



## Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial

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### Abstract

Biofertilizer has been identified as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming. The objective of this greenhouse study was to evaluate the effects of four biofertilizers containing an arbuscular mycorrhizal fungus (*Glomus mosseae* or *Glomus intraradices*) with or without N-fixer (*Azotobacter chroococcum*), P solubilizer (*Bacillus megaterium*) and K solubilizer (*Bacillus mucilaginosus*) on soil properties and the growth of *Zea mays*. The application treatments included control (no fertilizer), chemical fertilizer, organic fertilizer and two types of biofertilizer. The application of biofertilizer containing mycorrhizal fungus and three species of bacteria significantly increased the growth of *Z. mays*. The use of biofertilizer (*G. mosseae* and three bacterial species) resulted in the highest biomass and seedling height. This greenhouse study also indicated that half the amount of biofertilizer application had similar effects when compared with organic fertilizer or chemical fertilizer treatments. Microbial inoculum not only increased the nutritional assimilation of plant (total N, P and K), but also improved soil properties, such as organic matter content and total N in soil. The arbuscular mycorrhizal fungi (AMF) had a higher root infection rate in the presence of bacterial inoculation. By contrast, the AMF seemed to have an inhibiting effect on the P-solubilizing bacteria. The nutrient deficiency in soil resulted in a larger population of N-fixing bacteria and higher colonization of AMF.

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**Keywords:** PGPRs; *Azotobacter chroococcum*; *Bacillus megaterium*; *Bacillus mucilaginosus*; *Glomus mosseae*; *Glomus intraradices*; *Zea mays*; Greenhouse trial

### 1. Introduction

There are abundant microorganisms thriving in soil, especially in the rhizosphere of plants. It is well known that a considerable number of bacterial and

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fungus species possess a functional relationship and constitute a holistic system with plants. They are able to exert beneficial effects on plant growth (Vessey, 2003). Application of beneficial microbes in agricultural practices started 60 years ago and there is now increasing evidence that these beneficial microbial populations can also enhance plant resistance to adverse environmental stresses, e.g. water and nutrient deficiency and heavy metal contamination (Shen, 1997).

A group of bacteria are now referred to 'plant growth-promoting rhizobacteria' (PGPR), which participate in many key ecosystem processes such as those involved in the biological control of plant pathogens, nutrient cycling and seedling establishment, and therefore deserve particular attention for agricultural or forestry purposes (Weller and Thomashow, 1993; Glick, 1995; Elo et al., 2000). PGPR may colonize the rhizosphere, the surface of the root, or even superficial intercellular spaces of plants (McCully, 2001). It has been revealed that the effect of nitrogen fixation induced by nitrogen fixers is not only significant for legumes, but also non-legumes (Doebereiner and Pedrosa, 1987). Moreover, some strains have multiple functions for plant growth. The beneficial effect of *Azospirillum* may derive both from its nitrogen fixation and stimulating effect on root development (Doebereiner and Pedrosa, 1987; Lynch, 1990). Phosphate (P)- and potassium (K)-solubilizing bacteria may enhance mineral uptake by plants through solubilizing insoluble P and releasing K from silicate in soil (Goldstein and Liu, 1987). Some successful examples of inoculation with PGPR have been achieved both in laboratory and field trials. For example, strains of *Pseudomonas putida* and *Pseudomonas fluorescens* could increase root and shoot elongation in canola, lettuce and tomato (Hall et al., 1996; Glick et al., 1997). It has also been reported that wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculation (Klopper et al., 1991). Soil microorganisms are important components in the natural soil subecosystem because not only can they contribute to nutrient availability in the soil, but also bind soil particles into stable aggregates, which improve soil structure and reduce erosion potential (Shetty et al., 1994).

The majority of plants growing under natural conditions are associated with mycorrhizae (Smith

and Read, 1997). Mycorrhizal colonization of roots results in an increase in root surface area for nutrient acquisition. The extrametrical fungal hyphae can extend several centimeters into the soil and absorb large amounts of nutrients for the host root (Khan et al., 2000). There is well-documented evidence that arbuscular mycorrhizal fungi (AMF) contribute to increasing availability and uptake of P and micronutrients (Krishna and Bagyaraj, 1991). The mycorrhizal symbiosis, by linking the biotic and geochemical portions of the ecosystem, can also be regarded as a bridge connecting the root with the surrounding soil microhabitats (Toro et al., 1997).

The utilization of microbial products has several advantages over conventional chemicals for agricultural purposes: (i) microbial products are considered safer than many of the chemicals now in use; (ii) neither toxic substances nor microbes themselves will be accumulated in the food chain; (iii) self-replication of microbes circumvents the need for repeated application; (iv) target organisms seldom develop resistance as is the case when chemical agents are used to eliminate the pests harmful to plant growth; and (v) properly developed biocontrol agents are not considered harmful to ecological processes or the environment (Weller, 1988; Gloud, 1990; Shen, 1997).

Biofertilizers are products containing living cells of different types of microorganisms, which have an ability to convert nutritionally important elements from unavailable to available form through biological processes (Hegde et al., 1999; Vessey, 2003). In recent years, biofertilizers have emerged as an important component of the integrated nutrient supply system and hold a great promise to improve crop yields through environmentally better nutrient supplies. However, the application of microbial fertilizers in practice, somehow, has not achieved constant effects. The mechanisms and interactions among these microbes still are not well understood, especially in real applications.

