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Full Length Research Paper

Response of *Gliricidia sepium* tree to phosphorus application and inoculations with *Glomus aggregatum* and rhizobial strains in a sub-Saharian sandy soil

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A pot experiment was carried out in a green house at Bel Air station to determine effect of phosphorus on the growth of *Gliricidia sepium* in presence of rhizobial strains and an arbuscular mycorrhiza fungus. A factorial 3 factors block was designed with arbuscular mycorrhizal fungus *Glomus aggregatum*, phophorus fertilization as triple super phosphate and rhizobial inoculation strains ISRA 604 and ISRA 605 applied on *G. sepium* seedlings. A non nitrogen fixing tree (NFT), *Cassia siamea* was used as reference tree for estimating the nitrogen fixation using the ^{15}N isotope dilution technique. Mycorrhizal infection, nodulation, plant growth, P and N contents, ^{15}N atom % excess (^{15}Nae) and N fixed were determined. The results showed that the rhizobium strain ISRA 604 induced nodulation more than ISRA 605 in *G. sepium* with an increase of 38.28% for nodules number. Frequency (%F) and intensity (%M) of mycorrhization were highest at 0, 20 and 40 mg P kg^{-1} soil applied however rhizobial inoculation has influenced frequency of mycorrhization. In the rhizobium- AM fungus interaction, plant shoot total N content increased when trees were inoculated with *G. aggregatum* whatever the rhizobial strain inoculated. Roots N fixed (Ndfa) increased when plant was inoculated with ISRA 604 and amended with 20 mg P kg^{-1} soil. The inoculated plants exhibited the highest total nitrogen in whole plant as well as in shoots and roots than in non-inoculated and reference plants. *G. sepium* growth benefited from selected rhizobia and AM fungus inoculations and P application in a sandy soil.

Key words: Arbuscular mycorrhizal fungus, *Gliricidia sepium*, nitrogen fixation, phosphorus, rhizobium.

INTRODUCTION

In tropical and subtropical regions, nitrogen and phosphorus soil deficiency are common and constitute the main limiting nutrients in semi-arid and dry sub-humid Savanna (Graham, 1981; Buerkert et al., 2001; Bationo et al., 1992). Management practices are generally employed as part of many agro-ecosystems to improve soil productivity such as fertiliser application which is uneconomical and unsustainable in long-term (Baligar and Bennett, 1986). For sustainable land-use management and thereby mitigation of deforestation impacts, some works reported that woody legumes are important for re-vegetation of ecosystems that have low amounts of available N and P (Danso et al., 1992). The potential of leguminous such as nitrogen fixing trees (NFTs) are evidenced in the restoration and/or maintenance of soil fertility. Therefore these NFTs are very important in tropical soil management for solving the continuous soil fertility decreasing and improving sustainable agricultural productivity.

Gliricidia sepium, a fast-growing perennial leguminous tree is infected by rhizobial bacteria (Liyanage et al., 1994) and arbuscular mycorrhizal (AM) fungi (Habte and Turk, 1991). Native to Central America and Mexico (Simons and Steward, 1994), this introduced tree can be integrated into a farming multipurpose cropping system for soil nutrient enrichment. To play its expected role in farming methods, the N_2-fixing capability and P assimila-

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tion of this tree legume need to be improved. This could be done without application of large quantities of relatively expensive inorganic N and P fertilizers but through inoculation with selected highly specific rhizobial and mycorrhizal micro-symbiotic organisms. The establishment of rhizobial symbiosis increases the nitrogen nutrition of legumes and one of the main beneficial effects of the AM fungi is a better phosphorus uptake. When the symbiosis between the three partners legume, rhizobium and arbuscular mycorrhizal fungi is effectively established, their synergism oven contributes to improve soil fertility, which can be benefit to plant crops cultivated.

The objectives of this study was (i) to quantify the ability of *G. sepium* in nitrogen fixation and P uptake in sandy soils and (ii) to evaluate the impact of P fertilizer supply on rhizobial and mycorrhizal plant infection and plant growth, N_2 fixation and P assimilation.

MATERIAL AND METHODS

A green house pot experiment was carried out at Bel Air station in Dakar (latitude 14°44'N, longitude 17°30'W). The pots (24 cm diameter) contained 1.6 kg of non-sterile soil sampled in the station from the 0–30 cm topsoil. The soil was sieved through (1 mm) and homogenised. The sandy soil type (94% of sand) classified as an Arenosol presented the main following characteristics: 7.0 pH, 0.025% N, 26 ppm available P.

