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The mycorrhizal fungus *Glomus intraradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of *Acacia holosericea*

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Abstract

Plants inoculated with arbuscular mycorrhizal (AM) fungi utilize more soluble phosphorus from soil mineral phosphate than non-inoculated plants. However, there is no information on the response of soil microflora to mineral phosphate weathering by AM fungi and, in particular, on the catabolic diversity of soil microbial communities.

The AM fungus, *Glomus intraradices* was examined for (i) its effect on the growth of *Acacia holosericea*, (ii) plant-available phosphate and (iii) soil microbial activity with and without added rock phosphate.

After 4-months culture, AM fungal inoculation significantly increased the plant biomasses (by 1.78× and 2.23× for shoot and root biomasses, respectively), while mineral phosphate amendment had no effect in a sterilized soil. After 12-months culture, the biomasses of *A. holosericea* plants growing in a non-sterilized soil amended with mineral phosphate were significantly higher than those recorded in the control treatment (by 2.5× and 5× for shoot and root biomasses, respectively). The fungal inoculation also significantly stimulated plant growth, which was significantly higher than that measured in the mineral phosphate treatment. When *G. intraradices* and mineral phosphate were added together to the soil, shoot growth were significantly stimulated over the single treatments (inoculation or amendment) (1.45×). The P leaf mineral content was also higher in the *G. intraradices*+mineral phosphate treatment than in *G. intraradices* or rock phosphate amendment. Moreover, the number of fluorescent pseudomonads has been significantly increased when *G. intraradices* and/or mineral phosphate were added to the soil. By using a specific type of multivariate analysis (co-inertia analysis), it has been shown that plant growth was positively correlated to the metabolization of ketoglutaric acid, and negatively linked to the metabolisation of phenylalanine and other substrates, which shows that microbial activity is also affected.

G. intraradices inoculation is highly beneficial to the growth of *A. holosericea* plants in controlled conditions. This AM symbiosis optimises the P solubilization from the mineral phosphate and affects microbial activity in the hyphosphere of *A. holosericea* plants.
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Keywords: Arbuscular mycorrhizal symbiosis; Rock phosphate; *Acacia*; Microbial catabolic diversity

1. Introduction

In West Africa, agricultural practices over-exploiting the natural resources have decreased soil fertility particularly with deficiencies of nitrogen and phosphorus (Piéri, 1989). Phosphorus is an essential element for plant nutrition and it can be only assimilated as soluble phosphate. However, in natural conditions, most of phosphorus soil content (soil mineral phosphates (rock phosphates, RP) and organic phosphates) is poorly soluble.

Physical and chemical weathering of RP take place along plant roots into the rhizosphere that also supports large microbial communities. These microbes accelerate weathering of minerals by producing organic acids, phenolic compounds, protons and siderophores (Drever and Vance, 1994). Numerous studies have identified microbial groups

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which could solubilize mineral phosphates and improved plant phosphorus nutrition (Gadd, 1999).

Among soil microorganisms, arbuscular mycorrhizal (AM) fungi have been found to be essential components of sustainable soil-plant systems (Smith and Read, 1997; van der Hejden et al., 1998; Schreiner et al., 2003). They increased plant uptake of phosphorus (Bolan, 1991), micronutrients (Bürkert and Robson, 1994) and nitrogen (Barea et al., 1991). They enhanced water absorption (George et al., 1992) and act as antagonisms against some plant pathogens (Dehne, 1982). Bacterial communities could also greatly interact with establishment of mycorrhizal symbiosis (Andrade et al., 1998). Moreover, it has been demonstrated that plants inoculated with AM fungi utilize more soluble phosphorus from RP than non-inoculated plants (Manjunath et al., 1989; Antunes and Cardoso, 1991). The main explanation is that mycorrhizas developed an extramatrical mycelium which increased the root P absorbing sites (Bolan, 1991). This mycorrhizal effect has been frequently observed in RP amended soils and it induced spectacular stimulations of the plant growth and P foliar contents (Guissou et al., 2001).

Acacia is the largest mimosoid genus and includes nearly 1200 species (Pedley, 1986). They are abundant in savanas and arid regions of Australia, Africa, India and the Americas. Some of them prevent wind and rain erosion, control sand dunes, are sources of wood and provide fodder for browsing livestock. In addition, much of the nitrogen fixed in leaves and roots by the Rhizobial symbiosis is returned to the soil with the natural loss of leaves and the resulting humus improves the fertility of the soil and its physical properties. One of the fast-growing leguminous trees, *Acacia holosericea*, brought from Australia and introduced in Western Africa appears to be well adapted to these climatic conditions.

