GROWTH AND MINERAL NUTRITION RESPONSES OF MYCORRHIZAL AND NON-MYCORRHIZAL COWPEA, PIGEON PEA AND GROUNDNUT TO PHOSPHORUS SOURCES OF DIFFERENT SOLUBILITIES

B.D.K. Ahiabor[1] and H. Hirata[2]
[1,2]Laboratory of Plant Nutrition, Department of Biological Production,
Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan.
[1]Present address: CSIR-Savanna Agricultural Research Institute, P. O. Box 52, Tamale, Ghana.

ABSTRACT

The effects of a superphosphate (SP) and a rock phosphate (RP), with equal total P contents, on the growth and mineral nutrition responses of mycorrhizal and nonmycorrhizal cowpea, pigeon pea and groundnut were investigated in a pot experiment using an air-dry gamma ray-sterilized (1.5Mrad) Andisol subsoil. Adequate amounts of micronutrients were added to the pot soils as supplements. The plants were inoculated with surface-sterilized 100 spores/pot of Glomus etunicatum or none and with Rhizobium strains. At their maturities, dry weights of plant parts and nodule number, root length, arbuscular mycorrhizal fungal (AMF) colonization of roots and AMF spore production were also measured. Shoot nitrogen and phosphorus concentrations were determined by the Micro-Kjeldahl distillation and ammonium-molybdenum blue methods, respectively. Shoot copper and iron concentrations were measured by the atomic absorption spectrophotometry. Mycorrhiza formation was greater with superphosphate than with rock phosphate treatments. Rock phosphate enhanced mycorrhization in cowpea and pigeon pea but it decreased it in groundnut. Superphosphate-treated cowpea and pigeon pea plants were larger than RP-treated plants whether mycorrhizal or not but in groundnut growth increased only when the plant was treated with SP and inoculated with AMF. Mycorrhizal enhancement of shoot growth in superphosphate-fertilized groundnut could be a direct consequence of improved P nutrition resulting from increased hyphal uptake and/or enlarged absorptive root surface due to increased root fineness. There were similarities in shoot dry matter yields as well as shoot P uptakes of both mycorrhizal and non-mycorrhizal RP-treated plants suggesting that the legumes were so unresponsive to the RP that even mycorrhization could not trigger any response in them. The increased contents of N, Cu and Fe in the shoots of mycorrhizal SP-fertilized plants were observed to be the result of larger shoot sizes except with Fe in groundnut shoot whose enhancement could be related to a good mycorrhiza formation and a concomitant improvement in P nutrition which may further have been influenced by its fine root structure. There was a very close interdependency of fine root structure, AMF colonization, AMFspore production and improved P and Fe nutrition in groundnut as a result of soluble phosphate application.

Keywords: Growth, mineral nutrition, arbuscular mycorrhizal fungi, rock phosphate, grain legumes.

INTRODUCTION

Under similar growing conditions, plant species may differ in their ability to utilize soil phosphates or fertilizer phosphates especially in regions where the use of low-price but sparingly soluble phosphates, such as apatitic rock phosphates or calcined aluminium phosphates, is of importance. The high cost of soluble phosphate fertilizers such as single superphosphate (SSP) or triple superphosphate (TSP) has generated considerable interest in the utilization of rock phosphate (RP) and other P sources that require fewer inputs in their manufacture (Nnadi and Haque, 1988).

Infection of plants by arbuscular mycorrhizal fungi (AMF) to produce mycorrhizas has increased nutrient uptake especially that of phosphorus in soils with low phosphorus content (Mosse, 1973; Gerdemann, 1975). Application of phosphate fertilizer to soil, on the contrary, decreases the percentage of infection of roots with AMF (Hayman *et al*., 1975) and inhibit the ameliorating effect on plant growth (Thomson *et al*., 1986). The ability of arbuscular mycorrhizas to increase the uptake of P from phosphorus sources of different solubilities has generally been assessed at one or two rates of the particular applied P and comparisons have been based on top yield and P uptake at a particular rate of application (Powell and Daniels, 1978). In most of these studies, the rate of application of the insoluble P sources was inadequate for maximum plant growth (Powell and Daniels, 1978). Very little work has been done to compare the responses of mycorrhizal plants to phosphorus sources of different solubilities with equal levels of elemental P.

In this study, the relative effects of two P sources with varying solubilities (a superphosphate and a rock phosphate) but equal effective total P contents on mycorrhizal and nonmycorrhizal cowpea, pigeon pea and groundnut in an Andisol subsoil were estimated. Plant growth and N and P uptake responses as well as Fe and Cu nutrition were assessed and discussed. Treatment effects on root architecture were particularly examined.