Seeds of *G. sepium* provenance Bandia supplied by ISRA-Forest were surface scarified and sterilized for 15 min immersion in sulphuric acid and washed vigorously in sterile water and pregerminated on water agar at 30°C. Seedlings were then transferred into pots and watered daily with 100 ml with tap water.

Rhizobium strains ISRA 604 and ISRA 605 from Dakar MIRCEN collection separately cultured in yeast extract mannitol broth (Vincent, 1970) were used for inoculating *G. sepium* seedlings. The rhizobial inoculants, containing 10^9 cells ml^{-1}, were applied in liquid form at the rate of 10 ml pot^{-1}. Arbuscular mycorrhizal fungal inoculation was performed such that each seedling received 20 g of inoculum consisting of a mixture of spores, hyphae and millet root fragments from trap cultures containing *Glomus aggregatum* (Schenk and Smith emend. Koske; DAOM 227 128). AM fungal inoculum thoroughly mixed was added to each pot at about 2 cm depth around the root of seedling.

All pots were placed randomly in the green house in a factorial 3 factors block design with four replicates. The first factor was the rhizobial inoculation with two levels, i.e. strain ISRA 604 and strain ISRA 605; the second factor was the mycorrhizal (*G. aggregatum*) or non mycorrhizal inoculation; the third factor was the phosphorus fertilization as super triple phosphate at 0, 20, 40, 60 and 80 mg P kg^{-1} soil. A non NFT, *Cassia siamea* was used as reference tree for estimating the nitrogen fixation using the ^{15}N isotope dilution technique. ^{15}N fertilizer (ammonium sulphate containing 5 atom % ^{15}N excess) was applied to all plants at the rate of 20 mg N $plant^{-1}$. A separate treatment (four replicates) was set up without rhizobial and mycorrhizal inoculation and P fertilizer as absolute control.

At 105 days after sowing (DAS), the plants were harvested. The degree of root mycorrhizal infection was evaluated by the frequency (%F) and intensity (%M) of mycorrhization (Giovannetti and Mosse, 1980). The rhizobial infection was evaluated by counting the number of nodules (NN) formed on the roots and in addition, the size and the inside colour for nodulation index (NI). The number of nodules was recorded visually on carefully washed roots. The harvested plant materials were separated into different plant parts and dried at 70°C for 72 h. Plant shoots, roots and nodules dry weights were determined. Shoots and roots P contained were evaluated according to vanado-molybdate procedure. Nitrogen content (%N) and atom % ^{15}N excess (%^{15}Nae) were determined for each plant part by the International Atomic Energy Agency laboratory at Seibersdorf. Nitrogen fixation (%Ndfa) was calculated using the isotope dilution equation (Fried and Middelboe, 1977):

$$\%\text{Ndfa} = \left(1 - \frac{\%\ ^{15}\text{Nae in fixing crop}}{\%\ ^{15}\text{Nae in non fixing crop}}\right)$$

Data were statistically analysed and compared using Newman and Keul test when the F-test from the analysis of variance (ANOVA) was significant at P = 0.05.

RESULTS

Nodulation and mycorrhizal infection

The nodules number (NN) per plant depended significantly on the rhizobium strain used for plant inoculation (Table 1). The result (Table 2) showed that the rhizobial strain ISRA 604 induced nodulation of *G. sepium* more than ISRA 605 with an increase of 38.28% for NN. However, data analysed for nodulation index (NI) were not significantly different in any treatment since there was neither influenced by inoculations with rhizobial strains and arbuscular mycorrhizal fungus nor by application of phosphorus.

Mycorrhizal infection (frequency (%F) and intensity (%M)) varied significantly (p =0.05) when seedlings were inoculated with AM fungus in presence of P application (Table 1). Rhizobial inoculation has influenced frequency of mycorrhization. Table 2 showed that in presence of ISRA 605, frequency was 15.20% higher than in presence of ISRA 604. The interaction mycorrhizal inoculation and phosphorus fertilisation showed a significant effect on both %M and %F (Table 1). On average, the %M of plants inoculated with *G. aggregatum* (30.27) appeared higher than that of uninoculated plants (2.13) (Table 3). Similarly for the inoculated and uninoculated treatments with AM fungus, significant difference was observed for frequency of mycorrhizal colonisation at 78.92 and 30.10%, respectively. Native mycorrhizal infection in uninoculated plants is sensitive to P fertilizer increasing. The highest AM root colonisation parameters were observed when plant was inoculated with AM fungus and amended with 0, 20 and 40 mg P (Table 3). Like this, frequency and intensity appeared more sensitive to the low levels of P amendment.

