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NUTRIENT UPTAKE IMPROVEMENT OF FORAGE LEGUMES BY ROCK PHOSPHATE FERTILIZATION AND ARBUSCULAR MYCORRHIZAL FUNGI INOCULATION

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ABSTRACT

A series of experiments were carried out during 25 months consisting of greenhouse and field experiments and laboratory analysis. Centro (*Centrosema pubescens*) and puero (*Pueraria phaseoloides*) were inoculated with arbuscular mycorrhizal fungi (AMF) and without AMF and were fertilized with rock phosphate (RP) at dosage of 0, 100, 200, 300 and 400 kg P_2O_5/ha. Split in time in completely randomized design (CRD) in three replicates was used for greenhouse experiment and in completely randomized block design (CRBD) was used for field experiment. The significant difference among treatments was tested using Duncan Multiple Range Tests (DMRT). The results of greenhouse experiment showed that N and P uptake of puero were higher than centro. Nitrogen and P uptake of mycorrhizal legumes were higher than non-mycorrhizal legumes. Rock phosphate fertilization increased N and P uptake of mycorrhizal legumes approximately 9, and 22 times than non-mycorrhizal legumes respectively. The result of field experiment showed that N and P uptake of centro and puero increased after defoliation. However, N and P uptake of puero were higher than centro. Rock phosphate fertilization increased N and P uptake of legumes. Nitrogen and P uptake of legumes were not significantly different for both with or without AMF inoculation.

Keywords : *Centrosema pubescens*, phosphor, mycorrhiza, *Pueraria phaseseoloides*

Centro (*Centrosema pubescens*) and puero (*Pueraria phaseoloides*) are important forage legumes as protein and mineral sources for ruminant livestock in the tropics (Jones, 1990). However, most of the lands that are used for forage production belong to the non productive lands characterized by lack of P content and low soil pH.

The combination of persistent legumes and superphosphate fertilization have been used widely to improve the pasture productivity. However, the high cost of superphosphate fertilizer to be a limiting factor for the pasture productivity. Rock phosphate (RP) fertilization and arbuscular mycorrhizal fungi (AMF) inoculation become a promissing technique.

Centro and puero are suitable host plant for AMF culture (Lukiwati & Supriyanto, 1995) and persistent in non productive land (Latosolic soil) (Lukiwati *et al.*, 1994). Rock phosphate fertilization increased the productivity of mycorrhizal legumes (Lukiwati *et al.*, 1995). The constraint of mycorrhizal technology application in forage crops is caused by limited information on the role of AMF especially in the production and nutritive value improvement of forage crops in Indonesia.

The objective of the research is to study the effect of RP fertilization and AMF inoculation on N and P uptake of centro and puero.

MATERIALS AND METHODS

The experiments were carried out in the greenhouse and in the field conditions. Sterilized soil by gamma irradiation was used for greenhouse experiment. Each pot contained 4 kg air-dry weight soil inoculated with 50 *Glomus* sp spore according to the assigned treatment. Unsterilized soil was used for field experiment. Each plot (4 m x 5 m) was inoculated with 100 g of soil inoculum/hole plant according to the assigned treatment. Rock phosphate and KCl fertilizer (100 kg K_2O/ha) were applied at the time of planting. Urea was added (50 kg N/ha) at 3 weeks after planting for greenhouse and 4 weeks after planting for field experiment.

Split in time in Completely Randomized Design (CRD) for greenhouse and in Completely Randomized Block Design (CRBD) for field experiment in three replicates were used and tested using Duncan's Multiple Range Test (DMRT) by SAS program. The main plots consisted of three combination treatments as follows (1) legume species (centro and puero), (2) AMF inoculation (without and with AMF inoculation), (3) RP fertilizer (0, 100, 200, 300 and 400 kg P_2O_5/ha).

The defoliation period was used twice for greenhouse and three times for field experiment. The first defoliation was conducted three months after planting and the next defoliation was conducted every two months after defoliation. Variable observed were N and P uptake of centro and puero.

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RESULTS AND DISCUSSION

Greenhouse Experiment

The N (Table 1) and P uptake (Table 2) of mycorrhizal puero were higher than that of mycorrhizal centro. Performance of growth and root geometric (root number and distribution in the soil) of each species was difference and as well as the response to the treatments (Kerridge & Ratcliff, 1982; Lukiwati *et al.*, 1994). Greenhouse experiment was used gamma ray sterilized soil. Usually all of microorganism are dead at gamma ray 25-60 kGy level (Alexander, 1964). Legume belongs to the magnolioid roots plant. Therefore, their association with AMF will improved the growth and development of legume plant (Mosse, 1981). Nutrient uptake enhancement in mycorrhizal legumes is most likely due to the external fungal hyphae acting as an extension of the root system. External hyphae length prolong up to 7-10 m/g soil (Allen *et al.*, 1992). Accordingly, the mycorrhizal legumes will absorb the nutrient from the soil and will translocate the nutrient to the host root more efficient (more extensive and better distributed) than the non-mycorrhizal legume (Linderman, 1992). Mycorrhizal fungi usually increase the growth of plant solely by enhancing nutrient uptake (Marschner & Dell, 1994).