A biofertilizer containing N-fixer (*Azotobacter chroococcum*), P solubilizer (*Bacillus megaterium*) and K solubilizer (*Bacillus mucilaginosus*) and arbuscular mycorrhizal fungi (*Glomus mosseae* and *Glomus intraradices*) has been developed with great assistance from with our industrial partner (V-mark Resources, Hong Kong). The major objective of this experiment was to evaluate the effects of this biofertilizer on the promotion of plant growth (*Zea*

mays) and on the improvement of soil properties by means of a greenhouse study. The interactions among the microorganisms were also investigated.

## 2. Materials and methods

### 2.1. Soil

The soil used for this pot experiment was collected from Luk Tin Yuen, the New Territories, Hong Kong. The soil was air-dried and ground to pass through a 2-mm sieve and mixed thoroughly. The basic properties of the soil were as follows: pH 5.46, organic matter content 1.08%, total N 0.062%, total K 7408 mg kg<sup>-1</sup>, total P 1090 mg kg<sup>-1</sup>, available P (NaHCO<sub>3</sub>-extractable) 2.78 mg kg<sup>-1</sup> and water-soluble K 13.43 mg kg<sup>-1</sup>.

### 2.2. Fertilizer and microbial inocula

The biofertilizer used for this pot experiment was supplied by V-mark Resources. It consisted of chicken manure and powder of phosphate rock, with a final total N, P and K content of 13%, 4% and 8%, respectively.

Peat moss purchased from Gartengold, Germany, was chosen as the carrier for rhizobacteria inoculum. The peat moss was oven-dried at 70 °C for 72 h, and then ground and sieved to pass a 2-mm sieve, autoclaved at 121 °C for 20 min. The pH value of the peat moss was maintained at neutral with addition of CaCO<sub>3</sub>. Three strains, a nitrogen-fixing bacterium (*A. chroococcum*, HKN-5), a phosphate solubilizer (*B. megaterium*, HKP-2) and potassium solubilizer (*B.*

*mucilaginous*, HKK-2) were used. They were originally isolated from agronomic soils in Hong Kong, and kept at the Microbial Center of The Croucher Institute for Environmental Sciences, Hong Kong Baptist University. The P and K solubilizers were cultured with LB broth (USB, Life Science Amersham International) for 48 h in a shaking incubator under 28 ± 1 °C and 200 rpm. The N-fixing bacteria were cultured under the same conditions with Ashby liquid medium (mannitol 5.0 g, CaCO<sub>3</sub> 5.0 g, K<sub>2</sub>HPO<sub>4</sub> 0.5 g, NaCl 0.5 g, MgSO<sub>4</sub> · 7H<sub>2</sub>O 0.2 g, distilled water 1.0 l, pH 7.0). The density of each bacterial culture in the broth was counted using a haemocytometer. The bacteria were harvested at 6000 rpm and 4 °C using a centrifuge (Beckman, USA). The bacteria cells were then transferred into sterilized peat moss and well mixed. The mixture acted as the microbial inoculum, in which the final population sizes of N-fixing bacteria, P solubilizer and K solubilizer were 2.72 × 10<sup>8</sup>, 2.05 × 10<sup>8</sup>, 1.63 × 10<sup>8</sup> cfu g<sup>-1</sup> inoculum (wet weight), respectively. The inocula were sealed in sterile plastic bags and stored at 4 °C for further use, no longer than 3 months. The inocula of the two arbuscular mycorrhizal fungi (AMF) species, *G. mosseae* and *G. intraradices*, were purchased from Biorize Sarl, France. They were sand-based mycorrhizal inocula containing abundant chopped mycorrhizal root pieces, spores and hyphae.

### 2.3. Pot experiment

In this greenhouse trial, the effect of AMF and their combination with rhizobacteria on maize growth and soil properties was tested with two fertilization levels (Table 1). The high fertilization level (100%) for this

Table 1  
Design of pot experiment

Treatments	1	2	3	4	5	6	7	8	9
With bacteria inoculation	No	No	No	No	No	Yes	Yes	Yes	Yes
Mycorrhizal fungi species added	None	None	None	GM <sup>a</sup>	GI <sup>b</sup>	GM	GI	GM	GI
Fertilizer used	–	CF <sup>c</sup>	OM <sup>d</sup>	OM	OM	OM	OM	OM	OM
Fertilization level	0%	100%	100%	100%	100%	100%	100%	50%	50%
Code	control	chemical fertilizer	OM	OM+GM	OM+GI	BOM+GM	BOM+GI	50% BOM+GM	50% BOM+GI

<sup>a</sup> GM—*G. mosseae*.

<sup>b</sup> GI—*G. intraradices*.

<sup>c</sup> CF—chemical fertilizer.