Plant growth and P content

Plant growth was recorded as plant height and dry matter production. Plant height varied significantly (p =0.05) in both rhizobium-AM fungus and rhizobium-P fertilizer inte-

Table 1a. F ratios from analysis of variance (ANOVA) of the plant height (H), root (RDW), shoot (SDW) and nodules dry weights (NDW), nodules number (NN), frequency (%M) and intensity (%F) of mycorrhization, total ^{15}Nae, total Ndfa and total P of *Gliricidia sepium* cultivated in pots containing non sterile soil for 105 days in greenhouse.

Source of variation	dof	H	SDW	RDW	NDW	NN	%M	%F	Tot^{15}Nae	TotNdfa	Total P
Rhizobium (R)	1	4.15*	24.65*	0.05	**27.08***	**13.35***	1.31	**15.75***	16.12**	17.21*	28.08**
AM fungus (AM)	1	0.01	5.89	02.95	0.06	2.57	39.60*	63.11*	0.23	0.82	2.47
Phosphorus (P)	4	1.08	2.04	**12.10***	1.56	0.53	18.99*	21.42*	1.14	3.23*	0.58
R * AM	1	**7.19***	2.07	0.26	0.28	0.17	0.22	1.28	0.87	0.14	**3.48***
R * P	4	**2.56***	0.59	0.28	0.36	0.84	0.65	1.77	3.67*	2.89*	0.65
AM * P	4	1.02	2.08*	0.18	0.94	0.73	**13.63***	**3.21***	1.63	0.97	0.44
R * AM * P	4	0.80	**4.19***	0.20	0.56	0.86	1.46	1.94	**4.12***	**4.16***	1.64
CV(%)		11.5	10.6	23.6	29.9	38.9	39.3	8.68	19.16	20.06	19.8

*Significant, **Very significant.

Table 1b. F ratios from analysis of variance (ANOVA) of shoot (S) and root (R) %^{15}Nae, %Ndfa, Ndfa and root %N, total N and total P of *Gliricidia sepium* cultivated in pots containing non sterile soil for 105 days in green house.

Source of variation	dof	S%Nae	S%Ndfa	SNdfa	R%N	RtotalN	R%Nae	R%Ndfa	RNdfa	RtotalP
Rhizobium (R)	1	**15.50***	**15.45***	17.88*	21.79***	**6.19****	8.60*	8.60*	22.47***	0.02
AM fungus (AM)	1	0.17	0.17	0.91	1.07	**4.27****	0.64	0.64	0.13	0.45
Phosphorus (P)	4	1.67	1.67	2.58*	1.57	**11.16*****	0.19	0.19	4.51**	3.50*
R * AM	1	0.73	0.71	0.07	0.01	0.29	0.68	0.68	0.46	2.04
R * P	4	0.81	0.82	0.43	**3.35***	1.11	**3.43***	**3.43***	**5.68*****	**2.53***
AM * P	4	0.54	0.53	0.24	1.15	0.38	1.98	1.98	**2.27***	0.16
R * AM * P	4	0.65	0.66	**4.14***	1.19	0.33	0.27	0.27	0.45	2.00
CV(%)		24	7.5	24.7	13.0	26.5	17.5	33.9	41.1	16.6

*Significant, **Very significant, ***highly significant.

Table 2. Nodule number (NN), intensity of mycorrhization (%F), nodules dry weights (NDW) and shoot (SDW), %^{15}Nae and %Ndfa of *Gliricidia sepium* cultivated in pots containing non sterile soil for 105 days in greenhouse and inoculated with *Rhizobium* strains ISRA 604 and ISRA 605 and with *Glomus aggregatum*.

