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Response of Maize (*Zea mays*) to VAM Inoculation as Affected by Different Levels of Rock Phosphate Application

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ABSTRACT

To test the effects of rock phosphate levels (0, 100, 200, 300 and 400 kg/ha) and VAM inoculation on maize, the greenhouse experiments were carried out in sandy soil. The results revealed that the maize positively responded to the rock phosphate levels and VAM inoculation. The total root length, total dry matter, P and K uptake of most plants were increased as P increased in both nonVAM and VAM plants but VAM colonization were decreased as P increased. The maximum VAM colonization on the maize root, 31.80%, was found in the plants inoculated with VAM but no P application. Positive effect of VAM on maize growth in most treatments were shown at 10 weeks but not at 6 weeks. The VAM plants applied with 200 kg/ha of rock phosphate had maximum total dry matter, P uptake and K uptake (136.65 g/plant, 45.93 mg P/plant and 406.63 mg K/plant, respectively) which were higher than both non-inoculated plant at the same rock phosphate application level (10.00, 31.64 and 26.85%, respectively) and those of non-inoculated plant without rock phosphate application (286.72, 591.72 and 66.71%, respectively).

Key words : colonization, inoculation, maize, rock phosphate, VAM (vesicular-arbuscular mycorrhiza).

INTRODUCTION

Many documents are usually attributed to the availability of plant nutrients that will be decreased in both acid and alkaline soil. Phosphate, the most interesting one, is certainly included in this situation. Since the fixation or retention process of phosphate by Fe^{+2}, Al^{+3} in the acid soil and Ca^{+2}, Mg^{+2} in the alkaline soil occur rapidly after application of phosphate to the soil. These processes removed phosphate availability from soil solution, the plant can not take up the fixed P and probably show deficient symptom even in the soil with high level of P. These problems can be deleted by different means such as adjust the soil pH to the appropriated level, liming or utilization of phosphate solubilizing microorganisms. Among these, mycorrhizal fungi inoculation is usually attributed to enhance the plant growth and nutrient availability in soil low in available nutrient of various plants in different soils (Kothari *et al.*, 1990).

Enhancing the plant growth and P uptake were the first recognized beneficial effects of VAM associated with plant root. It was frequently shown that under the limit P condition, inoculation of VAM can improve the plant growth, due to an increased P uptake (Menge *et al.*, 1978., Pacovsky and Fuller, 1986). The increasing of P uptake by mycorrhizal plant root have been attributed to VAM hyphal extention beyond the root. Since VAM comprise the large portion of the soil microbial biomass and can penetrate in the excess of 90

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mm of the root free soil (Camel *et al.*, 1991) and was found that the concentration of inorganic phosphate inside the hyphae is approximately 1,000 times higher than that in the soil solution (Gianinazzi-Pearson and Gianinazzi, 1986). Secondly, reduced distance that phosphate must diffuse in the soil to the roots with hyphae acting as a bypass. Thirdly, increased soil phosphate availability due to the chemical modification of phosphate compound by fungi (Abott and Robson, 1984). Forthly, mycorrhizal roots may produce organic compounds such as citrate and oxalate which contribute to the desorption of adsorbed phosphate or dissolution of poorly soluble phosphorus (Bolan, 1991). However, the infection and effectiveness of VAM in the uptake of plant nutrient depend on various plant and soil conditions (Lambias and Cardoso,1993). Saif (1987) emphasized that plant species showed difference in root infection with VAM and no correlation between root infection and plant growth. There are frequent attributions about the effectiveness of VAM that , as the availability of phosphate in the soil is increased then the VAM infection and growth promoting effects decrease (Pacovsky and Fuller, 1986). In maize, Daniels Hetrick *et al.* (1984) suggested that root colonization of VAM was related to the soil P content. Similarly, cowpea root colonization of VAM was negatively correlated with P in the soil (Rajapakse *et al.*, 1989). On the other hand, VAM colonization on the leucaena root was increased when rock phosphate application increased (Manjunath *et al.*, 1989). Although growth responses of plant due to the infection of VAM frequently do not occur when soil phosphate is high, it is clear that inflow via hyphae can continue and give rise to high P concentration in plant tissue (Smith and Gianinazzi-Pearson, 1988). Thus, the level of P application should be examined with certain VAM species, locations and various plants.

