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PLANT MICROBE INTERACTIONS

Plant Growth-Promoting Rhizobacteria Allow Reduced Application Rates of Chemical Fertilizers

A. O. Adesemoye · H. A. Torbert · J. W. Kloepper

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Abstract The search for microorganisms that improve soil fertility and enhance plant nutrition has continued to attract attention due to the increasing cost of fertilizers and some of their negative environmental impacts. The objectives of this greenhouse study with tomato were to determine (1) if reduced rates of inorganic fertilizer coupled with microbial inoculants will produce plant growth, yield, and nutrient uptake levels equivalent to those with full rates of the fertilizer and (2) the minimum level to which fertilizer could be reduced when inoculants were used. The microbial inoculants used in the study were a mixture of plant growth-promoting rhizobacteria (PGPR) strains *Bacillus amyloliquefaciens* IN937a and *Bacillus pumilus* T4, a formulated PGPR product, and the arbuscular mycorrhiza fungus (AMF), *Glomus intraradices*. Results showed that supplementing 75% of the recommended fertilizer rate with inoculants produced plant growth, yield, and nutrient (nitrogen and phosphorus) uptake that were statistically equivalent to the full fertilizer rate without inoculants. When inoculants were used with rates of fertilizer below 75% of the recommended rate, the beneficial effects were usually not consistent; however, inoculation with the mixture of PGPR and AMF at 70% fertility consistently produced the same yield as the full fertility rate without inoculants. Without inoculants, use of fertilizer rates lower than the recommended resulted in significantly less plant growth, yield, and nutrient uptake or inconsistent impacts. The results suggest that PGPR-based inoculants can be used and should be further evaluated as components of integrated nutrient management strategies.

A. O. Adesemoye (✉) · J. W. Kloepper
Department of Entomology & Plant Pathology,
Auburn University,
209 Life Science Building,
Auburn, AL 36849, USA
e-mail: adeseao@auburn.edu

J. W. Kloepper
e-mail: kloepjw@auburn.edu

H. A. Torbert
USDA Agricultural Research Services,
National Soil Dynamics Laboratory,
411 South Donahue Drive,
Auburn, AL 36849, USA
e-mail: allen.torbert@ars.usda.gov

Introduction

Fertilizers are essential components of modern agriculture because they provide essential plant nutrients. However, overuse of fertilizers can cause unanticipated environmental impacts. One example of the negative impacts of fertilizer is the "dead zone" in the Gulf of Mexico where nutrients washing from fertilized farms across the Mississippi Basin cause oxygen starvation, leading to an almost lifeless area in the gulf [27]. One potential way to decrease negative environmental impacts resulting from continued use of chemical fertilizers is inoculation with plant growth-promoting rhizobacteria (PGPR). These bacteria exert beneficial effects on plant growth and development [5], and many different genera have been commercialized for use in agriculture. One of the important mechanisms for these beneficial effects is PGPR-elicited enhanced nutrient availability and nutrient use efficiency. In a recent review, Glick et al. [16] observed that some PGPR may influence plant growth by synthesizing plant hormones or facilitating uptake of nutrients from the soil through different direct mechanisms such as atmospheric nitrogen (N) fixation, solubilization of phosphorus (P), and synthesis

of siderophores for iron sequestration making nutrients more available to plants.

Chemical fertilizers often have low use efficiency, meaning that only a portion of the applied nutrients are taken up by plants [17]. For example, P is precipitated after addition to soil, thus becoming less available to plants [17]. In addition, applied N can be lost through nitrate leaching, resulting in contamination of groundwater [9]. Microbial inoculants have shown some promise in increasing nutrient availability. For example, previous reports have suggested positive impacts of microbes on N uptake involving nonlegume biological fixation [4, 13, 22, 37, 41]. Also, inoculation with some microbes, including arbuscular mycorrhiza fungi (AMF), resulted in P solubilization or enhanced plant uptake of fixed soil P and applied phosphate resulting in higher crop yield [2–4, 7, 10]. The main mechanism resulting in increased availability of inorganic P appears to be through the action of organic acids synthesized by inoculants [34].

