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EFFECT OF VESICULAR-ARBUSCULAR MYCORRHIZA ON KUDZU (*PUERARIA PHASEOLOIDES*) GROWTH IN PHOSPHATE FIXING KENYA SOILS

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ABSTRACT

A green house experiment was conducted on nine non-sterilized soils with different P sorption capacities, to test the effect of mycorrhiza *Glomus manihotis* on growth of Kudzu (*Pueraria phaseoloides*). A basal application of fertilizer P based on the amount of P required to establish 0.1 μg P ml^{-1} equilibrium soil solution concentration was applied before inoculation with *G. manihotis*. Plant dry matter yield and P uptake were positive in four soils and negative in the rest except two which had no significant differences. Root infection increased significantly in all but one soil. There was no relationship between these parameters and the soil P fixation index. The responses observed were probably related to the effectiveness of the indigenous VAM fungi populations in the soils.

***Key Words*: Mycorrhiza, Kudzu, P-fixation**

RÉSUMÉ

Un expérimentation a été conduite en serre sur 9 traitements de sols non stériles mais avec differentes capacités d'absorption du phosphore afin de tester l'effet de la mycorrhize, *Glomaus manihotis*, sur la croissance de *Pueraria phaseloides*. Une application basale de l'engrais en fonction de la quantité de P requise pour établir une concentration équilibre de 0.1 μg de P ml^{-1} dans le sol a été administrée avant l'inoculation de *G.manihotis*. La corrélation entre le poids sec de matière végétale et l'absorption de P était positive dans 4 sols et négative pour le reste à l'exception de 2 qui n'ont montré aucune différence significative. L'infection racinaire a augmenté de manière significative dans tous les sols à l'exception d'un seul. Il n'y a pas de relation entre ces paramètres et l'indice de fixation du phosphore. Les réponses observées étaient probablement liées à l'efficacité des populations de champignons dans les sols.

***Mots Clés*: Mycorrhize, Kudzu, fixation de phosphore.**

INTRODUCTION

Enhanced plant growth and phosphate uptake were the first recognized beneficial effects of vesicular-arbuscular mycorrhiza (VAM) association (Gerdemann, 1964). The available phosphate (P) status of a soil influences the infection of plant roots by VAM (Baylis, 1967). Widespread P deficiency in most Kenya soils has been attributed to strong P fixation (Hinga, 1973), and to lack of P containing minerals (Nyandat, 1980).

Diffusion of P is extremely slow in soils which adsorb large amounts of P. Mycorrhizae increase nutrient uptake by reducing the distance that nutrients must diffuse to plant roots (Rhodes and Gerdemann,1975). Only a few studies, such as those by Dodd *et al.* (1990), have assessed the effectiveness of VAM in non-sterile soils. Dodd *et al.* (1990) compared the effects of inoculation with VAM fungi of four crops and two phosphate sources and found that the combination of inoculation with VAM fungi and the less soluble P source (Phosphate rock) significantly increased plant yields and was as effective as the more expensive, more soluble, superphosphate alone.

The efficiency of P uptake attributed to the symbiotic association between the plant and VAM should be exploited in order to cut down on the need for fertilizers for the resource poor farmers.

The main objective of this study was to determine the effect of VAM inoculation on growth of Kudzu (*Pueraria phaseoloides*) in different P-fixing non-sterile Kenya soils.

TABLE 1. Soil chemical properties and classification of some Kenya soils.

Sites	-Simba	Kichaka Rongo	Rodi-Kitale	Kopany	Gachoka	Alupe	Kakamega	Kiamokama	Sosiot
Property									
$pH_1:_1H_2O$	5.4	5.2	4.8	4.8	5.2	5.4	5.0	4.6	4.8
CEC (cmol/Kg)	3.9	13.5	5.7	28.7	31.2	21.7	29.5	18.3	7.5
Exchangeable Bases (cmol/Kg)									
Na	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.2	0.2
K	0.3	0.5	0.1	0.4	0.6	1.2	2.0	0.1	1.2
Ca	0.4	2.5	0.9	1.9	4.3	2.1	3.5	0.9	1.0
Mg	0.2	1.3	0.4	1.1	1.4	1.2	1.8	0.2	0.6
Organic C (%)	0.59	1.12	1.76	1.30	1.40	1.57	2.42	2.16	3.33
total N (%)	0.04	0.10	0.17	0.10	0.07	0.12	0.32	0.23	0.34
$P_{ads.}$ maximum* (µg P/g)	55	193	366	380	552	634	660	1316	1465
Mehlich (cmol/Kg)Na	0.1	0.2	0.3	0.8	0.2	0.2	0.2	0.1	0.2
K	0.2	0.3	1.3	0.4	0.8	0.3	0.9	0.7	0.1
Ca	0.6	0.4	0.6	2.5	1.0	1.9	1.9	0.6	0.4
Mg	0.6	0.5	0.6	0.5	1.4	0.6	0.6	0.6	0.6
Mn	0.2	0.6	1.0	0.5	0.4	0.2	0.9	1.0	0.8
P (µgP/g)	2	4	6	9	2	4	6	8	12
Olsen P (µgP/g)	2	9	9	8	4	4	4	3	2
Soil Classification**	Orthic Acrisol	Humic Acrisol	Humic Ferralsol	Rhodic Ferralsol	Pellic Vertisol	Ferralo Orthic Acrisol	Dystro Mollic Acrisol	Dystro Mollic Nitosol	Humic-Nitosol