MATERIALS AND METHODS

Completely randomized design of pot experiment in the greenhouse with 4 replications were carried out at the Institute of Plant Nutrition, Justus-Liebig University, Giessen, Germany. Five levels of rock phosphate from hyperphos, 29.96% P_2O_5 (0, 100, 200, 300 and 400 kg/ha), and VAM inoculation (inoculation with *Glomus* sp. T6 and uninoculation) were examined in maize.

Soil and pot preparation

The Podzolic soil that showed very low not only pH but also the availabillity of plant nutrient particularly phosphorus was used as growth medium (pH 3.1, 6.25 mg P_2O_5/100 g, 4.7 mg K_2O/100g, 1.54 mg Mg/100 g, 1.80 mg Mn/kg, 5.00 mg Zn/kg, 0.16 mg Cu/kg and 5.13% humus). Each pot was filled with 6 kg of dried-soil. Before planting, the soil was treated with 11.10 g of $Ca(OH)_2$ in order to adjust the soil pH to 5.0. Two grams of K/pot and 30 mg Mg/pot from K_2SO_4 and $MgSO_4.7H_2O$ were also incorporated. Then, depending on the different treatments, the certain amount of rock phosphate was thoroughly incorporated with the soil.

There were 3 layers of the soil medium in every pot. Six hundred ml of soil was taken from the pot. Three hundred ml of soil mixed with 250 ml of infected expanded clay-VAM inoculum or sterilized expanded clay, for inoculated or uninoculated treatment, respectively,was layered on top of the lowest soil layer in the pot. The uppermost layer of the soil in the pot was filled with the rest of the soil (300 ml) and the soil was pressed firmly by hand. Then, certain amount of water was applied in order to keep the same level of the soil moisture in every pot. It must be considered in each step that the medium must be avoided from chemicals, pathogens and other VAM contamination during preparation.

Planting and cultural practices

The maize seeds (semu 16907; FAO 200) were surface-sterilized by soaking in 0.2% sportak solution for 30 minutes, and rinsed out by distilled water. Ten seeds were pressed downward 2 cm below the soil surface. A certain amount of distilled water was applied every day after planting and kept enough soil moisture level for appropriated plant growth. The plants were thinned to 5 plants at 12 days after planting. In every pot, nitrogen fertilizer was applied as much as 2 g N/pot as ammonium nitrate solution in a certain concentration at 2, 4 and 6 weeks after planting. During growing period, the parathion E 605 was sprayed 2 times at 4 and 6 weeks after planting.

Harvest and statistical analysis

The plants were harvested 2 times, 6 and 10 weeks after planting. After harvest, plant dry matter was evaluated after drying in the hot air oven at 100°C for 48 hours. After dry ashing, the plant nutrients were determined. P was determined using Carl Zeiss PM 7 Spectrophotometer at the wavelength of 450 nm and K was determined using Perkin Elmer 5000 Atomic Absorption Spectrophotometer at the wavelength of 766.5 nm.

The total root length of the plant was evaluated using Comiar Root scanner. VAM-root colonization was examined using Gridline Intersect Method after clearing and staining the root with Trypan Blue (Giovanetti and Mosse, 1980).

The analysis of variance of every parameter as affected by VAM inoculation and rock phosphate was determined. And then, the relevants average mean was also compared at 95% confidence using Duncan's new multiple range test.