MATERIALS AND METHODS

Growth medium

Air-dry subsoil samples of an Andisol (Melanudand) were used as the culture medium. The soil was passed through a 2mm-mesh sieve and was sterilized with gamma ray irradiation at 1.5Mrad. Some chemical characteristics of the soil type used are indicated in Table 1. Ground dolomitic limestone was added to raise the soil's original pH of 5.7 to pH 6.0 {1:2.5 (soil:water)} conducive to both legume growth and rock phosphate dissolution. The soil samples were packed into 16.4cm diameter Wagner pots at 2.4kg per pot. One week before planting, the pot soil was incubated with a soluble phosphate (SP), Ca $(H_2PO_4)_2$ (monocalcium phosphate) and South African rock phosphate (RP), EPL 86 at the rate of 400mg P per pot each. The rock phosphate (17.2% total P solubility and 2.1% solubility in 2% citric acid) was applied in a powder form {0.105mm (48%) – 0.5mm (52%)}. The amount of rock phosphate material added was calculated on total P solubility basis. Simultaneously, all pots received nutrient supplements of 300mg N (Urea), 400mg K (K_2SO_4 and KCl), 50mg Fe ($FeCl_3.6H_2O$), 10mg Cu ($CuCl_2.2H_2O$), 30mg Mn ($MnCl_2.4H_2O$), 25mg B (H_3BO_3), 100mg Zn ($ZnSO_4.7H_2O$), 10mg Mo [$(NH_4)_6Mo_7O_{24}.4H_2O$] and 5mg Co ($CoCl_2.6H_2O$). Treatments were in triplicates and controls (+AMF-SP-RP) were also established. In an earlier preliminary experiment (Ahiabor and Hirata, unpublished work) using the same soil type, absolute control plants (-AMF-SP-RP) died soon and could not complete their normal growth cycle and therefore such treatments were excluded in this work.

Microbial treatments

Each pot soil was pre-inoculated with either 100 spores of *Glomus etunicatum* which was isolated from the topsoil of the experimental farm field of

the Tokyo University of Agriculture and Technology, or none. These spores, which were precultured on clover in pots, were surface-sterilized (Ahiabor and Hirata, 1995) and had a germination rate of 52% (incubated at 28^{o}C for 8 days in a film of water), determined prior to inoculation. Differences in the soil microbiota were minimized by the addition of AMF-free filtrates (Ahiabor and Hirata, 1994; 1995).

Test plants

Cowpea (*Vigna unguiculata* L., cv Kuromame), pigeon pea (*Cajanus cajan* L. [Millsp.) cv ICPL 86009) and groundnut (*Arachis hypogaea* L., cv Nakateyutaka) were used. Surface-sterilized (by soaking in 0.5g L^{-1} NaOCl for 4 min followed by rinsing with sterilized distilled water) seeds of approximately equal weights of the respective crops were nursed on a vermiculite bed at room temperature. Partially germinated seeds with 5cm-long radicles were inoculated with compatible *Rhizobium* strains by immersing them in suspensions of the rhizobia for 30 minutes just before transplanting at two seeds per pot. Cowpea was inoculated with NOKO 704, pigeon pea with NOKO 705 and groundnut with NC 92. After adequate establishment, the plants were thinned to one per pot and grown in the greenhouse to reach their respective maturities: cowpea – 72 days, pigeon pea – 124 days and groundnut – 95 days. The pot soil water regime was maintained at 60% of the maximum water-holding capacity by daily adjustment with deionized water. Greenhouse micro-climate differences due to bench placement were minimized by changing the positions of pots at frequent intervals. Three replicates were harvested at each sampling and all data are reported as averages.

Shoot and root sampling

At maturity, shoots of plants were detached at the soil level and the different organs (stem, leaves and pods) were weighed separately after drying in a forced-air oven at 80^{o}C for 48 hours. The nodulated roots were carefully washed under a gentle jet of ordinary tap water. Fresh weights of roots were recorded after nodules were removed with a pair of forceps and counted. Dry weights of both nodules and roots were measured as for shoots. Before drying, 2g of fresh root samples with segments less than 2mm in diameter were cut into approximately 2cm pieces and stored in formalin acetic acid (FAA) solution (Kormanik and McGraw, 1984) for root length measurement and mycorrhizal analysis. Root lengths were determined with the Comair Root Length Scanner using the equation $A = 0.2246 + 0.9655E + 0.00123E^2$, where A denotes the actual root length and E the averages of three readings. 'A' was corrected for the bulk fresh root with the assumption of proportionality between root length and root fresh weight. The dry weights of these 2-gramme samples were corrected for in the total dry weight.

