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Impact of two fluorescent pseudomonads and an arbuscular mycorrhizal fungus on tomato plant growth, root architecture and P acquisition

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Abstract The ability of fluorescent pseudomonads and arbuscular mycorrhizal fungi (AMF) to promote plant growth is well documented but knowledge of the impact of pseudomonad-mycorrhiza mixed inocula on root architecture is scanty. In the present work, growth and root architecture of tomato plants (*Lycopersicon esculentum* Mill. cv. Guadalete), inoculated or not with *Pseudomonas fluorescens* 92rk and P190r and/or the AMF *Glomus mosseae* BEG12, were evaluated by measuring shoot and root fresh weight and by analysing morphometric parameters of the root system. The influence of the microorganisms on phosphorus (P) acquisition was assayed as total P accumulated in leaves of plants inoculated or not with the three microorganisms. The two bacterial strains and the AMF, alone or in combination, promoted plant growth. *P. fluorescens* 92rk and *G. mosseae* BEG12 when co-inoculated had a synergistic effect on root fresh weight. Moreover, co-inoculation of the three microorganisms synergistically increased plant growth compared with singly inoculated plants. Both the fluorescent pseudomonads and the myco-symbiont, depending on the inoculum combination, strongly affected root architecture. *P. fluorescens* 92rk increased mycorrhizal colonization, suggesting that this strain is a mycorrhization helper bacterium. Finally, the bacterial strains and the AMF, alone or in combination, improved plant mineral nutrition by increasing leaf P content. These results support the potential use of fluorescent pseudomonads and AMF as mixed inoculants for tomato and suggest that improved tomato growth could be related to the increase in P acquisition.

Keywords *Pseudomonas fluorescens* · *Glomus mosseae* · Mixed inoculum · Root architecture · Phosphorus acquisition

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Introduction

The rhizosphere has been defined as the volume of soil directly influenced by the presence of living plant roots, which, releasing an enormous amount of exudates, produce a significant stimulation of microbial density and activity (Hiltner 1904). Thus, the rhizosphere can be considered as a dynamic environment determined by reciprocal interactions between soil, plants and microflora associated with roots. On the other hand, depending on factors such as soil conditions, plant species, and age of the plant, microflora can influence plant growth and health, showing beneficial, deleterious or neutral effects. Plant beneficial microorganisms may be symbiotic or free-living. Among the symbiotic microorganisms, arbuscular mycorrhizal fungi (AMF) form mutual associations with more than 80% of plant species. Benefits to plants include improved mineral nutrition (Smith and Read 1997), protection against pathogens (Azcon-Aguilar et al. 2002) and enhanced resistance or tolerance to stress (Turnau and Haselwandter 2002). Moreover, according to the host plant and the myco-symbiont involved, AMF can show a strong impact on root morphogenesis. AMF-induced changes in root architecture generally include an increase in root branching and the development of a larger proportion of smaller diameter, higher order roots (Berta et al. 2002). Among the free-living microorganisms, plant

growth-promoting rhizobacteria (PGPR) have received special attention. PGPR can exert a beneficial effect on plant growth by suppressing soil-borne pathogens (Weller 1988), improving mineral nutrition (Kapulnik 1996), and phytohormone synthesis (Glick 1995). Since 1980, the ability of a large number of fluorescent *Pseudomonas* strains to suppress soil-borne diseases (Bakker et al. 1987; Mazzola 1999) and promote plant growth (Lifschitz et al. 1987; Glick 1995) has been reported. Plant growth enhancement by beneficial rhizobacteria is frequently evaluated as shoot and root fresh weight increase. In contrast, knowledge of the effects induced by PGPR on root architecture is scanty and contradictory (Glick 1995; Germida and Walley 1996; Gamalero et al. 2002).

Improvement of phosphorus (P) nutrition is one of the factors involved in plant growth promotion by AMF and PGPR. AMF are known to supply P to the plant by scavenging available P through the large surface area of their hyphae, by their high-affinity P uptake mechanisms, by organic acid production and by modifying root architecture (Ravnskov and Jakobsen 1995; Hofer 1996). Moreover, PGPR may improve plant P acquisition by solubilizing organic and inorganic phosphate sources through phosphatase synthesis or by lowering the pH of the soil (Rodriguez and Fraga 1999).

