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Soybean Response to Mycorrhiza, Rhizobium, P-Solubilizing Bacteria and Lime Application in Ultisol Soil

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***Abstract* – Availability of phosphorus (P) and nitrogen (N) in acid soils are generally low because P is transformed into the form of P that are not available to plants, while low of N can be caused by the slow process of organic N mineralization in acidic conditions. The study was aimed to assess the effect of lime and synergistic effect between biofertilizers mikorhiza, rhizobium, and P-solubilizing bacteria on soybean grown in Ultisol soil. The experiment was conducted under greenhouse conditions with a split plot design and six replications. The main plot was the equivalent dose of lime: 0 and 800 kg dolomite / ha. The sub plot was biofertilizer: 1) without biofertilizer, 2) mycorrhiza (VAM), 3) P-solubilizing bacteria (PSB), 4) Rhizobium, 5) VAM + PSB, 6) VAM + rhizobium, 7) PSB + rhizobium, and 8) VAM + PSB + rhizobium. The result showed a synergistic effect of Rhizobium, PSB and VAM in increasing growth, root nodules dry weight, N and P uptake, grain yield and yield components of soybean. The highest increased in grain yield (102%) over control (without biofertilizer and lime) was obtained from the combination of the three biofertilizers + lime application.**

***Keywords* – Lime, Rhizobium, P-solubilizing Bacteria, Mycorrhiza, Soybean, Ultisol.**

I. Introduction

Soybean productivity is determined by the availability of nutrients, mainly nitrogen (N) and phosphorus (P). Availability of P is highly dependent on soil pH with the optimum pH range 6.5-7.5 6,5-7,5 (Mitchell et al., 2000). In 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 (Alves, 2005). It is estimated that more than 80% of P fertilizer applied to the soil is quickly transformed into the form of P that are not available to plants. While the low of N can be caused by a decrease in the mineralization of organic-N to inorganic-N due to declining activity of microorganisms in acidic conditions (Mullen, 2005). An alternative way to overcome the deficiencies of N and P in acid soils is to improve the availability of nutrients and the ability of plants to reach and uptake nutrients by utilizing soil microbes such as rhizobium, P-solubilizing bacteria and mycorrhizal fungi.

Root-nodule bacteria, commonly known as rhizobia, have the ability to fix atmospheric nitrogen in the symbiosis with leguminous plants. Rhizobium inoculation on Ultisol was able to increase plant height, number of pods, 100 seed weight as well as grain yield of soybean (Tampubolon, 2008). The same thing was reported by Nisa et al. (2001), that inoculation of acid tolerant rhizobium in Ultisol increased soybean grain yield of Anjasmoro and Tanggamus variety by 135% and 97% respectively compared with non inoculation treatment. Combined inoculation of rhizobium and P-solubilizing bacteria on acid soils increased the number and weight of root nodules, seed yield and yield components of soybean and was more efficient than farmers conventional technologies, it was also reported that the technology saves 40-60 kg N and 60 kg P2O5 kg / ha and obtained economic efficiency (MBCR) to 43.98% (Ngoc Son et al., 2007).

Phosphate solubilization by microorganisms is an important process in natural ecosystems, especially in agricultural land. Some types of microbes such as bacteria, fungi and actinomycetes were reported active in the conversion of insoluble phosphate into soluble phosphates. Some researchers reported that 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 of the form is not available through the mechanism of solubilization and increase the availability of P for plants (Richardson 2001). Inoculation of P solubilizing Bacillus and Pseudomonas pieketti pantothenticus were able to increase rice grain yield by 55% and 76% over control (not inoculated). Khalil (2000) reported that inoculation of P-solubilizing microbe was able to increase the availability of P from rock phosphate applied into soil in twenty days, ie from 0.67 ppm to 17.78 ppm. Rock phosphate fertilization coupled with P-solubilizing microbial inoculation can save the use of P fertilizer on sugarcane by 25% (Sundara et al. 2002).

Vesikular arbuscular micorrhiza (VAM) has an important role in helping to increase the uptake of P by plants due to the increase in absorption capacity through the external hyphae of VAM which is infect plant roots (Mosse 1981). In P deficient soil inoculation of VAM on soybean plants, with or without P fertilizer was able to increase grain yields by 20% -50%, whereas the application of P fertilizer alone did not increase grain yield (Jalaluddin, 2005). Rhizobium inoculation on soybean plants increase mikorhiza fungal colonization in the root zone (Xie, 1995). VAM inoculation on peanut enhancing nodule weight and plant N levels (Devi and Reddy, 2001). This suggests a synergistic relationship between mycorrhizal and Rhizobium. The study was aimed to assess the effect of lime and synergistic effect between biofertilizers mikorhiza, rhizobium, and P-solubilizing bacteria on soybean grown in Ultisol soil.