Arbuscular mycorrhizal assays

Retrieved root samples from the root length measurement procedure were used to assess the extent of root colonization by AMF. The grid-line intercept method (Giovanetti and Mosse, 1980) was used. Cowpea and pigeon pea root samples were cleared in 100g L^{-1} KOH solution (which allows ready stain penetration) at 90^{o}C on a hot plate for 1h but 3h in the case of groundnut roots as its cytoplasm and nuclei were difficult to clear. Prior to staining root samples with 0.5g L^{-1} trypan blue in Lactoglycerol (modified from Kormanik and McGraw, 1984), the adhering KOH solution was removed by neutralizing it with HCl but at the same time ensuring a small amount of acidity as this enhances proper staining. Staining was accomplished at 90^{o}C for 20min for cowpea and pigeon pea and 40min for groundnut roots.

Spores of AM fungi in the post-harvest soils were also estimated. Hundred millilitres (100ml) of tap water was added to 5g samples of air-dry post-harvest soil and stirred vigorously with a sonicator at 100 watts for 2 min to dislodge AMF spores from both soil particles and organic debris. The soil suspension was leached through a 0.053mm-

mesh sieve and spores were retrieved by sucrose (60% solution) density-gradient centrifugation (modified from Daniels and Skipper, 1984) and counted in a film of water under a stereomicroscope.

Tissue N, P, Cu, and Fe analyses

Dried stems and leaves were finely homogenized with a mechanical grinder and digested with concentrated (18 M) H_2SO_4 using 300g L^{-1} H_2O_2 as an oxidant. N and P concentrations in respective aliquots were determined by the Micro-Kjeldahl distillation and ammonium-molybdenum blue (Murphy and Riley, 1962; modified by Watanabe and Olsen, 1965) methods, respectively whereas those of Cu and Fe were measured by the atomic absorption spectrophotometry.

Statistical analysis

Analysis of variance (ANOVA) was conducted on all data using Statistix 7 and treatment means were compared using Duncan's Multiple Range Test (DMRT) at $P = 0.05$.

RESULTS

Degrees of AMF colonization of the three legumes were comparatively high with the more soluble phosphate, $Ca(H_2PO4)_2$ (hereafter referred to as SP) with cowpea being the most vulnerable plant (Table 2). Rock phosphate (RP) application was suppressive on AMF colonization in groundnut.

The AM fungus produced significant high populations of spores only when in association with SP-plants (Table 2). However, in the absence of SP, pigeon pea and groundnut could still support the production of a sizeable amount of spores – about 25% of that measured in SP treatments. Sporulation of the fungus on cowpea was relatively low, especially with RP treatment.

Simultaneous application of SP and AMF promoted a significant biomass production in all legumes (Table 3). AMF inoculation was irrelevant to increased biomass production unless P was in a readily soluble form. A significant nodule biomass was produced when plants were treated with SP and AMF (Tables 3). Pod yield was significant in all mycorrhizal plants treated with SP. Furthermore, in groundnut, irrespective of RP application or AMF inoculation, pod production was not affected (Table 3). Root biomass formed quite a large portion (about 21%) of the total plant biomass in pigeon pea. In all legumes, the form in which P was applied was important in total root length and specific root length responses especially when plants were mycorrhizal (Figs. 1, 2 and 3). In the presence of SP, root elongation in mycorrhizal pigeon pea (Fig. 2) and groundnut (Fig. 3) was remarkably extensive. When P was added as rock phosphate, there was a significant tendency of root shortening in all legumes compared to the no P treatment. In non-mycorrhizal cowpea (Fig. 1) and pigeon pea (Fig. 2) addition of SP increased root fineness but in groundnut this effect was absent. Figures 2 and 3 show the presence of a general positive relationship between total root lengths and specific root lengths (S.R.L.) in pigeon pea and groundnut. This relationship was highly positively correlated in groundnut (R = 0.79).

Mineral nutrition

The concentrations of phosphorus and nitrogen in the stems and leaves of the test legumes are shown in table 4. In the presence of SP, only in groundnut did mycorrhiza significantly increase concentration of P in the plant parts analyzed. Even though application of RP had no effect on shoot P concentrations of the legumes, among RP-fertilized plants only in pigeon pea was mycorrhization generally important in raising the shoot P concentration.

When plants received both SP and AMF inoculation, concentration of N in their tops was comparatively generally lower than in all the other treatments (Table 4). In both mycorrhizal and non-mycorrhizal plants, N concentration in RP-fertilized plants was higher than in SP-treated plants.