In the present work, we studied the impact of *Pseudomonas fluorescens* 92rk and P190r, isolated from the ectomycorrhizosphere and rhizosphere, respectively, and the AMF *Glomus mosseae* BEG12, alone or in combination, on tomato growth and root architecture. The effect of the three microorganisms on plant P acquisition was also assessed and discussed in relation to root system architecture.

Materials and methods

Microorganisms and microbial inoculants

Pseudomonas fluorescens 92 was isolated from basidiomes of *Suillus grevillei* (Varese et al. 1996) and was shown to promote cucumber (*Cucumis sativus* L.) plant growth (Gamalero et al. 2003). *P. fluorescens* P190 was isolated from tomato (*Lycopersicon esculentum* Mill.) rhizosphere and was effective in protecting eggplant and tomato seedlings from damping-off caused by *Rhizoctonia solani* AG-4 (Bucki et al. 1998). A rifampicin- and kanamycin-resistant mutant (92rk) of strain 92 was obtained by plating onto King's B agar (KB, Fluka) (King et al. 1954) supplemented with rifampicin and kanamycin (100 μg ml^{-1}). A rifampicin-resistant mutant (P190r) of the strain P190 was obtained by plating on KB supplemented with rifampicin (100 μg ml^{-1}). Cultures of bacterial cells grown in Luria-Bertani (LB) broth medium (Miller 1972) supplemented with antibiotics were stored at −80°C in 50% glycerol. *P. fluorescens* 92rk and P190r inoculants were produced on KB agar plates at 28°C for 48 h. Bacteria were scraped from the medium and suspended in 0.1 M $MgSO_4$. $7H_2O$, pelletted by centrifugation (4500 *g*, 20 min), washed twice and suspended in 0.1 M $MgSO_4$. $7H_2O$. The bacterial density of the suspension was determined by turbidity (λ = 600 nm) and adjusted to 10^7 cfu ml^{-1}.

Glomus mosseae BEG12 was shown to promote tomato plant growth (Gamalero et al. 2002) and suppress root rot due to *Phytophthora parasitica* (Trotta et al. 1996; Cordier et al. 1996). Mycorrhizal inoculum consisting of infected leek roots, sporocarps, spores and hyphae of *G. mosseae* BEG12 on quartz sand was obtained from Biorize (Dijon, France).

Metabolic characterization of Pseudomonas strains

Metabolic fingerprints of bacterial strains were obtained with the Biolog system (Biolog Inc., Hayvard, Calif., USA). Bacteria were grown on KB agar, transferred twice on BUG (Biolog Universal Growth) agar and incubated at 28°C for 24 h. Colonies were scraped from the medium, suspended in 0.4% saline solution and the inoculum density adjusted to a turbidity of 52±3%. Aliquots (150 μl) of this suspension were pipetted into the wells of a Biolog GN MicroPlate, incubated at 28°C and measured manually after 48 h. Data were collected and analysed by Biolog Microlog 2 software.

Treatments and plant growth conditions

A mixture of 0.3–0.7 mm coarse grade quartz sand (Punto Elle, Turin, Italy) was sterilized at 200°C for 2 h and distributed into 600-ml containers. Tomato seeds (*L. esculentum* Mill. cv. Guadalete), kindly provided by Dr. Scaravonati (Solanum c.s.a, Lodi, Italy), were surface sterilized by gently shaking in a 5% NaClO solution for 3 min and rinsed six times for 5 min and four times for 20 min in sterile demineralized water. The seeds were pregerminated on moist sterile filter paper at 24°C for 3 days. Inoculation of *P. fluorescens* 92rk and P190r was performed by dipping the germinated seeds for 20 min in a bacterial suspension (10^7 cfu ml^{-1}) before sowing. Inoculation of *G. mosseae* BEG12 was performed by incorporating 25% (v/v) of the inoculum-quartz sand mix into the culture substrate. One germinated seed was sown in each pot. Five containers were uninoculated (control) or inoculated with *G. mosseae* BEG12 and 25 containers were inoculated with *P. fluorescens* 92rk or P190r, 92rk+P190r, 92rk+BEG12, P190r+BEG12 and 92rk+P190r+BEG12, respectively. Plants were cultivated in a growth chamber with a 16/8 h light/dark photoperiod, 24/20 C light/dark thermoperiod, 150 μE m^{-2} s^{-1} light irradiance at pot height, 60% RH, and were watered to saturation three times per week with a modified Long Ashton nutrient solution containing 32 μM phosphate (Trotta et al. 1996).