II. Materials and Method

The study was conducted in the greenhouse of Indonesian Legumes and Tuber Crops Research Institute Malang during May to August 2012. Treatment was arranged in a split plot design with six replications (three replicates were for destructive sampling at the vegetative phase and the rest three replicates were maintained until harvest at the mature age of plants). The main plots were the equivalent dose of dolomitic lime: 0 and 500 kg /ha. Subplots was inoculation treatment: 1) without inoculation, 2) mycorrhiza (VAM), 3) P-solubilizing bacteria (PSB), 4) rhizobium, 5) VAM + PSB, 6) VAM + rhizobium, 7) PSB + rhizobium, and 8) VAM + PSB + rhizobium.

The soil used was taken from upland Ultisol of East Lampung Province. 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 and then watered to field capacity. Isolates in a solution of "nutrient broth" was applied at planting by pouring into the planting hole. Base fertilizer equivalent to 50 kg urea, 75 kg SP36 and 100 kg KCl per hectare was applied at planting closed to the planting hole.

The inoculants of acid tolerant rhizobium (*Bradyrhizobium japonicum*) and P-solubilizing bacteria (*Pseudomonas sp*) is the collection of ILETRI Laboratory of Soil Microbiology, while the mycorrhizal inoculant was obtained from the Microbiology Laboratory of Gadjah Mada University. Rhizobium and P-solubilizing bacteria inoculant in a solution of "nutrient broth" was applied at planting by pouring into the planting hole. Each inoculant at a dose of 10 ml / pot containing 10^9 cfu (colony forming units) per milli liter solution of "nutrient broth". While mycorrhizal inoculant was applied at a dose of 50 g per pot. Soybean of Argomulyo 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. Data were collected at the vegetative phase (45 dap) for plant dry matter, nodule dry weight, N and P content. At harvest samples were measured for plant height, number of pods per plant and grain yield.

III. Result and Discussion

Ultisol soil of East Lampung was very acidic (pH: 4), very low organic matter content (<2%), low level of N and P, and medium K (Tabel 1). Al saturation (> 20%) was high above the tolerance limit of soybean plants against Al saturation which is about 20% (Arya, 1990).

Table 1: Chemical properties of Ultisol soil of East Lampung Province,

pH H2O	C-org %	N %	P2O5 ppm	K	Ca	Na	Mg	Al-dd	Al saturation (%)
				Me/100 g					
3,95	1,13	0,10	8,18	0,41	0,80	0,10	0,49	1,10	37,9

Nodule dry weight was significantly affected by lime application. Average weight of nodule in soil treated with lime was higher than those of without lime (Figure 1). Without lime, combine inoculation of Rhizobium + PSB showed higher nodule weight compared to those of other treatments (Figure 1). This suggests that both bacteria inoculants were tolerant to acidity and were able to synergize with each other, resulted in positive effects better than its single inoculation. The same thing was reported by Ngoc Son et al. (2007) that the application of Rhizobium bacteria (*Bradyrhizobium japonicum*) and phosphate solubilizing bacteria (*Pseudomonas spp.*) was able to increase number and dry weight of soybean root nodules. The highest nodule weight was obtained in the treatment of Rhizobium + PSB + VAM + lime. Synergism between Rhizobium with PSB and VAM in nodulation can be due to the need of P nutrient for root nodule formation was met by the activity of P solubilization and uptake by the PSB and VAM (Saber et al., 2005). The influence of P on root nodule is indirect, ie deficiency of P will reduce the supply of photosynthate to root for root nodule formation, thus P deficiency will result in a decrease in the number and weight of nodules (Tsvetkova and Georgiev, 2003).

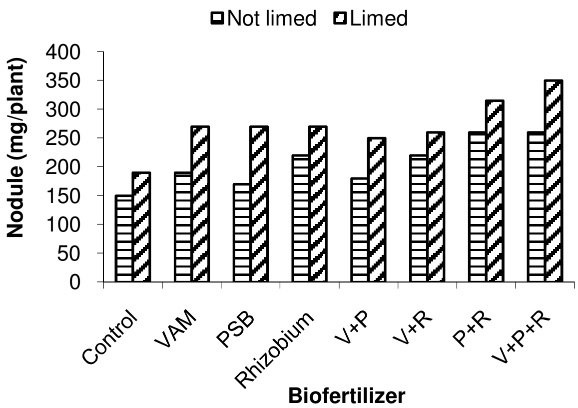


Fig.1. Root nodule dry weight of soybean as influenced by VAM (V), PSB (P), and Rhizobium (R) biofertilizer.

Same as the variable weight of root nodules, there was interaction between lime and biofertilizer on soybean plant height (Table 2). In the treatment without lime each of biofertilizer was able to increase plant height over control, moreover when the biofertilizer was combined with each other. The highest plant height was obtained in the combination treatment of Rhizobium + PSB + VAM, in which the application of lime becomes not useful in increasing plant height.

The increase in plant dry matter was showed a similar pattern with the increase in plant height. On treatment without dolomite, each of the biofertilizer increased plant dry matter over control, moreover when it was combined with each other, the higher increase in plant dry matter was obtained. However, the highest plant dry matter was obtained in the combination of Rhizobium + PSB + VAM, accompanied by lime application.

Table 2: Effect of biofertilizer and lime application on plant height and dry matter of soybean on Ultisol soil.