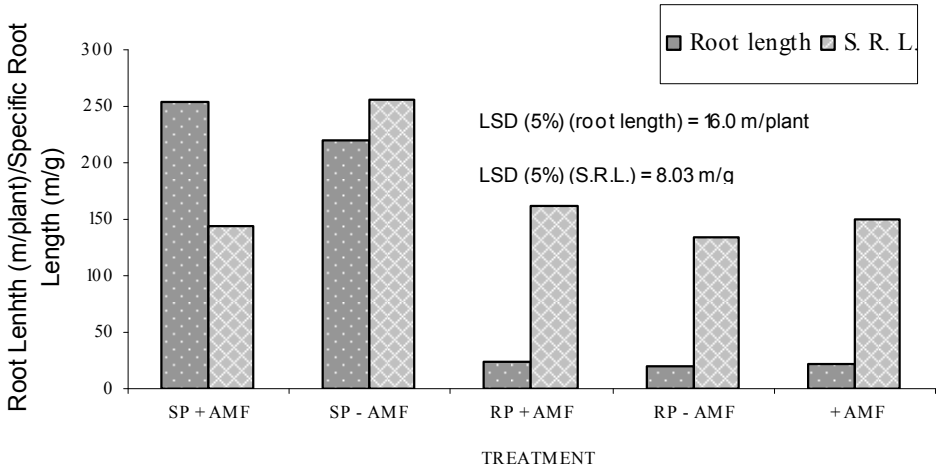

Fig. 1. Root length (m/plant) and specific root length (S. R. L.) (m/g) of cowpea as affected by AMF colonization and phosphorus fertilizer type.

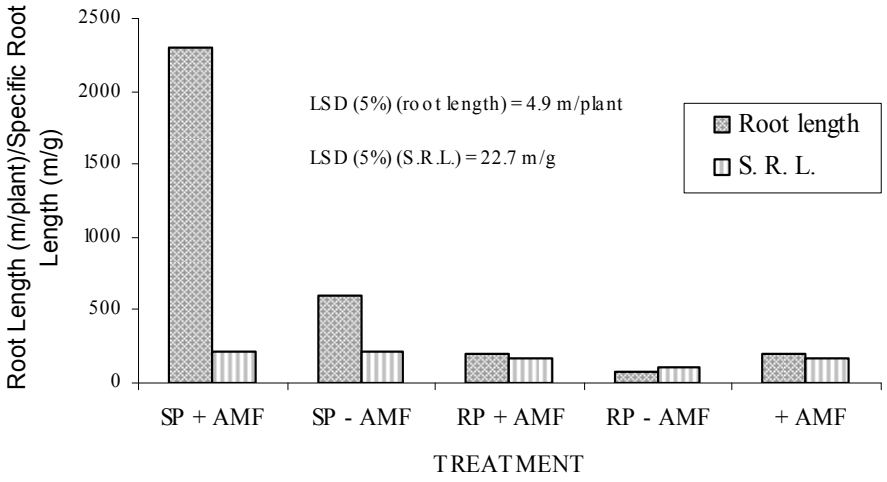

Fig. 2. Root length (m/plant) and specific root length (S. R. L.) (m/g) of pigeon pea as affected by AMF colonization and phosphorus fertilizer type

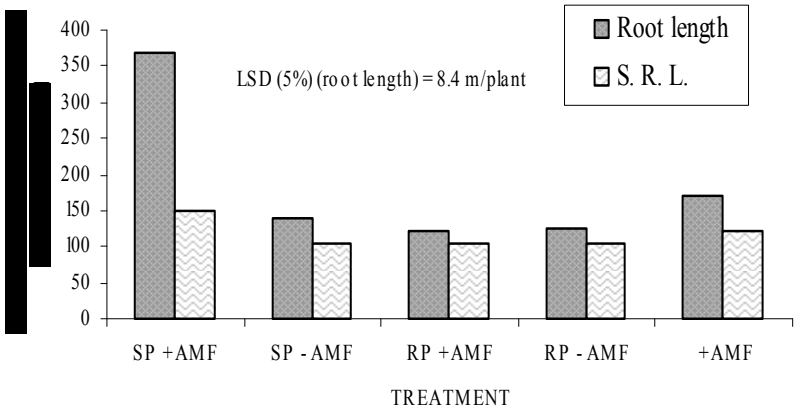

Fig. 3. Root length (m/plant) and specific root length (S. R. L.) (m/g) of groundnut as affected by AMF colonization and phosphorus fertilizer type.

