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Contribution of *Pseudomonas mendocina* and *Glomus intraradices* to aggregate stabilization and promotion of biological fertility in rhizosphere soil of lettuce plants under field conditions

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Abstract

A field-plot experiment was undertaken to compare the effect of inorganic fertilizer with that of inoculation with an arbuscular mycorrhizal fungus, *Glomus intraradices*, or with a plant-growth-promoting rhizobacterium, *Pseudomonas mendocina*, alone or in combination with inorganic fertilizer, on plant growth and nutrient uptake by lettuce. The influence of the microbial inoculation treatments on soil physical, biochemical and biological properties was also assessed. Two months after planting, fertilizer and inoculation with *G. intraradices* or *P. mendocina* had significantly increased shoot and root biomass and foliar nutrient contents (P, Fe). The inoculation with *G. intraradices* or *P. mendocina* both increased the soil water-soluble carbohydrates and the percentage of stable aggregates. In this study, we provide the first evidence of the beneficial effect of a plant-growth-promoting rhizobacterium on soil aggregate stabilization under field conditions. Only inoculation with *P. mendocina* had a significant effect on the dehydrogenase and phosphatase activities, 21 and 89%, respectively, compared with the control. Inorganic fertilization alone did not increase aggregate stability or enzyme activities in soil, even though this treatment produced the largest increases in mass of lettuce.

Keywords: Aggregate stability, arbuscular mycorrhizal fungi, enzymatic activities, microbial biomass C, plant-growth-promoting rhizobacterium

Introduction

The beneficial rhizosphere micro-organisms are important determinants of plant health and soil fertility because they participate in many key ecosystem processes such as those involved in the biological control of plant pathogens, nutrient cycling and seedling establishment (Jeffries *et al*., 2003). However, the natural roles of rhizosphere micro-organisms have been marginalized due to conventional farming practices such as tillage and high inputs of inorganic fertilizer and pesticides (Mäder *et al*., 2002). Improving crop yields by stimulating the growth and activity of such beneficial micro-organisms appears to be a promising alternative to agricultural practices involving extensive usage of pesticides and fertilizer. Thus, micro-organisms beneficial to plants are expected to play an important role in the sustainability of agroecosystems.

There are several groups of beneficial rhizosphere micro-organisms, which may be symbiotic or free-living. Among the symbiotic micro-organisms, arbuscular mycorrhizal (AM) fungi form mutual associations with more than 80% of plant species, including agronomic plants. AM fungi are known to enhance mineral uptake (particularly P), tolerance to water-stress (Ruíz-Lozano, 2003), and soil aggregation (Caravaca *et al*., 2002). However, AM symbiosis is influenced by various management practices, such as the degree and type of fertilization, the host plant species or cultivar, the mycorrhizal species, the type of host-plant root system and the crop rotation or soil tillage (Oehl *et al*., 2003). In addition, many environmental factors, such as soil water and aeration (Augé, 2001) and soil micro-organisms (Johansson *et al*., 2004) have a major

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impact on AM distribution and effectiveness. In particular, the so-called mycorrhiza helper bacteria are known to stimulate mycelial growth of mycorrhizal fungi and/or to enhance mycorrhizal formation (Toro *et al*., 1997). Among free-living micro-organisms, the plant-growth-promoting rhizosphere bacteria (PGPR) have received special attention. PGPR can exert a beneficial effect on plant growth due probably to a combination of N fixation, mobilization of nutrients in the soil and excretion of plant hormones (Vessey, 2003). The use of PGPR may permit reductions in chemical fertilizer inputs to inoculated plants or increase the efficiency of uptake of applied fertilizer nutrients, especially for nutrients of low mobility such as iron and phosphorus. In particular, on soils of high pH, iron forms insoluble hydroxides and the availability of free Fe is often too low to meet the plant demand. Some authors have found that the production of siderophores by bacteria of the genus *Pseudomonas* increases Fe solubility, contributing to the overall iron requirements of plants, especially in calcareous soils (Masalha *et al*., 2000). However, the application of microbial fertilizers in the field has given contradictory results because environmental factors contribute significantly to the biosynthesis of siderophores.