It has been previously demonstrated that *A. holosericea* growth was greatly enhanced by *G. intraradices* (Duponnois and Plenchette, 2003). Similar positive effects have been obtained with other AM fungi such as *G. mosseae* (Cornet and Diem, 1982), *G. fasciculatum* (Senghor, unpublished thesis) and *G. aggregatum* (Duponnois et al., 2001). *A. holosericea* is usually considered to be endomycorrhizal dependent (Reddell and Warren, 1987).

Since AM fungi are obligate endosymbionts and live on carbohydrates obtained from the root cells, all soil factors affecting plant growth and physiology such as fertility, will also modify fungal activity and, in terms, influence the structure and functioning of bacterial communities (Azaizeh et al., 1995; Andrade et al., 1998).

Although the mechanisms involved in RP weathering and their consequences on plant growth are well studied, there are no information on the response of soil microflora to RP weathering by AM fungi and, in particular, on the catabolic diversity of soil microbial communities.

In the present study, the AM fungus, *Glomus intraradices* was examined for (i) its effect on the growth of *A. holosericea*, (ii) its RP solubilizing activity and (iii) the impact of RP weathering on soil microbial activity.

2. Materials and methods

2.1. Plant and fungal inoculum

Seeds of *A. holosericea* were surface-sterilized with concentrated sulphuric acid (36 N) for 60 min. The acid solution was then decanted and the seeds rinsed for 12 h in four rinses of sterile distilled water. Seeds were then transferred aseptically in Petri dishes filled with 1% (w/v) water agar. The plates were incubated for 4 days at 25 °C. The germinating seeds were planted when rootlets were 1–2 cm long.

The AM fungus *Glomus intraradices* Schenk and Smith (DAOM 181602, Ottawa Agricultural Herbarium) was multiplied on leek (*Allium porrum L.*) for 12 weeks under greenhouse conditions on Terragreen™ substrate. This calcined clay (particule size average 5 mm), Oil-Dri US-special Ty/IIIR (Oil-Dri Company, Chicago, USA) is an attapulgite from Georgia used as substrate for propagation of AM fungi (Plenchette et al., 1996). Before inoculation of the *Acacia* seedlings, the leek plants were uprooted, gently washed and roots cut into 0.5 cm long pieces bearing around 250 vesicles cm^{-1}. Non-mycorrhizal leek roots, prepared as above, were used for the control treatment without AM inoculation. It has been previously demonstrated that this AM fungus significantly improved the growth of tree species such as Australian *Acacia* species, *Gmelina arborea*, etc. (unpublished data).

2.2. Experimental design

The germinated seeds were individually grown in 0.5 l polythene bags filled with an sterilized sandy soil (140 °C, 40 min) collected in an experimental station localized at Gampella (20 km from Ouagadougou, Burkina Faso). After autoclaving, the physico-chemical characteristics of the soil were as follows: pH (H_2O) 5.6; clay (%) 4.6, fine silt (%) 0.0; coarse silt (%) 0.8; fine sand (%) 25.5; coarse sand (%) 69.1; carbon (%) 2.04; total nitrogen (%) 0.04; Olsen phosphorus 4.3 mg kg^{-1}; total phosphorus 116 mg kg^{-1}.

The chemical characteristics of the RP used in this experiment, Kodjari rock phosphate (Burkina Faso, KRP), are as follows: Ca (%) 32.0; CO_2 (%) 1.0; K (%) 0.119; Na (%) 0.605; Mg (%) 1.06; Fe (%) 0.375; Al (%) 0.488; S (%) 0.025; Cl (%) 0.043; F (%) 3.2; total P (%) 13.2; soluble P (%) 0.032. Before use, the KRP was ground with a pestle and mortar and passed through a 90 μm sieve. Then, the cultural substrate was mixed with 0.1% (w/w) KRP powder, whereas treatments without KRP did not receive any RP amendment.