Because essential plant nutrients are taken up from the soil by roots [29], good root growth is considered a prerequisite for enhanced plant development. Many PGPR systems cause stimulation of root growth [9, 25], sometimes via production of phytohormones by the plant or the bacteria [25, 38]. If promotion of root growth by PGPR could be achieved with high frequency in the field, PGPR may be potential tools for increasing nutrient uptake.

Two key questions arise from some of the past studies: Is it possible to reverse the current trend of applying large amounts of fertilizers by supplementing reduced fertilizer with inoculants? Can the potential benefits of PGPR and/or AMF in plant nutrient uptake be utilized by combining them with reduced levels of fertilizers? The overall hypothesis is that PGPR or combinations of PGPR and AMF with fertilizers will improve the use efficiency of fertilizers and lead to a reduction in the amount of fertilizer usage.

The objectives in this study were to determine (1) if reduced rates of inorganic fertilizer coupled with microbial inoculants (PGPR or PGPR plus AMF) will produce plant growth, yield, and nutrient uptake levels equivalent to those with full rates of the fertilizer and (2) the minimum level to which fertilizer could be reduced when inoculants were used. To achieve these objectives, experiments were designed using single strains as well as formulated PGPR products with or without AMF coupled with different fertilizer regimes. For the PGPR strains, a two-strain mixture was used which included *Bacillus amyloliquefaciens* IN937a and *Bacillus pumilus* T4. The strains were previously reported to elicit significant effects on root development, plant growth, biocontrol, and/or induced systemic resistance [23, 32, 36, 42].

Results from some past studies have suggested ineffectiveness of PGPR using single-strain inoculations [14, 25], but mixtures of strains provided more consistency [8, 18, 36]. Some levels of interactions have been reported by co-inoculating PGPR with AMF [4, 6, 7, 31, 39]. Some studies, mostly with single elements, have suggested that PGPR are more effective when nutrients become limiting [12, 38]. Reported here are the results of a study that included single elements (N and P) as well as conventional water-soluble NPK fertilizer and the interaction of a two-strain mixture of PGPR with AMF.

Materials and Methods

Sources of Inoculants

Plant growth-promoting rhizobacteria used included two single strains that have been used in previous studies [26, 36, 42]. The two PGPR strains, *B. amyloliquefaciens* IN937a and *B. pumilus* T4, were obtained from the culture collection of the Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, USA and used as spore preparations. Another inoculant used was a commercial PGPR formulation, which consisted of many PGPR *Bacillus* strains with the trade name Plant Growth Activator (PGA; Organica, Norristown, PA, USA). In addition, the arbuscular mycorrhiza fungi used was *Glomus intraradices* obtained from Becker Underwood (Ames, IA, USA).

Test for Nitrogen Fixation by PGPR Strains

The two PGPR strains were tested for their capacity to fix N using JNFb medium [30]. The strains were streaked onto JNFb agar plates and stab-inoculated into JNFb semisolid medium in test tubes. After incubation for 48 h at 28°C, the color of agar and liquid medium was examined in comparison to a positive control consisting of the N-fixing strain *Azosprillum brasilense* obtained from the bacterial culture collection at Auburn University.

Experimental Design and Preliminary Studies

All assays reported in this paper were conducted in the greenhouse at the Plant Science Research Center, Auburn University using tomato (*Solanum lycopersicum*, formerly *Lycopersicon esculentum*) cultivar Juliet (Park Seed, Anderson, SC, USA). The growth medium was a mixture of one part field soil and three parts sand. After seeding, water was applied twice daily, and the greenhouse temperature was maintained at 21–25°C.

