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Effectiveness of P-Solubilizing Bacteria as Biofertilizer for Soybean in Acid Soil

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Abstract – **The study was conducted in the greenhouse and in the field on the Ultisol of East Lampung Province during the rainy season of 2011. The objective was to obtain an effective isolate of phosphate solubilizing bacteria that able to improve the productivity of soybean in acid soil. In the greenhouse, treatments were arranged in a split plot design with three replications. The main plot was the equivalent dose of lime: 0, 400, and 800 kg dolomite / ha. Subplots consisted of factor I which was P fertilizer equivalent to 0, 100, and 200 kg SP36/ ha, and factor II inoculation of phosphate-solubilizing bacteria (*Pseudomonas sp*), namely without inoculation, isolate P1, isolate P2 and isolate P3. The field experiment used split plot design with three replications. The main plots were the SP36 doses: 0, 100 and 200 kg SP36/ha. The subplots were phosphate solubilizing inoculation: without inoculation, isolate P1, isolate P2, isolate P1 + P2 and a commercial inoculant. Results of the greenhouse showed that isolates P2 increased grain yield by 19% and when combined with 800 kg dolomite/ha increase yield by 42% compared to control (without fertilizer P and P-solubilizing). Whereas P1 isolate was effective in dose of 400 kg/ha dolomite, with a yield improvement of 26%, followed by P2 (21%) and P3 (17%). Without P fertilizer, P2 isolate was able to increase yield by 23% equivalent to grain yield obtained from 200 kg SP36/ha. The highest yield increase (50%) was obtained by the treatment of P2 isolates + 200 kg SP36/ha. On the field experiment at fertilization rate of 100 kg SP36/ha, P2 isolate was able to increase yield by 48.7% followed by the commercial inoculant of 33%, isolates of P1+P2 of 20% and P1 isolate of 16% over the control treatment. Fertilizer alone at a dose of 100 kg SP36/ha did not improve yield, whereas yield increased at dose of 200 kg SP36/ha was 24.8%.**

Keywords – **P-Solubilizing Isolates, P-Fertilizer, Lime, Soybean, Acid Soils.**

I. INTRODUCTIONS

Soybean production area in Indonesia will have to be expanded to the newly opened area on acid soils available outside Java island, especially on the island of Sumatra. Constraints faced on acid soil is the low availability of nutrients, especially phosphorus (P) which is the second major nutrient after nitrogen needed for plant growth and grain yield. P deficiency decreases the activity of photosynthesis per unit leaf area by 30% and whole plant leaf area by 90% (Qiu and Israel 2006) and photosynthate supply to the roots which in turn inhibits root nodule formation and activity (Tsvetkova and Georgiev 2003).

P nutrient availability to plant is highly dependent on soil pH with the optimum pH range of 6.5-7.5 (Mitchell et al. 2000). On acid soils with high level of Al, Fe and Mn, the P fixation occurs at a form of Al-P, Fe-P and Mn-P which is poorly soluble and causes P becomes unavailable to plants. When the low soil P availability is due to a high soil fixation, then fertilizer alone would not be effective. Tawari et al. (1980) reported that fixation of fertilizer P on Alluvial soil rich in Al and Fe reached 81 to 93% and greatly reduced the efficiency of use of P fertilizer. The same case was reported by Goldstein (1986) stating that effectiveness of P fertilization in acid soils was low because most of the P (70-90%) was transformed into the form that was not available.

Solubilizing of phosphate by microbes is an important process in natural ecosystems, especially on agricultural land. Some types of microbes such as bacteria, fungi and actinomycetes were reported active in the conversion of insoluble phosphates into soluble phosphates (Chabot et al., 1996; Pal, 1998). In acid soil there are some microbes that effectively solubilize P but are less useful because the amount is not high enough to compete with other microbes that are also located around the plant roots (Jose et al. 2001). Therefore the use of effective P solubilizing microbial P as an inoculants (applied in the amount higher than the number of indigenous microbes in the soil) would be expected to overcome the problems of P fixation in acid soil.