<sup>d</sup> OM—organic fertilizer (autoclaved biofertilizer).

pot experiment was 300 mg ammonium-N, 92.3 mg P and 184.6 mg K per kilogram of dry weight of soil, respectively. In the eighth and ninth treatments, the fertilization level was reduced to half of those of treatments of 6 and 7. Two grams of peat moss-based bacterial inoculum and/or 15.0 g sand-based mycorrhizal inoculum were inoculated into the pots according to the treatment design. All the treatments without microbial inoculation received the same amounts of the autoclaved carrier-based inocula in order to keep similar soil texture and other properties among all the pots.

Three further treatments were set up for comparison, including chemical fertilizer (added as urea,  $\text{KH}_2\text{PO}_4$  and KCl), organic fertilizer (autoclaved biofertilizer) and no fertilization (control). The detailed information is listed in Table 1.

The seeds of maize (*Z. mays*) were surface disinfected for 30 min in 10% peroxide solution. Four seeds were then sown in a plastic pot ( $\varnothing_{\text{bottom}}$  10 cm  $\times$  18.5 cm  $\times$   $\varnothing_{\text{top}}$  15 cm) containing 1.5 kg autoclaved loamy soil. One week after seed germination, the seedlings were thinned to two in each pot. All the pots were randomly placed in a greenhouse ( $20 \pm 4$  °C). There were four replicates for each treatment. The seedlings were watered daily with deionized water to maintain the moisture at approximately 60% water holding capacity of the soil. The crop was harvested after 87 days.

#### 2.4. Biological and chemical analyses

After the plants were harvested, the growth parameters (height and fresh biomass) of plants under different treatments were recorded. The harvested plants were rinsed with deionized water and oven-dried at 75 °C for 72 h. The dried shoot tissues were ground and then digested using concentrated  $\text{HNO}_3$  (Page et al., 1982) for the determination of K using an atomic absorption spectrometer. Total N and P were extracted by digesting shoot tissue with 3 ml concentrated  $\text{H}_2\text{SO}_4$  and 1 ml  $\text{H}_2\text{O}_2$  at 360 °C, and determined by the Berthelot reaction and molybdenum blue method, respectively (Page et al., 1982). The soil samples were collected to determine the nutrient concentrations (N, P, K) using methods mentioned above. The amount of  $\text{NaHCO}_3$ -extractable P (available inorganic P) from the soil was

determined by extracting samples with 0.5 M  $\text{NaHCO}_3$  (pH 8.5) at a solution/solid ratio of 20:1 for 30 min (Olsen and Sommers, 1982). Total organic carbon (TOC) in soil was analysed by an acid dichromate method (Page et al., 1982).

Enumeration and isolation of N-fixing bacteria, P- and K-solubilizing bacteria from the fresh soil sample was conducted using suspension dilution techniques on agar plates with differentiating media [for *N-fixing bacteria*: glucose 10.0 g, NaCl 0.2 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.2 g,  $\text{K}_2\text{HPO}_4$  0.5 g, 2 drops of 1%  $\text{FeCl}_3$  and 1%  $\text{MnCl}_2$  solution, 1% Congo Red solution 5 ml (after pH modification), agar 20.0 g, distilled water 1.0 l, pH 7.0; for *P solubilizer*: glucose 10.0 g,  $(\text{NH}_4)_2\text{SO}_4$  0.5 g, NaCl 0.3 g, KCl 0.3 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.5 g,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.03 g,  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  0.03 g,  $\text{Ca}_3(\text{PO}_4)_3$  10.0 g, agar 20.0 g, distilled water 1.0 l, pH 7.0; for *K solubilizer*: sucrose 5.0 g,  $\text{Na}_2\text{HPO}_4$  2.0 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.5 g,  $\text{FeCl}_3$  0.005 g,  $\text{CaCO}_3$  0.1 g, glass powder 1.0 g, agar 20.0 g, 1.0 l distilled water, pH 7.0]. Roots were washed and stained for analysis of colonization by AMF using a modified procedure described by Philips and Hayman (1970). The roots were kept in cold 10% (w/v) KOH for 24 h and then heated in 10% KOH for 1 h before being cleared with alkaline  $\text{H}_2\text{O}_2$  for 30 min. The roots were then rinsed with tap water and neutralized with 10% (w/v) HCl for 5 min and stained with boiling glycerol–trypan blue solution (0.05%) for 10 min. Newman's intersection method was used to measure root colonization by AMF (Giovannetti and Mosse, 1980).

#### 2.5. Data analysis

Analysis of variance (ANOVA) was performed on all experimental data and means were compared using the Duncan's multirange test with SigmaStat software. The significance level was  $p < 0.05$  unless otherwise stated.

### 3. Results and discussion

#### 3.1. Inoculum establishment in the plant rhizosphere

The mycorrhizal inoculum significantly increased the extent of AMF colonization of the root system compared to the uninoculated control treatments