*P Fixation Index

**FAO/UNESCO Soil Classification based on FURP (1983).

MATERIALS AND METHODS

Nine bulk top soil (0–20 cm) samples from different locations in Kenya, representing different agro-ecological zones were collected and characterised (Table 1). The samples were collected from Fertilizer Use Research Project (FURP) sites. The soils comprised of three Acrisols, two Ferralsols, three Nitosols, and one Vertisol (FURP,1988).

Soil pH was determined in water in a ratio of 1:1. Organic carbon was determined by the Walkley and Black (1934) method and total nitrogen by the Micro-Kjeldahl method (Bremner,1965). Cation exchange capacity and exchangeable bases were determined after sodium and ammonium saturation respectively using solutions of Sodium acetate and Ammonium acetate buffered at pH 8.2 (Chapman,1965). Available Sodium, Potassium, Calcium, Magnesium, Manganese and Phosphorus were determined after soil extraction by the Mehlich *et al.*(1962) method. Sodium, Potassium, and Calcium were determined by flamephotometry. Phosphorus, Manganese, and Magnesium were measured by spectrometry using a Novaspec LKB. Available P was determined by the method of Olsen and Dean (1965). Phosphorus adsorption isotherms (Fig. 1) were constructed for each soil using data generated according to the procedure of Fox and Kamprath (1970) and the maximum P adsorbed calculated from the Langmuir equation for each soil by the Woodruff and Kamprath (1965) method.

The Langmuir equation is expressed as: $C/X = 1/aX_m + C/X_m$,

where:

C= concentration of P in soil solution (µg P ml)

X= P adsorbed /unit weight of soil (µg P/g soil)

a= affinity term

X_m= maximum P adsorbed (µg P/g soil)

Both a and X_m can be used as P fixation indices. The latter was used in this study.

A green house experiment was conducted in a completely randomised design (CRD). Nine soils and two VAM (+VAM and -VAM) inoculation levels constituted the treatments. Each treatment combination was replicated four times. Plastic pots of 12 cm diameter, each filled with 300 g of soil were used. Kudzu was the test crop.

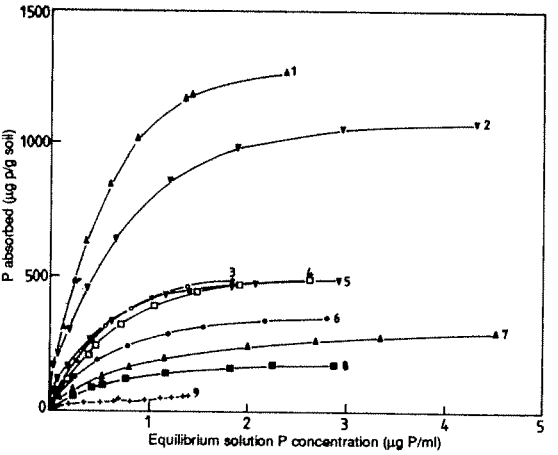

Figure 1. Phosphorous adsorption isotherms for Sosiot (1), Kiamokama (2), Kakamega (3), Rodi-Kopany (4), Alupe (5), Gachoka (6), Kitale (7), Rongo (8), and Kichaka-Simba (9) soils.