For endomycorrhizal inoculation, one hole (1 cm by 5 cm) was made in each pot and filled with 1 g fresh leek

root (mycorrhizal, or not for the control treatment without fungus). The holes were then covered with the same sterilized soil. Plants were daily watered with tap water (pH 6.0) without fertiliser. The pots were arranged in a randomised complete block design with 12 replicates per treatment. They were placed in a glasshouse under natural light (daylight approximately 12 h, mean temperature 25 °C day).

After 4-months culture, eight plants were randomly chosen from each treatment. They were uprooted and the root systems gently washed. The oven-dried (1 week at 65 °C) of the shoots was measured. The roots were cleared and stained according to the method of Phillips and Hayman (1970). They were placed on a slide for microscopic observation at 250× magnification (Brundrett et al., 1985). About 100 1 cm root pieces were observed per plant. The extent of mycorrhizal colonization was expressed as the number of mycorrhizal root pieces/number of non-mycorrhizal root pieces×100. Then the dry weight of roots was measured (65 °C, 1 week). With this fungal strain, the morphology of these vesicles was very different from external spores. Internal spores were never observed under our cultivation conditions.

For each combination, the remaining four plants were transferred into 20 l pots filled with the same non-sterilised soil. Plants were grown under nursery conditions at ambient temperature from 15 to 40 °C with daily watering. After 12-month culture, the height of the plants was measured. Then they were uprooted and the root systems gently washed. The soil from each pot was carefully mixed and 2 kg sub-samples were taken and kept at 4 °C for further analysis. The leaves and the stems of each plant were divided and their oven-dried weights (2 weeks at 65 °C) were measured. After drying, 1 g of leaf tissue from each plant was ground, ashed (500 °C), digested in 2 ml HCl 6 N and 10 ml HNO_3N and then analysed by colorimetry for P (John, 1970). Another 1 g sub-sample of leaf tissue was ground and digested in 15 ml H_2SO_4 36 N containing 50 g l^{-1} salicylic acid for N (Kjeldhal) determination. Root nodules induced by indigenous rhizobia were detected and counted. Two grams of fresh root were randomly sampled along the root system and the intensity of AM symbiosis was determined as described before. Then the roots were oven-dried (1 week, 65 °C) and weighed.

2.3. Measurement of changes in the catabolic diversity of microbial communities in soil treatments

Microbial functional diversity in soil treatments was assessed by measurement of the patterns of in situ catabolic potential (ISCP) of microbial communities (Degens and Harris, 1997). Thirty four substrates, comprising a range of amino acids, carbohydrates, organic acids and amides, were screened for differences in SIR responsiveness between soil treatments. The substrate concentrations providing optimum SIR responses were as follows: 15 mM for amino acids (L-phenylalanine, L-glutamine, L-serine, L-arginine, L-asparagine, L-histidine, L-lysine, L-glutamic acid, L-tyrosine L-cysteine), 75 mM for carbohydrates (D-glucose, D-mannose, sucrose), 15 mM for amides (D-glucosamine, *N*-methyl-D-glucamine, succinamide), 100 mM for carboxylic acids (ascorbic acid, citric acid, fumaric acid, gluconic acid, quininc acid, malonic acid, formic acid, α-ketoglutaric acid, α-ketobutyric acid, succinic acid, tartaric acid, uric acid, oxalic acid, gallic acid, malic acid, tri-citrate, DL-a-hydroxybutyric acid) and 100 mM for cyclohexane (polymer) (Degens and Harris, 1997). One gram equivalent dry weight soil was mixed to each substrate suspended in 2 ml sterile distilled water (West and Sparling, 1986) in 10 ml bottles. CO_2 production from basal respiratory activity in the soil samples was also determined by adding 2 ml sterile distilled water to 1 g equivalent dry weight of soil. After the addition of the substrate solutions to soil samples, bottles were immediately closed and kept at 28 °C for 4 h. CO_2 fluxes from the soils were measured using an infrared gas analyser (IRGA) (Polytron IR CO_2, Dräger™) in combination with a thermal flow meter (Heinemeyer et al., 1989). Results were expressed as μg CO_2 g^{-1} soil h^{-1}.