Table 1: Some chemical properties of the Andisol subsoil used

Property	Amount
CEC (meq/100g)	20.7
Exchangeable cations (meq/100g):	
K	0.67 (= 26.1 mg/100g)
Ca	7.30 (= 145.6 mg/100g)
Mg	2.30 (= 27.3 mg/100g)
Soluble cations (meq/100g):	
K	0.11 (= 4.19 mg/100g)
Ca	0.39 (= 7.72 mg/100g)
Mg	0.18 (= 2.23 mg/100g)
Total N (%)	0.08
Total C (%)	0.99
Total P (mg/g)	0.092
Bray I-P (µg/g)	1.04
Bray II-P (µg/g)	2.67

Table 2: Arbuscular mycorrhizal fungal (AMF) colonization rate (%) of cowpea, pigeon pea and groundnut and AMF spore production (no./5 g a.d.s.) in their rhizospheres as affected by P fertilizer type

TREATMENT	COWPEA		PIGEON PEA		GROUNDNUT	
	Colonization rate	Spore number	Colonization rate	Spore number	Colonization rate	Spore number
SP + AMF	54a[1]	365a	40a	789a	41a	780a
SP - AMF	nd[2]	nd	nd	nd	nd	nd
RP + AMF	34b	25b	28b	210b	12c	205b
RP - AMF	nd	nd	nd	nd	nd	nd
+AMF	7c	40b	18c	150b	30b	215b

[1]Values (means of three replicates) with similar letter(s) within a column are not significantly different at *P* = 5% by Duncan's Multiple Range Test (DMRT).
[2]not detected.

Table 3: Dry matter distribution in cowpea, pigeon pea and groundnut as affected by AMF colonization and phosphorus fertilizer type.

		NODULE	ROOT	STEM	LEAVES	POD	TOTAL
CROP TYPE	**TREATMENT**	**(mg/plant)**	**(g/plant)**	**(g/plant)**	**(g/plant)**	**(g/plant)**	**(g/plant)**
COWPEA	SP + AMF	351.20a[1]	1.74a	5.35a	6.81a	8.27a	22.52a
	SP - AMF	9.57b	0.86b	1.40b	2.70b	1.89b	6.87b
	RP + AMF	0.53b	0.18c	0.22b	0.79c	nd	1.19bc
	RP - AMF	0.57b	0.13c	0.13b	0.53c	nd	0.80c
	+ AMF	0.63b	0.16c	0.18b	0.72c	nd	1.06bc
PIGEON PEA	SP + AMF	1273.33a	10.76a	23.19a	11.63a	2.87a	49.73a
	SP - AMF	10.77b	2.55b	3.85b	2.58b	0.19b	9.17b
	RP + AMF	0.07b	0.86c	0.82c	1.35bc	nd	3.02bc
	RP - AMF	nd[2]	0.25c	0.18c	0.31c	nd	0.74c
	+ AMF	nd	0.86c	0.74c	1.37bc	nd	2.98bc
GROUNDNUT	SP + AMF	306.73a	2.47a	8.14a	16.09a	24.40a	51.41a
	SP - AMF	0.80b	1.30b	1.64b	3.06b	2.18b	8.18b
	RP + AMF	0.25b	1.12b	0.89b	2.48b	2.15b	6.64b
	RP - AMF	0.03b	1.16b	1.12b	2.18b	0.77b	5.23b
	+ AMF	0.10b	1.35b	1.24b	3.16b	2.80b	8.55b

[1]For each crop, values (means of three replicates) with similar letter(s) within a column are not significantly different at $P = 5\%$ by Duncan's Multiple Range Test (DMRT).

[2]nd = not detected.

Mycorrhizal SP-treated plants had the highest amounts of P and N in their shoots (Table 5). Unlike in pigeon pea, application of SP alone could not enhance shoot uptake of P in cowpea and groundnut if they were not mycorrhizal. Whether plants were mycorrhizal or not, rock phosphate applications did not have any effect on shoot accumulations of P and N. In pigeon pea, for example, the depressed N content of shoots of non-mycorrhizal RP-fertilized plants was due to a significantly low leaf nitrogen.

Table 6 shows that in all crops concentrations of Cu were considerably lower than Fe concentrations. In mycorrhizal pigeon pea, application of SP remarkably reduced Cu concentration in the shoot (Table 6). However, in inoculated cowpea and groundnut, RP-fertilized plants had higher leaf Cu concentrations than SP-treated ones (Table 6) whereas stem Cu concentration of cowpea remained unchanged irrespective of treatment. There was also no treatment effects on Fe concentration in cowpea tops and in groundnut leaves (Table 6). Whereas the addition of SP or RP together with AMF raised the concentration of Fe in the stem of groundnut compared to the AMF only treatment, the opposite was the case with pigeon pea.