Maintenance of soil quality is an integral part of agricultural sustainability. Soil organic matter influences a wide range of physical, chemical and biological properties of soil and is considered by some authors as the most important indicator of soil quality (Bolinder *et al*., 1999). Early changes in total soil organic matter may be small and detectable only by monitoring the active fractions of SOM such as the labile C fractions and microbial biomass C. Microbial activity in the rhizosphere is a major factor that determines the availability of nutrients to plants and has a significant influence on plant health and productivity. Soil enzyme activities and microbial biomass have been shown to be sensitive indicators of changes produced by management practices, crops, fertilizers or environmental conditions (Roldán *et al*., 2005). Soil structure is crucial to the success of sustainable agriculture. On all but the coarsest soils aggregation is essential to maintain soil porosity; it facilitates water infiltration, provides adequate habitat space for soil organisms and an adequate oxygen supply to roots and soil organisms, and helps avoid soil erosion (Díaz-Zorita *et al*., 2002). We hypothesize that introduced micro-organisms can have a significant effect on soil properties and quality as they can interact with natural micro-organisms in the rhizosphere. However, there are relatively few studies on the use of such biological and physical parameters as indicators of soil quality in agroecosystems treated with microbial inoculants (Caravaca *et al*., 2002).

The objectives of this study were to: (1) compare the effectiveness of inorganic fertilizer with that of inoculation with an AM fungus or with a plant-growth-promoting rhizobacterium, alone and in combination with inorganic fertilizer in increasing plant growth and nutrient uptake in lettuce seedlings grown in a field trial and, (2) ascertain the changes in soil physical, biochemical and biological properties related to soil microbial activity induced by microbial inoculation.

Material and methods

Study sites and plant

The research was conduced on an experimental field, belonging to the University of Murcia (SE Spain), that had frequently grown lettuce. The climate is semi-arid Mediterranean with an average annual rainfall of 300 mm and a mean annual temperature of 19.2 °C; the potential evapo-transpiration reaches 1000 mm year^{-1}. The main characteristics of the field soil were: pH (1:5 H_2O) 8.5; electrical conductivity 0.82 dS m^{-1}; TOC 0.63%; total N 0.52 g kg^{-1}; available P 10 μg g^{-1}; extractable K 166 μg g^{-1}; $CaCO_3$ 40.0%; cationic exchange capacity 10 cmol kg^{-1}.

The plant used in the experiment was lettuce (*Lactuca sativa* L. cv. Focea). The plants were grown from seed for 15 day in peat without any fertilizer prior to transplanting into field plots.

Micro-organisms

The AM fungus *Glomus intraradices* (Schenk & Smith) was obtained from the collection at the experimental field station of Zaidín, Granada (EEZ1), Spain. AM fungal inoculum consisted of a mixture of rhizospheric soil from pot cultures (sorghum sp.) containing spores, hyphae and mycorrhizal root fragments.

The *Pseudomonas mendocina* strain was obtained from Probelte, S.A., Murcia, which was selected on the basis of its ability to produce siderophores. *Pseudomonas mendocina* was grown in a medium (nutrient broth, Scharlau Chemie, Spain) composed of meat and yeast extracts, peptone and sodium chloride, for 2 days at room temperature on a Heidolph Unimax1010 shaker. The bacterial culture was centrifuged at 2287 *g* for 5 min at 2 °C and the sediment was re-suspended in sterilized tap water. The bacterial suspension contained 10^9 colony forming units (CFU) mL^{-1}.

Experimental design and layout

The experiment was conducted in a fully randomized block design with five replicate blocks for each treatment. Blocks measured 20 m^2 (2 m wide and 10 m long). Five treatments were established: untreated control, plants inoculated with the AM fungus *G. intraradices*, soil inoculated with the bacteria *P. mendocina*, soil fertilized with inorganic fertilizer and the combination of soil inoculated with the bacteria *P. mendocina* and fertilized with an inorganic fertilizer. At least 40 seedlings per block were planted (eight plants × five treatments in each block).

