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Effects of rockphosphate and arbuscular mycorrhizal fungi on growth and nutrition of *Sesbania sesban* and *Gliricidia sepium*

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A greenhouse experiment was conducted to evaluate the effects of different indigenous arbuscular mycorrhizal (AM) fungi on the mobilization of phosphorus from Senegalese natural rock phosphate (NRP) for growth of *Gliricidia sepium* and *Sesbania sesban* seedlings. Levels of tested NRP were compatible with high AM fungal proliferation but modified pattern of root colonization according to plant cultivar and fungal species. NRP applications and AM inoculation positively stimulated growth parameters and shoot mineral mass of *G. sepium* and *S. sesban* after four months cultivation. More than 200% of weight gains in *S. sesban* were recorded with all AM fungi combined with 600 or 800 mg NRP. With Gliricidia, only *Glomus aggregatum* in presence with these high NRP levels induced the same tendency. *Glomus fasciculatum* enhanced twice height growth of Sesbania in presence of 400, 600 and 800 mg NRP. The impact of dual application of AM fungi and NRP on nutritional content was more marked with *Sesbania* than in *Gliricidia* seedlings.

Key words: Rock Phosphate, AM fungi, *Ghricidia sepium, Sesbania sesban.*

INTRODUCTION

A large proportion of Sahelian soils are generally deficient in available phosphorus. According to Perri (1989), the property of these soils in P, major element that conditions the agriculture and forest yields, characterizes West African regions. To raise the level of assimilated soil P, one can think of a better exploitation of natural rock phosphate (NRP) which overflows most countries in West Africa.

In Senegal, during the last decades, policy makers strongly recommended to farmers, a large scale use of NRP, to recapitalize the soil P. However, mitigated results were obtained on plant productivity due to lack of consideration of water availability, organic matter and microbial components.

Agroforestry systems often use perennial trees to obtain high quantities of litterfalls. However, the organic matter content recycled in soils is generally very weak and the litter quality is also too low to add sufficient available P to the soil to enhance crop growth (Sanchez, 1995). In this context of decrease of soil fertility, a biological way exploiting symbiotic and associated microorganisms, could lead to a better use of NRP in agriculture and forestry. Several works showed that combination of NRP and AM fungi enhanced P availability in rhizosphere and its translocation to plants (Siquiera et al., 1998; Asimi et al., 1989; Vanlauwe et al., 2000).

Besides improving uptake of poorly mineral elements, AM fungi enhance drought tolerance and protection of plants against plant pathogens (Dehne, 1982; Caron,

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1989; Garcio-Garrido and Ocampo, 1988) and soil stability (Miller and Jastrow, 1992). Otherwise, in most cases of P-deficient soils, the growth of cultivated plants is highly dependent to mycorrhizal status (Duponnois et al., 2001; Diop et al., 2003).

The present study was undertaken to assess the degree of effectiveness of arbuscular mycorrhizal fungi and different NRP levels in culture of two multipurpose trees, *Gliricidia sepium* and *Sesbania sesban.*

MATERIALS AND METHODS

Fungal materials

Three AM fungi: *Glomus aggregatum* (Schenk and Smith emend. Koske; DAOM 227 128), *G. fasciculatum* (Thaxter sensu Gerdemann; DAOM 227 127) Gerd and *Glomus mosseae* (Nicolson et al Gerd. et al. Trappe; DAOM 227 131) were used as inoculum. Their efficiency in stimulating tropical plants growth was proved (Diop et al., 2003; Ndiaye et al., 2003). Fungal isolates were propagated in association with *Zea mays* in plastic pots containing 1 kg of sterile coarse sandy soil. For each AM fungus, inoculum consisted of a soil mixture containing heavily colonized roots of *Z. mays*, spores and mycelium. After 3 months of cultivation in a glasshouse, *Z. mays* seedlings were harvested.

Seed germination, fungal inoculation and growing conditions

Seeds of *S. sesban* and *G. sepium* were provided by the Senegalese Institute of Agricultural Research. They were surface sterilized in 8% sodium hypochlorite for 5 min to release their dormancy, rinsed with sterilized distilled water and pregerminated on 1% water agar at 28 °C in the dark. One pregerminated seed was planted in each pot (25 x 10 cm) filled with 1 kg of a sterilized soil collected at Nioro. The soil was a sandy clay loam, slightly acidic (pH: 5.5), with 27 ppm available P and 2.4% organic C.

Inoculation was simultaneously achieved at transplantation using 5 g of soil containing infected root pieces (80%) and spores (35) of each AM fungal isolate.