(Fig. 1). The inoculation with beneficial bacteria and low fertilization level increased root colonization by both *G. mosseae* and *G. intraradices*. It has already been noted that the rhizobacteria can act as ‘mycorrhization helper bacteria’, which improve the ability of mycorrhizal fungi to colonize plant roots (Fitter and Garbaye, 1994). The mechanisms by which these bacteria stimulate AM colonization are still poorly understood. Specialized bacterial activities such as the production of vitamins, amino acids, and hormones may be involved in these interactions (Barea et al., 1997). The presence of rhizobacterial inoculation might have assisted in the germination of a large number of spores thus leading to a higher infection percentage (Tandon and Prakash, 1998). Some PGPR endophytic species are known to have cellulase and pectinase (Kovtunovych et al., 1999; Verma et al., 2001) and these activities could not doubt aid in mycorrhizal infection. The present results demonstrated that the population size of the inoculated rhizobacteria varied in accordance with the levels of fertilization and AMF colonization in the rhizosphere (Table 2; Fig. 1). The low level of fertilization (50% BOM+GM and 50% BOM+GI) resulted in a higher level of mycorrhizal root infection and a larger community of *A. chroococcum* in the rhizosphere, compared to the treatments with a high level of fertilization (BOM+GM and BOM+GI). According to our preliminary results (in preparation),

Table 2

The population size of introduced beneficial bacteria in the rhizosphere of maize after 87 days of growth<sup>a</sup>

Treatment	N-fixing bacteria ( $10^4$ cfu/g dry soil)	P solubilizers ( $10^6$ cfu/g dry soil)	K solubilizers ( $10^6$ cfu/g dry soil)
Control	1.09±0.53 a	0.94±0.04 b	0.65±0.04 ab
Chemical fertilizer	1.22±0.12 a	0.36±0.01 a	0.72±0.03 b
OM	1.58±0.04 b	1.00±0.05 b	0.57±0.01 a
OM+GM	1.57±0.34 ab	0.97±0.31 b	0.67±0.03 ab
OM+GI	1.65±0.30 bc	0.90±0.20 b	0.75±0.12 b
BOM+GM	58.8±4.20 de	24.1±0.08 c	34.1±1.70 d
BOM+GI	54.5±6.40 d	18.6±3.95 d	20.9±3.40 c
50% BOM+GM	81.4±14.5 g	13.9±1.06 c	25.4±7.26 cd
50% BOM+GI	63.1±11.5 ef	23.5±3.59 e	24.6±3.81 cd

<sup>a</sup> For each response variable, values (means of four replicates) not sharing a common letter differ significantly ( $P<0.05$ ) from each other (Duncan’s multirange test).

the propagation of *A. chroococcum* was seriously inhibited when the Ammonium N concentration exceeded  $200 \text{ mg kg}^{-1}$ . This is in agreement with Wani (1990) who noted that the population size of N-fixing bacteria in soil decreased significantly after N fertilizer was used. However, a sharp decline of the number of P and K solubilizers (with a decrease rate of 42.3% and 25.5%, respectively) was observed with the increase of *G. mosseae* colonization level. It implies that maize is likely more dependent on the symbiosis with *G. mosseae* than P solubilizers under the condition of insufficient nutrient supply, e.g.

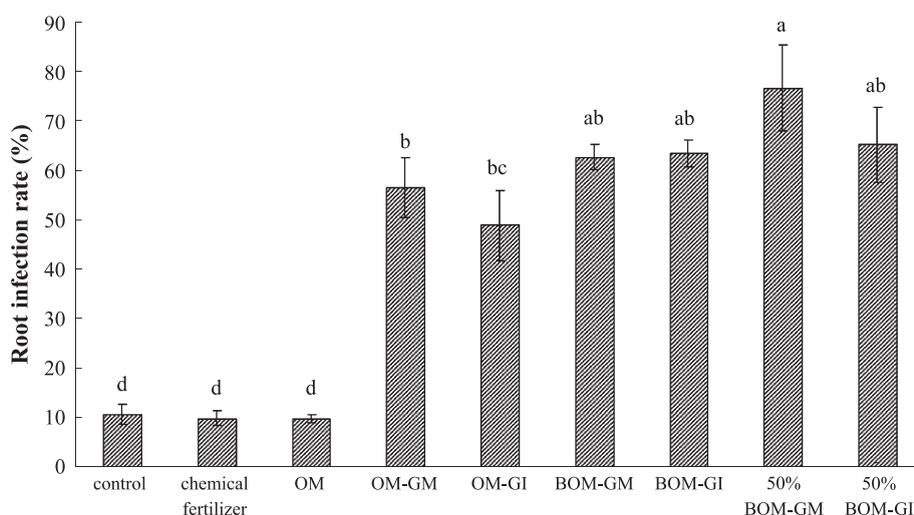


Fig. 1. Plant root infection rate by arbuscular mycorrhizal fungi in different treatments. Different letters above bars indicate a significant difference at  $p<0.05$  according to Duncan’s multirange test.