2.4. Mycorrhizal soil infectivity measurement

The mycorrhizal soil infectivity (MSI) was determined from each soil sub-sample taken from each pot. It was calculated with a bioassay based on a dose (quantity of a non-disinfected soil)—response (mycorrhizal status of test plants) according to the biological assay principle (Plenchette et al., 1989). Cultivation of a population of mycotrophic plantlets was done on a range of concentrations of natural soil diluted with the same disinfected soil. Six dilutions of each soil samples were realised by mixing the original soil in various quantities (100, 48, 24, 12, 6 and 3%, w:w) with the same autoclaved soil (140 °C, 40 min) to give a range of concentrations. There were five replicates per dilution. Seeds of millet (*Pennisetum typhoides* L.) were pre-germinated for 2 days in Petri dishes on humid filter paper. Ten germinated seeds were transplanted into plastic pots (5.5 cm diameter; 6 cm high) filled with 100 g of each dilution. Pots were placed in a greenhouse (30 °C day, 20 °C night, 10 h photoperiod) and watered daily with tap water. Plants were only grown for 2 weeks to detect the first hyphal entries and not the secondary mycorrhizal infections. Then the entire root system of each seedling was collected, gently washed under tap water, clarified in 10% KOH for 30 min at 90 °C and stained for 15 min with acid fuchsin (0.05% in lactoglycerol). Each entire root system was mounted on a microscope slide and checked at a 250× magnification for the presence of endomycorrhizal structures. A single AM hyphal entry was considered as a record of mycorrhizal infection to give an all or nothing quantitative response. The infected plants were counted and the results were expressed as percentages of mycorrhizal plants per pot.

For each soil treatment, the percentage of mycorrhizal plants was plotted against the logarithm of non-sterilised soil concentration. Regression curves (model $Y = BX + A$) were calculated for each soil treatment and variance analysis was performed to test the non-equality of their slope. The mycorrhizal soil infectivity (MSI) unit was calculated using a regression line equation (Duvert et al., 1990) and defined as the minimum dry weight (g) of soil required to infect 50% (MSI_{50}) of a plant population under the bioassay conditions and calculated for $Y = 50\%$. Anovas were carried out to compare slopes of regression lines between non-sterile soil concentrations and percentages of mycorrhizal seedlings. Soils with statistically similar slopes were grouped and a common slope was calculated to compare their *y*-intercepts using a *t*-test (Dagnelie, 1975). This software has been created by André Carteron (INRA, Station de Génétique et d'Amélioration des plantes, Dijon, France).

2.5. Bacteria CFU

Bacteria belonging to the group of fluorescent pseudomonads have been only enumerated since it has been previously demonstrated that these bacteria could act as RP solubilizers (De Freitas et al., 1997) and interact with AM fungi (Meyer and Linderman, 1986). Soil subsamples (1 g fresh weight) were suspended in 10 ml sterile magnesium sulfate. Then serial dilutions of homogenised suspensions were plated on King's B medium (King et al., 1954) to isolate fluorescent pseudomonads and incubated for 48 h at 30 °C. The King's B medium plates were examined under UV light and fluorescent colonies were counted.

2.6. Statistical analysis

Data were treated with one-way analysis of variance. Means were compared using PLSD Fisher test ($p < 0.05$). Mycorrhizal indexes were transformed by arcsin (sqrt) before statistical analysis.

Co-inertia analysis (COIA) was used to analyse the relationships between the plant and soil microbial characteristics, and the SIR responses. Co-inertia analysis (Dray et al., 2003) is a multivariate analysis technique that describes the relationships between two data tables. Classical methods like principal components analysis (PCA), or correspondence analysis (CA), aim at summarizing a table by searching orthogonal axes on which the projection of the sampling points (rows of the table) have the highest possible variance. This characteristic ensures that the associated graphs (factor maps) will best represent the initial data. To extract information common to both tables, canonical correlation analysis (CANCOR) searches successive pairs of axes (one for each table) with a maximum correlation. The problem is that it often leads to axes with a very high correlation, but with very low percentages of explained variance. To overcome this difficulty, COIA searches pairs of axes with maximum covariance (instead of correlation). Computations are based on the cross-table between SIR responses and plant variables. The importance of each axis is given by the percentage of total co-inertia, which is similar to the percentage of explained variance for each canonical axis.

Another problem of CANCOR and also of canonical correspondence analysis (CCA, Ter Braak, 1986), is that the number of rows of both tables must be high compared to the number of columns of the independent variables table. When the number of rows is low, CANCOR cannot be used, and CCA is reduced to a plain CA (see for example Dray et al., 2003). COIA does not suffer from this problem, and it can be used even if the number of rows is lower than the number of columns, which is the case in this paper. A simple PCA-like standardisation was applied to both data tables before computing COIA.