Root colonization by P. fluorescens 92rk and P190r and G. mosseae BEG12

Bacterial densities on roots were assessed 0, 7, 14, 21 and 28 days after sowing. Whole rootlets (day 0) and root systems (days 7, 14, 21 and 28) were aseptically cut from five germinated seeds and five plants, respectively. Sand loosely adhering to roots was removed by gently washing the roots with sterile water. Rootlets and roots were vortexed for 15 min in $MgSO_4$. $7H_2O$ buffer (0.1 M). Suspensions obtained were serially diluted and plated on solid KB supplemented with rifampicin and/or kanamycin (100 μg ml^{-1}). After incubation for 48 h at 25°C, the cfu number was determined and expressed per gram of root fresh weight (Gamalero et al. 2002).

Mycorrhizal infection (M%) was evaluated 28 days after sowing on five root systems, according to Trouvelot et al. (1986). Briefly, 30 randomly chosen 1-cm-long pieces were cut from each root system, cleared for 30 min at 60°C in 10% KOH, stained with 1% methyl blue in lactic acid and mounted on a slide. The intensity of mycorrhizal colonization was evaluated microscopically.

Plant growth and root architecture

Five plants per treatment were collected 28 days after sowing and the following parameters determined: root and shoot fresh weight, total root length, total root surface area, total root volume, number of tips and degree of root branching, represented by root tip number divided by total root length. For this purpose, whole root systems

were placed in a transparent water container and scanned using a dedicated Desk Scan II scanner, equipped with a special lighting system for root measurement. Digitized root images were analysed by MacRhizo V 3.9 software (Régent Instruments, Canada) and morphometric parameters evaluated.

P content

Dry leaf material was wet-digested and analysed for total P content as described by Trotta et al. (1991). Briefly, pulverized samples of 30–40 mg were digested on a microburner in 100-ml Kjedahl flasks with 1 ml of 10 N H_2SO_4 and 10–20 drops of 30% H_2O_2. After neutralization and addition of an ammonium molybdate-sulphuric acid solution, samples were boiled for 1 min with 100 mg of ascorbic acid, diluted to 50 ml and the optical density determined with an Ultrospec 3000 spectrophotometer (Pharmacia) at 660 nm. P-content values were obtained from a 10–350 µg P calibration curve.

Statistical analysis

Since bacterial populations approximate an exponential normal distribution (Loper et al. 1985), bacterial density values were logarithmically transformed before analysis. Non-transformed and transformed values were submitted to analysis of variance and to Fisher's least significant difference test (P=0.05) using a Statview statistics package. All experiments were duplicated and the results shown are from one representative experiment.

Results

Bacterial metabolic fingerprinting

Metabolic fingerprints of *P. fluorescens* 92rk and P190r obtained by Biolog analysis was very similar (data not shown). A wide variety of substrates were utilized by the strains: 70 and 67 positive reactions on 92 substrates were recorded for *P. fluorescens* 92rk and P190r, respectively. In detail, both strains were able to use molecules typically found in tomato exudates, such as fructose, glucose, sucrose and acetic, citric, lactic, α-ketoglutaric, cis-aconitic, propionic, L-pyroglutamic and succinic acid (Kravchenko et al. 2003).