Some researchers reported that certain bacteria were more active than other types of microbes in the conversion of P (Igual et al. 2001; Sadia et al. 2002; Thakuria et al. 2004). Bacteria of the genus Bacillus and Pseudomonas can mobilize P previously is not available to the plant through the mechanism of dissolution which then increases the availability of P to plants (Richardson, 2001). Inoculation of *Bacillus pantothenticus* and *Pseudomonas pieketti* was able to increase rice grain yield by 55% and 76% over yield of control (Thakuria et al. 2004). Khalil (2000) reported that inoculation of P solubilizing microbes increased the availability of P from rock phosphate applied to the soil in twenty days, i.e. from 0.67 ppm to 17.78 ppm. In sugarcane fertilization with rock phosphate fertilizers accompanied by P-solubilizing microbial could save the use of P fertilizer by 25%, and 50% use of expensive fertilizers superphosphat could be replaced with a cheaper rock phosphate (Sundara et al. 2002). On soybean, the use of P-solubilizing biofertilizer was able to increase the available soil P, P uptake and grain yield (Suryantini and Kuntyastuti 1998; Suryantini 1999). The objective of the present research was to identity an effective isolates of phosphate solubilizing bacteria that in turn be able to improve the productivity of soybean in acid soils.

II. MATERIALS AND METHOD

The first study was conducted in the greenhouse of Indonesian Legumes and Tuber Crops Research Institute Malang to evaluate the effectiveness of P-solubilizing bacterial isolates and the second study was conducted in the field as a further test to obtain an effective isolates on the natural environment to increase solubility of soil phosphorus and the productivity of soybean in acid soil.

Research in the greenhouses used soil taken from the same location of field trials, it was upland acid Ultisol in the eastern part of Lampung province, the southern part of Sumatra island. Before planting, the soil was analyzed to obtain the data of pH, organic C, P, Al, Fe, Mn and natural population of P-solubilizing microbes. Treatment was arranged in a split plot design with three replications. The main plots were the equivalent dose of lime: 0; 400; and 800 kg dolomite/ha. Subplots consisted of factor I which was P fertilizer equivalent to 0; 100; and 200 kg SP36/ ha, and factor II inoculation of phosphate-solubilizing bacteria (*Pseudomonas sp*) i.e. without inoculation, isolate P1, isolate P2 and isolate P3.

Soil was air-dried and pounded, then put into each pot, at amount 5 kg / pot. Lime was applied three days before planting by mixing it with the soil in pot. Isolates in a solution of "nutrient broth" was applied at planting by pouring into the planting hole. Each isolate at a dose of 10 ml / pot containing 10^9 cfu (colony forming units) per milli liter solution of "nutrient broth". P fertilizer and base-fertilizer equivalent to 50 kg Urea + 100 kg KCl / ha, was applied at planting closed to the planting hole. Soybean of Anjasmoro variety was planted 4 seeds/pot then at 10 days later were thinned to 2 plants/pot. Watering was done regularly to keep the soil moisture. The variable measured were plant height, number of pods and seed weight per plant.

The field experiment was conducted in upland acid Ultisol in East Lampung during the rainy season of 2011. Treatment was arranged in a split plot design with three replications. The main plots were P fertilizer consisted of: A) no fertilized, B) 100 kg SP36/ha and C) 200 kg SP36/ha. The subplots were P-solubilizing inoculation consisted of: 1) without inoculation, 2) isolates P1, 3) isolates P2, 4) isolates P1 + P2 and 5) commercial inoculant. Treatment plot size was 4 mx 5 m, plant spacing of 40 cm x 15 cm, two plants per hill. Soybean variety planted was Anjasmoro.

Data were collected at the vegetative phase (45 dap) from sample of 5 plants per plot for plant dry weight and P content in the plants. At harvest samples of 10 plants per plot were measured for plant height and number of pods per plant, while the harvested plots of 3 m x 4 m was observed for number of harvested plants and grain yield.

III. RESULT AND DISCUSSION

Soil analysis of samples from research sites in Desa Sukadana of East Lampung indicated marginal land; from the soil is very acidic (pH<4), very low organic matter content (<2%), N, P and K is low (Table 1). Population of P-solubilizing bacteria is low, below the effective number. According to Sabarudin et al. (2011) P-solubilizing bacteria was able to increase the availability of P in the soil when the population reached 1 x 10^9 cfu / g soil.

