SELECTION FOR EFFECTIVE *AZOSPIRILLUM* ASSOCIATED WITH MAIZE IN ACID SOIL

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ABSTRACT

Rapid screening of effective *Azospiriluum* associated with maize in the greenhouse is needed to predict the right combination of for effective association in the field. To achieve this purpose activities in greenhouse were carried out to select the effective single isolates, preliminary evaluation on mixed inoculant and host specificity. Twenty six isolates originated from different maize and rice rhizosphere of different locations, were evaluated for it's effectiveness on plant perfomance within 3 experiments. Six isolates were considered effective and selected for further evaluation. One experiment with 15N technique was carried out to evaluate single isolates vs multi isolates in plant perfomance, N yield and fertilizer use efficiency. The mixed inoculant *Azospirillum* (LS 2+BtJ. No. 18 + BtJ. No. 52-b)were superior as compared to mixed inoculant comprises of 2 isolates, and single isolates. This combination resulted highest N yield and N fertilizer use efficiency (14%). No specificity between three maize genotypes and 2 *Azospirillum* tested.

Keywords : Azospirillum isolates –effectivity- maize – acid soil-host specificity

INTRODUCTION

Maize is one of the important foodcrops in Indonesia after rice and soybean. The government gave most attention by carrying intensification program since 1974. These efforts resulted in the increase of cultivation area, productivity and grain yield. Within 20 years, from cultivation area of 2.572.000 ha became 3.109.000 ha with productivity two times in 1994. Eventhough , maize production of 6.868.885 ton was reached, an amout of 1.118.000 ton was imported to fulfil the demand for maize in the same year (Set Bimas, Dept. of Agriculture, 1998).

To obtain high grain yield high amount of N fertilizer is needed to obtain high grain yield, depending on cultivar or hybrids an amount of 200 – 300 kg of urea used

The use of *Azospirillum* and other genera of plant growth promoting rhizobacteria (PGPR) as biofertilizer to increase production in cereals, such as wheat, rice, and maize has been investigated. The mixed PGPR inoculant for rice and wheat had been produced and used widely by farmers in Pakistan (NIBGE, 1998), while *Azospirillum lipoferum* strain CRT 1 for maize had been marketed as Azogreen in France (Fages, 1994) and it's inoculation increased maize grain yield in Togo was reported (Okon and Labandera-Gonzales, 1994)..

Extensification of agriculture land out of Java island which predominated by red yellow podsolic exposed foodcrop cultivation to soil acidity. Rauf, *et al.,* (1998) evaluating *Azospirillum* inoculation on maize grain yield in several soil types,

indicated that the lowest grain yield was obtained in Bone-Bone podsolic. Furthermore, Gandanegara, *et al*. (2001), found out different plant response to different *Azospirillum* isolates in term of grain and N yield in acid soil. These results emphasized the important study on the selection of effective *Azospirillum* associated with maize in acid soil.

To predict the successful plant bacteria combination, rapid screening in the greenhouse is needed prior to testing in the field as suggested by Bashan, *et al.*, 1990.

This paper reported some activities in the greenhouse in selecting the effective *Azospirillum* associated with maize in acid soil.

Selection of effective isolates

The high ability to survive and adaptability of microorganism in acid soil will determine the successful of bacterial interaction with plant host. Experiments with other soil microorganism, *Rhizobium* showed that bacterial with such capability attached easily to plant root. This result was supported by Vargas and Graham 1989, which showed that strain *Bradyrhizobium* tolerant to acid soil proliferate faster and attached easier to plant roots than sensitive strains.

Twenty five isolates originated from maize root and one isolate from rice root washed were used for evaluation in 3 experiments. Maize seedlings cv. Bisma inoculated individually with these isolates and grown in acid soil from Karangrejo with pH 4.5 and Al saturation 68%. Plants were harvested at 3 – 4 weeks depending on the experiment. The effect of *Azospirillum* inoculation on plant growth were measured in fresh and dry weight, and increase in total plant dry weight.

In general inoculation of *Azospirillum* isolates enhanced maize plant growth, either on roots or shoot weight. Some isolates stimulated more on root growth while the others stimulated more on shoot growth. Increase in these part were ranging from 1-56% depending on the experiments (Figure 1, 2, and 3). The increase in plant weight due to the enhancement of mineral and water uptake followed the morphological and physiological changes in roots by the inoculation as suggested by Fallik, *et al*., 1994 ; Okon and Labandera-Gonzales, 1994.

In experiment 1, the effect of inoculation was shown in the increase of total plant and shoot dry weight ($P<0.10$), with the increase of shoot weight ranging 30 - 90 % over control. The highest increase obtained by inoculation with isolate BtJ. 21 followed by BtJ. 18 and BtJ. 24. The bigger volume of soil used in the experiment 2 and 3 resulted better of plant growth and smaller increase of plant dry weight which ranging 1 - 19% at experiment 2. The effect of inoculation could be observed on root and shoot fresh weight ($P<0.10$) and shoot dry weight ($P<0.05$). Plant inoculated with isolates BtJ. 46-c and BtJ. 52-b showed higher shoot fresh weight (8.27 and 8.05 g/pot) and shoot dry weight (0.88 and 0.89 g/pot) as compared to control with 5.42 g/pot and 0.75 g/pot of shoot dry weight, respectively.