In early October 2004, *L. sativa* seedlings were transplanted in individual holes. The AM inoculum was mixed with the soil, at a rate of 5% (inoculum:soil, v:v). A filtrate (Whatman No. 1 paper) of the pot culture of fungal inoculum was added to control plants to provide the microbial populations accompanying the mycorrhizal fungi. *Pseudomonas mendocina* was inoculated three times during the 2 months of the growth period. The dose of inoculum applied corresponded to 10^{10} CFU per plant and per application. The same amount of the autoclaved bacterial inoculum was added to control plants, to provide any nutrients added with the PGPR. Fertilized plants received 10 mL of Long Ashton Nutrient Solution on two occasions (μg mL^{-1}): 175.9 N, 156.2 K, 160.2 Ca, 98.4 S, 11.7 Na, 5.4 Cl, 5.0 Fe-chelate sequestrene, 0.54 Mn, 0.54 B, 0.10 Cu, 0.06 Zn, 0.006 Mo and 40 P. The plants were watered well throughout the experiment.

Two months after planting, five plants were harvested and five soil samples (one per block) were collected from each treatment (25 soil samples in total). Each sample consisted of eight bulked subsamples (200 cm^3 soil cores), collected randomly at 0–20 cm in the rhizospheres of individual plants. Soil strongly adhering to roots and collected at 0–4 mm from the root surface was defined as rhizosphere soil. Soil samples, sieved to <2 mm, were divided in two; one subsample was stored at 2 °C for microbiological analysis and the other subsample was allowed to dry at room temperature for physical and chemical analyses.

Plant analysis

Fresh and dry (105 °C, 5 h) mass of shoots and roots were recorded. The foliar concentrations of P and Fe were determined after digestion in nitric-perchloric acid (2:1) for 2 h. The foliar P was determined by colorimetry (Murphy & Riley, 1962) and foliar Fe was measured by atomic adsorption spectroscopy on a UNICAM 969 AA-Spectrometer.

The percentage of root length colonized by AM fungi was calculated by the gridline intersect method (Giovannetti & Mosse, 1980), after staining with trypan blue (Phillips & Hayman, 1970).

Soil analyses

Water-soluble carbon in a 1:5 extract (soil:water, w:v) was determined with an automatic Carbon Analyser for liquid samples (Shimadzu TOC-5050A) and water-soluble carbohydrates were determined by the method of Brink *et al.* (1960). Available P (with sodium bicarbonate) (Olsen *et al.*, 1954) was determined by colorimetry, according to Murphy & Riley (1962).

The percentage of stable aggregates was determined by the method described in Lax *et al.* (1994). A 4 g aliquot of soil sieved between 0.2 and 4 mm was placed on a small 0.250 mm sieve and wetted by spraying with water. After 15 min the soil was subjected to an artificial rainfall of 150 mL with energy of 270 Jm^{-2}. The remaining soil on the sieve was placed in a previously weighed capsule (T), dried at 105 °C and weighed (P1). The soil was then soaked in distilled water and, after 2 h, passed through the same 0.250 mm sieve with the assistance of a spatula to break the remaining aggregates. The residue remaining on the sieve, made up of plant debris and sand particles, was dried at 105 °C and weighed (P2). The mass of stable aggregates as a percentage of the total aggregates was calculated by $(P1 - P2) \times 100/(4 - P2 + T)$.

Microbial biomass C was determined using the fumigation-extraction method (Vance *et al.*, 1987). Ten grams of soil at 60% of its field capacity was fumigated in a 125-mL Erlenmeyer flask with purified $CHCl_3$ for 24 h. After removal of residual $CHCl_3$, 40 mL of 0.5 M K_2SO_4 solution was added and the sample was shaken for 1 h and filtered. The K_2SO_4-extracted C was measured as for water-soluble carbon. Microbial biomass C was calculated as the difference between the carbon of fumigated and non-fumigated samples divided by the calibration factor ($K_{EC} = 0.38$).