Results from the greenhouse experiment showed a significant difference in effectiveness between isolates. There were interactions between isolates, lime and P fertilizer treatments of all observed variables. On acid soil, soybean tolerance Al toxicity at the Al saturation ≤ 20% (Arya, 1990), in this study to reduce the Al saturation to 20% requires an addition of dolomite limes as 4 grams per pot, or equivalent to 800 kg per hectare as dolomite dose recommendation. Apparently no effect of lime on Anjasmoro soybean variety, but the interaction of lime with P-solubilizing isolates was significant on all observed variables.

Plant growth

Plant height was influenced by interactions between P-solubilizing bacteria and dose of dolomite (Table 2). Application of dolomite up to 800 kg / ha did not increase plant height, however bacterial isolates P2 and P3 on treatment without dolomite increased plant height compared to that of control treatment. Highest plant height were obtained from a treatment 400 kg dolomite / ha + P2 isolates, followed by that of 400 kg dolomite / ha + P1 isolate, whereas the combination of dolomite with P3 isolates did not increase plant height.

Plant height was also affected by the interaction between P-solubilizing bacteria and dose SP36 fertilizer. Without P fertilizer, all isolates were able to increase plant height resulted in higher than plants in the control treatment (Table 3). However, the highest plant height was obtained from the combination treatment of 100 kg SP36/ha + P2 isolate, followed by treatment of 100 kg SP36/ha + P1. While the combination of P3 isolate with SP36 did not give benefit in increasing plant height.

Component of yield and grain yield

There was an interaction between P-solubilizing bacterial isolate with the rate of dolomite for number of pods per plant. Application of dolomit up to 800 kg/ha did not increase the number of pods. P-solubilizing isolate P2 without dolomite increased number of pods by 18% from 21 pods/plant (control) to 25 pods /plant (Table 2). The combination of isolates with dolomite generated higher number of pods and the highest (41 pods / plant) was obtained from treatment of isolate P2 + 800 kg dolomite / ha, an increase of 49% compared to controls. Whereas P1 isolate was effective in combination with doses of dolomite 400 kg / ha with 39 pods / plant or an increase of 38%, followed by P3 isolate increased 18% compared with that of control.

Number of pods was also influenced by the interaction between isolates with doses of P-fertilizer. Without P-fertilizer, the three isolates (P1, P2 and P3) increased the number of pods compared to the control treatment, but the increases was relatively low. The highest number of pods was obtained from the combination treatment of 100 kg SP36/ha + P1 isolate and 100 kg SP36/ha + P2 isolate, with an increase of about 30%, from 30 pods / plant (control) to 36 and 38 pods per plant. Number of pods of those combination treatment equivalent to that obtained from 200 kg SP36/ha fertilization (Table 3).

Increases in grain yield showed a similar pattern with the increase in number of pods per plant and was influenced by the interaction between P-solubilizing isolates with a rate of dolomite (Table 2). Treatment without dolomite, isolates P2 and P3 increased grain yield by 19% and 6% over control treatment, whereas P1 isolates did not affect grain yield. However, at dose of 400 kg dolomite per hectare isolate P1 was more effective than other isolates and was able to increase grain yield by 26%, followed by isolates P2 and P3 with 21% and 17% respectively. At a rate of 800 kg dolomite / ha P2 isolates showed the highest yield increase (42%), whereas two other multi isolates showed yield decreased. This suggests that isolates P2 had an advantage, effective in acid soil either limed or not, compared to other isolates.

P-solubilizing isolates also showed significant interaction effect with P fertilization on grain yield (Table 3). Treatment without P-fertilizer, isolates P1, P2 and P3 demonstrated their effectiveness by improving grain yield 4.4%, 23% and 9% over the control treatment. At fertilization of 100 kg SP 36/ha, all isolates improved yield higher than that obtained from fertilized P alone. Soybean yield on treatment isolates P2 was equivalent to yield at 200 kg SP36/ha fertilized. More efficient use of fertilizer P was indicated by combination of 100 kg SP36/ha with P1 and P2 isolates which was able to provide higher yields than the yield at 200 kg SP36/ha fertilization. At 200 kg SP36/ha P2 isolate gives the highest yield increased of 50% compared to that of control and 25% higher than that fertilized with 200 kg SP36/ha. Conversely the two other isolates showed effect of a decrease in yield.