Survival of *P. fluorescens* 92rk and P190r

Survival of *P. fluorescens* 92rk and P190r in the tomato rhizosphere, measured as the logarithm of cfu g^{-1} root fresh weight, differed significantly. The densities of 92rk in the rhizosphere of 7-, 14- and 28-day-old plants (8.37, 7.66 and 5.79 cfu g^{-1}, respectively) were significantly (P=0.001, 0.008, 0.009) higher than those of strain P190r (7.59, 7.17 and 4.94 cfu g^{-1}, respectively). Densities of *P. fluorescens* 92rk in the rhizosphere of 7- and 14-day-old plants inoculated with both bacterial strains (8.13 and 7.37 cfu g^{-1}, respectively) were significantly (P=0.033 and <0.0001) higher than those shown by *P. fluorescens* P190r (7.66 and 6.81 cfu g^{-1}, respectively). *G. mosseae* BEG12 apparently increased, but not significantly, the density of the strain 92rk (6.14 cfu g^{-1}) in 28-day-old plants, compared with single-inoculated plants (5.79 cfu g^{-1}). Moreover, both 92rk (7.31 and 6.71 cfu g^{-1}, respectively) and P190r (6.78 and 6.31 cfu g^{-1}, respectively) decreased in 7- and 14-day-old plants inoculated with 92rk+P190r+BEG12.

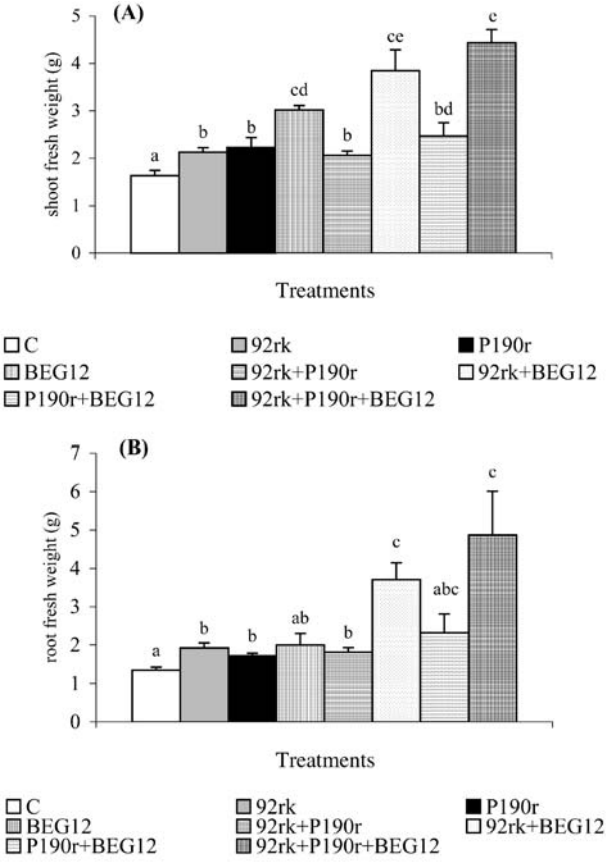

Fig. 1 Shoot fresh weight (**A**) and root fresh weight (**B**) of tomato plants inoculated or not with *Pseudomonas fluorescens* 92rk, P190r and *Glomus mosseae* BEG12, alone or in combination. Means with the same letters are not significantly different ($P \leq 0.05$) according to Fisher's least significant difference test

Glomus mosseae BEG12 mycorrhizal colonization

Mycorrhizal colonization (M%) after 28 days of culture was 34.7% in plants inoculated with BEG12; this value was significantly (P=0.050 and 0.023) increased in plants co-inoculated with the strain 92rk alone (41.0%) or in combination with P190r (42.3%). *P. fluorescens* P190r alone did not increase mycorrhizal colonization (34.8%).

Effects of *P. fluorescens* 92rk, P190r and *G. mosseae* BEG12 on plant growth

When singly inoculated, *P. fluorescens* 92rk or P190r significantly increased both shoot and root fresh weights (Fig. 1A, B), while *G. mosseae* BEG12 increased only