RESULTS

VAM root colonization

The percentages of VAM root colonization or infection on the maize root were illustrated in Figure 1 and Table 1. There was no infection of VAM on the maize root in uninoculated plants of both 6 and 10 weeks. The colonizations were proliferated in VAM inoculated plants. At 6 weeks, the colonization of VAM on the maize root was very low, 1.04 - 2.03%, and showed no statistical significance at every rate of P application. But at 10 weeks, level of rock phosphate application had highly significant effect on VAM colonization. The colonization of VAM in plant without rock phosphate application was maximum at 31.8%, and decreased as rock phosphate application increased, 5.58, 3.29, 2.70 and 2.48% at 100, 200, 300 and 400 kg/ha of rock phosphate, respectively.

Total root length

The total root length of maize as affected by VAM inoculation and phosphate application in this experiment (Figure 2 and Table 1) were highly significant for both 6 and 10 weeks of tested plants and similar to the effect of rock phosphate level on the root length of plant. VAM, on the other hand, had no effect at 6 weeks but showed highly significant effect on the total root length at 10 weeks.

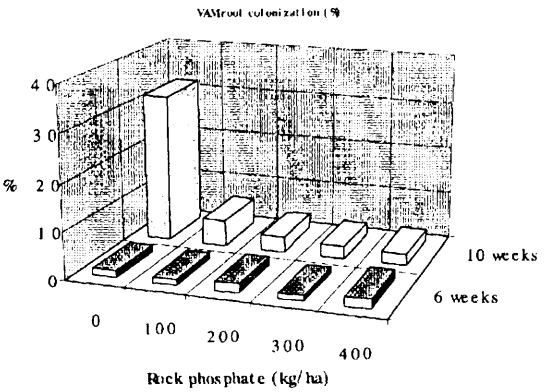

Figure 1 VAM root colonization of maize as affected by VAM and rock phosphate application.

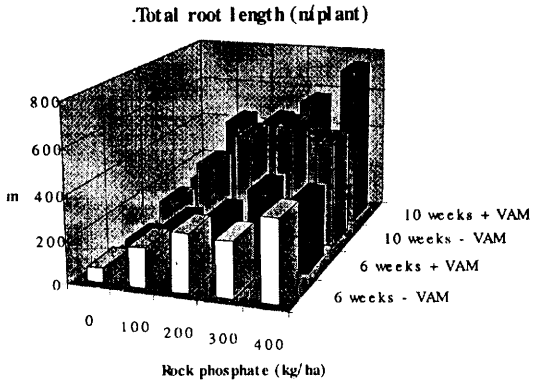

Figure 2 Total root length of maize as affected by VAM and rock phosphate application.

It was clearly shown that the total root length of both VAM and nonVAM plants were increased as rock phosphate increased to reach the maximum at 200, 400 kg/ha for both 6 and 10 weeks. In case of VAM, the total root length of maize was increased in VAM plant at 10 weeks. The maximum total root length of 747.49 m/plant clearly shown the beneficial effect of VAM in inoculated plant over non-inoculated plant, 263.30 m/plant, which was applied with 400 kg/ha of rock phosphate.

Total dry matter

The total dry matter of maizes (shoot and

Table 1 VAM root colonization, total root length and total dry matter of maize applying with VAM and rock phosphate.