Fertilizer P addition was based on the amount of P required to establish an equilibrium P soil solution concentration of 0.1 µg P ml^{-1}. This is considered the highest concentration which can not inhibit mycorrhizal development (Howeler *et al.*,1982). Phosphorus requirement for each soil was calculated from it's P adsorption isotherm and supplied as $Ca(H_2PO_4)_2$ solution in the pot. This was done with the first water application to ensure uniform P distribution in the entire soil. The amount of P required to establish 0.1 µg P ml^{-1} equilibrium soil solution concentration increased with increase in P fixation index. The P fixation indices were greatest in the soils from Kiamokama and Sosiot, moderate in Kakamega, Alupe and Rodi-Kopany soils, low in Gachoka, Kitale and Rongo soils and lowest in Kichaka-Simba soil. A blanket application of potassium (15 mg K_2SO_4/pot) and a cocktail of trace elements was done in each pot. The cocktail of trace elements was supplied by ABM chemicals Limited, Stockport, United Kingdom, and contained Fe, Zn, Cu, Mn, B and Mo. It was applied at a rate of 0.75 mg per pot (5.0 kg ha^{-1}) as recommended by the manufacturers for application to arable crops when the pH is less than 6.5.

The field capacities of the soils were determined in the laboratory. The pots filled with soils were then placed on shallow saucers and watered with distilled water every three days to 60% field capacity and fortnightly to field capacity. This

was ensured by weighing the pots and supplying just enough water to establish the required moisture status. The watered pots were left to stand in the green house for four days before pre-germinated Kudzu seedlings were transplanted. Two Kudzu seedlings of similar size were selected for uniformity and transplanted to each pot, and then thinned after one week to one plant per pot.

VAM inoculum was prepared in open pots filled with steam sterilised sand using mycorrhiza fungus of the species *G.manihotis* and onion and leek as host plants. The spore count for the soil VAM inoculum was 126 spores per 10 g soil. Soil VAM inoculum was sieved and the mixture of spores, mycelia, and chopped roots suspended in 100 ml distilled water. This was then used as VAM inoculum in the planting hole at a rate of 2 ml pot^{-1}. This method of inoculation was justified since direct contact between the roots and the inoculum is essential for mycorrhizal infection to occur.

Kudzu seedlings were also inoculated with *Bradyrhizobium* sp. (Arachis strain NC92) at the time of transplanting to cater for their nitrogen requirement through biological nitrogen fixation. Kudzu plants were harvested eight weeks after transplanting. Shoots were separated from the roots and the latter saved after washing off the soil. Fresh roots were sampled for mycorrhizal infection scores. They were cleared and stained by the method of Phillips and Hayman (1970) and VAM infection assessed by the slide method of Mosse and Giovanetti (1980). Yield components were obtained initially by taking the fresh weights of roots and shoots and the weights after drying at 60°C for 48 hours. Dry matter yield was the sum of the dried root and shoot weights. The dried ground shoots were digested in perchloric acid and the P in tissue determined by spectrophotometry (Allen,1984). Phosphorus uptake in the shoots was then calculated.

One week after transplanting Kudzu seedlings in pots, untreated sub-samples of each of the nine soils were wet-sieved for the recovery and quantitative estimation of indigenous VAM propagules a described by Daniels and Skipper (1984) method. The procedure followed, involved triplicate suspension of 100 g air dry soil samples in tap water in 500 ml beakers and decanting the supernatant solution through a series of sieves with openings of sizes 200, 106, and 63 µm respectively. Suspension and decantation was repeated six times for each soil sample. The material held in the 106 and 63 µm sieves were transferred into petri-dishes and the VAM fungi spores counted under a travelling microscope with X40 magnification.

Analysis of variance was done for dry matter yields, shoot P uptake and percent root VAM infection using a GENSTAT statistical program.

TABLE 2. Effect of VAM inoculation of Kudzu on DMY, shoot P uptake, and root infection, and counts of indigenous VAM fungi spores in the soils with different P fixation indices.

Sites	P-fixation Index (µg g^{-1} soil)	DMY Change (mg pot^{-1})	Root P-uptake (mg P pot^{-1})	Indigenous Infection[a] (%)	VAM counts (no/10 g soil)
Kichaka-Simba	55	-43	-21	+62	7
Rongo	193	+348	+763	+15	10
Kitale	366	+297	+1219	+9	22
Rodi-Kopany	380	-10	-71	+26	23
Gachoka	552	-75	-186	+33	16
Alupe	634	-179	-439	+1 ns	32
Kakamega	660	-137	-33 ns	+42	48
Kiamokama	1316	+272	+315	+32	21
Sosiot	1465	+331	+668	+44	28
Mean	-97	+246	+29	23	
SED	-24	-84	-4	-1	
CV (%)	16.6	25.8	45.0	6.2	

[a]ns-not significant; ANOVA for root infection data was done with arc-sine transformation (changes presented are based on back-transformed data).