The effect of inoculation were more pronounced at longer observation time (4 weeks) in experiment 3, and significant increase of fresh and dry weight of shoot were seen at $P<0.05$. *Azospirillum* isolates Az LS 2, Az 1 and 7, BtJ. 45, and 50 resulted plant growth and 31-55 % of increase of shoot weight was obtained.

^{15}N experiment

One experiment was carried out to evaluate different *Azospirillum* inoculants on plant growth, N yield, and N fertilizer use efficiency. Three selected isolates from previous experiments were used as single isolate inoculants (Az LS 2, BtJ. 18, and BtJ.

No. 52-b) and their combination in mixed inoculants. ^{15}N dilution technique was used to measure the N fertilizer use efficiency in maize plants at 6 weeks.

The effect of inoculation was shown in Table 1 and 2. Control plants had low root and shoot dry weight, 0.97 g/pot and 2.09 g/pot, respectively. Significant increase in shoot dry weight were obtained by inoculation with any single isolates, mixed inoculant with two or three isolates as compared to control. Mixed inoculant consisted of (Ls 2 + BtJ. 18 + BtJ. 52-b) was superior in plant perfomance, N yield and the most efficient in the use of N fertilizer. The lowering effectiveness of dual inoculants (Ls 2 + BtJ. 18) and (BtJ. 18 + BtJ. 52-b) were probably attributed to antagonism action of isolate BtJ. 18 when mixed with two isolates (LS2 or BtJ. 52-b).

Host specificity

Reports mentioned that the host specificity between *Azospirillum* isolates and maize genotypes is still controversial. The general opinion mentioned that *Azospirillum lipoferum* predominantly colonizes root of C4 plants, while *Azospirillum brasilense* predominantly colonize C3 root plants (Dobereiner, *et al*., 1986). Similar host preference was found in 3 maize genotypes with A. *lipoferum* and *A. brasilense* (El-Komy, et al., 1997). On the other hand, studies with *Azospirillum* inoculation of wheat and maize indicated no differences between different cultivars in growth yield under field condition (Zambre, *et al.,* 1984).

Our result indicated no host specificity between 2 selected *Azospirillum* isolates (BtJ. 46-c and BtJ. 52-b) and three maize cultivars (Ardjuna, Bisma, and local variety) using rainfed Seputih Raman ultisol at 3 weeks old plants. Differences on growth parameters measured due to plant genotypes ($P<0.05$) and inoculation ($P<0.10$). To confirm this phenomenon of host specificity further study is needed.

CONCLUSION

1. Results from 3 experiments on selecting the effectivity of *Azospirillum* associated with maize showed that six out 26 *Azospirillum* isolates were effective and will be used for further evaluation
2. ^{15} N study on evaluating single isolates vs mixed inoculants showed that the mixed *Azospirillum* isolates (LS2+BtJ. 18+BtJ.52-b) was the most effective and efficient in using N fertilizer.
3. Preliminary study indicated no host specificity between isolate BtJ. No. 46-c and 52-b with 3 maize varieties tested.

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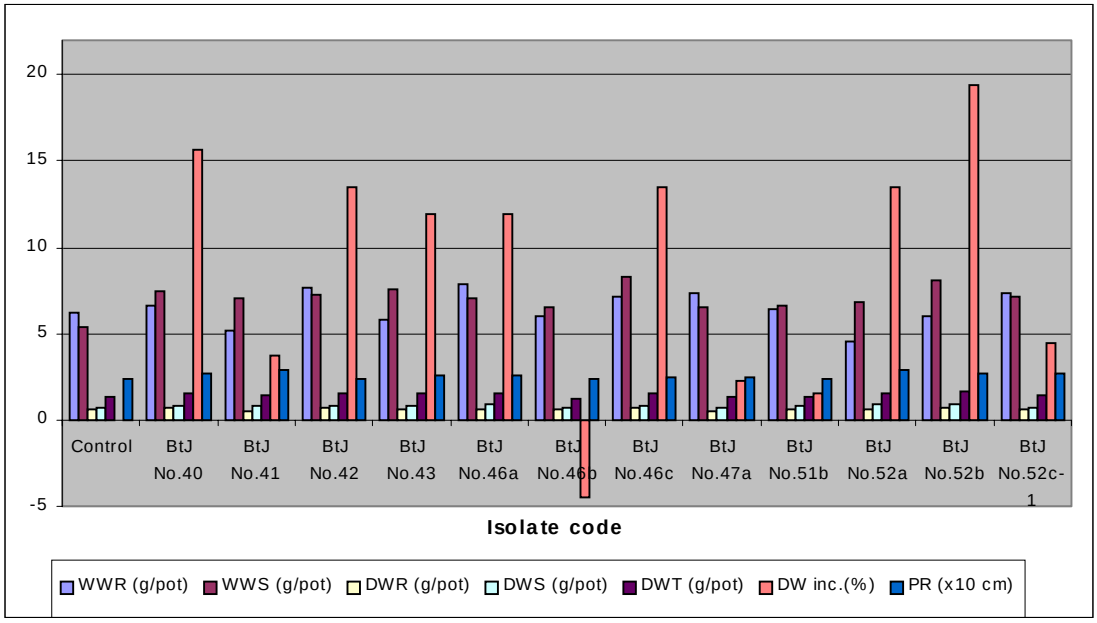
Figures 1, 2, and 3. The effect of *Azospirillum* isolates on plant growth.