The overall experimental design was a randomized complete block with variations in the number of blocks depending on each test. The main blocks were inoculant types, while fertilizer rate was the subfactor. Inoculant type included PGPR alone, PGPR+AMF, AMF alone, and no inoculants. The fertilizer used was water-soluble Peters Professional® 20:10:20 Peat-Lite Special (Buddies Plant Food, Ballinger, TX, USA). The 100% fertilizer rate was 1.25 g L^{-1} which was the manufacturer's recommended rate. Additional fertilizer rates were tested as detailed in the following sections.

Establishing a Plant Growth Curve Using Different Rates of Fertilizer Without Inoculation

A plant growth curve was used to establish the response of tomato to different fertilizer rates without any inoculation. Experiments were set up by planting one tomato seed directly into each 10-cm-diameter pots containing the growth medium. Fertilizer was applied without microbial inoculation. Fertilizer treatments included 100%, 80%, 70%, 60%, and 50%. The fertilization of planted pots was carried out by applying 25 ml of solution of the appropriate treatment per plant. Each treatment had 20 replicates to allow two-time destructive sampling of ten replicates each. The first sampling was done at 4 weeks after planting (WAP) and the second at 6 WAP. Plants were removed from the pot. Roots were washed in slow-running water to remove adhering soil and laid on paper towels to drain. Plant height, stem caliper (taken at the oldest leaf position), and wet weight were recorded. Samples were dried for 7 days in the dryer at 70°C, and dry weights were taken. Growth index was estimated by multiplying height by width, and the growth index was plotted against fertilizer rates.

Tests with Inoculants and Water-Soluble Fertilizer

The different rates of fertilizer combined with PGPR or PGPR plus AMF were compared to the full rate of fertilizer (100%) without inoculants (positive control). The design was a 5×3 factorial randomized complete block. The five fertilizer treatments were 100%, 80%, 75%, and 70%, and an application rate of 50% was used as the negative control. The three inoculant treatments were no inoculation, PGPR, and PGPR plus AMF. Apart from the addition of inoculants (PGPR or PGPR plus AMF), methods were similar to those used in establishing the growth curve. The PGPR formulation used in this study (PGA) was prepared at the label rate of 3.78 g L^{-1} (1 tbsp gal^{-1}). In assays where a two-strain PGPR mixture was used, inoculation was carried out as explained below. In assays that involved AMF, *G. intraradices* was applied directly to seeds at planting before filling the holes.

Tests with a Two-Strain Mixture, AMF, and Hoagland Solution

The spore suspension of the two PGPR strains (*B. amyloliquefaciens* IN937a and *B. pumilus* T4) were diluted appropriately and mixed together. The concentration was adjusted to log 5 cfu/ml and used for inoculation. At planting, 1 ml of the bacterial suspension was applied onto each seed in a 10-cm pot containing a 1:3 mixture of field/sand soil. A follow-up inoculation was carried out at 1 week after planting by applying 1 ml of PGPR drench per pot around the base of each plant.

The study, with preceding assays, left some questions unanswered. Were there specific effects on uptake of any of the two growth limiting nutrients (N and P) in the plants? Was the effect, if any, related to growth promotion? To answer these questions, tests were conducted with Hoagland solution [28] as the fertilizer, which allowed the varying of each element and the precise tracking of changes that occurred. Experiments were done for N and P, and assays on each element were repeated. The design was a randomized complete block with inoculant type as the main block and fertilizer rate as the subfactor because, in this study, fertilizer rate was more important in the statistical interaction between fertilizer rate and inoculant type. There were three inoculant types: (1) no inoculant, (2) a mixture of two *Bacilli* PGPR strains, and (3) two *Bacilli* PGPR strain mixture plus AMF, *G. intraradices*. Fertilizer rates reported here include 100% (full-strength Hoagland solution), 80%, 75%, 70%, and 50% (negative control). The different fertilizer rates were made by appropriately varying the amount of N and P in Hoagland solution [20, 28]. More details about preparing and applying Hoagland solution as used in this study are shown below.