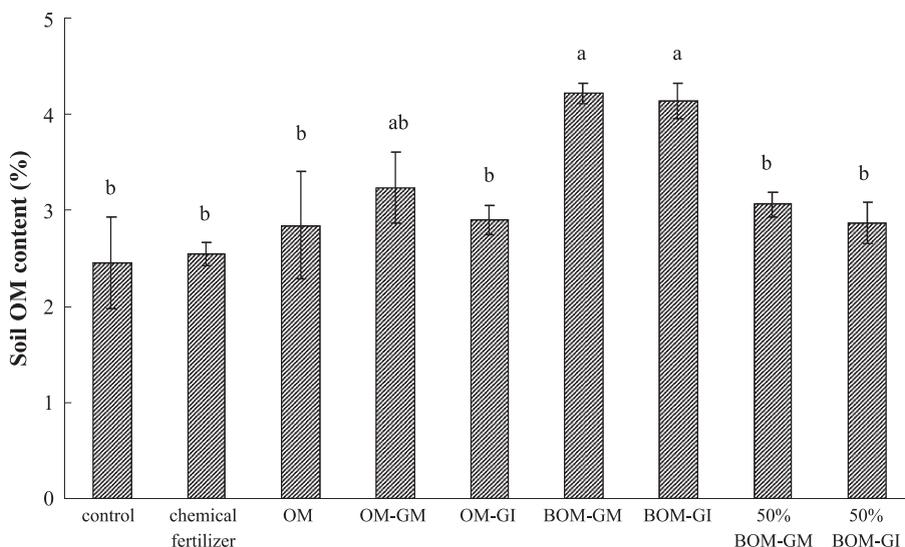
when P deficiency occurs. [Toro et al. \(1997\)](#) reported a drop of density in the introduced P-solubilizing bacteria from  $10^6$  to  $10^3$  cfu  $g^{-1}$  soil in the rhizosphere with the increase of root mycorrhizal colonization. By contrast, the colonization with *G. intraradices* showed a strong stimulating effect on the propagation of these two solubilizers. [Raj et al. \(1981\)](#) stated that mycorrhizal colonization with *G. mosseae* allowed the introduced populations of beneficial soil microorganisms like *Azotobacter*, *Azospirillum* and phosphate-solubilizing bacteria to maintain a higher abundance than non-mycorrhizal plants and thereby exerted a synergistic effect on plant growth. In practice, good indigenous species are preferable to be selected as inocula in order to ensure a successful colonization of mycorrhizae since the possible competition may occur between introduced and autochthonous populations in unsterilized field soil conditions.

### 3.2. Soil properties

The dual inoculation of rhizobacteria and mycorrhizae resulted in a significant increase of soil organic matter content ([Fig. 2](#)). The OM content in treatments of BOM+GI and BOM+GM increased by 75%, in comparison to the uninoculated control. However, the results imply that the increase was not directly

induced by the activity of soil microorganisms. The treatments with low fertilizer levels exhibited a larger population size of N-fixing bacteria and higher mycorrhizal root colonization, but a relatively low OM content. Most soil microorganisms consume a considerable amount of organic matter, e.g. carbohydrates, to generate the energy for maintenance and growth. Thus, some organic carbon (C) is lost with the production of carbon dioxide. The significant correlation ( $p < 0.05$ ) between soil organic matter content and plant dry biomass ([Table 3](#)) suggests that the organic matter content in the rhizosphere was mainly influenced by plant growth, especially root exudates through the root metabolism and physiological activities. It has been reported that *Azotobacter* not only provides nitrogen, but also produces a variety of growth-promoting substances ([Hegde et al., 1999](#)), among them indole acetic acid, gibberellins and B vitamins ([Rao, 1986](#)). These substances stimulate, at least to some degree, the production of root exudates.

In addition, another important characteristic of *Azotobacter* associated with plant improvement is excretion of ammonia in the rhizosphere in the presence of root exudates ([Narula and Gupta, 1986](#)), which could explain why the dual inoculation treatments with bacteria and AMF resulted in a slightly higher ( $p > 0.05$ ) total N content in soil ([Fig. 3](#)) compared to those with AMF inoculation only. The



[Fig. 2](#). Organic matter content in the soil receiving different fertilizer treatments. Different letters above bars indicate a significant difference at  $p < 0.05$  according to Duncan's multirange test.

Table 3  
Pearson's correlation matrix for plant, soil and microbial parameters

	Biomass	P in plant	K in plant	N in plant	Mycorrhizal infection ratio	Soil OM content	Soil avail. P	Soil total N	Pop. size of N-fixer	Pop. size of P solubilizer	Pop. size of K solubilizer
Biomass	1.000										
P in plant	0.911**	1.000									
K in plant	0.841**	0.941**	1.000								
N in plant	0.497*	0.449	0.455	1.000							
Mycorrhizal infection ratio	0.854**	0.723*	0.708*	0.449	1.000						
Soil OM content	0.844*	0.756	0.712*	0.430	0.557	1.000					
Soil avail. P	0.822*	0.850**	0.714*	0.377	0.465	0.810*	1.000				
Soil total N	0.704*	0.582*	0.613*	0.513*	0.518*	0.643*	0.563	1.000			
Pop. size of N-fixer	0.591*	0.378	0.444	0.098	0.788*	0.417*	0.085	0.473*	1.000		
Pop. size of P solubilizer	0.674*	0.502*	0.510*	0.180	0.706*	0.614*	0.286	0.504*	0.871**	1.000	
Pop. size of K solubilizer	0.695*	0.519*	0.546*	0.060	0.753*	0.580*	0.290	0.535*	0.942**	0.935**	1.000

Levels of significance: \* $p < 0.05$ , \*\* $p < 0.01$ .

application rate of organic fertilizer also influenced soil N content. It could be attributed not only to N but also organic C contained in the fertilizer. Wani et al. (1988) reported that the use of suitable farmyard manures, green manures and other organic manures and fertilizers may enhance the benefits of *Azotobacter* inoculation. This is due to the fact that the N-fixation reaction needs a lot of energy from available organic C to break the bonds between nitrogen atoms (Postage, 1998).

