Growth and Phosphorus Uptake Responses of Bambara Groundnut (*Vigna subterranea* (L.) Verde) to Inoculation with Arbuscular Mycorrhizal Fungi in two Acid Soils

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Résumé
Asmah, A. E. & Boateng, E. *La croissance et les réponses de l'absorption de phosphore d'arrachide de Bambara (Vigna subterranea* (L.*) Verde) pour inoculer avec les fungus mycorhizes arbuscules dans deux sols d'acides.* L'effet de mycorhize arbuscule sur l'absorption de croissance et de nutriments par arachide de bambara (*Vigna subterranea*) cultivé dans les deux sols acides {les séries *Basachia, (Haplustox)* et *Udu (Gleyic Luvisol*)} a été étudiée dans un phytotron. L'objectif était de déterminer l'ampleur de l'infection dans les racines de plantes de *V. subterranea* par les fungus mycorhizes arbuscules indigènes et son effet sur l'absorption de phosphore et de la production de matière sèche. Les sols ont été analysés afin de déterminer les cations échangeables, échangeables en aluminium d'ammonium, azote nitrique et phosphore disponible. Inoculum fongique mycorhiziens de mycorhizienne a été produite à partir de cultures de pot de fungus mycorhiziens indigènes pour les sols à l'aide d'oignon hautement mycorhiziens plantes dans des échantillons de sols sans stériles. Arachide de bambara a été planté dans les sols avec et sans l'application de l'inoculum. Inoculation des sols avec les fungus mycorhizes arbuscules ont améliorées de manière significative l'absorption du phosphore dans les sols. La production de matière sèche dans les usines mycorhiziens a été sensiblement plus élevée que les plantes non-mycorhiziens. La dépendance de *V. subterranea* sur la condition mycorhiziens pour une meilleure croissance évaluée dans les séries Basachia et Udu sols, étaient 16.95 % et 20 %, respectivement. Il est suggéré que l'association de fungus mycorhizes arbuscules en arachide de bambara pourrait être mobilisée pour améliorer l'absorption des éléments nutritifs tels que phosphore dans les sols avec la fertilité marginale pour accroître les rendements.

Mots-clés : Les fungus mycorhizes arbuscules,*Vigna subterranea*, dépendance mycorhize, absorption de phosphore.

Abstract
The effect of arbuscular mycorrhiza on growth and nutrient uptake by bambara groundnut (*Vigna subterranea)* grown in two acid soils (Basachia series, a Haplustox and Udu series, a Gleyic Luvisol) was investigated in a phytotron. The aim was to determine the extent of infection in the roots of *V. subterranea* plants by indigenous arbuscular mycorrhizal fungi, and its effect on the uptake of phosphorus and dry matter production. The soils were analyzed

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to determine exchangeable cations, exchangeable aluminium ammonium, and nitrate nitrogen and available phosphorus. Arbuscular mycorrhizal fungal inoculum was produced from pot cultures of mycorrhizal fungi indigenous to the soils using highly mycorrhizal onion plants in non-sterile samples of the soils. Bambara groundnut was planted in the soils with and without the application of inoculum. Inoculation of soils with arbuscular mycorrhizal fungi significantly enhanced the uptake of phosphorus in both soils. Dry matter production in mycorrhizal plants was significantly greater than non-mycorrhizal plants. The dependency of *V. subterranea* on the mycorrhizal condition for improved growth evaluated in Basachia series and Udu series soils, were 16.95% and 20% respectively. It is suggested that the arbuscular mycorrhizal association in bambara groundnut could be harnessed to improve the uptake of nutrients such as phosphorus in soils with marginal fertility to increase yields.

Keywords: Arbuscular mycorrhizal fungi, *Vigna subterranea*, mycorrhizal dependency, phosphorus uptake.

Introduction

Mycorrhizas are mutualistic associations between plant roots and soil fungi although they have also been described by Hackskaylo (1972) as a physiologically well-balanced parasitism. Among the different types of mycorrhizas found in nature, the endomycorrhizal type which involves fungi in the *Zygomycota* are the most abundant. The mutualistic association is rare in plant families such as the *Cruciferae, Cyperaceae, Polygonaceae, Chenopodiaceae, Jucanaceae and Proteaceae.*

Mycorrhizas have been recognized as being essential for nutrient cycling and plant survival in terrestrial ecosystems. An important aspect of the symbiosis which is vital for productivity in agricultural systems is the transfer of phosphate via fungal hyphae to the plant facilitated by plasma membrane-spanning transporter-proteins (Nagy *et al.,* 2005). Various workers have reported enhanced uptake of N, P, Zn and Mn by plants (Faber *et al.,* 1990; Chen *et al.*, 2004; Whitfield *et al*., 2004), and protection of roots from pathogenic organisms (Newsham *et al.*, 1995). Under limiting moisture conditions in soils, plant growth has also been found to be greatly improved in the presence of mycorrhizas (Kucey *et al*., 1989; Al-Karaki *et al*., 2004).