The combined treatment of AMF and SP remarkably raised the contents of Cu and Fe in the shoots of all crops (Table 7) but without mycorrhiza, this fertilizer enhanced total shoot uptake of Cu in cowpea only although stem content

Table 4: Concentrations (mg/g) of phosphorus and nitrogen in the stems and leaves of cowpea, pigeon pea and groundnut as affected by arbuscular mycorrhizal fungal (AMF) colonization and P fertilizer type

	COWPEA				PIGEON PEA				GROUNDNUT			
	Phosphorus		Nitrogen		Phosphorus		Nitrogen		Phosphorus		Nitrogen	
TREATMENT	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves
SP + AMF	0.104a[1]	0.817a	9.72b	10.69c	0.093c	0.546a	4.18b	8.75d	0.208a	0.815a	4.15c	9.46c
SP - AMF	0.033a	0.487a	18.66b	19.92b	0.18ab	0.622a	12.20ab	18.60c	0.094bc	0.326b	12.29b	11.95bc
RP + AMF	0.045b	0.843a	37.82a	30.42a	0.183ab	0.584a	22.13a	31.12ab	0.085c	0.380b	14.52b	14.68ab
RP - AMF	0.056a	0.557a	37.98a	34.46a	0.148bc	0.412b	22.01a	26.31b	0.149b	0.315b	19.11a	16.59a
+AMF	0.175a	0.773a	36.64a	30.74a	0.21a	0.613a	16.59ab	37.26a	0.133bc	0.385b	11.54b	13.02abc

[1]Values (means of three replicates) with similar letter(s) within a column are not significantly different at $P = 5\%$ by Duncan's Multiple Range Test (DMRT).

Table 5: Shoot nitrogen (N) and phosphorus contents (mg/plant) of cowpea, pigeon pea and groundnut as affected by arbuscular mycorrhizal fungal (AMF) colonization and P fertilizer type.

	NITROGEN			PHOSPHORUS		
Treatment	Cowpea	Pigeon pea	Groundnut	Cowpea	Pigeon pea	Groundnut
SP + AMF	115.51a[1]	199.67a	145.5a	5.4a	8.46a	14.77a
SP - AMF	71.81b	81.95b	50.93b	1.32b	2.23b	1.16b
RP + AMF	30.59c	56.07bc	31.93b	0.7b	0.9c	1.01b
RP - AMF	23.63c	11.95c	26.22b	0.31b	0.15c	0.85b
+AMF	28.41c	60.02b	32.08b	0.61b	0.99c	1.39b

[1]Values (means of three replicates) with similar letter(s) within a column are not significantly different at $P = 5\%$ by Duncan's Multiple Range Test (DMRT).

Table 6: Concentrations (µg/g) of copper (Cu) and iron (Fe) in the stems and leaves of cowpea, pigeon pea and groundnut as affected by arbuscular mycorrhizal fungal (AMF) colonization and P fertilizer type.

	COWPEA				PIGEON PEA				GROUNDNUT			
	Cu		Fe		Cu		Fe		Cu		Fe	
Treatment	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves
SP + AMF	8.21	16.11b[1]	402.1	301.6	5.30c	16.18c	143.13b	197.70b	5.96ab	8.31b	1204.83a	438.2
SP - AMF	7.21	14.15b	128.8	299.4	7.24c	18.83c	173.10b	189.03b	3.93b	13.08ab	1042.53ab	363.6
RP + AMF	4.14	23.07a	199.2	343.8	18.42a	24.87bc	365.33b	172.87b	7.64a	13.88a	823.75b	458.33
RP - AMF	5.52	15.00b	394.3	326.6	17.24a	34.35a	872.63a	315.95a	5.85ab	10.88ab	408.57c	424.54
+AMF	8.8	19.29ab	379.3	377.5	17.87a	30.10ab	847.80a	218.20ab	7.39ab	8.65b	434.93c	375.86

[1]Values (means of three replicates) with similar letter(s) within a column are not significantly different at $P = 5\%$ by Duncan's Multiple Range Test (DMRT).

Table 7: Total shoot copper and iron contents (µg/plant) of cowpea, pigeon pea and groundnut as affected by arbuscular mycorrhizal fungal (AMF) colonization and P fertilizer type.

	COPPER			IRON		
Treatment	**Cowpea**	**Pigeon pea**	**Groundnut**	**Cowpea**	**Pigeon pea**	**Groundnut**
SP + AMF	147a[1]	311a	182a	3903a	5595a	16829a
SP - AMF	49b	77b	44b	1030b	1064b	2728b
RP + AMF	19.1c	47b	40bc	272b	478c	1841b
RP - AMF	8.2c	37b	29c	229b	433c	1344b
+AMF	15.3c	54b	36bc	341b	851bc	1725b

[1]Values (means of three replicates) with similar letter(s) within a column are not significantly different at $P = 5\%$ by Duncan's Multiple Range Test (DMRT).

Table 8: Contents (µg/plant) of copper and iron in the stems and leaves of cowpea, pigeon pea and groundnut as affected by arbuscular mycorrhizal fungal (AMF) colonization and P fertilizer type.