Dehydrogenase activity was determined according to García *et al.* (1997). One gram of soil at 60% of field capacity was exposed to 0.2 mL of 0.4% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) in distilled water for 20 h at 22 °C in the dark. The INTF (iodo-nitrotetrazolium formazan) formed was extracted with 10 mL of methanol by shaking vigorously for 1 min and filtering through Whatman No. 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

Urease and *N*-α-benzoyl-L-argininamide (BAA) hydrolysing protease activities were determined in 0.1 M phosphate buffer at pH 7; 1 M urea and 0.03 M BAA were used as substrates respectively. Two millilitres of buffer and 0.5 mL of substrate were added to 0.5 g of soil, which was incubated at 30 °C (for urease) or 39 °C (for protease) for 90 min. Both activities were determined as the NH_4^+ released by hydrolysis (Nannipieri *et al.*, 1980).

Phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate. Two millilitres of 0.1 M maleate buffer at pH 6.5 and 0.5 mL of substrate were added to 0.5 g of soil and incubated at 37 °C for 90 min. The reaction was stopped by cooling at 2 °C for 15 min. Then, 0.5 mL of 0.5 M $CaCl_2$ and 2 mL of 0.5 M NaOH were added, and the mixture was centrifuged at 2287 *g* for 5 min. The *p*-nitrophenol (PNP) formed was determined with a spectrophotometer at 398 nm (Tabatabai & Bremner, 1969). Controls were made in the same way, except that the substrate was added after incubation was stopped, before $CaCl_2$ and NaOH were added.

Statistical analysis

All data were subjected to an analysis of variance and comparisons among means were made using the least significant difference (LSD) test calculated at $P < 0.05$. Statistical procedures were carried out with the software package SPSS 10.0 for Windows.

Results

Labile C fractions, microbial biomass and aggregate stability

The rhizosphere soil of plants inoculated with *G. intraradices* or *P. mendocina*, alone or combined with fertilization, contained more water-soluble carbohydrates than that of the control plants. The fertilized and control soils contained similar quantities of water-soluble carbohydrates (Table 1). The fertilization and the inoculation with *P. mendocina* or *G. intraradices* had no significant effect on soil water-soluble C or microbial biomass C (Table 1). The inoculation with *G. intraradices* or *P. mendocina* significantly increased the aggregate stability of the rhizosphere soil of the lettuce plants (Table 1). The combination of fertilization and inoculation with *P. mendocina* was even more effective in increasing the percentage of stable aggregates (about 84% more than the control soil). The fertilized soil without inoculation and the control soil had the lowest aggregate stability.

Enzyme activities

Rhizosphere soil inoculated with *P. mendocina* had significantly greater dehydrogenase activity than the control soil (Table 2). However, neither mycorrhizal inoculation nor fertilization had any significant effect on dehydrogenase activity. The urease and protease-BAA activities of the rhizosphere soil were not affected by the fertilization or the inoculation with *P. mendocina* or *G. intraradices* (Table 2). Only the inoculation with *P. mendocina*, alone or combined with the fertilization, significantly increased the phosphatase activity (Table 2), producing an average increase of about 117% over the control soil.

Lettuce growth, foliar nutrients and mycorrhization

Two months after planting, all treatments assayed had significantly increased the shoot dry mass of lettuce seedlings, over that of the control plants (Table 3). Shoot dry mass was similar for plants inoculated with *G. intraradices* and plants grown in the soil inoculated with *P. mendocina*. The largest effect on plant growth was observed in the fertilization treatment, alone or in combination with the bacterial inoculation of *P. mendocina* (about 48% greater than the control plants). The lettuce root dry weight (Table 3) showed a similar trend to the shoot dry weight but there were no significant differences between fertilized plants and plants inoculated with *P. mendocina* (on average, 140% greater than the control plants).