Rhizobium	**NN**	**%F**	**NDW (g.pl^{-1})**	**SDW (g.pl^{-1})**	**%^{15}Nae**	**%Ndfa**
ISRA 604	242 a	50.66 b	0.90 a	43.7 a	0.04 b	78.58 a
ISRA 605	175 b	58.36 a	0.63 b	38.9 b	0.05 a	73.54 b

In each column, values are significantly different at p = 0.05.

interactions (Table 1). Thus there was a difference in plant growth. Table 2 showed that dry weight of nodules was increased when plants were inoculated with ISRA 604 compared to ISRA 605 for 42.88%. The dual inoculation with rhizobium and AM fungi produced an increase of 7.25% on plant height when inoculated with rhizobium ISRA 604 strain (Table 4). In contrast plant height was not higher when it was inoculated with mycorrhizal and rhizobium ISRA 605 strain. In the presence of rhizobium ISRA 604 strain, the height of plant was higher at 0 and 20 mg P fertilizer than that of *Rhizobium* ISRA 605 which was higher at 40 and 60 mg P fertilizer. Plant shoots and roots total P content was significant at p =0.05 in rhizobium-AM and rhizobium-P fertilizer, respectively (Table 1). Root total P content was lower when plants inoculated with ISRA 605 did not receive P fertilizer (Table 5).

Main effects of rhizobial inoculation and P fertilizer were observed on Table 1 on plant nodules dry weight and root dry weight respectively (p =0.05). Low rate P application had high positive effect on plant root development (Table 5). Thus plant RDW was increased with phosphorus application up to 20 mg P kg^{-1} soil. Over this rate, there was a decrease of plant RDW (result not showed).

Plant nitrogen content and fixed nitrogen

Total nitrogen in whole plant as well as in shoot and root was significantly higher in inoculated seedlings than in

Table 3. Frequency (%F) and intensity (%M) of mycorrhization of *Gliricidia sepium* cultivated in pots containing non sterile soil for 105 days in greenhouse and inoculated with *Glomus aggregatum*, strains ISRA 604 and ISRA 605 and amended with phosphorus.

Interactions : Phosphorus*AM fungus	**%F**	**%M**
Without *G. aggregatum*		
0 mg p	37.50 d	3.59 d
20 mg p	36.67 d	2.97 d
40 mg P	29.13 de	1.99 d
60 mg P	28.42 de	1.43 d
80 mg P	18.79 e	0.60 d
With *G. aggregatum*		
0 mg P	87.50 a	38.66 a
20 mg P	89.00 a	42.21 a
40 mg P	84.83 ab	35.96 a
60 mg P	76.25 b	23.21 b
80 mg P	57.00 c	11.32 c

For each interaction, values with the same letter are not significantly different at p = 0.05.

Table 4. Height (H) and shoot total P content of *Gliricidia sepium* cultivated in pots containing non sterile soil for 105 days in greenhouse and inoculated with *Rhizobium* strains ISRA 604 and ISRA 605 and with *Glomus aggregatum*.

Interactions : *Rhizobium* * AM fungus	**H (cm)**	**Total P(g.pl^{-1})**
ISRA 604		
With *G. aggregatum*	128.7 a	2.49 b
Without *G. aggregatum*	120.0 b	1.99 c
ISRA 605		
With *G. aggregatum*	114.0 b	2.92 a
Without *G. aggregatum*	122.1 a	2.29 b

For each interaction, values are significantly different at p = 0.05.

uninoculated and reference plants. Significant different on total nitrogen were observed on plant roots for main factors but not on shoot and whole plant (Table 1). Plant roots N content was significantly different due to rhizobial inoculation effect (Table 2) whereas nitrogen content was similar between treatments in shoots (Table 6). The rhizobial strain ISRA 604 increased the amount of N for 18.18% in comparison to ISRA 605 (result not showed). However in each plant part, the $\%^{15}N$ atom excess proportion was higher in reference tree than in the fixing tree *G. sepium* indicating the occurrence of nitrogen fixation in *G. sepium*. The ^{15}N enrichment was significant (p =0.05) for rhizobial inoculation in shoot however in roots it was different for phosphorus and rhizobial interaction (Table 1). In shoot, rhizobial strain ISRA 604 had higher %Ndfa than ISRA 605 with 78.58 and 73.54% respectively (Table 2) ; at that time, Table 5 showed that in roots the interaction of the same strain ISRA 604 inoculation and 20 mg P application shown the best %Ndfa. The nitrogen fixed in shoots was higher than that in roots with 76.06 and 34.06%, respectively. In whole plant such as in plant shoot, the tripartite rhizobial and mycorrhizal inoculations and phosphorus application interaction for Ndfa was highly significant (p =0.05; Table 1). Table 6 exhibited that the highest amount of total nitrogen (1.21 g.pl^{-1}) was fixed when *G. sepium* seedlings was inoculated both with ISRA 604 and AM fungus *G. aggregatum* and amended with 20 mg P. The amount of nitrogen fixed in shoot was higher than that reported in root and represented 94% of the N fixed in the whole plant.