	COWPEA				PIGEON PEA				GROUNDNUT			
	Copper		Iron		Copper		Iron		Copper		Iron	
TREATMENT	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves
SP + AMF	39a[1]	108a	1943a	1960a	123a	188a	3296a	2299a	48a	134a	9714a	7115a
SP - AMF	11b	38b	197b	833b	29b	48b	613b	451b	6b	38b	1624b	1104b
RP + AMF	1.1c	18bc	41b	231bc	12bc	35b	231b	247bc	6b	34b	718c	1123b
RP - AMF	0.5c	7.7c	56b	173c	3c	34b	326b	97c	6b	23b	413c	931b
+AMF	1.3c	14bc	73b	268bc	12bc	42b	542b	309bc	9b	27b	546c	1179b

[1]Values (means of three replicates) with similar letter(s) within a column are not significantly different at $P = 5\%$ byDuncan's Multiple Range Test (DMRT).

of Fe was also increased in groundnut (Table 8). In cowpea, this enhanced shoot uptake of Cu was observed to be due to an increased uptake of this micronutrient in the stem (Table 8). Whereas accumulations of Cu and Fe were generally higher in the leaves than in the stems of cowpea (Table 8), in pigeon pea Fe was generally more absorbed in the stems (Table 8).

DISCUSSION

It has frequently been shown that mycorrhizal plants are much more efficient than non-mycorrhizal plants in utilizing rock phosphate fertilizers applied to soil (Singh and Singh, 1993; Graham and Timmer, 1985). It is also well established that application of soluble phosphate fertilizers to soils reduces the growth benefits from arbuscular mycorrhizal fungal infection (Powell and Daniel, 1978; Mosse, 1973). This study, however, showed that mycorrhiza formation was greater with the monocalcium phosphate (calcium dihydrogen phosphate) than with rock phosphate. Rock phosphate addition also increased mycorrhiza formation in cowpea and pigeon pea compared to the no P control but it decreased it in groundnut. This suggests that some amount of external phosphate was necessary for efficient AM formation in cowpea and pigeon pea and this benefit increased when the applied P was in a more readily soluble form (Ahiabor and Hirata, 2003). However, Mosse *et al*. (1976) observed that mycorrhiza infection in *Stylosanthes*, *Centrosema*, clover and onion in soils that ranged from pH 5.3 to 8.1 was unaffected by added rock phosphate.

All SP-treated plants were larger than the counterpart RP-treated plants except in groundnut whose growth increased only when such plants were mycorrhizal. This suggests that unlike cowpea and pigeon pea, growth of groundnut was independent of the type of phosphate fertilizer applied. Groundnut growth was therefore highly mycorrhiza-dependent under the conditions of this experiment. Mycorrhizal enhancement of shoot growth in SP-fertilized groundnut could be

a direct consequence of improved P nutrition (a significantly high concentration of P was obtained in mycorrhizal SP-treated groundnut) occasioned either by increased hyphal uptake or enlarged absorptive root surface due to increased root fineness (i.e. high specific root length values) observed with this treatment, or both. This is confirmed by the observation by Jarell and Beverly (1981) and Cooper (1984) that an increase in both shoot weight and shoot nutrient concentration is evidence of a treatment effect. In the absence of improved P nutrition, caused by the unavailability of the added P to the plants either because of lack of AM fungi to exploit the SP or because of the sparse solubility of the RP, groundnut developed a coarse root structure (low specific root values). This contradicts the contention by Fitter (1985) that plants grown in low fertility soils possess small root diameters.