The content of Hoagland solution prepared for the study on P was different from that for N. For P, Hoagland solution (formulation I) was prepared with the components as originally formulated in 1933 by Hoagland and Snyder [28]. One liter of our 100% P solution was made by using 1 ml of 1 M potassium dihydrogen phosphate (KH_2PO_4), 5 ml of 1 M potassium nitrate (KNO_3), 5 ml of 1 M calcium nitrate tetrahydrate ($Ca[NO_3]_2{\cdot}4H_2O$), 2 ml of 1 M magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$), 1 to 2 ml of Fe-ethylenediamine tetraacetic acid (EDTA), and 1 ml of micronutrient stock. The Fe stock solution was covered with aluminum foil to prevent light degradation. The micronutrient stock solution was made of 2.86 g L^{-1} boric acid (H_3BO_3), 1.82 g L^{-1} manganese chloride tetrahydrate ($MnCl_2{\cdot}4H_2O$), 0.22 g/L zinc sulfate heptahydrate ($ZnSO_4{\cdot}7H_2O$), 0.02 g/L molybdic acid (85% of $Na_2MoO_4{\cdot}2H_2O$), and 0.08 g L^{-1} copper (II) sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$). The percentage of P was

varied by changing only the volume of KH_2PO_4 as appropriate.

For N, Hoagland solution was prepared using a slightly different composition [20] that had only one nitrogen source (formulation II). One liter of 100% N solution was prepared by using 7.5 ml of 1 M calcium nitrate tetrahydrate (Ca $[NO_3]_2{\cdot}4H_2O$), 10 ml of 0.05 M monocalcium phosphate (Ca $[HPO_4]_2$), 20 ml of 0.1 M calcium sulfate dihydrate ($CaSO_4{\cdot}2H_2O$), 5 ml of 0.5 M potassium sulfate (K_2SO_4), 2 ml of 1 M magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$), 2 ml of Fe-EDTA, and 1 ml of micronutrient stock. The percentage of N was varied by changing only the volume of Ca $(NO_3)_2{\cdot}4H_2O$ as appropriate. The solutions were autoclaved and adjusted to pH 5.8 with NaOH. Approximately 25 ml of each solution of varying nutrient content were then applied per pot according to the experimental plan. The first fertilizer application was carried out on the day of seeding. Using the volume of 25 ml maintained a low salt index level to avoid complications with germination.

Measurement of Plant Growth and Nutrient Content of Plant Tissues and Soil

Destructive sampling was done at 4 WAP. This time was chosen for nutrient analysis because in preliminary tests, it was observed that concentration of nutrients decreased with age of tissue. In each test, the height of tomato, fresh weight, and dry weight of tissue were measured. Also, in four experiments, root development or architecture was analyzed for each root before drying. Root architecture was measured with a scanner model LA 1600+ and WinRhizo software version 2004a (Regent Instruments, Sainte-Foy, Quebec, Canada). Parameters analyzed in the root system included total root length, surface area, volume, projected area, number of tips, mean diameter, and numbers of roots with diameters of 0–0.5 mm and 0.5–1 mm. Dry plant samples were analyzed for N and P contents (two growth-limiting nutrients). The methods used for nutrient analysis were the same as previously presented [1]. Nutrient (N and P) uptake of plants per treatment was estimated through uptake per gram of plant tissue multiplied by total yield per treatment (i.e., yield × percent nutrient per gram of plant tissue).

Data Analysis

Data were analyzed using GLM procedure, and Fisher's protected least significant difference (LSD) was used to separate treatment differences [24]. Statistical significance was considered at α=0.05. Regression fitting was carried out for relationships among variables. These analyses were done using Statistical Analysis System 9.1 (SAS Institute, Cary, NC, USA).