The fertilization and the inoculation with *P. mendocina* or *G. intraradices* significantly increased the foliar P content, with fertilized plants and *G. intraradices*-inoculated plants having the highest contents (Table 4). The highest foliar Fe contents were in the plants inoculated with *P. mendocina*, followed by the fertilized without inoculation and the *G. intraradices*-inoculated treatments (Table 4). The plants grown in the soil which was both fertilized and inoculated with *P. mendocina* contained similar concentrations of foliar Fe to the control plants.

Only the mycorrhizal inoculum significantly increased the extent of AM fungal colonization of the root system (Table 4). The natural colonization observed in the control plants (about 30% at the end of the experiment) was similar to that in the two other treatments.

Discussion

Effectiveness of the bacterial inoculation

The selected PGPR, *P. mendocina*, was very effective in promoting plant growth although to a lesser extent than inorganic fertilizer. Several mechanisms have been described for the action of PGPR (Kloepper *et al.*, 1991). The bacterial effect on plant growth can be attributed to an increase in nutrient availability in the rhizosphere. The mechanisms by which these increases take place involve siderophore production, which helps facilitate the transport of certain nutrients,

Table 1 Labile C fractions, microbial biomass C and aggregate stability of rhizosphere soil of lettuce plants in response to microbial inoculation treatments 2 months after planting ($n = 5$)

	Water-soluble CH ($\mu g\ g^{-1}$)	Water-soluble C ($\mu g\ g^{-1}$)	Microbial biomass C ($\mu g\ g^{-1}$)	Aggregate stability (%)
Control	4 ± 1 a	66 ± 3 a	102 ± 8 a	11.0 ± 0.4 a
Fertilized (F.)	7 ± 1 ab	67 ± 4 a	94 ± 5 a	12.4 ± 0.6 ab
G. intraradices	11 ± 1 c	58 ± 4 a	91 ± 5 a	15.0 ± 0.6 bc
P. mendocina	9 ± 0 bc	65 ± 2 a	86 ± 2 a	16.0 ± 0.3 bc
F. + *P. mendocina*	11 ± 0 c	56 ± 2 a	118 ± 5 a	20.2 ± 0.6 c

Values are presented as mean ± standard error. CH, carbohydrates. Values in columns followed by the same letter are not significantly different (LSD, $P < 0.05$).

Table 2 Enzyme activities of rhizosphere soil of lettuce plants in response to microbial inoculation treatments 2 months after planting ($n = 5$)

	Dehydrogenase (μg INTF g^{-1} soil)	Urease (μmol NH_3 g^{-1} h^{-1})	Protease-BAA (μmol NH_3 g^{-1} h^{-1})	Phosphatase (μmol PNP g^{-1} h^{-1})
Control	23.1 ± 0.8 a	0.27 ± 0.01 ab	0.14 ± 0.02 a	0.18 ± 0.03 a
Fertilized (F.)	24.6 ± 0.9 ab	0.25 ± 0.01 ab	0.15 ± 0.01 a	0.12 ± 0.02 a
G. intraradices	20.2 ± 1.4 a	0.26 ± 0.06 ab	0.15 ± 0.06 a	0.12 ± 0.04 a
P. mendocina	27.9 ± 1.1 b	0.35 ± 0.02 b	0.18 ± 0.03 a	0.34 ± 0.02 b
F. + *P. mendocina*	23.1 ± 1.6 a	0.14 ± 0.03 a	0.07 ± 0.01 a	0.44 ± 0.03 b

Values are presented as mean ± standard error. INTF, iodo-nitrotetrazolium formazan. Values in columns followed by the same letter are not significantly different (LSD, $P < 0.05$).

Table 3 Growth of the lettuce plants in response to microbial inoculation treatments 2 months after planting ($n = 5$)

	Shoot (g DM $plant^{-1}$)	Root (g DM $plant^{-1}$)
Control	7.4 ± 0.24 a	0.86 ± 0.03 a
Fertilized (F.)	11.3 ± 0.16 c	1.25 ± 0.03 c
G. intraradices	9.5 ± 0.16 b	1.08 ± 0.02 b
P. mendocina	9.6 ± 0.13 b	1.14 ± 0.02 bc
F. + *P. mendocina*	10.6 ± 0.20 bc	1.22 ± 0.02 bc

Values are presented as mean ± standard error. Values in columns followed by the same letter are not significantly different (LSD, $P < 0.05$).