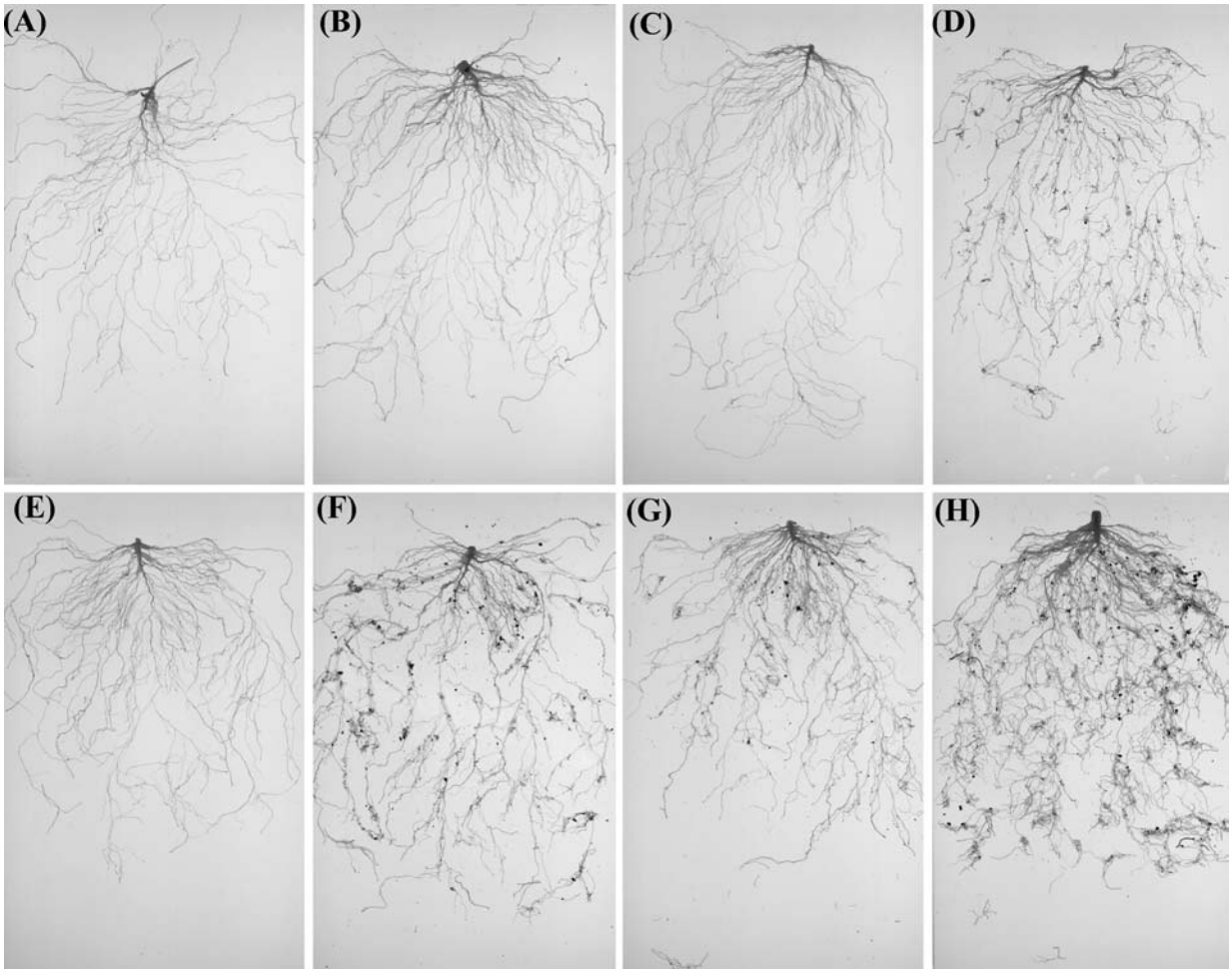

Fig. 2 Root systems of tomato: uninoculated (**A**), inoculated with *P. fluorescens* 92rk (**B**), *P. fluorescens* P190r (**C**), *G. mosseae* BEG12 (**D**), *P. fluorescens* 92rk and P190r (**E**), *P. fluorescens* 92rk and *G. mosseae* BEG12 (**F**), *P. fluorescens* P190r and *G. mosseae* BEG12 (**G**) and *P. fluorescens* 92rk, P190r and *G. mosseae* BEG12 (**H**)

shoot fresh weight (Fig. 1A). Moreover, comparable increases in shoot and root fresh weights were observed in plants inoculated with 92rk+P190r (Fig. 1A, B). Co-inoculation of 92rk+BEG12 induced a significant increase in shoot fresh weight compared with all other treatments, with the exception of BEG12- and 92rk+P190r+BEG12-inoculated plants (Fig. 1A), as well as a similar enhancement of root fresh weight, significantly higher than that observed in BEG12-treated plants (Fig. 1B). In contrast, inoculation with P190r+BEG12 only increased shoot fresh weight compared with untreated plants (Fig. 1A). Finally, co-inoculation of the three microorganisms increased shoot and root fresh weights relative to all other treatments, with the exception of 92rk+BEG12-inoculated plants (Fig. 1A, B).