The field experiment

Best of isolates obtained in greenhouse experiment was further tested on the field of dry land Ultisols in East Lampung. The same soybean variety Anjasmoro. Lime was applied at a rate of half recommendation (400 kg dolomite / ha) based on the best results in the greenhouse. The results showed significant interaction between P-solubilizing biofertilizer with dose of P-fertilizer for variable of plant height, plant dry weight, number of pods per plant and grain yield yield.

Plant growth

Plant height increased with increasing the rate of P fertilizer, but the highest value was obtained at 100 kg SP36/ha combined with P2 isolates, followed by the combination of 100 kg SP36/ha with P1 (Table 4). The increase in plant dry weight also showed the same pattern with the increase in plant height. The highest weight of plant dry matter was obtained from treatment of 100 kg fertilizer SP36/ha + P2 isolate, followed by treatment of 100 kg SP36/ha + P1 isolates (Table 4).

Component of yield and grain yield

The use of P fertilizer increased the number of pods per plant with increasing dose applied, but the highest number of pods obtained at 100 kg SP36/ha combined with P2 isolates, followed by combination with P1 isolates (Table 4). Commercial inoculant increased the number of pods at fertilizer of 0-100 kg SP36/ha but the increase is lower than the other two isolates.

Increase in grain yield of soybean same as in the greenhouse experiment was supported by the improvement of the vegetative (plant height and plant dry weight) and number of pods per plant that showed the same pattern of increase with an increase in grain yield. In the treatment without P-fertilizer, P1 and P2 isolates able to increase grain yield 13% and 11% compared to controls. However, the highest yield obtained in combination treatment 100 kg SP36/ha + P1 isolates P1 and 100 kg SP36/ha + P2 isolates with increased respectively 23% and 26.8% compared to controls. This result is equivalent to grain yield at 200 kg SP46/ha fertilization. Unlike the isolates tested, commercial inoculum conversely not be able to increase soybean yields.

Significant interaction between P-solubilizing isolates with P fertilization on increasing grain yield is closely related to plant P uptake, indicated by a significant positive correlation ($R^2 = 0.81$) between seed yield with plant P uptake (Figure 1). Effective P solubilization from inorganic forms of P-to P-insoluble inorganic soluble so easily absorbed by plants led to increased P uptake and grain yield. Sandeep et al. (2008) reported that increased crop productivity is also due to the role of P nutrients in the root growth and with increasing levels of P then the more extensive root causing uptake of the elements other than P also increased.

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Table 1: Biological and chemical analysis results of Ultisol East Lampung before planting.

P-solubilizing bacteria cfu/g tanah	pH H2O	C-org %	N %	P2O5 ppm	K	Ca	Mg	Al-dd
					Me/100 g			
56 x 10^5	3,95	1,13	0,10	8,18	0,06	1,01	0,49	0,87

Table 2: Effect of dose of dolomite and P-solubilizing isolates on plant height and dry weight, number of pods per plant and grain yield of soybean in acid upland soils. Pot experiment. Malang 2010.

Treatment		Plant height	Plant dry	Podfilled	Grain yield
Dolomite (kg/ha)	Isolate	(cm)	matter (g)	number/ plant	(g/plant)
0 (control)	Without	51 f	4.38 def	31 de	6.10 ef
	P1	54 e	4.72 bcd	30 e	6.05 f
	P2	59 bcd	5.22 a	35 c	7.25 c
	P3	58 cd	4.48 cdef	32 d	6.45 d
400 kg/ha	Without	55 e	4.20 f	31 de	6.50 d
	P1	61 b	4.78 bc	39 ab	7.70 b
	P2	63 a	4.98 ab	37 b	7.40 c
	P3	55 e	4.56 cde	35 c	7.15 c
800 kg/ha	Without	55 e	4.28 ef	33 d	6.35 de
	P1	59 bcd	5.12 a	35 c	6.45 d
	P2	60 bc	4.76 bc	41 a	8.65 a
	P3	57 d	4.36 ef	32 de	6.60 d

Values followed by different letters in a column were significantly different ($P < 0.05$), using Duncan's multiple range test.

Table 3: Effect of dose of P fertilizer (SP36) and P-solubilizing isolates on plant height and dry weight, number of pods per plant and grain yield of soybean in acid upland soils.