Plants were grown in a glasshouse under natural light (day length 12 h, mean temperature 30°C and 60% relative humidity). During the experiment, the pots were weighed daily and water loss was compensated for by top watering.

Natural rock phosphate material

The natural rock phosphate (NRP) extracted at Taiba (100 Km from Dakar) containing 12.2% of total P was used in this experiment. Fine NRP powder was distributed uniformly on the top of pots two weeks before experimentation and regular watering was achieved to facilitate solubilization of rock P.

Experimental design and assessments

S. sesban and *G. sepium* were examined separately in factorial, two- factor pot experiments. The first factor was “AM inoculation” with four levels (*G. aggregatum, G. mosseae, G. fasciculatum* and control). The second factor was “NRP fertilization” with levels (0, 200, 600 and 800 mg/pot). Each treatment was replicated 10 times resulting in 160 pots per species.

Harvest was made after 4 months and the roots washed and cleaned with tap water. Mycorrhizal root colonization was estimated using Trouvelot et al. (1986) method after staining with trypan blue (Philips and Hayman, 1970).

Plant growth was regularly measured and at harvest, shoots were separated from roots, dried at 65°C for three days and weighed individually. For the analysis of shoot mineral content, standard methods were used. Potassium was determined by flame emission spectrophotometry, and colorimetry for nitrogen (after Kjedahl digestion) and, phosphorus according to the vanado-molybdate procedure.

The Newman-Keuls multiple range test ($p<0.05$) was used for statistical analysis. AM colonization data were transformed to arcsine square before statistical analysis

RESULTS

Mycorrhizal root colonization

The rate of root infection varied according to plant species, fungal species and natural rock phosphate levels (Tables 1 and 2). Rock Phosphate did not inhibit root colonization of both plants by *Glomus* sp., but affect the pattern of colonization.

Sesbania sesban root infections

Typical AM structures symbolizing different fungal development were recorded in stained roots. However, NRP levels positively stimulated intensity of root colonization by *G. fasciculatum* and slightly decreased AM development of *G. mosseae. G. aggregatum* benefited more high NRP levels for colonizing *S. Sesban* roots.

Gliricidia sepium root infections

NRP did not influence the intensity of root colonization by *G. aggregatum*. *G. fasciculatum* alone caused 18% of root infection and this was greatly increased in the presence of 800 mg NRP, reaching more than 50%. Root colonization of *G. sepium* by *G. mosseae* was appreciably increased with addition of NRP up to 400 mg.

Influence on development of *Sesbania sesban* seedlings

NRP application and AM inoculation positively increased shoot dry weight of *S. Sesban* (Table 1) . NRP at rate of 800 mg was less suitable for shoot dry weight even if it induced 2X yield comparatively to control plants. Among the AM fungi tested, *G. aggregatum* gave the highest biomass when inoculated alone. Addition of NRP stimulated the effects of all AM fungi on biomass production. Supplementation of NRP at rate of 600 and 800 mg allowed AM fungi to induce a gain shoot dry weight more than 200%.

Growth of *S. Sesban* was significantly ameliorated by NRP and AM fungi. The highest growth stimulation (more than 2X) was obtained by *G. fasciculatum* in combination with all NRP rates and by *G. mosseae* supplemented with 800 mg NRP.

Table 1. Shoot weight, growth and intensity of mycorrhizal colonization of *Sesbania sesban* as influenced by AM fungi and natural rock phosphates levels

Treatments		Shoot weight (g)	Height (cm)	Intensity of mycorrhization (%)
NRP (mg)	0	0.54 d	30.6 d	
	400	1.26 b	46.2 c	
	600	1.42 b	54b	
	800	1.1 c	48.8 c	
Glomus aggregatum		1.26 b	56.6 b	47.32 a
G. agg + 400		1.35 b	50 c	29.02 b
G.agg + 600		1.72 a	58.6 b	50.7 a
G.agg + 800		1.64 a	56.9 b	39.72 a
Glomus fasciculatum		0.88 c	48.5 c	31.22 b
G. fasc + 400		1.32 b	61.6 a	49.72 a
G. fasc + 600		1.68 a	60 a	37.82 a
G. fasc + 800		1.82 a	70.4 a	37.76a
Glomus mosseae		1.1 c	51.6 c	39.76 a
G. moss + 400		0.82 c	57 b	33.48 b
G. moss + 600		1.66 a	55.4 b	28.82 b
G. moss + 800		1.65 a	66.4a	31.16 b

Values sharing the same letter in each column, are not significantly different (P = 0.05)