DISCUSSION

Soil nitrogen and phosphorus contents are two important factors in the N_2 fixing process (Wall et al., 2000) particularly in plant infection and growth. Plant's need of high mineral N and P application for growth have to be substituted or reduced by both rhizobial strains and AM fungi in biological process (Diouf et al., 1999; Wall et al., 2000). However, responses to microbial infection were variable depending in some cases on species and soil conditions (Stamford et al., 1997; Manjunath et al., 1989). Mycorrhizal colonization of *G. sepium* growth on a sandy soil classified as an Arenosol was influenced by the application of P in contrast to the results on *L. leucocephala* growth on an Oxisol (Osorio and Habte, 2001). High P rates decreased %M and %F on *G. sepium* inoculated with AM fungus *G. aggregatum*.

G. sepium plant height, shoot dry weight, nodule number and nodule dry weight showed a positive effect to rhizobial inoculation, when only mycorrhization index and percentage of infection are significantly different for AM fungus inoculation and P fertilizer. These results agree with those of some authors on rhizobial inoculation efficiency and P added and inoculation with vesicular arbuscular mycorrhizal fungi (Jasper et al., 1989; Stamford et al., 1997). On our works, the substantially increased differences in NN of *G. sepium* plant were attributed to rhizobial inoculation whereas, in contrast in *Alnus incana* plants, high P level stimulated nodules number and dry matter (Wall et al., 2000). Mycorrhizal colonisation evaluated with %F and %M was highest at 20 mg P application and reduced with increasing phosphorus application. The natural mycorrhizal infection was lower and slightly decreasing with high P added. Indigenous mycorrhizal population is sensitive to high P application with a decreasing of - 45.43% in %M when 20 mg P were yet added. This indicates that *G. sepium* plant colonisation with native AM fungi are more sensitive to soil P contained than in presence of *G. aggregatum*. These results are in contrast to those obtained by Stamford et al. (1997). For these authors, P fertilizer increased native mycorrhizal infection in no-inoculated

Table 5. Plant height (H) and root total P, %N, %^{15}Nae, %Ndfa, and Ndfa of *Gliricidia sepium* cultivated in pots containing non sterile soil for 105 days in greenhouse and inoculated with *Rhizobium* strains ISRA 604 and ISRA 605 and amended with phosphorus.

Interactions : *Rhizobium* * Phosphorus	**H (cm)**	**Total P (g.pl^{-1})**	**%N**	**%^{15}Nae**	**%Ndfa**	**Ndfa (g.pl^{-1})**
ISRA 604						
0 mg P	126.1 a	2.40 a	2.30 a	0.05 ab	35.16 ab	0.10 b
20 mg P	128.3 a	2.89 a	2.36 a	0.04 b	46.09 a	0.16 a
40 mg P	122.5 ab	2.63 a	2.18 ab	0.05 ab	35.63 ab	0.09 b
60 mg P	125.3 ab	2.44 a	2.08 ab	0.05 ab	39.58 ab	0.08 b
80 mg P	119.7 b	2.88 a	2.30 a	0.05 ab	32.81 ab	0.08 b
ISRA 605						
0 mg P	17.5 ab	2.18 b	1.69 c	0.06 a	29.22 ab	0.10 b
20 mg P	103.5 b	2.70 a	2.09 ab	0.06 a	22.66 b	0.10 b
40 mg P	124.6 a	2.77 a	1.98 abc	0.05 ab	31.88 ab	0.08 b
60 mg P	125.1 a	2.85 a	2.08 ab	0.06 a	29.06 ab	0.09 b
80 mg P	119.5 ab	2.53 a	1.86 bc	0.05 ab	38.59 ab	0.08 b

For each interaction, values with the same letter are not significantly different at p = 0.05.

Table 6. Shoot dry weight (SDW) and Ndfa of *Gliricidia sepium* cultivated in pots containing non sterile soil for 105 days in greenhouse and inoculated with *Rhizobium* strains ISRA 604 and ISRA 605, with *Glomus aggregatum* and amended with phosphorus.