	Rock phosphate kg/ha					
	0	100	200	300	400	Average
VAM root colonization (%)						
6 weeks	1.64	1.08	1.95	1.04	2.03	1.55
10 weeks	31.80^{a}	5.58^{b}	3.29^{b}	2.70^{b}	2.48^{b}	9.17
Total root length (m/plant)						
6 weeks -VAM	66.71^{d}	182.15^{c}	268.74^{abc}	254.99^{abc}	375.50^{a}	229.58
+VAM	60.21^{d}	165.75^{cd}	221.09^{bc}	356.41^{a}	331.40^{ab}	226.97
average	63.46^{c}	173.95^{bc}	244.92^{ab}	305.60^{a}	353.45^{a}	228.28
10 weeks -VAM	130.31^{d}	334.78^{c}	490.08^{cb}	546.44^{b}	484.19^{bc}	397.16^{b}
+VAM	132.81^{d}	450.74^{bc}	466.69^{bc}	576.17^{b}	747.49^{a}	474.78^{a}
average	131.56^{c}	329.76^{b}	478.38^{ab}	561.30^{a}	615.84^{a}	435.97
Total dry matter (g/plant)						
6 weeks -VAM	1.75^{g}	6.05^{c}	7.43^{cd}	8.37^{b}	9.51^{a}	6.61^{a}
+VAM	1.70^{g}	5.20^{f}	7.15^{d}	8.08^{bc}	8.90^{ab}	6.20^{b}
average	1.72^{e}	5.62^{d}	7.29^{c}	8.22^{a}	9.20^{a}	6.41
10 weeks -VAM	8.96^{d}	27.78^{c}	31.50^{b}	32.96^{ab}	33.02^{ab}	26.83^{b}
+VAM	8.11^{d}	28.89^{c}	34.65^{a}	34.41^{a}	34.92^{a}	28.19^{a}
average	8.53^{c}	28.33^{b}	33.06^{a}	33.66^{a}	33.96^{a}	27.51

root dry weight) showed in Figure 3 and Table 1 were highly significant for plants of either 6 or 10 weeks. Similarly, the effect of rock phosphate showed highly significance on the total dry matter. On the other hand, the effect of VAM on the total dry matter in this experiment was significant at 10 weeks. Interestingly, nonVAM plant had higher total dry matter than that of VAM plant at 6 weeks. At 10 weeks, the total dry matter of VAM plant turn to be higher. P application stimulated the growth of maizes at both periods. As rock phosphate level was increased, the total dry matter was increased to reach the maximum weight in 6 weeks at 400 kg/ha, but in 10 weeks, the maximum total dry weight was shown at 200 - 400 kg/ha. This phenomenon showed the beneficial effect of VAM drastically on stimulating the growth of plant illustrated as total dry matter. These enhanced effect were 3.15, 1.45 and 1.90 g/plant at 200, 300 and 400 kg/ha, respectively, when the maximum total dry matter was reached as high as 34.41-34.92 g/plant.

P uptake

VAM and rock phosphate application had highly significant effect on P uptake of plant at both growing stages. At 6 weeks, nonVAM plant took up higher P than VAM plant, but 4 weeks later, the effect of VAM on enhancing P uptake was clearly illustrated. In case of P application, the plant took up more P as P application increased (Figure 4 and Table 2). And the contributed P uptake of the VAM plant at 10 weeks was in the order of 2.57, 11.22, 11.14, 4.81 and 0.78 mg P/plant at 0, 100, 200, 300 and 400 kg/ha of rock phosphate, respectively. Therefore, the maximum effectiveness of VAM in this experiment was found to be at the level of 200 kg/ha of rock phosphate to stimulate the P uptake at 45.93 mg P/ plant

K uptake

The K uptake of maizes at different growing stages as affected by VAM inoculation and rock phosphate rate were highly significant and moderately significant at 6 and 10 weeks, respectively. The VAM had nonsignificant effect on K uptake at both growing stages. For P application, the effect were highly significant and moderately significant at 6 and 10 weeks, respectively (Figure 5 and Table 2).

At different rock phosphate levels, the plant took up K in different quantities. The K uptake of plant was increased and reached the peak when 200

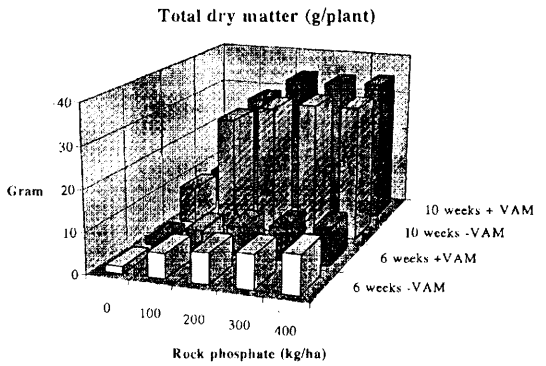

Figure 3 Total dry matter of maize as affected by VAM and rock phosphate application.