Table 2. Shoot weight, growth and intensity of mycorrhizal colonization of *Gliricidia sepium* as influenced by AM fungi and natural rock phosphates levels

Treatments		Shoot weight (g)	Height (cm)	Intensity of mycorrhization (%)
NRP (mg)	0	1.01 d	12 d	
	400	1.58 c	14.6 d	
	600	1.92 c	15.4 d	
	800	2.08 c	16.10 c	
Glomus aggregatum		2.01 c	18.4 c	41.04 b
G. agg + 400		2.3 b	20.7	31.06 b
G.agg + 600		3.12 a	23.6 a	34.22 b
G.agg + 800		3.26 a	23.8 a	39.66 b
Glomus fasciculatum		2.33 b	22.1 a	17.9 d
G. fasc + 400		2.52 b	22.84 a	21.66 c
G. fasc + 600		2.84 b	23.75 a	34.76 b
G. fasc + 800		2.6 b	22 a	51.62 a
Glomus mosseae		1.58 d	19.7 c	43.42 b
G. moss + 400		2.21 c	14.6 d	53.4 a
G. moss + 600		2.66 b	21.2 b	51.62 a
G. moss + 800		2.18 c	20.6 c	66.90 a

Values sharing the same letter in each column, are not significantly different (P = 0.05)

Influence on development of *Gliricidia sepium* seedlings

NRP Application or inoculation with each AM fungus increased significantly shoot dry matter of *G. sepium* (Table 2). Gain weights of more than 56, 99 and 130% were respectively induced by *G. mosseae*, *G. aggregatum* and *G. fasciculatum* comparatively to uninoculated plants without NRP application. NRP fertilizers did not influence the ability of *G. fasciculatum* to stimulate shoot

Table 3. Shoot mineral content of *Sesbania sesban* as influenced by AM fungi and natural rock phosphate levels

Treatments		N (mg)	P (mg)	K (mg)
NRP (mg)	0	14.31 e	0.68 c	17 c
	400	27.47 d	2.1 b	24.7 b
	600	38.77 c	2.1 b	24.7 b
	800	25.96 d	2.56 b	20.6 b
Glomus aggregatum		30.24 c	2.3 b	34.78 a
G. agg + 400		29.9 c	2.99 b	30 a
G.agg + 600		65.93 a	3.82 a	31.75a
G.agg + 800		42.08 b	3.46 a	31.04 a
Glomus fasciculatum		34.68 b	2.03 b	26.5 b
G. fasc + 400		31.15 c	3.37 a	25.87 b
G. fasc + 600		41.55 b	3.33 a	32.23 a
G. fasc + 800		36.95 c	3.97 a	32.76 a
Glomus mosseae		23.62 d	2.01 b	30.36 a
G. moss + 400		46.42 b	2.88 b	29 a
G. moss + 600		45.44 b	3.14 a	33.02 a
G. moss + 800		44.24 b	3.34 a	29.32 a

Values sharing the same letter in each column, are not significantly different (P = 0.05)

Table 4. Shoot mineral content of *Gliricidia sepium* as influenced by AM fungi and natural rock phosphate levels.

Treatments		N (mg)	P (mg)	K (mg)
NRP (mg)	0	81.80 b	2.34 d	10.20 d
	400	55.74 d	1.73 e	13.51 d
	600	51.01 e	1.9 e	27.58 c
	800	65.76 c	1.58 e	18.76 d
Glomus aggregatum		97.42 a	5.15 a	29.30 c
G. agg + 400		68.51 c	3.59 b	50.40 a
G.agg + 600		98.30 a	4.15 b	51.79 a
G.agg + 800		89.42 b	3.85 b	42.38 a
Glomus fasciculatum		89.55 b	5.28 a	29.69 c
G. fasc + 400		78.28 c	3.18 c	41.28 a
G. fasc + 600		78.17 c	3.58 b	35.66 b
G. fasc + 800		68.05 c	2.96 c	39.54 b
Glomus mosseae		86.25 b	3.2 b	17.40 d
G. moss + 400		65.94 c	3.74 b	22.48 c
G. moss + 600		88.80 b	4.04 b	36.74 b
G. moss + 800		89.75 b	2.66 c	23.46 c

Values sharing the same letter in each column, are not significantly different (P = 0.05)

dry mass. *G. aggregatum* in association with either 600 or 800 mg NRP resulted in a high increase of shoot dry weight (+ 208%) in comparison to plants cultivated alone.

Application of NRP alone did not influence growth of *G. sepium.* Maximum growth seedlings were recorded with *G. fasciculatum* alone or in combination with NRP level or when *G. aggregatum* was in presence with 600 or 800 mg NRP.