Monte-Carlo tests can be used to check the significance of the relationship between the two tables. This method consists in performing many times a random permutation of the rows of one table (or of both), followed by the re-computation of the total co-inertia. By comparing the total co-inertia obtained in the normal analysis with the co-inertia obtained after randomisation, we get an estimation of the probability to meet a situation similar to the observed situation, without relationship between the two tables (i.e., a significance test of the relationship). Computations and graphical displays were made with the free ADE-4 software (Thioulouse et al., 1997), available on Internet at http://pbil.univ-lyon1.fr/ADE-4/

3. Results

After 4-months culture, AM fungal inoculation (*G. intraradices*) with or without KRP increased significantly the height, shoot and root biomasses (Table 1). The positive fungal effect on *A. holosericea* growth was not modified when KRP was added together with the fungus.

After 12-months culture, the height, steam and total shoot biomasses of *A. holosericea* plants growing in a soil amended with KRP, were significantly higher than those recorded in the control treatment (Table 1). The fungal inoculation had also significantly stimulated plant growth (height, leave, stem and total shoot biomass) which was also significantly than that measured in the KRP treatment (Table 1). When *G. intraradices* and KRP were added together to the soil, shoot growth (leave and stem biomasses) were significantly stimulated over the single inoculation or amendment treatments (Table 1). The same positive effects were recorded for the root biomass (Table 1). No significant differences were found between the treatments for the number of nodules per plant and the mycorrhizal colonisation (Table 1).

Compared to the control, P and N contents were significantly higher in the KRP treatment (Table 2). This

Table 1
Growth response of *Acacia holosericea* seedlings, mycorrhizal colonization and rhizobial establishment in soils inoculated with *G. intraradices* and/or rock phosphates after 4-months culture in a disinfected soil and 12-months culture in a non-disinfected soil

	Treatments			
	Control	KRP[a]	*G. intra.*[b]	*G. intra.* + KRP
Four-months culture				
Height (cm)	10.7a[c]	11.7a	16.2b	16.8b
Shoot biomass (mg dry weight)	445a	450a	790b	810b
Root biomass (mg dry weight)	220a	245a	490b	510b
Mycorrhizal colonization (%)	0	0	22.2a	20.8b
Twelve-months culture				
Height (cm)	60a	104b	137c	151c
Leave biomass (g dry weight)	12.1a	6.1a	143.1b	212.2c
Steam biomass (g dry weight)	7.5a	42.6b	101.2c	143.1d
Total shoot biomass (g dry weight)	19.6a	48.7b	244.3c	355.3d
Root biomass (g dry weight)	2.07a	10.4b	28.3c	43.6d
Root biomass/total biomass	0.095a	0.175a	0.103a	0.109a
Number of nodules per plant	7a	37a	64a	3a
Mycorrhizal colonization (%)	76.7a	86.7a	76.7a	80.1a

[a] KRP, Kodjari Rock Phosphate.
[b] *G. intra.*, *G. intraradices*.
[c] Data in the same line followed by the same letter are not significantly different according to the one-way analysis of variance ($P<0.05$).

KRP effect was also significantly lower than that recorded with AM fungal inoculation. The highest values of mineral contents were calculated when *G. intraradices* and KRP were inoculated together to the soil (Table 2). The MSI_{50} was significantly higher in the *G. intraradices* treatment than those determined in the control and in the *G. intraradices* + KRP treatment (Table 3). Fluorescent pseudomonads were detected when AM fungal inoculation was performed with or without KRP. In contrast, there were no bacteria belonging to this group in the soil of the control treatment (Table 3).

Table 2
Effect of *G. intraradices* inoculation and/or Kodjari Rock Phosphate amendment on leaf mineral content of *A. holosericea* plants after 12-months culture in a non-disinfected soil

Treatments	P (mg per plant)	N (mg per plant)
Control (not inoculated)	18.5a[a]	245.9a
KRP[b]	48.5b	1431.1b
G. intraradices	148.6c	3450.3c
G. intraradices + KRP	270.9d	5865.6d

[a] Data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance ($P<0.05$).
[b] KRP, Kodjari Rock Phosphate.