Phosphorus is also a major nutrient for plants and microorganisms. The soil for this experiment is fairly poor in available P. However, available P (Olsen-P) in soil was significantly increased with the inoculation of AM fungi alone or in combination with rhizobacteria (Fig. 4). The efficiency of utilization of P fertilizer usually remains very low (20–25%) because a large portion of the soluble inorganic phosphate applied to soil as chemical fertilizer is rapidly immobilized soon

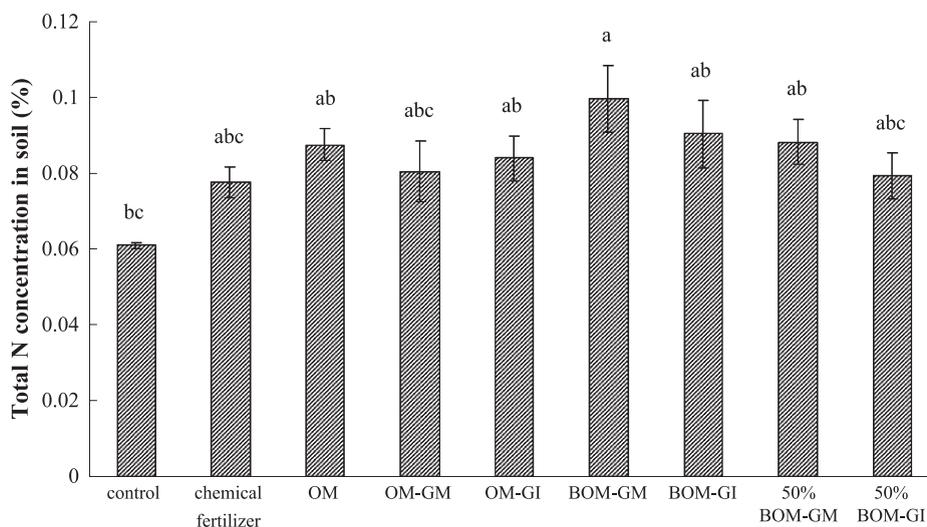


Fig. 3. Total nitrogen in the soil receiving different fertilizer treatments. Different letters above bars indicate a significant difference at  $p < 0.05$  according to Duncan's multirange test.

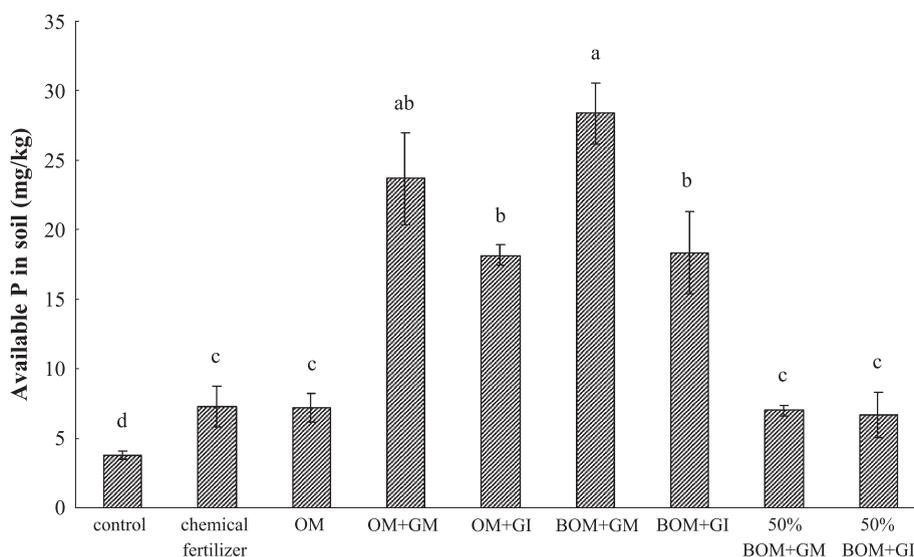


Fig. 4. Available phosphorus (Olsen-P) status in soils receiving different treatments. Different letters above bars indicate a significant difference at  $p < 0.05$  according to Duncan's multirange test.

after application, and becomes unavailable to plants due to chemical fixation in the soil (Dey, 1988; Hilda and Reynaldo, 1999). Besides, native soil P is mostly unavailable to crops because of its low solubility. Therefore, the AMF colonization and P-solubilizing bacteria can play an important role in improving P bioavailability. However, the effect of P-solubilizing bacteria was less significant when compared with AMF according to present results.

No significant difference of available K in soil between the treatments (data not shown) was observed. However, the inoculation of beneficial microbes exerted a stimulating effect on K uptake by the plants (Fig. 7).