Effects of *P. fluorescens* 92rk and P190r and *G. mosseae* BEG12 on root architecture

The effects of *P. fluorescens* 92rk and P190r and *G. mosseae* BEG12 on root architecture were evaluated by measuring several morphometric parameters. Plants treated with the three microorganisms, alone or in combination, clearly showed more developed root systems than the control (Fig. 2). *P. fluorescens* 92rk significantly increased total root length, total root surface area and volume. The same parameters were apparently increased, although not significantly, by P190r (Table 1). *G. mosseae* BEG12 significantly increased total root surface area and volume, number of tips and degree of root branching. The co-inoculation of 92rk+P190r induced a significant enhancement of total root length compared with controls (Table 1). Co-inoculation with 92rk+BEG12 significantly increased all parameters considered; in particular, total root length was significantly higher than that of singly inoculated plants (Table 1). On the contrary, co-inoculation with P190r+BEG12 did not influence root

Table 1 Effect of *Pseudomonas fluorescens* 92rk and P190r and of *Glomus mosseae* BEG12 on total root length, total root area, total root volume, number of tips and root branching of tomato. Values (mean value ± standard error) with the same letter are not significantly different ($P \leq 0.05$) according to Fisher's least significant difference test

Treatment	Total root length (cm)	Total root area (cm^2)	Total root volume (cm^3)	Number of root tips	Root branching degree
Control	594.7±35.2 a	105.9±4.6 a	1.4±0.09 a	548.5±41.4 a	0.9±0.04 ac
92rk	798.3±32.9 b	157.7±5.8 bcd	2.4±0.1 bc	697.0±67.2 ab	0.8±0.06 a
P190r	716.1±35.3 ab	120.6±3.8 ab	1.6±0.04 a	693.8±52.9 ab	0.9±0.03 acd
BEG12	758.4±65.5 ab	170.4±14.4 cd	3.0±0.2 cd	958.8±92.6 bc	1.2±0.1 b
92rk+P190r	783.3±19.7 b	144.1±2.5 abc	2.1±0.03 ab	623.4±29.9 a	0.7±0.03 a
92rk+BEG12	967.5±37.6 c	195.4±4.8 de	3.3±0.1 d	1121.5±62.6 cd	1.1±0.02 bd
P190r+BEG12	681.2±93.9 ab	131.0±19.8 abc	2.0±0.3 ab	740.20±92.48 ab	1.1±0.06 bc
92rk+P190r+BEG12	1349.1±128.6 d	233.7±34.5 e	3.7±0.6 d	1301.2±213.2 d	1.1±0.03 b

Table 2 Leaf phosphorus (P) content (μg/plant) in tomato plants inoculated or not with *P. fluorescens* 92rk and P190r and with *G. mosseae* BEG12, alone or in combination. Values (mean value ± standard error) with same letter are not significantly different ($P \leq 0.05$) according to Fisher's least significant difference test

Treatment	Leaf P content
Control	271.6±1.7 a
92rk	334.1±0.07 b
P190r	354.8±1.2 bd
BEG12	509.8±0.7 c
92rk+P190r	414.3±2.5 d
92rk+BEG12	822.8±4.9 e
P190r+BEG12	615.1±4.03 f
92rk+P190r+BEG12	988.0±3.3 g

architecture (Table 1). The co-inoculation of the three microorganisms significantly increased all parameters analysed when compared to the controls. Moreover, total root length and surface area and the number of tips were significantly higher than in singly inoculated plants. In particular, total root length was significantly higher than that of plants treated with 92rk+BEG12 (Table 1).