Table 3
Effect of *G. intraradices* inoculation and/or Kodjari Rock Phosphate amendment on the mycorrhizal soil infectivity and on the number of fluorescent pseudomonads per gram of soil after 12-months culture in a non-disinfected soil

Treatments	MSI 50[a] (g)	Fluorescent pseudomonads (CFU g^{-1} of soil)
Control (not inoculated)	12.1a[b]	0a
KRP[c]	20.3ab	167b
G. intraradices	35.6b	157b
G. intraradices + KRP	12.7a	467b

[a] Quantity of soil required to infect 50% (MSI50) of a plant population.
[b] Data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance ($P<0.05$).
[c] KRP, Kodjari Rock Phosphate.

Co-inertia analysis of the relationships between SIR responses and plant variables were shown in Fig. 1. The Monte-Carlo permutation test on the total co-inertia between the two tables was highly significant ($p\sim0.005$), which suggested that there was a strong relationship between both data sets. In the four graphics A, B, C and D, the *x*-axis was the first co-inertia axis (77% of the total co-inertia), and the *y*-axis was the second co-inertia axis (8% of the total co-inertia). For the factor map of SIR responses substrates (Fig. 1A), the following six substrates were in the left part of the map (negative values of first co-inertia axis): phenylalanine, citrate, glutamine, ascorbic acid, formic acid, and cyclohexane. On the opposite side of the map, ketoglutaric acid had a high positive coordinate on the first axis. This opposition showed that theses substrates were metabolised in different soil treatments. On the second axis, tartaric acid is opposed to sucrose and succinamide. In the factor map of plant variables (Fig. 1B), on the first axis, variables correlated to plant growth (height, stem and leaf biomass, root biomass, number of fluorescent pseudomonads) had high positive coordinates, so they were far in the right part of the map. Total leaf phosphorus is opposed to nodules and mycorrhizal colonization on the second axis. The factor map of SIR responses soil samples showed the distribution of the four treatments as follows: control (C), addition of Kodjari rock phosphate (K), inoculation of *G. intraradices* (G) and addition of both (GK). There were four repetitions for each treatment. The four control points had highly negative coordinates on the first factor. This showed that the six substrates on the left on Fig. 1A were more actively metabolised in the control than in the other treatments. Conversely, ketoglutaric acid was more actively metabolized in the G and G + K treatments than in the control. In the factor map of plant variables soil samples, the four treatments were ordered in the same way as in Fig. 1C,