Biofertilizer	Plant height (cm)		Plant dry matter (g)	
	Not limed	Limed	Not limed	Limed
Control	51,3 h	58,6 cde	6,34 f	8,23 cde
VAM	53,8 g	58,8 cde	6,92 ef	8,25 cde
PSB	55,0 fg	59,7 bcd	8,23 cde	8,45 bcd
Rhizobium	55,0 fg	58,8 cde	7,89 de	8,87 bcd
VAM + PSB	57,3 def	60,8 ab	8,95 bcd	9,26 bcd
VAM + Rhizobium	56,9 ef	60,2 abc	8,68 bcd	9,71 bc
PSB + Rhizobium	58,0 cde	60,8 ab	9,12 bcd	9,78 b
VAM + PSB + Rhizobium	61,0 ab	62,2 a	9,74 b	10,38 a

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

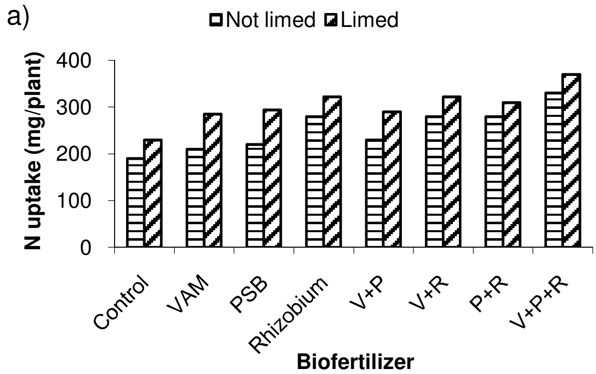
As in the previous variables, there was an interaction between biofertilizers and lime on grain yield. In the treatment without lime, each of the biofertilizers was able to increase grain yield, and when combined with each other the yield was likely to be higher, especially in combination with Rhizobium. It can be seen from the grain yield in the treatment of VAM + Rhizobium and PSB + Rhizobium, which was tends to be higher than in the treatment of VAM + PSB. When the VAM + PSB treatment was added with Rhizobium significantly higher grain yield was obtained with an increase of 55% over control, moreover when it was accompanied by lime application then the highest grain yield was obtained with an increase of 102% compared to that of control (Table 3).

Table 3: Effect of biofertilizer and lime on number of pods and grain yield of soybean on Ultisol soil.

Biofertilizer	Pods number / plant		Grain yield (g/plant)	
	Not limed	Limed	Not limed	Limed
Control	36.2 f	39,2 ef	5,8 f	7,6 cd
VAM	39.5 ef	40,3 de	6,1 de	8,8 bc
PSB	38.8 ef	41,5 cde	6,5 de	8,9 bc
Rhizobium	39.1 ef	45,5 bc	6,2 de	8,6 bc
VAM + PSB	40.7 de	44,2 bcd	7,0 d	8,7 bc
VAM + Rhizobium	41,5 cde	47,3 ab	7,5 cd	9,4 b
PSB + Rhizobium	42.7 cde	47,0 ab	7,6 cd	9,0 b
VAM + PSB + Rhizobium	45.4 bc	50,5 a	9,0 b	11,7 a

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

This is presumably due to low soil N levels (Table 1), thus fulfillment of P only through the application of P fertilizers, PSB and VAM cannot increase grain yield. Urea application of 50 kg / ha appears not been able to meet crop N needs that require a supply of symbiosis N fixation by Rhizobium. This can be seen from the increase in weight of nodules and N uptake in Rhizobium inoculation treatment than those without inoculation (Figur 1 and 2). While the positive effect of lime on soybean productivity improvements could be due to liming not only increased the microbial activity in the supply of P and N fixation but also increased mineralization of soil organic matter so that more crop nutrient needs were met (Fageria et al., 1995; Anetor and Akinrinde, 2006). The increase in grain yield was supported by an increase in the number of pods per plant which showed the same pattern of increase with an increase in seed yield of soybean (Table 3), as well as supported by the increased uptake of N and P (Figure 2).



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b)

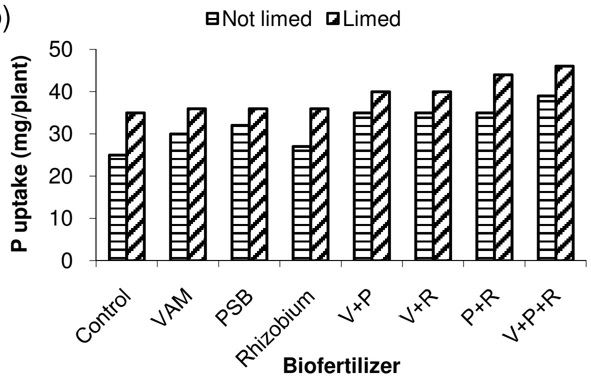

Fig.2. Uptake of N (a) and P (b) of soybean as influenced by Vam (V), PSB (P), and Rhizobium (R) biofertilizer.

IV. Conclusion

Combined inoculation of Rhizobium, P-solubilizing bacteria (PSB) and VAM on soybean grown in Ultisol soil showed a synergistic effect in increasing growth and productivity, moreover when accompanied by lime application.

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