### 3.3. Plant biomass accumulation

The dry matter of the plants after the growth period of 87 days ranged from 0.58 to 9.04 g pot<sup>-1</sup> (Fig. 5). The control plants showed very poor growth, which may be attributed to nutrient deficiency, e.g. the lack of available P in the unfertilized soil. In addition, sterilization of the soil killed the native microflora which assisted plant growth and nutrient uptake. Moderate increases in plant biomass were observed due to the increase of nutrients either in chemical or organic form. The fertilizer effect on plant growth was much more pronounced after inoculation of AMF and

its combination with beneficial bacteria. The maximum yield of 9.04 g pot<sup>-1</sup> was obtained with the treatment of BOM-GM while the BOM-GI treatment achieved the greatest height of 102 cm. With regards to the increase in plant biomass, *G. mosseae* seemed to be more effective than *G. intraradices* at the recommended fertilization level with or without rhizobacterial inoculation. However, the effect of the two mycorrhizal fungi at 50% of the recommended level on plant yields was not significantly different ( $p > 0.05$ ). It was noted that even plants grown on the 50% fertilization level produced almost three times the dry matter produced by plants grown on the chemical fertilizer and organic fertilizer treatments. These results suggest that the dual inoculation of beneficial bacteria and AMF could, at least to some extent, compensate the nutrient deficiency in soils. The unexpectedly low biomass of plants grown on the chemical and organic fertilizer treatments could also be attributed to the disappearance of indigenous microbes, which may be essential to increase nutrient bioavailability and uptake in the rhizospheric soil. Stimulation of different crops by rhizobacterial inoculation has also been demonstrated by other studies both in laboratory and field trials. For example, it was reported that wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculants (Kloepper et al., 1989);

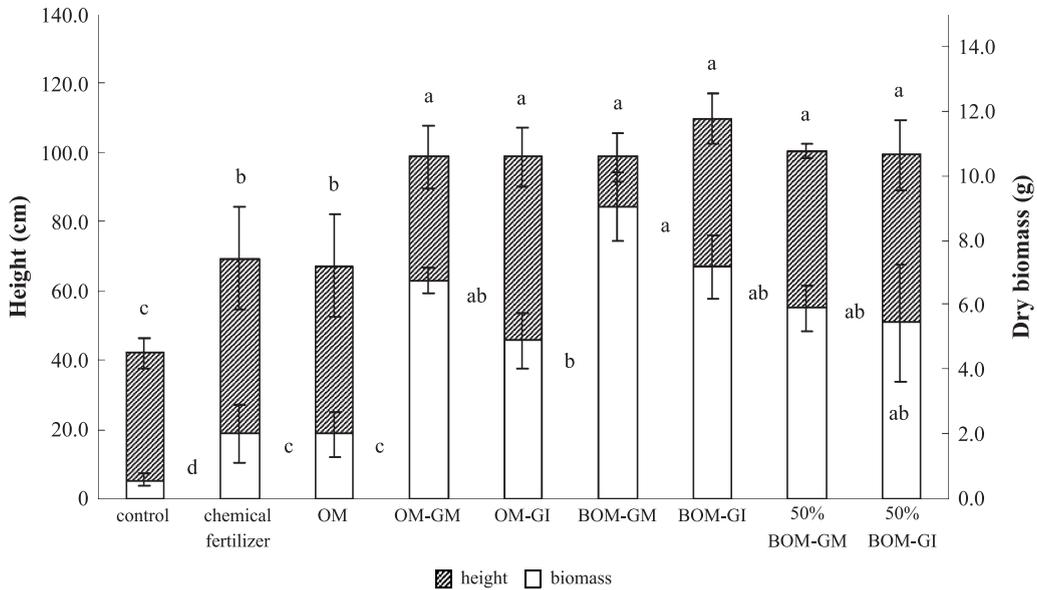


Fig. 5. Effect of inoculation with beneficial microbes on the growth parameters of *Z. mays*. Different letters above bars indicate a significant difference at  $p < 0.05$  according to Duncan's multirange test.

and a 10–20% yield increase in the same crop was reported in field trials using a combination of *B. megaterium* and *A. chroococcum* (Brown, 1974). Strains of *Pseudomonas* have increased root and shoot elongation in canola, lettuce and tomato (Hall et al., 1996; Glick et al., 1997).

### 3.4. Nutrient acquisition

Dual inoculation with AMF and rhizobacteria seemed to be the most effective treatment combination to improve plant nutrient uptake (Figs. 6 and 7). N concentration in plants under different treatments