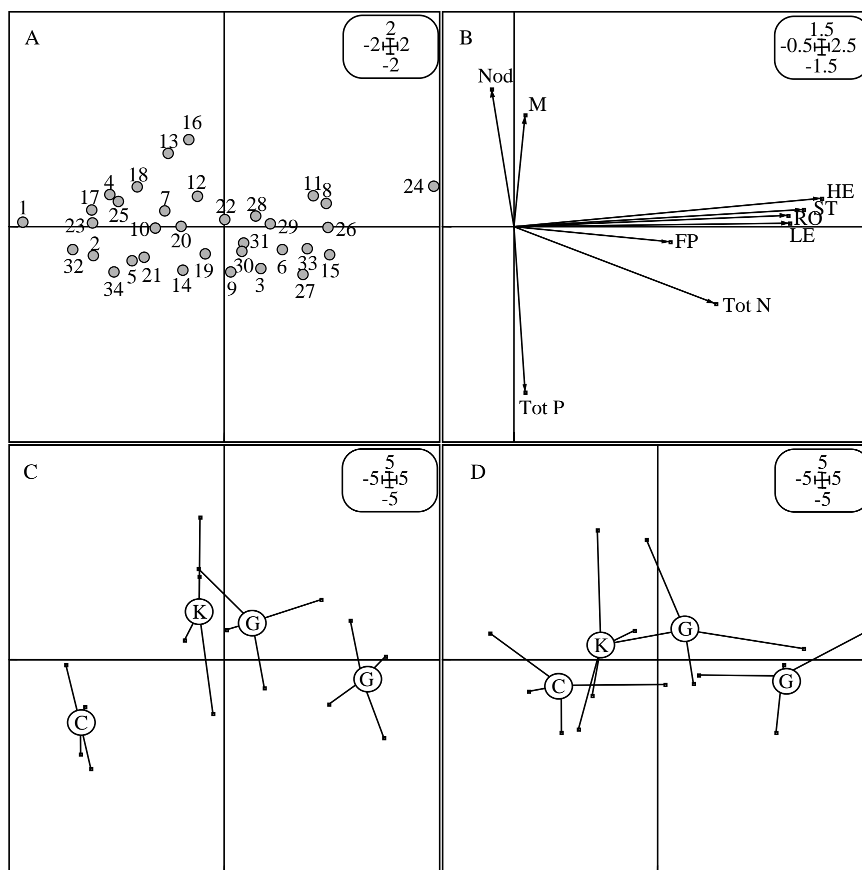

Fig. 1. Co-inertia analysis of the SIR responses of the soils inoculated with *G. intraradices* and/or Kodjari Rock phosphate, plant growth and microbiological variables. (A) Factor map of SIR responses (L-phenylalanine, 1; L-glutamine, 2; L-serine, 3; L-arginine, 4; L-asparagine, 5; L-histidine, 6; L-lysine, 7; L-glutamic acid, 8; L-tyrosine, 9; L-cysteine, 10; D-glucose, 11; D-mannose, 12; sucrose, 13; D-glucosamine, 14; *N*-methyl-D-glucosamine, 15; succinamide, 16; ascorbic acid, 17; citric acid, 18; fumaric acid, 19; gluconic acid, 20; quinic acid, 21; malonic acid, 22; formic acid, 23; α-ketoglutaric acid, 24; α-ketobutyric acid, 25; succinic acid, 26; tartaric acid, 27; uric acid, 28; oxalic acid, 29; gallic acid, 30; malic acid, 31; tri-citrate, 32; DL-a-hydroxybutyric acid, 33; cyclohexane, 34. (B) Factor map of plant growth and microbial variables (nod, number of nodules per plant; MC, mycorrhizal colonisation; HE, height; ST, stem biomass; RO, root biomass; LE, leaf biomass; FP, fluorescent Pseudomonads; Tot P, P leaf content; Tot N, nitrogen leaf content; MC, mycorrhizal colonization). (C) Factor map of SIR responses soil samples (C, control (not inoculated); K, Kodjari rock phosphate amendment; G, *G. intraradices* inoculation; GK, *G. intraradices* inoculation and Kodjari rock phosphate amendment). (D) Factor map of plant growth and microbial variables soil samples (for the legend, see (C)).

with the Control on the left, the K and G treatments in the middle, and GK on the right. This suggested that (i) the addition of rock phosphate, and of *G. intraradices* had a positive effect on plant growth, (ii) this effect was cumulative, since the GK treatment had a still stronger positive effect on plant growth and (iii) plant growth was positively linked to the metabolization of ketoglutaric acid, and negatively linked to the metabolisation of the six substrates underlined in Fig. 1A.

4. Discussion

From this study, it could be concluded that AM fungal inoculation with *G. intraradices* was effective in a non-sterilized soil, (ii) this plant growth stimulation could be improved with Rock Phosphate amendment, (iii) *G. intraradices* could decrease the MSI and (iv) high levels of keto-glutaric acid SIR responses were associated with the *G. intraradices* + KRP soil.

Whereas rock phosphate amendment had no effect on *A. holosericea* during the 4-months culture in a sterilized soil, it increased significantly plant growth after 12-months culture in non-sterilized soil. This implied that the contribution of indigenous soil microflora to plant P nutrition by absorbing from the inorganic P pool was significant. It has been previously demonstrated that legumes could immediately benefit from application of Rock Phosphate (Vanlauwe et al., 2000). Traditionally, benefits of legumes to a subsequent crop have been translated into N-fertilizer replacement values of the legume biomass (Sanginga et al., 1999). However, it has been suggested that other effects than the increased N supply such as the improvement of soil physical and biological characteristics after the legume crop could be also important (Kahm et al., 1999). In the present research work, two biological factors (AM fungi and fluorescent

pseudomonads) have been studied. It has been previously shown that fluorescent pseudomonads could solubilize organic or inorganic phosphates (Duponnois, 1992, unpublished thesis). In addition, a lot of studies have demonstrated that AM fungi could mobilize P from RP (Bâ and Guissou, 1996; Bâ et al., 1996).