Treatment		Plant height (cm)	Plant dry matter (g)	Podfilled number/ plant	Grain yield (g/plant)
SP36 (kg/ha)	Isolate				
0 (kontrol)	Without	49 e	4.26 e	30 d	5.70 h
	P1	56 c	5.16 a	32 cd	5.95 g
	P2	58 bc	5.28 a	34 c	7.00 c
	P3	57 c	4.34 de	33 c	6.20 fg
100 kg/ha	Without	54 d	4.32 de	30 d	6.45 ef
	P1	60 b	4.66 bcd	36 b	7.55 b
	P2	62 a	4.96 ab	37 ab	7.80 b
	P3	57 c	4.56 cde	34 c	7.00 c
200 kg/ha	Without	57 c	4.26 e	36 b	6.85 cd
	P1	57 c	4.78 bc	37 ab	7.00 c
	P2	62 a	4,72 bc	40 a	8.55 a
	P3	56 c	5,50 cde	32 cd	7.00 c

Values followed by different letters in a column were significantly different ($P < 0.05$) using Dunkan's multiple range test.

Table 4: Effect of dose of P fertilizer (SP36) and P-solubilizing inoculant on plant height and dry weight, number of pods per plant and grain yield of soybean in acid upland.

Treatment		Plant height (cm)	Plant dry matter (g)	Podfilled number/ plant	Grain yield t/ha
SP36 (kg/ha)	Inokulant				
0 (control)	Without	59 e	2.45 d	27,6 i	1,60 c
	P-1	60 de	2.62 d	29,9 gh	1,81 b
	P-2	61 d	3.52 c	31,6 f	1,78 b
	Comersial	62 d	3.31 c	30,5 g	1,71 bc
100 kg/ha	Without	59 e	2.51 d	29,1 h	1,72 bc
	P-1	68 b	4.64 b	36,8 b	1,97 a
	P-2	70 a	5.23 a	40,8 a	2,03 a
	Comersial	65 c	3.41 c	31,8 f	1,67 bc
200 kg/ha	Without	61 d	3.59 c	36,8 b	2,00 a
	P-1	60 de	2.67 d	34,6 c	1,67 bc
	P-2	67 b	4.74 b	37,0 b	2,03 a
	Comersial	60 de	3.68 c	33,0 de	1,75 bc

Values followed by different letters in a column were significantly different ($P < 0.05$) using Dunkan Duncan's multiple range test.

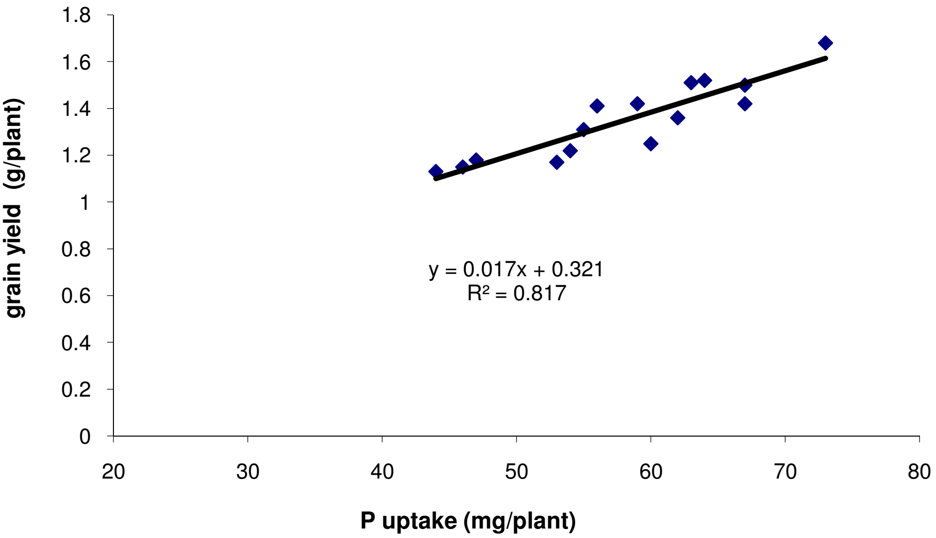

Fig.1. Relationship between P uptake and grain yield of soybean