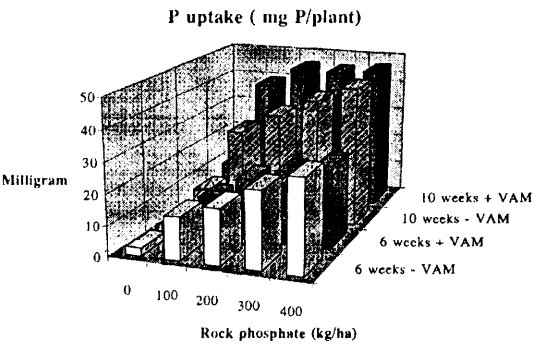

Figure 4 P uptake of maize as affected by VAM and rock phosphate application.

kg/ha of rock phosphate was applied then decreased drastically. Turning to the VAM plant at 10 weeks, even there was no significant effect, it seemed that the K uptake of VAM plant showed higher quantity than nonVAM plant did. The trends of contributed K uptake by VAM were 7.57, 22.29, 86.06, 68.70 and 8.85 mg K/plant at 0, 100, 200, 300 and 400 kg/ha of rock phosphate, respectively. This result suggested that the maximum effectiveness of VAM on K uptake could be found at 200 kg/ha of rock phosphate and ultimately reached the highest K uptake of 406.63 mg/plant.

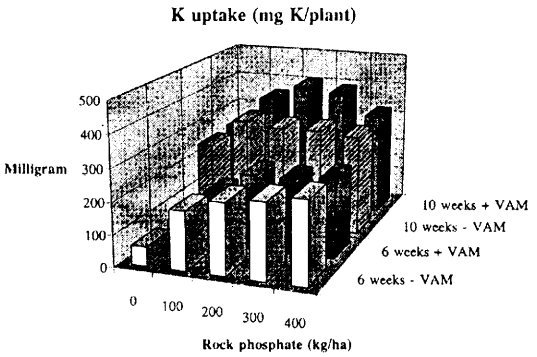

Figure 5 K uptake of maize as affected by VAM and rock phosphate application.

DISCUSSION

From the early growing period to 6 weeks, it has been show that the total root length , P uptake and K uptake of nonVAM plants were higher than those of VAM plants. These might result from the insufficient amount of nutrients used to stimulate their growth which dued to the incomplete infection of VAM. The VAM infection on the plant root occur in many steps, from spore germination, hyphae growing from propagules to appressoria forming at the root surface and the infection units develop within the root cortex to arbuscules or vesicles. These steps are time consuming which depend on

Table 2 P and K uptake of maize as applying with VAM and rock phosphate.

	Rock phosphate kg/ha					
	0	100	200	300	400	Aerage
P uptake (mg P/plant)						
6 weeks -VAM	2.57^{l}	14.08^{g}	18.16^{f}	25.08^{c}	30.33^{a}	18.04^{a}
+VAM	2.53^{l}	12.42^{h}	19.99^{e}	23.56^{d}	26.81^{b}	17.06^{b}
average	2.55^{e}	13.25^{d}	19.08^{c}	24.32^{b}	28.57^{a}	17.55
10 weeks -VAM	6.64^{d}	28.34^{c}	34.89^{b}	40.83^{ab}	45.90^{a}	31.32^{b}
+VAM	9.21^{d}	39.56^{ab}	45.93^{a}	45.64^{a}	46.68^{a}	37.16^{a}
average	7.92^{c}	33.95^{b}	40.41^{ab}	43.23^{a}	46.29^{a}	34.24
K uptake (mg K/plant)						
6 weeks -VAM	60.38^{c}	184.59^{b}	223.93^{a}	239.04^{a}	259.48^{a}	193.48
+VAM	58.82^{c}	165.00^{b}	234.09^{a}	230.87^{a}	246.04^{a}	186.96
average	59.60^{c}	174.79^{b}	229.01^{a}	234.95^{a}	252.76^{a}	190.22
10 weeks -VAM	243.92^{b}	333.66^{ab}	320.57^{ab}	322.38^{ab}	312.26^{ab}	306.55
+VAM	251.49^{b}	355.95^{a}	406.63^{a}	391.08^{a}	321.11^{ab}	345.25
average	274.70^{b}	344.80^{a}	363.60^{a}	356.73^{a}	316.68^{ab}	325.90