Results

The Growth Response Curve

The results obtained in tests to develop a standard response curve of tomato plants to different fertilizer rates showed that the growth of tomato was significantly greater with 100% fertility than with any other lower rates across all parameters (plant height, shoot and root, fresh and dry weights; Table 1). Figure 1 shows the model growth curves of tomato plant under the different rates of fertilizer, which is a plot of growth index against fertilizer rates at 4 weeks after planting.

Growth, Yield, and Nutrient Content for Tests with Water-Soluble Fertilizer

Results indicated that plant heights resulting from treatment with PGPR plus 80% or 70% of fertilizer were statistically equivalent to the heights with 100% fertility without PGPR. The effects were slightly different for co-inoculation of PGPR and AMF. Although 100% fertilizer without microbial inoculants produced statistically similar plant height as 80% or 70% of fertilizer plus PGPR and AMF, plants that

Table 1 Some growth parameters for response of tomato plant to different fertilizer treatments

Treatments	Fresh weight		Dry weight	
Percent fertilizer	Fresh shoot	Fresh root	Dry shoot	Dry root
100	9.13 a	4.07 a	2.09 a	0.53 a
90	7.37 b	3.09 b	1.31 b	0.31 b
80	6.43 c	2.82 b	1.16 b	0.22 c
70	5.39 d	2.29 c	0.89 c	0.20 c
60	4.28 e	2.04 c	0.78 c	0.17 c
50	1.03 f	0.59 d	0.18 d	0.06 d
$LSD_{(0.05)}$	0.89	0.69	0.29	0.06

Values in each column with different letter(s) are significantly different at p=0.05. Fertilizer was water-soluble 20:10:20; 100%=1.25 g L^{-1}

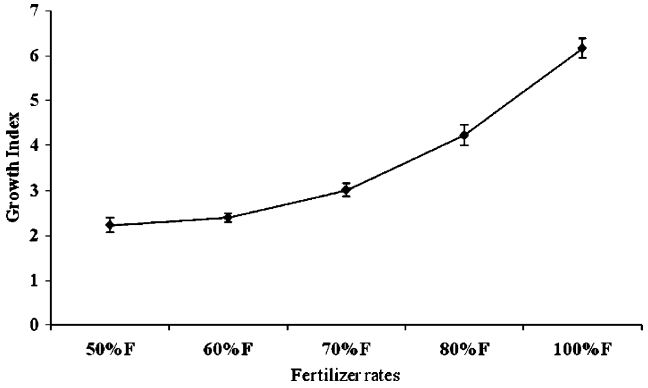

Figure 1 Growth response curve of tomato to different fertilizer rates at 4 WAP. *F* fertilizer, *WAP* weeks after planting

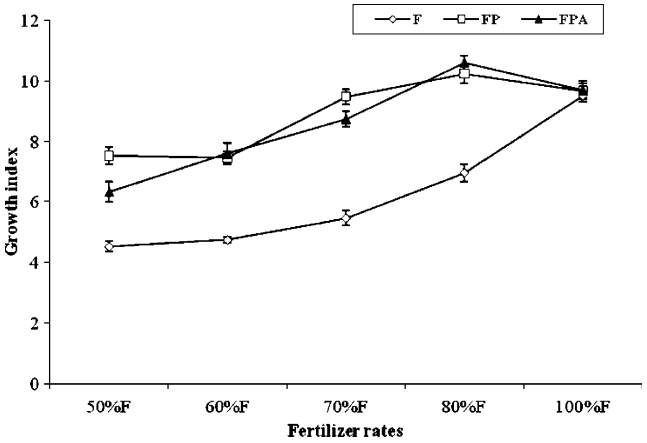

Figure 2 Growth index of tomato at different fertilizer rates with or without inoculants. *F* fertilizer, *P* plant growth-promoting rhizobacteria, *A* arbuscular mycorrhiza fungi. Growth index is height of plant multiplied by width

receive 80% of fertilizer with PGPR and AMF grew significantly taller than those with 70% fertilizer with PGPR and AMF (Table 2). After multiplying height by width to arrive at growth index, the comparison between uninoculated and inoculated plants showed that the inoculants significantly enhanced the growth of the plants, even at suboptimal fertilizer rates. Also, there were no differences among the growth index for plants that received 70% fertilizer plus PGPR, 80% fertilizer plus PGPR, or 100% fertilizer without PGPR. However, 100% fertility without PGPR was significantly greater than plants that received 70% fertilizer plus co-inoculation of PGPR and AMF (Fig. 2).