Plant P acquisition

The influence of the three microorganisms, alone or in combination, on plant P acquisition, measured as total P content in leaves, was assayed on 28-day-old tomato plants. As shown in Table 2, *P. fluorescens* 92rk and P190r and *G. mosseae* BEG12, alone or in combination, significantly increased leaf P content. P content of BEG12-inoculated plants was twice that of untreated plants. The co-inoculation of 92rk+BEG12 and P190r+BEG12 tripled and doubled the P content value relative to controls, respectively. The highest leaf P content was recorded in plants co-inoculated with all three microorganisms (Table 2).

Discussion

The populations of both *P. fluorescens* 92rk and P190r decreased in time and with plant growth. Reduction of rhizospheric density of fluorescent pseudomonads has been reported many times (Bakker et al. 1987; Frey-Klett et al. 1997; Chiarini et al. 1998; Gamalero et al. 2002) and was usually attributed to both biotic (predation, competition, root exudation) and abiotic (temperature, pH, soil texture and mixture) factors (Van Veen et al. 1997). Moreover, a series of other factors (ability to grow on root exudates, to synthesize amino acids, vitamins and the O-antigen of LPS, to firmly adhere to the root and motility) defined as "rhizospheric competence" is involved in the establishment of effective and enduring root colonization by an introduced bacterium (Lugtenberg and Dekkers 1999). As shown by Biolog metabolic fingerprinting, both *Pseudomonas* strains were able to grow on molecules detected in tomato rhizosphere (Kravchenko et al. 2003), indicating a good fitness of the strains to the rhizospheric environment. However, quantitative and qualitative variation in root exudates during plant growth could affect the dynamics of introduced rhizobacteria (Lynch and Whipps 1990). The density of *P. fluorescens* 92rk on tomato roots was higher than that of P190rk, suggesting more efficient root colonization or better exploitation of the root zones producing higher amount of exudates. The development of the mycorrhizal symbiosis influenced bacterial dynamics. A significant reduction in bacterial density was observed in 7- and 14-day-old plants inoculated with 92rk+P190r+BEG12. In the earlier stages of plant growth and mycorrhizal symbiosis development, competition between the microorganisms, perhaps for root exudates, may be stronger. On the contrary, in older plants, a higher photosynthetic capacity could more easily support a complex microflora (Paulitz and Linderman 1989).

P. fluorescens 92rk, alone or co-inoculated with P190r, increased mycorrhizal colonization of tomato roots by *G. mosseae* BEG12. This result suggests that strain 92rk behaves as a mycorrhiza helper bacterium (MHB) in *L. esculentum.* MHB have been described for ectomycorrhizal symbiosis (Garbaye 1994) and only a few examples of MHB have been reported for AM symbiosis (Toro et al. 1997; Singh and Kapoor 1998). The mechanisms by which bacteria stimulate AM colonization are still poorly understood, but certain bacterial activities, such as the production of metabolites increasing root cell permeabil-

ity and hormone synthesis, are expected to be involved (Barea et al. 1997).

P. fluorescens 92rk and P190r, and *G. mosseae* BEG12, inoculated alone, promoted tomato plant growth. Plant growth promotion by fluorescent pseudomonads has been ascribed to the suppression of phytopathogenic soil-borne microorganisms (Weller 1988; Mazzola 1999) and to direct effects on plant physiology (Glick 1995; Ravnskov and Jakobsen 1999). AMF are known to affect plant growth and health by improving mineral nutrition (Ravnskov and Jakobsen 1995; Clark and Zeto 2000) and by increasing resistance to or tolerance of biotic (Cordier et al. 1996; Trotta et al. 1996) and abiotic stress (Turnau and Haselwandter 2002). In our case, although the presence of microflora associated with the mycorrhizal inoculum can not be ruled out, plant growth promotion by the bacterial strains and the AMF could be related mainly to a direct effect and to an improvement of mineral nutrition, respectively. The few studies in the literature comparing the efficacy of combinations of beneficial rhizobacteria to single strain inoculation showed that mixed inocula can lead to a competitive process by which plant growth may be reduced (Chiarini et al. 1998; Probanza et al. 2002). The lack of additive or synergistic effects of the co-inoculation of 92rk+P190r on plant growth suggests the presence of competition for root exudates, and this is supported by the similar metabolic profiles obtained. In contrast, co-inoculation of 92rk+BEG12 showed an additive effect on shoot fresh weight and a synergistic effect on root fresh weight, whereas P190r+BEG12 increased shoot fresh weight relative to controls. Moreover, co-inoculation of the three microorganisms showed synergistic effects compared with single inoculated plants. Some reports demonstrate additive effects on plant growth of AMF and rhizobacteria (Edwards et al. 1998; Galleguillos et al. 2000), while others have reported neutral effects (Andrade et al. 1997; Walley and Germida 1997). To our knowledge, synergistic effects have been observed in only few cases (Ravnskov and Jakobsen 1999). Interestingly, competition between the two pseudomonads strains seems to be reduced by the presence of *G. mosseae* BEG12. Indeed, it is feasible that AM symbiosis, by inducing variation in plant physiology and root morphology, plays a key role in the establishment of environmental conditions favourable to the growth and the activity of a complex microflora.