RESULTS

Inoculation of Kudzu with *G.manihotis* significantly ($P<0.01$) increased dry matter yields and shoot-P uptake (Table 2) in soils from Rongo, Kitale, Sosiot and Kiamokama, but decreased them in Kichaka-Simba, Rodi-Kopany, Gachoka, Alupe and Kakamega soils. The decrease was not significant for both dry matter yields and shoot-P uptake in two soils and for shoot-P uptake alone in one soil.

Comparison between the proportion of root infected by VAM in Kudzu plants inoculated with *G.manihotis* and those not inoculated was used to assess the effect of inoculation (Table 2). Vesicular-arbuscular mycorrhiza fungal spores were common in all the soils sampled. Counts of indigenous VAM fungi and percent root infection varied significantly in the different soils. The spore counts ranged from 7 to 48 spores per 10 g soil with a mean of 23 spores per 10 g soil. There was no significant correlation between per cent root infection and the counts of indigenous VAM fungi spores. However, a close relationship was observed between the P fixation index and counts of indigenous VAM fungi. Increase in counts of indigenous VAM fungi was associated with increase in P fixation index in all soils except three which included the two most strongly P fixing soils. With the exception of the Alupe soil, *G.manihotis*, significantly increased percent root infection in all the other soils.

The viability of the indigenous VAM fungi spores was not attempted in this study. It was however noted that VAM fungi of the genus *Glomus* was predominant in all soils.

DISCUSSION

This study demonstrates that response to inoculation of Kudzu with *G. manihotis* in different soils is variable, hence the negative mean change in dry matter yields and positive change in mean P uptake, and proportion of root infected.

Soils from Rongo, Kitale, Sosiot, and Kiamokama in which significantly greater dry matter yield changes were obtained were also associated with significant increases in P uptake in the Kudzu plants. It can be inferred from these results that the introduced *G.manihotis* was more effective than the indigenous VAM fungi. The positive response of Kudzu to VAM inoculation in these soils could be attributed to the ability of *G.manihotis* to form extensive mycelia within the soil. This conforms to the observation by Sanders and Sheikh (1983) who found that P uptake by whole root systems partly depends on the extent to which the external mycelium explore the soil volume and the proportion of the roots that are mycorrhizal.

Lack of a response to VAM inoculation in the Kichaka-Simba soil when it had the biggest increase in the proportion of root infection could be due to parasitic effect of the fungi. A parasitic effect occurs when there is competition between the roots and VAM fungi for photosynthates and is associated with greater root infection. The parasitic effect leads to reduced growth of the plant and small dry matter yields. Hypeparasitic fungi depresses sporulation (Schenck and Nicolson, 1977), and decreases the effectiveness of VAM fungi, hence the non-significant change in P uptake found for the Kudzu plants grown in the Kichaka-Simba soil.

The Rodi-Kopany soil is a Ferralsol characterised by poor drainage. The non-significant change in dry matter yield and P uptake obtained for Kudzu in this soil could be attributed to lack of adaptation of *G.manihotis* to the soil conditions especially when the soil moisture status was adjusted to 60% and 100% Field Capacity. This agrees with Gerdemann (1968) who worked with some poorly drained soils of the temperate regions and found that plants grown in these soils rarely had mycorrhizae.

The indigenous VAM fungi in the soils from Gachoka, Alupe, and Kakamega were more effective than *G.manihotis* and caused the negative response obtained in change in dry matter yields and P uptake. These results agree with Kang *et al.* (1980) who showed that soils in which the indigenous VAM fungi are more effective tend to show no response to introduced VAM fungi. In these soils it is probable that competition between *G.manihotis* and the indigenous VAM fungi in infecting Kudzu roots and extending in the soil was a factor in determining the response to inoculation. Other studies such as those by Bethlenfalvay *et al.* (1985) have singled out competition between different VAM fungi species

in the soil as important in the use of VAM fungi in agriculture.

Counts of indigenous VAM fungi spores in the soils had no relationship with the dry matter yields. This conforms to Kucey and Paul (1983) whose results showed that the extent to which VAM fungi in a soil reflects the inoculum potential is uncertain.

The observed increase in indigenous VAM fungi spore counts with increase in P fixation index can be explained by the inverse relationship between the beneficial effects of VAM fungi and the amount of available P in the soil (Sanders and Tinker, 1973). As the P fixation index increases, available P decreases and an environment is created which favours the activities and multiplication of VAM fungi spores.

This study has shown that in a range of dissimilar soils it is not possible to predict plant response to VAM inoculation. However, future studies should evaluate effective VAM fungi in groups of similar soils with similar previous management as this determines VAM fungi population in the soil.

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