Chemical fertilizers, which are an indispensable component of modern soil and crop management practices has not been affordable by many resource-poor peasant farmers in Ghana and this has resulted in the continued practice of shifting cultivation with its long term detrimental effects on the environment (Kleinman *et al*., 1995). Arbuscular mycorrhizas can play a role in such instances by boosting the uptake of poorly available nutrients from soils as soil fertility declines with continuous

uptake of nutrients by crops and via crop residue removal.

On land that has received inorganic fertilizers such as phosphate, the uptake and utilization of the nutrient by a crop stand may only be a fraction of the amount applied during the growing season. The residual nutrients from previous applications could be made available to plants through effective mycorrhiza formation which could ultimately result in reductions in previous rates of application. *Vigna subterranea*, (bambara groundnut) an edible legume grown in all the agroecological zones of Ghana has in recent years received attention as regards its potential to augment farmers incomes, to improve nutrition and to restore soil fertility through nitrogen fixation in an intercrop systems (Ofori *et al.*, 2001; 2005).

Considering the role that arbuscular mycorrhizas play in crop production systems and the benefits that could accrue to farmers with limited financial resources, a short-term investigation was conducted to determine the effect of indigenous arbuscular mycorrhizal fungal infection in the roots of *Vigna subterranea* on plant phosphorus concentration and uptake, dry matter production and the dependency of the plant on the mycorrhizal condition for improved growth.

Materials and methods

Soil samples of two soil series, Udu series (Gleyic Luvisol) and Basachia series (Typic Haplustox), which were selected on the basis of their low concentrations of available phosphorus, low CEC and the differences in soil reaction were used in this investigation. The soils were analyzed to determine some chemical properties (Table 1). Exchangeable cations and effective CEC were determined by the method of Gillman (1979). Ammonium and nitrate nitrogen were determined by Kjeldahl analysis (Keeney and Nelson, 1982). Available phosphorus was determined colorimetrically (Murphy and Riley, 1967) after extraction with anion exchange resin (Biorad AG 21K 16-20 mesh). Exchangeable aluminum was determined after KCl extraction (Pritchard, 1967).

To produce the fungal inoculum, a modification of various techniques described in literature (Dehne *et al*., 1986; Sylvia and Jarstfer, 1992; Munro *et al.*, 1999; Singh, 2003) was used and pot cultures of mycorrhizal fungi indigenous to the soils were established on highly mycorrhizal onion plants in non-sterile sub-samples of the soils. Quartz sand was treated with 2M hydrochloric acid to remove ionic species and biological organisms. The sand was saturated with the acid for 2 hrs after which the acid was drained and washed with distilled water till the effluent pH was the same as that of the

Table 1. Some chemical characteristics of the soils used.

Soil series	pH	CEC	Chemical characteristics Fxchangeable cations Al	Ca	Mg	K	P	Available NH_4-N	NO_3-N
			cmol kg^{-1}					mg kg^{-1}	
Udu	5.31	4.36	0.11	2.32	0.39	0.20	8.78	37.84	23.52
Basachia	4.65	3.27	0.32	1.14	0.33	0.23	4.85	31.33	21.22

distilled water. Samples (500 g) of each of the two soils then were mixed with autoclaved, hydrochloric acid-washed sand in a 3:1 soil to sand ratio and placed in polyethylene pots (15 cm diameter x 15 cm depth). Distilled water was added to bring the soils to saturation and the pots were left for 24 hrs. Seeds of onion (*Allium sepa* cv. *Ailsa Craig*) were surface sterilized by first washing with a surfactant (Tween 80) for 2 minutes and then immersing in a 0.5% sodium hypochlorite (NaOCl) solution for 5 min. The seeds were then washed with several changes of sterile distilled water and sown at four seeds per pot. The pots were placed in a growth chamber (Phytotron) set at 25°C , 70% relative humidity and 12 hr photoperiod.

After eight weeks of growth, plant tops were cut with a scapel and the roots separated from the soil by immersion in four litres of water in a polypropylene containers. Root sub-samples were cleared in 10% KOH, washed with distilled water and acidified with 1% HCl by immersion in the acid for 1 hr. The acidified roots were stained in 0.05% trypan blue in acidic glycerol (Koske and Gemma, 1989). The stained roots were examined microscopically for mycorrhizal fungal infection using the method of Biermann and Lindemann, (1981). The remaining roots after sub-sampling were cut into 0.5 cm pieces and mixed with the soil in which they had been growing to constitute the inoculum which consisted of a mixture of infected roots, spores and soil. The inoculum was then stored in a cold room at 5°C. Samples of the two soils were put through a wet-sieving and decantation procedure to ascertain the presence of spores (Janos, 1984; Norris, 1992).