Table 4 Foliar nutrients and arbuscular mycorrhizal colonization of the lettuce plants in response to microbial inoculation treatments 2 months after planting ($n = 5$)

	Foliar P (mg $plant^{-1}$)	Foliar Fe (mg $plant^{-1}$)	Mycorrhization (%)
Control	22.5 ± 0.1 a	1.43 ± 0.01 a	33.4 ± 1.3 a
Fertilized (F.)	50.5 ± 0.2 c	2.54 ± 0.02 bc	40.0 ± 1.7 a
G. intraradices	44.1 ± 0.2 bc	2.25 ± 0.02 bc	60.0 ± 1.2 b
P. mendocina	38.3 ± 0.2 b	3.02 ± 0.04 c	30.8 ± 1.5 a
F. + *P. mendocina*	37.7 ± 0.1 b	1.65 ± 0.01 ab	35.8 ± 1.0 a

Values are presented as mean ± standard error. Values in columns followed by the same letter are not significant different (LSD, $P < 0.05$).

notably ferric iron (Vessey, 2003). Iron is an essential nutrient of plants, but it is relatively insoluble in soil. Thus, iron can be crop limiting in calcareous soils, such as the soil used in our experiment. Some rhizospheric bacteria produce soluble organic compounds (siderophores) which bind Fe^{3+} and help maintain it in solution. There is evidence that a number of plant species can absorb bacterial Fe^{3+}-siderophore complexes (Wang *et al*., 1993). In previous studies, it was shown that *P. mendocina* is an iron-solubilizing rhizobacterium, which is able to obtain Fe for growth from several insoluble Fe minerals in aerobic environments (Hersman *et al*., 2001). In this study, we have observed an increase in the foliar Fe of plants inoculated with *P. mendocina*, which was similar to that produced by an iron chelate. Bacteria-mediated increases in root weight are commonly reported responses to PGPR inoculations, resulting in a better access of plants to nutrients in the soil. In our study, the bacterial inoculation with *P. mendocina* appeared effective in improving the content of foliar Fe, which could be related to a greater root development of these plants with respect to the control plants. However, the fertilizer treatment increased the content of foliar P to a greater extent than the bacterial inoculation despite similar root growth in both treatments. It is worth noting that there was a negative interaction, with respect to the content of foliar Fe, between the fertilizer treatment and the bacterial inoculation.

PGPR sometimes enhance plant growth indirectly by stimulating the relationship between the host plant and beneficial rhizospheric fungi, such as arbuscular mycorrhizae. However, the PGPR used in this study did not behave as mycorrhizal helper bacteria (Garbaye, 1994), because they did not promote natural colonization of plants, but neither did they exhibit an inhibitory effect on native endophytes.

The reactivation of microbial activity in the rhizosphere can increase plant nutrient availability, as the soil microbial community mediates the processes of organic matter turnover and nutrient cycling. Enzyme activities are sufficiently sensitive to indicate perturbations caused by microbial inoculation (Naseby & Lynch, 1997). Oxidoreductases, such as dehydrogenase, are involved in oxidative processes in soils and their activity mainly depends on the metabolic state of the soil biota; thus, they are considered as good indicators of the soil microbial activity (García *et al*., 1997). The inoculation with *P. mendocina* was the most effective treatment with respect to increasing dehydrogenase activity. The measurement of hydrolases provides an early indication of changes in soil fertility, as they are related to the mineralization of important nutrient elements required for both plant and

microbial growth (Alguacil *et al*., 2005). The activity of phosphatase, which is capable of hydrolysing organic phosphate esters, was increased by bacterial inoculation. However, Naseby & Lynch (1997) found that phosphatase activity of the rhizosphere soil of wheat decreased with inoculation with a genetically-modified *Pseudomonas fluorescens* strain. Urease, a hydrolase related to the hydrolysis of urea to CO_2 and NH_3, and protease-BAA, which catalyses the hydrolysis of simple peptidic substrates to plant-available ammonium, did not increase with bacterial inoculation.