Shoot mineral status of *Sesbania sesban*

Phosphorus uptake by *S. Sesban* increased by at least 3X by NRP applications and AM fungi (Table 3). No significant differences were noted between NRP rates in stimulating P nutrition. Same tendency was recorded when *G. fasciculatum* was combined with any NRP level. High NRP levels stimulated P uptake by *S. Sesban* seedlings colonized by *G. aggregatum* or *G. mosseae*.

N uptake was also ameliorated with NRP and AM inoculation. More than 4.60 X N uptakes were caused by dual combination of *G. aggregatum* and 600 mg NRP. N nutrition of *S. sesban* was identically regulated by NRP levels in presence of *G. mosseae*.

K uptake was also ameliorated with NRP and AM inoculation. There were no significant differences between effects of NRP levels on K nutrition. *G. aggregatum* and *G. mosseae* raised similarly K uptake even they were cultivated in presence of all NRP rates. Application of 600 or 800 mg NRP also increased K uptake of *S. Sesban* colonized by *G. fasciculatum.*

Shoot mineral status of *Gliricidia sepium*

P uptake by *G. sepium* decreased by NRP application alone (Table 4). AM inoculation allowed stimulation of P nutrition with or without “phosphating”. There were no significant differences on P uptake when *G. aggregatum* was combined with NRP. High NRP level (800 mg) inhibited impact of *G. fasciculatum* and *G. mosseae* on P absorption.

NRP application alone inhibited N uptake and did not allowed *G. fasciculatum* to overcome this inhibition. Highest N stimulation was recorded with *G. aggregatum* alone and when it is combined with 600 mg NRP.

NRP application stimulated K uptake of G. seedlings inoculated with each AM fungus. However, no significant differences on K absorption were recorded when *G. fasciculatum* was cultivated with 600 and 800 mg NRP.

DISCUSSION

S. sesban and *G. sepium* were selectively colonized by *Glomus sp*. in presence of NRP applications. Previous works (Diop et al., 1992; Azcon et al., 2003) showed that AM infections units could be used as a sensitive marker of the physiological state of inoculated plants. NRP rates did not inhibit root colonization but affected its pattern of development and probably the optimal functioning of AM symbiosis as already related (Sharma et al., 1996). High variability in AM root colonization due to fertilizers also indicates that NRP rates can be used as tool for selection of best symbiotic partners.

AM inoculation usually enhanced growth and shoot biomass of plants (Duponnos et al., 2001; Diop et al., 2003). Our results agreed with this assertion but showed variable responses according to AM isolates and plants. This

is not surprising as relative mycorrhizal dependency is a key factor for success of inoculation (Plenchette et al., 1983). Under our conditions, NRP fertilizers profited more plant growth in presence of introduced *Glomus* spp. particularly in the rooting zone of *G. sepium*. The slight acidity of used soil was probably enhanced during period of cultivation. Changes to the pH of rhizosphere are also involved in absorption/assimilation process. Under low soil pH, NRP is solubilised more and consequently AM fungi mobilized P for plant growth (Khaliq and Sanders, 1997; Azcon et al., 2003). Our results also showed a lack of relationship between intensity of mycorrhizal root colonization and shoot biomass.

NRP fertilizers and AM fungi positively stimulated shoot mineral content of *S. sesban*. High NRP rates did not inhibit this increase. Mycorrhizal inoculation could explain this increase of shoot mineral noted in *Sesbania* due to greater exploration of the soil by fungal hyphae (Abbot and Robson, 1986). Difference on N, P, K uptakes recorded within *Glomus* spp.; and *S. Sesban* associations confirmed that genetic factors play a role in translocation of mineral elements (Khalil et al., 1994; Diop et al., 2003). Under our conditions, AM inoculation is necessary to stimulate shoot mineral mass of *G. sepium*. Probably phosphatase enzyme activity is lower in the rhizosphere of *G. sepium* than in *S. sesban* rooting zone. Previous works (Radersma and Grierson, 2004) indicated phosphatase enzymes are important in mineralization of organic P.

In conclusion, this study clearly shows an efficient biological way of exploiting natural rock phosphate for growth of *S. sesban* and *G. sepium* in a short period. However other works must be undertaken with select efficient solubilising rhizobacteria and AM fungi and to compare their effects in mineralisation and mobilization P of rock P in different cultivated soils. The results obtained could be vulgarized for a better use of the solubilising rock phosphate currently exploited in the northern region of Senegal to develop a sustainable culture.

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