Soil samples of Basachia (Typic Haplustox) and Udu (Gleyic Luvisol) series which had been sterilized by irradiation with gamma rays (2.5Mrad) at a nuclear facility were amended with N as urea at 8.8 mg kg^{-1} and P as KH_2PO_4 at 2.2 mg kg^{-1}. The low rates of application were chosen to provide nutrients for the initial establishment of

the plants taking into account the low levels of the nutrients in the soils and also the inhibiting effects of high concentrations of the nitrogen and phosphorus on root infection which is well documented (Jasper *et al.,*1979; Amijee *et al.,* 1989; Smith *et al*., 2000; Huat, 2002; Blanke *et al.,* 2004).

Surface-sterilised seeds of bambara groundnut (*Vigna subterranea* L. Verde) were used for the test and treatments consisted of two irradiated soils (Basachia series and Udu series) soils with and without mycorrhizal inoculum. The soils were mixed with the inoculum of indigenous mycorrhizal fungi at the rate of 40g of inoculum per kilogram of soil and watered to a matric potential of –30 kPa by the addition of 200 ml deionised water per kilogram soil at 48 hour intervals. The seeds were sown in similar polyethylene pots as described above and placed in a growth chamber with the following conditions: Temperature – 27°C/24°C (day/night), relative humidity - 70%, photoperiod – 12 hr, luminous intensity – 20,000 lux. Another set of irradiated soils was treated similarly but without inoculation. A sample of the mixed inoculum used was filtered using a mixed-ester membrane filter (pore size, 20um) to block mycorrhizal propagules. The filtrate containing other microbiota was added to the uninoculated soils to minimize differences in soil microbiota between inoculated and uninoculated soils.

Plants were harvested after 35 days of growth when plants had flowered and just before the commencement of pod-filling which is a period of increased nutrient uptake in bambara groundnut. To determine plant dry weight, pots with plants were immersed in water and the whole plants were separated from soil. Fresh weights were determined and root samples were taken after which the plants were oven-dried at 65°C for 72 hrs and weighed. Phosphorus in whole plants was determined colorimetrically by the vanado-molybdate method after perchloric-sulphuric acid digestion (Cresser and Parsons, 1979). Shoot, root phosphorus concentrations and phosphorus uptake were calculated.

To assess root infection, roots were cleared in 10% KOH solution, thoroughly rinsed in distilled water and acidified with 1% HCI. Roots were stained with a solution of 0.05% trypan blue in acidic glycerol (Koske and Gemma, 1989), and stained roots were examined microscopically for mycorrhizal fungal infection using the method of Biermann and Lindemann (1981).

Mycorrhizal dependency was evaluated by expressing the difference in dry weights between mycorrhizal and non-mycorrhizal plants as a percentage of the dry weight of non-mycorrhizal plants (Plenchette *et al.,* 1983).

MINITAB statistical software was used for all statistical analyses. Data were

analysed using analysis of variance procedures and mean comparisons were computed using the least significant difference test.

Results and Discussion

The soils differed in various physical and chemical characteristics (Table 1). Basachia series soil was more acidic in the reaction than Udu series soil and this was a reflection of the ecological zones from which the samples were taken. High acidity in the Basachia series soil from the high rainforest ecological zone is as a result of depletion of basic cations through plant uptake and leaching by rainfall which is over 2000 mm annually (Greenland and Kowal, 1960). Both soils had low CEC and available phosphorus which constitute a major constraint to plant growth. Based on their external morphological characteristics, spores isolated from the soils were identified to be predominantly *Glomus* species and *Gigaspora* species. Quantitative counts of spores are usually not indicative of the infectiveness or effectiveness of the fungal partners to form mycorrhizas since other infective propagules such as fungal mycelia or root pieces with embedded fungal material could also be present.

The mean dry weights of plants grown in inoculated Basachia and Udu soils were greater ($P<0.01$) than those of plants grown in the respective soils without AM fungal inoculation (Table 2). However between the two soils there were no significant differences in dry weights of plants whether they were mycorrhizal or not (Table 2). Increases in plant biomass of many crop plants with arbuscular mycorrihizas have also been reported (Liu *et al*., 2005; Vestberg *et al*., 2005). Growth responses in mycorrhizal plants have been attributed to many factors including changes in hormonal status induced by mycorrhizal fungi infection (Estrada-Luna and Davies, 2003; Fitze, 2005) as well as increased phosphorus nutrition (Schroeder and Janos, 2005).