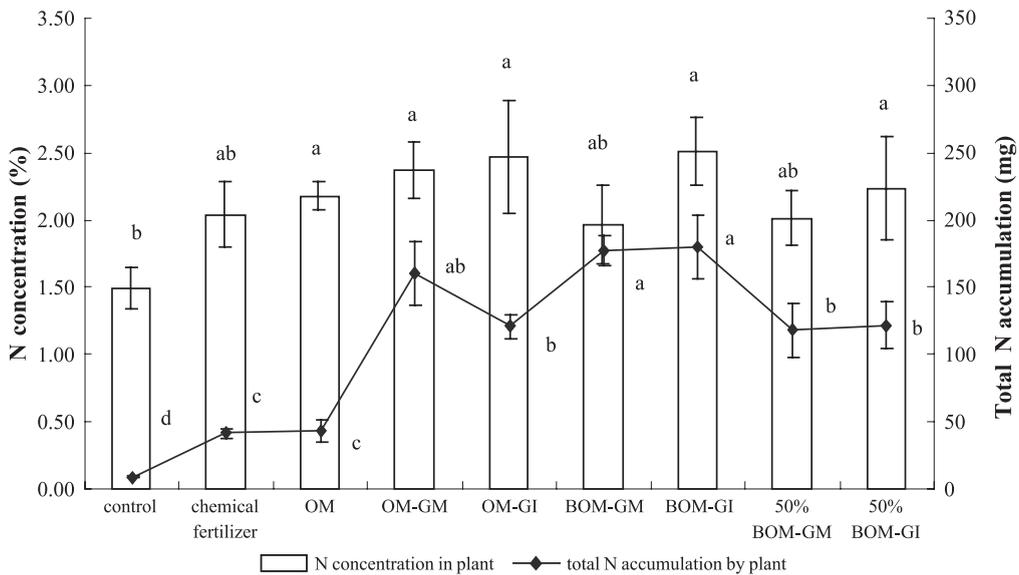


Fig. 6. Nitrogen assimilation by *Z. mays*. Different letters indicate a significant difference at  $p < 0.05$  according to Duncan's multirange test.

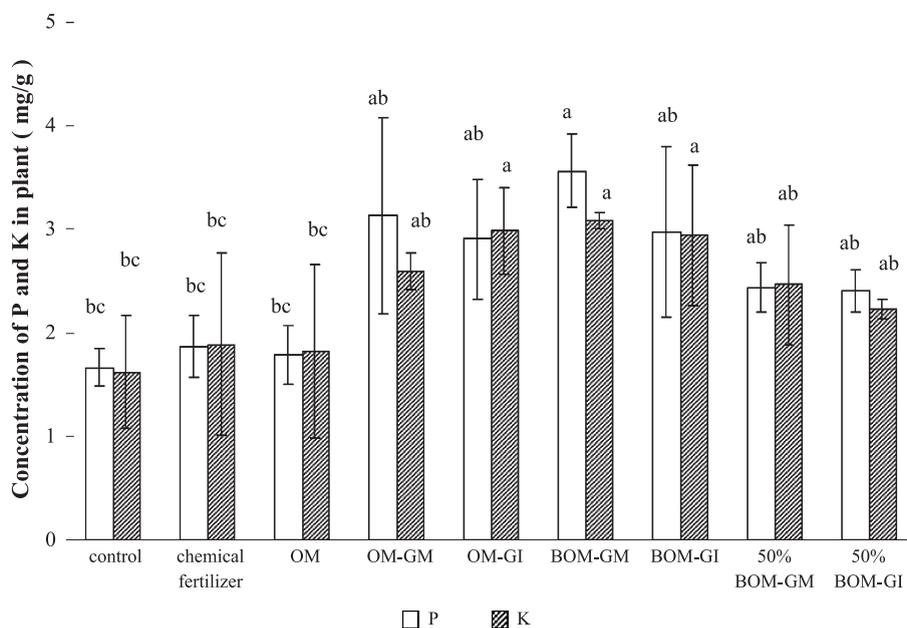


Fig. 7. Phosphorus and potassium uptake by *Z. mays*. Different letters above bars indicate a significant difference at  $p < 0.05$  according to Duncan's multirange test.

ranged from 1.49 (control) to 2.50% (dual inoculation with rhizobacteria and *G. intraradices*). Although dually inoculated plants (with rhizobacteria and *G. mosseae*) showed unexpectedly low N concentrations in the plant tissue, the fungi still assisted the host to assimilate the maximum total N and resulted in a higher biomass. The inoculation with *G. mosseae* had a more stimulating effect on the assimilation of N than *G. intraradices* in the absence of bacterial inoculation. However, *G. intraradices* performed better than *G. mosseae* in stimulating N and P uptake, when combined with bacterial inoculation, especially at lower nutrient level. The pattern of P and K uptake by plants under different treatments was similar to N assimilation. The lowest P and K uptake was detected in plants grown in uninoculated and unfertilized pots. Either single treatment of chemical or organic fertilization, or with AMF/bacteria inoculation resulted in an increase in P and K uptake to different degrees when compared with the control. The maximum P and K assimilation were obtained with the dual inoculation of *G. mosseae* and rhizobacteria.

The present results show that inoculation with microorganisms may increase the efficiency of fertil-

izer use at both high and low fertilization levels. Amending soil with beneficial microbes could compensate for nutrient deficiency and maintain, at least partly, a normal plant development. The biofertilizer therefore may have a potential to decrease the input cost of agricultural production, and be applied to the revegetation of low commercial value sites, such as metal tailings ponds (Carlot et al., 2002).

#### 4. Conclusion

The application of biofertilizer containing beneficial microbes showed a promoting effect on the growth of *Z. mays* and improvement of soil properties through an 87-day greenhouse study. The inoculation with rhizobacteria can significantly increase the root mycorrhizal colonization. Low fertilization level resulted in an increase of root infection by the mycorrhizal fungi, which indicated plants might be more dependent on mycorrhizal symbiosis than on P solubilizers under a P-deficient condition. The presence of mycorrhizal fungi had different influence on the population of rhizobacteria, and *G. intraradices* was able to stimulate the introduced beneficial

bacterial growth in the rhizosphere soil. However, the high mycorrhizal infection with *G. mosseae* showed a strong inhibition of P and K solubilizers.

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