Interactions: *Rhizobium* *AM fungus*Phosphorus	**SDW (g.pl^{-1})**	**Ndfa (g.pl^{-1})**	**Total N**	**15Nae**	**Tot Ndfa (g.pl^{-1})**
ISRA 604					
Without *G. aggregatum*					
0 mg P	45.9 ab	0.90abc	1.37a	0.047bc	0.95 ab
20 mg P	43.9 ab	0.84abc	1.47a	0.041c	0.93 ab
40 mg P	39.1 bc	0.90abc	1.22a	0.048ab	0.94 ab
60 mg P	41.3 a	0.86abc	1.40a	0.043bc	0.89 ab
80 mg P	39.0 bc	0.88abc	1.28a	0.042bc	0.93 ab
With *G. aggregatum*					
0 mg P	45.8 ab	0.93abc	1.35a	0.044bc	0.99 ab
20 mg P	46.6 ab	1.15a	1.24a	0.042bc	1.21 a
40 mg P	51.6 a	0.87abc	1.24a	0.042bc	0.92 ab
60 mg P	40.8 bc	1.02ab	1.21a	0.048ab	1.07 ab
80 mg P	43.3 ab	0.93abc	1.23a	0.040c	0.96 ab
ISRA 605					
Without *G. aggregatum*					
0 mg P	38.7 bc	0.77abc	1.10a	0.051ab	0.79 bc
20 mg P	35.7 c	0.78abc	1.17a	0.048ab	0.80 bc
40 mg P	40.6 bc	0.79abc	1.22a	0.056a	0.83 ab
60 mg P	41.6 bc	0.64c	1.04a	0.051ab	0.68 c
80 mg P	40.3 bc	0.77abc	1.12a	0.049ab	0.80 bc
With *G. aggregatum*					
0 mg P	40.0 bc	0.75abc	1.09a	0.049ab	0.78 bc
20 mg P	43.3 ab	0.86abc	1.30a	0.052a	0.90 ab
40 mg P	34.6 c	0.63c	1.03a	0.059a	0.75 bc
60 mg P	38.2 bc	0.73abc	1.06a	0.056a	0.80 bc

For each interaction, values with the same letter are not significantly different at p = 0.05.

Mimosa caesalpiniaefolia plants. The application of increasing levels of P reduced mycorrhizal colonisation of *G. sepium* plant. Similar observations have been reported by Ingleby et al. (2001) on *Calyandra calothyrsus* seedlings. However these authors indicated that high levels P fertilizer eliminated growth benefits attributable to mycorrhizal inoculation. In this study even though mycorrhizal inoculation enhanced plant growth when co-inoculated with rhizobial strain ISRA 604 it decreased in presence of ISRA 605. For this last rhizobial strain high P application increased more plant growth.

The effect of rhizobial inoculation on mycorrhizal infection (%F) was observed when compared the two rhizobial strains. Plants P content had been increased for plant inoculated with both rhizobium and AM fungus. Mycorrhizal inoculation did not affect N fixation in our study even thus Barea et al. (1987) reported a greater N fixation in mycorrhizal than in non-mycorrhizal plants. Significant increases in the proportion of N derived from atmosphere (%Ndfa) due to low P application (20 mg P kg^{-1}) were not observed within *Gliricidia* provenance (Sanginga, 1992). When plant was inoculated with *Rhizobium* in presence of AM fungi, increasing of P application did not increase Ndfa of plant shoot.

Total N_2 fixed varied significantly due to combined significant main effect of *Rhizobium* inoculated and P application. In contrast, Sanginga et al. (1989) attributed the difference in total N_2 fixed to difference in the effect of P on plant growth. Inoculation with ISRA 605 in presence of AMF *G. aggregatum* increased significantly N_2 fixed when 20 mg P were applied. In the same conditions the increasing of N_2 fixed observed for ISRA 604 was not significant. These results are in accordance with that of Sanginga et al. (1991) showing that the %Ndfa of *G. sepium* did not change with increasing levels of P application. Plant inoculated with both rhizobium and AM fungus and amended with P exhibited similar shoot Ndfa in each treatment. The potential nitrogen fixation and P uptake and growth of *G. sepium* can be positively influenced when rhizobium and AM fungus inoculations and P level fertiliser are adequately selected.

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