Under semi-arid conditions, it is well known that AM fungal inoculum potential is very low and it has been already been shown that AM inoculation of plants is very efficient in establishing plants on disturbed soils (Estaun et al., 1997). Our results corroborate these conclusions. But, *A. holosericea* plants inoculated with *G. intraradices* could modilize P from RP more efficiently than those mycorrhized with indigenous AM fungi which suggest that Rock Phosphate weathering is highly dependent to the fungal symbiont.

Surprisingly, *A. holosericea* plants previously inoculated with *G. intraradices*, had significantly decreased the MSI of their cultural soils compared to the control. In soils, AM fungi are found as spores, hyphae and infected root pieces. All these propagules are sources of inoculum and extraradical mycelium is thought to be the main source (Sylvia and Jarstfer, 1992). Soil disturbance by grazing or erosion results in loss of AM propagules (Mosse, 1986). Other biological agents can damage the extraradical mycelium such as free living nematodes (Villenave et al., 2003). Since it has been demonstrated that AM fungal inoculation could increase the abundance of saprophagous nematodes (bacterial- and fungal-feeding nematodes) (Villenave et al., 2003), these microorganisms could decrease the number of fungal propagules in the soil and, consequently, limit mycorrhizal soil infectivity potential.

It is now well established that AM fungi modify root functions (i.e. root exudation) (Marshner et al., 1997), change carbohydrate metabolism of the host plant (Shachar-Hill et al., 1995) and influence rhizosphere populations (Azaizeh et al., 1995; Andrade et al., 1998). Furthermore, AM fungi can exude substances that have a selective effect on the microbial community in the rhizosphere and in the soil (Hobbie, 1992). As these microbial communities differ from those of the rhizosphere (zone of soil subjected to the influence of living roots), this microbial compartment is commonly named 'mycorrhizosphere' (Linderman, 1988). The mycorrhizosphere is usually divided in two different zones, one under the dual influence of root and fungal partners of the mycorrhiza (the mycorrhizosphere) and the other subjected to the AM hyphae (the mycosphere or hyphosphere) which supports different bacterial activities from those recorded in the mycorrhizosphere (Andrade et al., 1998). Microorganisms in the hyphosphere of AM fungi may affect mycorrhizal functions such as nutrient and water uptake carried out by the external hyphae of AM fungi. In contrast, fungal activities could also modify the structure and the biofunctionning of microbial communities.

Rock phosphates can be solubilized or weathered under the influence of water, acids, complexing agents and oxygen. Biological weathering or biochemical weathering is made by microorganisms which produce organic acids, phenolic compounds, protons and siderophores (Drever and Vance, 1994). Soluble organic acids affecting rock phosphate weathering in soils could be of high molecular weight (i.e. humic substances) or low molecular weight produced by plant roots and soil microorganisms (Ochs, 1996). These low molecular weight organic acids produced by plant roots and soil microorganisms are very effective in promoting mineral dissolution.

Mycorrhizal fungi can solubilize surrounding weatherable minerals through excretion of organic acids such as α-ketoglutaric acid. This organic compound could exert a selective influence on soil microbial communities though a multiplication of α-ketoglutarate catabolizing microorganisms (higher SIR response to α-ketoglutaric acid).

The fluorescent pseudomonad populations were also more important in the soils inoculated with *G. intraradices* and/or rock phosphate. It has been previously demonstrated that the mycorrhizosphere activity exerts a significant stimulating effect on the populations of fluorescent pseudomonads (Founoune et al., 2002). This mycorrhizosphere effect on fluorescent pseudomonads was not AM fungal specific while it has been detected in the KRK treatment with the indigenous AM fungi. In this last case, root biomass being significantly higher than in the control, it suggested that plant roots could intensively interact with native AM fungi and promote this mycorrhizosphere effect.

These microorganisms using organic acids excreted by AM fungi, could also act as solubilizing agents against Rock Phosphates. This result suggests also that Rock Phosphate weathering is not limited to the AM fungus and the host plant but could be enlarged to the soil microbiota.

Above results clearly show that *G. intraradices* inoculation is highly beneficial to the growth of *A. holosericea* plants in controlled conditions. This AM symbiosis optimises the P solubilization from the Kodjari Rock Phosphate and affects microbial activity in the hyphosphere of *A. holosericea* plants. More researches must be undertaken to precise the ecological relevance of AM symbiosis and their associated microbial communities induced weathering.

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