There was a high correlation between the growth index and the treatments (Fig. 2) with $y=1.218x+2.592$, $R^2=0.874$; $y=0.707x+6.751$, $R^2=0.749$; and $y=0.975x+5.665$, $R^2=0.8333$ for fertilizer, fertilizer plus PGA, and fertilizer plus PGA and AMF, respectively. Comparison of the yield in tomato fruits showed that 70% or 80% fertilizer plus PGPR and AMF were comparable to 100% fertilizer without inoculants (Fig. 3). For the treatment of fertilizer plus PGPR, only inoculant-supplemented 80% fertilizer produced the same yield as 100%. The inoculant-supplemented 70% fertilizer treatment produced significantly lower yield. The results indicated that 80% of fertilizer plus inoculants produced comparable results with 100%, but similar treatment with 70% fertilizer was not consistent.

Growth and Nutrient Content for Tests with a Two-Strain Mixture

With Hoagland solution, it was possible to track changes that occurred in growth and N and P uptake. The growth of plants that received 75% to 90% of fertilizer plus inoculation of PGPR or PGPR and AMF was comparable to the full fertilizer rate without inoculants. Also, the inoculation of PGPR or co-inoculation of PGPR and AMF produced similar effects (Fig. 4). The amount of N per gram of tomato shoot and root tissues were statistically the same for 100% fertilizer without inoculants and 75% fertilizer supplemented with PGPR (Figs. 5 and 6 for shoot and root, respectively). Also, plants that received 70% fertilizer with inoculants produced comparable amount of N in shoot as those with 100% fertilizer without inoculants (Fig. 5). On a whole-tissue basis, 75%, 80%, or 90% fertilizer plus inoculants gave results that were significantly equivalent to 100% fertilizer (Fig. 7). The fluctuation that occurred in

Table 2 Plant height of tomato at different fertilizer treatments with inoculation

Percent fertilizer	Fertilizer	Fertilizer+PGPR	Fertilizer+PGPR+AMF
100	19.9 a	21.5 a	20.4 ab
80	17.8 b	22.2 a	21.2 a
70	16.5 c	21.3 a	19.4 b
60	14.9 d	18.9 b	17.8 c
50	14.6 d	19.0 b	15.8 d
$LSD_{(0.05)}$	1.09	1.09	1.19

Values in each column with different letter(s) are significantly different at $p=0.05$. Fertilizer was water-soluble 20:10:20; 100%=1.25 g L^{-1}

AMF arbuscular mycorrhiza fungi, *PGPR* plant growth-promoting rhizobacteria

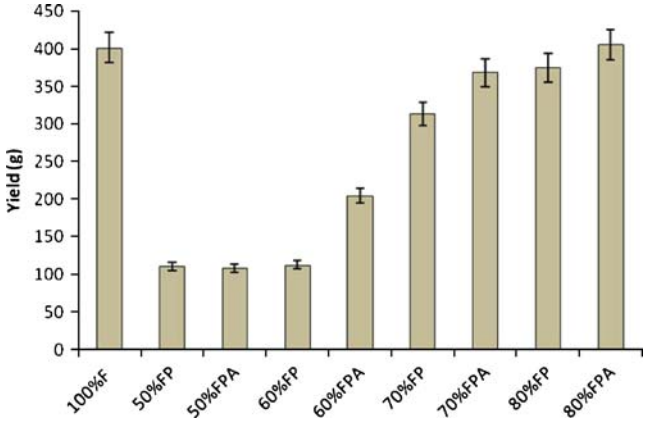

Figure 3 Yield of tomato with or without inoculant. *F* fertilizer, *P* plant growth-promoting rhizobacteria, *A* arbuscular mycorrhiza fungi