Besides promoting plant growth, the two bacterial strains and the AM fungus, depending on the inoculum combination, modified root architecture. *P. fluorescens* 92rk increased total root length, surface area and volume. This is in agreement with the effects of *P. fluorescens* A6RI (Gamalero et al. 2002) and 92r (Gamalero et al. 2003) on the development of tomato and cucumber root, respectively. Longer root systems are more adapted to soil exploration and exploitation (Berta et al. 2002). As shown by Glick (1995), auxin synthesis by rhizobacteria is involved in the enhancement of root length. Although indole-3-acetic acid production by strain 92rk has been reported in vitro (Gamalero et al. 2003), this hypothesis remains to be tested *in planta*.

Glomus mosseae BEG12 increased total root surface area and volume, number of tips and degree of root branching. Although AMF can affect root plasticity in a variety of ways (Berta et al. 2002), the most common effect is an increase in root branching with an improved ability to absorb and transport nutrients. The mechanisms by which these changes come about are not well understood; however, the improvement of mineral nutrition, phytohormone synthesis, hyperpolarization of cortical cell membrane potential and proton extrusion are involved (Berta et al. 2002).

A synergistic effect on total root length and additive effects on total root surface area, volume and tip number were observed in plants inoculated with 92rk+BEG12. In particular, the increases in total root length and tip number could be attributed to 92rk and BEG12 activity, respectively. Moreover, the mycorrhizospheric origin of strain 92rk could be one of the factors involved in the positive interaction with the AMF. The highest values of root architecture parameters were recorded in plants inoculated with the three microorganisms: total root length, total root surface area and number of tips were synergistically increased, while an additive effect was observed on total root volume and degree of root branching. Longer and more-branched root systems could be considered more efficient both in soil exploration and in nutrient uptake and transport, favouring the successful establishment of many plant species.

Comparison of the effects of 92rk+BEG12 and 92rk+P190r+BEG12 on root architecture highlights the role of *P. fluorescens* P190r. This bacterial strain seems to express more clearly its beneficial traits when co-inoculated with *P. fluorescens* 92rk and the myco-symbiont. We can suppose that inoculation of 92rk+BEG12, by inducing modification of plant growth, root development and exudation, provides a rhizospheric environment favourable for the beneficial activity of strain P190r.

The modification of root architecture parameters induced by the bacterial strains could be related to increased P acquisition: root systems with higher root surface area and volume are indeed characterized by a higher absorptive surface. AMF can provide P to plants not only by scavenging the available P through the large surface area of their hyphae, but also by their high affinity P-uptake mechanisms (Ravnskov and Jakobsen 1995). Moreover, the increase in the degree of root branching induced by the AM symbiosis results in enhancement of root hairy zones where P acquisition mainly occurs (Hofer 1996). The synergistic increase in P content in plants co-inoculated with 92rk+P190r+BEG12 could be ascribed both to modification of root architecture, due to the bacterial strains and to the myco-symbiont, and to a greater absorptive surface, due to the mycelial development of *G. mosseae* BEG12.

Overall, our results illustrate the complexity of the interactions within the rhizosphere and mycorrhizosphere. These interactions can result in negative or positive

effects depending on the microorganisms involved and plant growth conditions. The use of two fluorescent pseudomonads combined with an AMF, by exploiting different modes of action, can benefit host plants by contributing to plant growth promotion and by positively affecting root system architecture.

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