No significant differences were observed in P concentration of plants grown in Basachia soil compared with those grown in Udu soils. Significantly greater P concentrations were found in bambara groundnut plants grown in Udu soils with inoculation compared to plants grown in uninoculated soil (Table 2). Significant differences in P concentrations of mycorrhizal and non-mycorrhizal plants have also been reported for other plants (Bucking and Shacker-Hill, 2005; Mozafar *et al.,* 2000).

Phosphorus uptake by mycorrhizal bambara groundnut plants was significantly greater than by non-mycorrhizal plants (Table 2). Such increase has been attributed to an enhanced absorption and translocation of P from areas distant from roots by mycorrhizal hyphae. The absorption of phosphate is therefore enhanced by the extension of fungi hyphae beyond the P

Table 2. Effect of inoculation with AM fungi on growth parameters, and mycorrhizal dependency in *Vigna subterranea.*

Soil series	*Dry weight g pot^{-1}*	*Root length cm*	*Root infection %*	*P concentration mg g^{-1}*	*P uptake mg pot^{-1}*	*Mycorrhizal dependency %*
Basachia	0.59	487		1.15	0.67	
Udu	0.60	467		1.16	0.69	
Basachia (I)	0.69	511	45	1.65	1.14	16.95
Udu (I)	0.72	499	47	1.73	1.25	20.00
LSD	0.04	35		0.50	0.17	

LSD: Least Significant Difference
(I): Inoculated

depletion zone (Bieleski,1973; Koide, 1991). The uptake of less mobile elements such as phosphorus is limited by the rate of diffusion through the soil. The potential of arbuscular mycorrhizas to increase the uptake of phosphorus from soils deficient in the element has been found to vary considerably, depending on the fungi isolate, the plant species and its genotype.

There was no AM mycorrhizal fungal infection in the roots of bambara groundnut plants grown in soils without inoculation. Infection levels in plants grown in inoculated Basachia and Udus soils were 45% and 47% respectively and there was no significant differences in root lengths of plants grown in Udu and Basachia soils (Table 2). Root infection per se may not necessarily imply a beneficial interaction between the mutualistic partners since different fungal partners may differ in their effectiveness in conferring known benefits such as phosphorus translocation from areas farther away from host roots. Increased phosphorus concentration as observed in plants grown in Udu soils is an indication of a beneficial interaction and this may probably be due to a favourable soil environment for a specific species or combination of fungal species in comparison with the soil environment in the Basachia series. The existence of functional diversity among arbuscular mycorrhizal fungi suggests that a combination of several species of fungi could increase the effectiveness of phosphate extraction from soil (Koide and Mosse, 2004). It has been observed that under natural conditions in agricultural soils, and in undisturbed sites, VAM fungi present usually belong to more than one genus; many species of

the same genus have also been observed in soils from the same location (Stribley, 1987; Escudero and Mendoza, 2005).

The mycorrhizal dependency of plants grown in Udu series and Basachia series soils were 20.00% and 16.95% respectively and the observation of arbuscular mycorrhizal (AM) fungal structures in the roots of *Vigna subterranea* plants was therefore indicative of the ability of the legume to form mycorrhizas with indigenous AM fungi present in the soils. Plant species differ in their nutrient requirements and root architecture and consequently their dependency on AM fungi vary considerably from one crop to another. This range of plant response to arbuscular mycorrhizas has to be taken into account in the management of cropping systems or a crop rotations (Dalpe and Monreal, 2004). Many factors have been reported to influence the mycorrhizal dependency of plants. These factors include soil type (Menge *et al*., 1978), endophyte species (Jaisme-Vbega and Azcon, 1995) as well as availability and concentration of phosphorus and other nutrients (Siqueria and Saggin-Junior, 2001; Tanaka and Yano, 2005).

Mycotrophy in *Vigna subterranea* plants as evidenced in this investigation implies that yield reductions could occur in the absence of effective mycorrhizal associations. Phosphorus uptake in mycorrhizal plants was greater when compared to those without mycorrhizas in soils with low fertility used in this investigation. In such soils, the manipulation of indigenous strains of VAM fungi to favour the most effective fungal species or the introduction of known effective strains through inoculation procedures could therefore be a viable option to enhance nutrient uptake and yield in *Vigna subterranea*. The potential benefits of arbuscular mycorrhizas could therefore be harnessed in the field under natural conditions to enhance the growth of the legume in soils with marginal fertility.

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