the previous test using water-soluble fertilizer for results on 70% fertilizer plus inoculants was also seen for the two-strain mixture on Hoagland solution for N uptake. Results for 70% treatment were not consistent. For P where AMF was one of the treatments, P uptake was significantly the same on total plant basis but not on a per gram of tissue basis (Fig. 8). Co-inoculation of PGPR and AMF with 70% fertilizer gave the best result, resulting in P uptake equivalent to that with 100% fertility without inoculant. Compared to the positive control, significantly more P was taken up by plants treated with 90% fertilizer and inoculants (PGPR plus AMF; Fig. 8).

Discussion

The results presented here support the hypothesis that PGPR or combinations of PGPR and AMF can improve the nutrient use efficiency of fertilizers. When the percentage of recommended fertilizer was reduced and inoculants were used, plant height, shoot dry weight, root dry weight, yield,

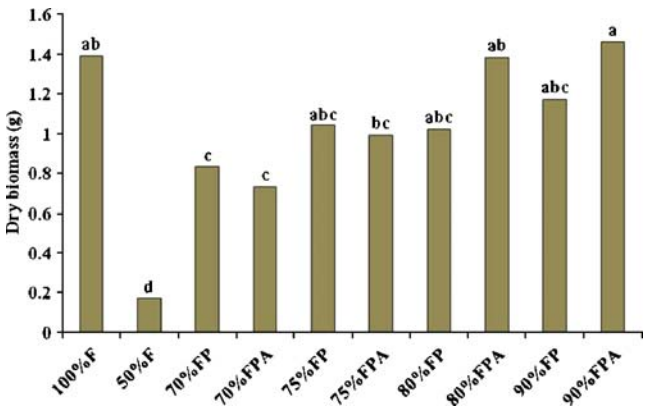

Figure 4 Dry biomass of plants with or without inoculants. *F* fertilizer, *P* plant growth-promoting rhizobacteria, *A* arbuscular mycorrhiza fungi

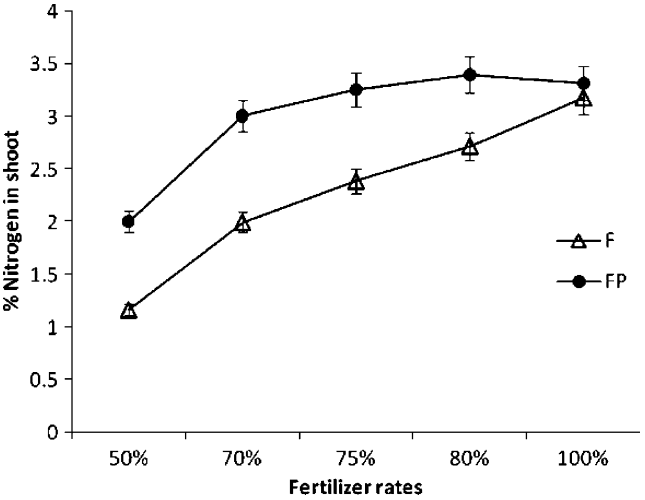

Figure 5 Nitrogen uptake per gram of tomato shoot with or without PGPR. *F* fertilizer, *P* plant growth-promoting rhizobacteria

and nutrient uptake were comparable to those with the full rate of fertilizer without inoculants (Table 2 and Fig. 2). After testing different reduced fertilizer rates, under these experimental conditions, 75% fertilizer was the stable minimum to which fertilizer could be reduced if supplemented with PGPR to achieve growth equivalent to 100% fertilizer without PGPR. Results also show that 100% fertilizer produced plant growth that was greater than all other lower rates if inoculants were not added (Table 1 and Fig. 1). This agrees with Biswas et al. [9] who suggested an interdependence of fertilizer N inputs and inoculants for optimal gain in rice productivity