host plants and environments. In harmony with the conclusion of Manjunath and Habte (1988) that the infection of leucaena plant in sand-soil mixture in greenhouse was very low at early growing period but increased rapidly and reach a peak of 84% at 30 days after planting. But, Malibari *et al.* (1988) found that the maximum infection of VAM on the maize root was appeared at 55 days after planting. Therefore, the VAM probably parasitized and competed with the plant for nutrients and photosynthates partially from the plant. Consistent with the suggestion of Buwalda and Goth (1982) that the asssociation between VAM and plant called symbiosis may even become parasitic, probably due to the competition for photosynthates between them. However, after the root were completely infected in the appropriate percentages, the beneficial effects of VAM were illustrated that VAM enhanced the plant to have most growth parameters higher than in nonVAM plant. The beneficial effects of VAM from this experiment confirmed the conclusion of many researchers that the increasing of plant growth dued to the beneficial effect of VAM in increasing nutrient uptake particularly P at the appropriate period (Menge *et al.*, 1978., Pacovsky and Fuller, 1986., Sylvia *et al.*, 1933). However, the beneficial effects of VAM in this experiment was decreased as rock phosphate level increased even the plant growth enhancement of VAM was found at high rock phosphate level. They were similar to the reason explained by Sylvia *et al.* (1993) that in the soil with high P, at 66 ppm, VAM made an important condition to the yield of maize.

It was also shown that P uptake of most plants was pararelled to the P application. However, the total dry matter of the VAM plant reached the peak at 200 kg/ha of rock phosphate and then decreased. While the non VAM plant reached the peak of growth at 400 kg/ha of rock phosphate, and the plant dry matter and P uptake were the same as in VAM plant. Therefore, the luxury consumption of P in the VAM plant may occur at the high P levels. Similarly, the length of plant root were still developed further at the high P level meanwhile the plant growth did not develop even with VAM inoculation. These may due to the limiting of nutrient availability in pot experiment and also the decreasing in beneficial effect of VAM at high P level. These concepts have been studied and concluded that the beneficial effects of VAM were affected by the high P level (Lambias and Cardoso, 1993., Raju *et al.*, 1990).

At high P level, K uptake in the plant seemed to be maximized at the peak of plant growth and declined later. This result is agreed with the experiment conducted by Raju *et al.* (1990). With VAM and low P level, the K uptake increased. However, with VAM but high P level, the K uptake become lower.

The infection of VAM on maize roots was decreased as rock phosphate level increased. These may due to the plants received enough available P from the high level of rock phosphate that caused the low important condition and infection of VAM later. This study confirmed the conclusion of many studies that when applied high level of P , the infection of VAM will be decreased (Furlan and Bernier-Cardou, 1989., Rajapakse et al., 1989). However, the percentage of VAM infection was not related to the plant growth, P uptake and K uptake, but showed the capability of VAM at any P level. At the certain level of P, particularly low level, high percentage of VAM infection will enhance the plant growth and therefore, the nutrient uptake of the plant will be higher than that of the low percentage one. Similar to the suggestion of Manjunath and Habte (1988) that, in the soil with low nutrient availability, the pattern of immobile nutrient uptake and accumulation are closely related to those of the development of VAM infection.

CONCLUSION

This experiment showed the advantages of VAM utilization that can reduce a certain amount of P fertilizer particularly in the green house. However, it should be further studied in the field in order to harvest and evaluate the grain yield of various plants in different locations.

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