Table 1. Nitrogen uptake at two period of defoliation

	Defoliation Period			
	I		II	
	- AMF	+ AMF	- AMF	+AMF
	mg/pot			
Legume Species				
Centro	5.17 c	111.61 b	5.87 c	104.40 b*
Puero	21.26 c	244.87 a	28.70 c	111.86 b
Rock Phosphate				
0	3.29 e	113.12 cd	1.81 e	68.93 cde
100	14.84 e	125.74 cd	12.14 e	56.24 de
200	19.59 e	127.10 cd	20.76 e	144.63 bc
300	14.35 e	220.24 b	25.16 e	124.04 cd
400	14.00 e	305.00 a	26.53 e	146.81 bc

Means followed by a common letter at each treatment are not significantly different at 5% level (DMRT)

Combination of RP fertilization and AMF inoculation caused a higher the N (Table 1) and P uptake (Table 2) than RP fertilization without AMF inoculation. The nitrogen and P uptake of mycorrhizal legumes were higher than non-mycorrhizal one at the same level of RP fertilization. In this case, the effect of AMF inoculation and RP inoculation was synergistic and increased N and P uptake of mycorrhizal legumes approximately 9 and 22 times more than did non-mycorrhizal legumes respectively. Treatments combination of AMF inoculation and RP fertilization could increase plants growth, especially if P nutrient is a major limiting factor to the plant production (Dodd *et al.*, 1990).

The nitrogen (Table 1) and P uptake (Table 2) of mycorrhizal centro and puero decreased at the second defoliation while those of non-mycorrhizal was not significantly affected by defoliation period. Under these condition there is probably competition between the plant and the fungus in photosynthate utilization for host plant regrowth and AMF development. The development of AMF may normally be controlled by photosynthate supply from host plant (Azcon-Aquilar & Bago, 1994).

Table 2. Phosphorus uptake at two period of defoliation

	Defoliation Period			
	I		II	
	- AMF	+ AMF	- AMF	+AMF
	mg/pot			
Legume Species				
Centro	0.09 e	6.17 b	0.12 e	5.33 b*
Puero	0.55 e	12.92 a	0.64 e	6.77 b
Rock Phosphate (kg P_2O_5/ha)				
0	0.03 f	4.02 def	0.05 f	3.24 ef
100	0.31 f	6.42 cde	0.29 f	3.30 ef
200	0.54 f	7.81 cd	0.52 f	7.78 cd
300	0.41 f	12.31 b	0.50 f	7.11 cde
400	0.31 f	17.14 a	0.54 f	8.82 bc

Means followed by a common letter at each treatment are not significantly different at 5% level (DMRT)

Field Experiment

The nitrogen (Table 3) and P upatke (Table 4) of puero were higher than centro after first and second defoliation. Performance of plant growth and root geometric (root number and distribution in the soil) of each plant species are different, as well as their response to the treatments (Kerridge & Ratcliff, 1982; Lukiwati *et al.*, 1994). The nitrogen (Table 3) and P uptake (Table 4) increased after first and second defoliation. Centro and puero have stolon and thereby defoliation promoted new stolon growth (Bogdan, 1977). Defoliation promoted vegetative regrowth of legume as shown in the N and P uptake.

Table 3. Nitrogen uptake at three period of defoliation

Treatments	Defolistion Period		
	I	II	III
	g/m²		
Legume Species :			
Centro	1.96 d	2.57 c	3.99 a*
Puero	1.52 e	3.22 b	3.92 a
Rock Phosphate (kg P_2O_5/ha)			
0	1.53 g	2.38 ef	3.57 c
100	1.84 fg	2.96 de	3.43 cd
200	1.75 g	2.90 de	3.90 a
300	1.87 fg	2.81 e	4.25 b
400	1.71 g	3.41 cd	4.92 a

Means followed by a common letter at each treatment are not significantly different at 5% level (DMRT)

Table 4. Phosphorus uptake of three defoliation period

Treatment	Defoliation Period I	II	III
	..... g/m^2		
Legume Species :			
Centro	0.07 e	0.18 d	0.29 b*
Puero	0.05 e	0.24 c	0.33 a
Rock Phosphate (kg P_2O_5/ha) :			
0	0.06 f	0.18 e	0.28 bc
100	0.07 f	0.22 de	0.28 bc
200	0.06 f	0.22 de	0.30 a
300	0.06 f	0.19 e	0.35 a
400	0.06 f	0.25 cd	0.37 a

Means followed by a common letter at each treatment are not significantly different at 5% level (DMRT)

The effect of RP fertilization was not significantly increased the N and P uptake of forage legumes at the first defoliation. That was because of the legumes plant were still at the initial growth, while RP belong to slow available source of phosphorus.

Rock phosphorus fertilization increased the nitrogen and phosphorus uptake of mycorrhizal legumes. However, both of mycorrhizal and non-mycorrhizal legumes showed a similar respons at the same level of RP fertilization. Response to the AMF inoculation in the field greatly varried (Powell, 1984) or no response (Lin & Hao, 1991). This is because most of agricultural soils already contain mycorrhizal fungi indigenous population. Mycorrhizal inoculation will only be successful in the field if low population and low effectivity of indigenous mycorrhizal fungi or depending upon AMF inoculum potential (Mitiku-Habte & Fox, 1993).

Field experiment was carried out on the unsterilized soil. A year before, cassava had been harvested from the field, since then the field was followed. Cassava plant belong to mycorrhizal-obligat type. Cassava rhizosphere could increase effectivity of indigenous-mycorrhizal fungi (Potty, 1988; Sieverding, 1991). Spore isolation in the beginning of field experiment, there was indigenous population of AMF up to 496 spore/100 g in the soil sample. While a result of spore isolation of soil inoculum we found some spores infected by pathogen fungi that could decreased the affectivity of AMF inoculum (data not presented). Mycorrhizal spore could be infected by pathogen fungi in the storage of soil inoculum (Bagyaraj, 1988). Legume roots in the field experiment could also be infected by non-mycorrhizal fungi.

The results lead to a recommendation that the high potential and effectivity of AMF inoculum combined with RP fertilizer can improve nutrient uptake of forage legumes in the non-productive lands.

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