutions and reduce the input for rice cultivation in the tidal swamp ricefields of South Kalimantan.

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