The organic C released by roots promotes a dense microbial community in the immediate environment of the root which, in turn, produces exocellular mucilaginous polysaccharide material that has the capacity to stabilize soil aggregates (Jastrow *et al*., 1998). The release of more soluble carbohydrates into the rhizosphere soil of plants treated with PGPR probably increased aggregate stability. On the other hand, the fact that the greatest microbial activity was in the rhizosphere soil of the plants grown in the soil inoculated with *P. mendocina* might be due to the increase in stable aggregates, which protect the organic fraction (on which extracellular enzymes and soil micro-organisms are immobilized) from microbial degradation (Nannipieri, 1994). As far as we are aware, this is the first study showing evidence of the effect of a plant-growth-promoting rhizobacterium on soil aggregates stabilization under field conditions.

Effectiveness of the mycorrhizal inoculation

Inoculation with the AM fungus *G. intraradices* proved to be an effective means of encouraging lettuce seedling growth. The local AM fungi community from the experimental soil was much less effective than the added *Glomus* inoculum at stimulating host-plant growth. Plant development, biomass production and nutrient concentration and content, as affected by AM colonization, should all be considered together. Thus, total nutrient content can be taken as indicative of mycorrhizal effectiveness because it takes into account the balanced effect of nutrient acquisition/biomass production. Our findings show that mycorrhizal inoculation increased root growth and favoured the uptake of P and Fe in *G. intraradices*-colonized seedlings. Mycorrhizae increase nutrient uptake by altering the development and morphology of the root system, yielding a more extensive absorbing area, which may be considered a mechanism of tolerance to nutrient deficit (Jeffries *et al*., 2003).

The present study confirms the influence of mycorrhizal inoculation on the aggregate stability of the rhizosphere soil of lettuce plants. The mechanisms involved in aggregate stabilization are based on the enmeshment of soil particles by hyphae and roots, and the exudation of polysaccharides (Bearden & Petersen, 2000). Mycorrhizal fungi do not consume organic C from the soil due to their symbiotic condition, but their effect on the host's physiological processes affects the quantity and quality of organic C delivered to the rhizosphere environment via fungal hyphae (Marschner *et al*., 1997). Indeed, the concentrations of soluble carbohydrates were higher in the rhizosphere soil of the plants inoculated with *G. intraradices*. Only soil carbohydrates of microbial origin seem to play a principal role in soil stabilization. They are considered transitory binding agents and are in continual equilibrium between excretion and decomposition. The increased levels of stable aggregates resulting from mycorrhizal inoculation can also be attributed to the proliferation of fungal hyphae in the rhizosphere soil (Roldán *et al*., 1994; Jeffries & Barea, 2000). According to Roldán *et al*. (1994), the binding effect of roots and hyphae is long-lived (months). AM fungi also produce a glycoprotein, glomalin, that acts as an insoluble glue to stabilize aggregates (Wright & Anderson, 2000).

The extensive root system of the fertilized plants did not improve aggregate stability of the rhizosphere soil. This could be due to a lesser ability of native AM fungi to enmesh particles of soil into aggregates and/or less secretion of microbial polysaccharides in the rhizosphere soil.

Conclusions

The treatments involving inoculation with an AM fungus or a rhizobacterium (PGPR) were effective for improving the nutrient status of plants and soil structural stability, leading to enhanced plant growth. The greater effectiveness of mycorrhizal plants in increasing aggregate stability is based on the proliferation of AM fungi and secretion of polysaccharides. In this study, we provide the first evidence of the beneficial effect of a plant-growth-promoting rhizobacterium on soil aggregate stabilization, which was possibly due to proliferation of the same rhizobacterium or of other microorganisms in the rhizosphere soil. The inorganic fertilizer alone did not increase aggregate stability.

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