The similarities in shoot dry matter yields and shoot P uptakes of both mycorrhizal and non-mycorrhizal plants with RP amendment may suggest that the legumes were so unresponsive to the RP that even arbuscular mycorrhiza formation could not trigger any response in the crops. These results are in sharp contrast to the observation by Murdoch *et al.* (1967) that treatments with a readily available source of P produced no significant differences in P uptake or dry matter yield between mycorrhizal and non-mycorrhizal maize plants but with RP treatments, they obtained a much higher dry matter yield and P accumulation when plants were mycorrhizal. These workers and others (Hall *et al*., 1977; Mosse *et al*. 1976) concluded that mycorrhizal plants could utilize rock phosphate fertilizers whereas non-mycorrhizal ones could not. This conclusion could not be affirmed in this investigation. Mosse *et al*. (1976), however, observed that in acid soils addition of RP generally improved growth of the non-mycorrhizal plants and inoculation with AM endophytes greatly improved its utilization whereas in neutral and alkaline soils RP was unavailable to non-mycorrhizal plants and remained so after inoculation with AM fungi. Solubility and availability of added rock phosphates to plants, whether mycorhizal or not, may therefore depend on the pH status of the growth medium as reported by Barnes and Kamprath (1975) and Khasawneh and Doll (1978). In this work, the lack of response to the added RP, even when plants were mycorrhizal, may partly be due to the absence of a more conducive soil pH regime than what was maintained to enhance the dissolution of the RP. Further reasons for the relative unavailability of applied RP to plants may be due to the fact that in some experiments the rates of RP applied have been so low that neither mycorrhizal nor non-mycorrhizal plants derived any phosphate from them (Ross and Gilliam, 1973; Mosse *et al*., 1976) whilst in others rates of the added RP have been such that only mycorrhizal plants have benefited (Azcon *et al*., 1976; Powell and Daniels, 1978). This has led to the erroneous general conclusion by some authors (Powell and Daniels, 1978) that "mycorrhizal plants can utilize rock phosphate whereas non-mycorrhizal plants cannot." Though only a single rate of rock phosphate was used in this study and therefore no specific conclusion as to whether mycorrhization could lead to increased growth response to, or utilization of, the added RP could be made, it was clear that under the conditions of this experiment the RP used did not promote plant growth or P uptake even when plants were mycorrhizal despite a stimulation of AMF colonization in cowpea and pigeon pea.

The increased content of N in the tops of mycorrhizal SP-fertilized plants was not due to an improved N nutrition as nitrogen was highly diluted in such plants but was the result of larger shoot sizes. Furthermore, in the absence of mycorrhiza, SP addition drastically reduced the N concentration in the shoot of cowpea compared with other plants of equal or smaller dry weights. Whereas Raju *et al*. (1990) observed equal concentrations of N in both mycorrhizal and non-mycorrhizal maize plants, Nielsen and Jensen (1983) reported only AMF-induced increases in

shoots of Lucerne. The increased content of N despite the reduced shoot concentrations of this element observed in this work may therefore be explained on the basis of the "dilution effect" phenomenon which results from dissimilar rates of plant growth and nutrient absorption. In this case, there was a more rapid accumulation of shoot dry matter than of nitrogen in cowpea.

In pigeon pea and groundnut, mycorrhiza was more effective in raising the Cu concentrations in the stem and leaves, respectively when RP was applied compared to SP treatment. Whereas a direct relationship between the concentrations of P and Cu in the stem may explain this observation in pigeon pea, in groundnut there was a P-Cu antagonism in the leaves. Increased Cu concentration in other mycorrhizal plants have been reported by other workers (Packovsky, 1986; Gildon and Tinker, 1983). Although this high level of Cu (14 μg g^{-1}) in the leaves of RP-treated mycorrhizal groundnut cannot be attributed to AM fungal colonization, it compares favourably with the optimum concentration (11 μg g^{-1}) observed by Packovsky and Fuller (1986) in the leaves of soybean inoculated with *Glomus fasciculatum* without any P amendment. This did not, however, result in any increased Cu uptake.

The high Fe concentration in the stem of mycorrhizal RP-fertilized groundnut could not be due to the influence of AMF colonization nor to any improved P assimilation or any increased root fineness. Some unknown mechanism(s) may have been responsible.

Except with Fe in groundnut, the significantly high contents of the micronutrients found in the shoots of these legumes when inoculated with AMF and treated with SP were not due to any increased mineral concentration or a favourable mycorrhizal establishment but mainly to the higher production of shoot biomass. In groundnut, the enhanced shoot (stem) Fe uptake could be related to a good mycorrhiza formation as well as a concomitant improvement in P nutrition which may further have been influenced by the fine root structure.

CONCLUSION

Although AM fungal colonization of cowpea and pigeon pea was moderate in treatments receiving rock phosphate application, growth and P uptakes were very poor. This could be explained by the fact that before any effective mycorrhization can take place, photosynthates and nutrients must be made available by the host plant to the mycorrhizal endophyte. The lack of photosynthate supply to the AM fungus due to the poor growth of the mycorrhizal RP-fertilized cowpea and pigeon pea could be the cause of the non-significant spore production in the rhizosphere of such plants even though AM fungal colonization was favourable. Hence before plants, through mycorrhization, can mobilize rock phosphates, a certain quantity of P might be necessary, especially with less reactive rock phosphates, for the formation of the mycorrhizal apparatus. In practical terms, this means that a small quantity of easily available P in the form of a starter fertilizer might be needed for a priming action in order to allow these legume plants to create conditions under which they can develop an effective mycorrhiza to mobilize the sparsely soluble rock phosphate for growth. The results also showed that among the three legumes there was an existence of a very close interdependency of fine root structure, AM fungal colonization, spore production and improved P and Fe nutrition in groundnut as a result of soluble phosphate application.

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