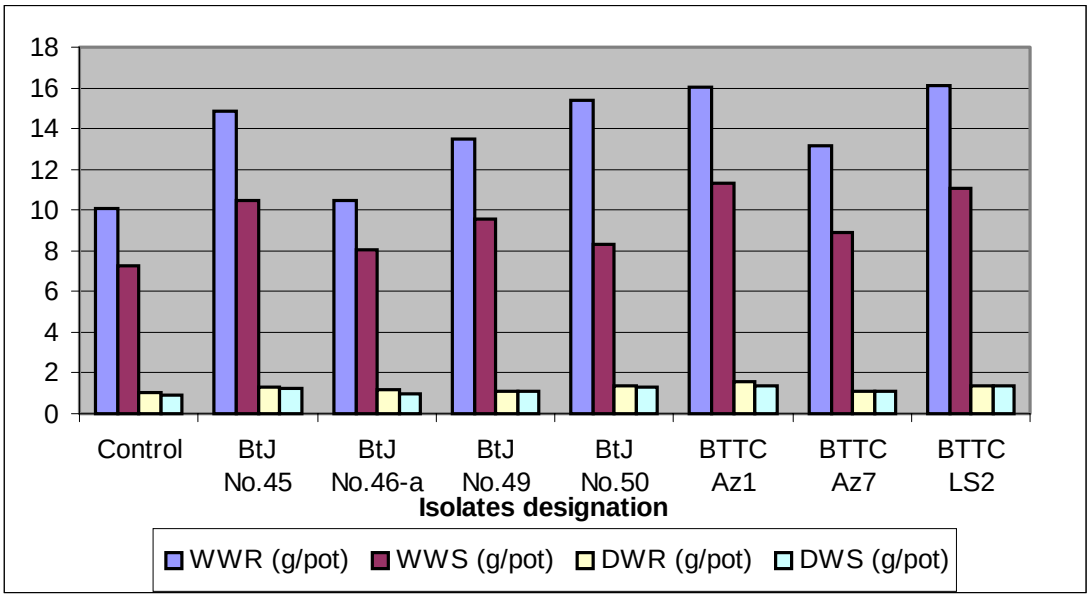


Table 1. The effect of inoculation of single and multi isolates of *Azospirillum* on plant perfomance.

Inoculation	Root DW, g/pot	Shoot DW, g/pot	%N	N yield, mg N/pot
Az LS 2	1.61	4.13	2.40	98
BtJ. 18	1.46	3.20	2.39	75
BtJ. 52-b	2.03	4.52	2.33	105
Az Ls 2+ BtJ. 18	1.14	2.90	2.68	76
Az LS 2 + BtJ. 52-b	2.74	5.10	2.24	113
BtJ. 18 + BtJ. 52-b	0.44	3.15	2.49	76
Az LS2+BtJ. 18+BtJ. 52-b	2.64	5.49	2.31	183
Control	0.97	2.09	2.51	33

Table 2. Parameters 15N isotopic

Inoculation	^{15}N a.e.	Ndff,		N FUE,
		%	mg N/pot	%
Az LS 2	2.55	54	53	12
BtJ. 18	2.44	52	39	8
BtJ. 52-b	2.39	51	54	11
Az LS 2 + BtJ. 18	2.41	52	53	11
LS 2 + BtJ. 52-b	2.31	49	55	12
BtJ. 18 + BtJ. 52-b	2.27	49	38	8
Az LS 2+BtJ. 18 + BtJ. 52-b	2.38	51	65	14
Control	2.35	50	25	4

Table 3. The effect of plant genotypes and *Azospirillum* isolates on some growth parameters.

Genotypes	Inoc.	RootFR, g/pot	RootDW, g/pot	ShootFR, g/pot	ShootDW, g/pot
Arjuna	control	14.05	8.28	0.65	1.19
	BtJ. 46-c	15.50	8.98	0.76	1.33
	BtJ. 52-b	14.63	8.55	0.76	1.30
Bisma	control	13.16	6.38	0.56	1.10
	BtJ. 46-c	14.13	7.83	0.76	1.29
	BtJ. 52-b	14.40	7.19	0.70	1.24
Local	control	10.90	3.96	0.47	0.91
	BtJ. 46-c	12.79	4.89	0.48	1.05
	BtJ. 52-b	13.47	5.44	0.47	1.22