When 70% fertilizer rate or lower was supplemented with PGPR or co-inoculation of PGPR and AMF, lower growth of tomato was observed or inconsistent growth compared to the 100% fertilizer control. In some instances, inoculant-supplemented 70% fertilizer gave

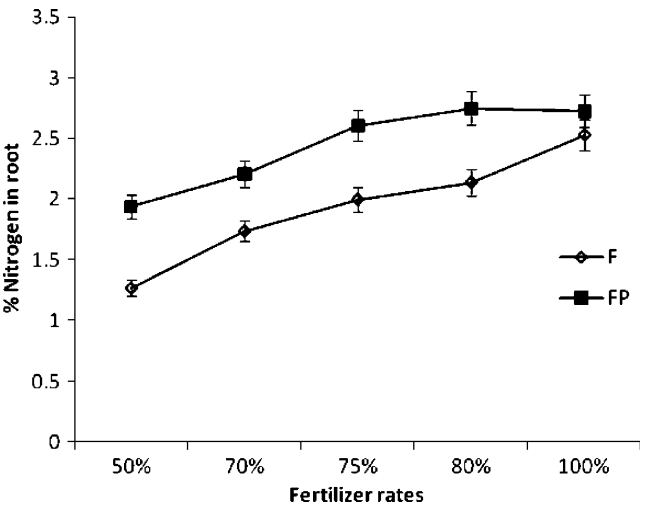

Figure 6 Nitrogen uptake per gram of root tissue with or without PGPR. *F* fertilizer, *P* plant growth-promoting rhizobacteria

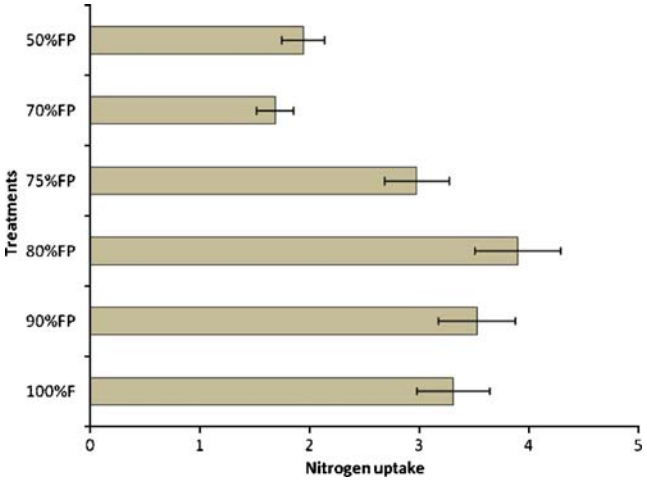

Figure 7 Nitrogen uptake on dry whole plant basis at 4 WAP with PGPR. Uptake was estimated by multiplying plant dry weight by % N per gram of tissue. *F* fertilizer, *P* plant growth-promoting rhizobacteria

growth that was comparable to 100% fertilizer without PGPR (Fig. 2 and Table 2) or comparable yield (Fig. 3, PGPR plus AMF bar). In the current system, the results support reduced fertilizer rates down to 75% if PGPR was added because that is the minimum at which results were consistent. This is different from the observations of Canbolat et al. [10] and Elkoca et al. [15], who reported no significant difference in root and shoot biomass of barley or seed yield and biomass of roots and shoots of chickpea, respectively, when inoculant alone or fertilizer alone was used. Based on those results, it was suggested that inoculants could be an alternative to fertilizer for chickpea [15]. In contrast, these current results demonstrate that, for tomato, inoculants may allow reduced rates of fertilizer but that they will not replace fertilizer.

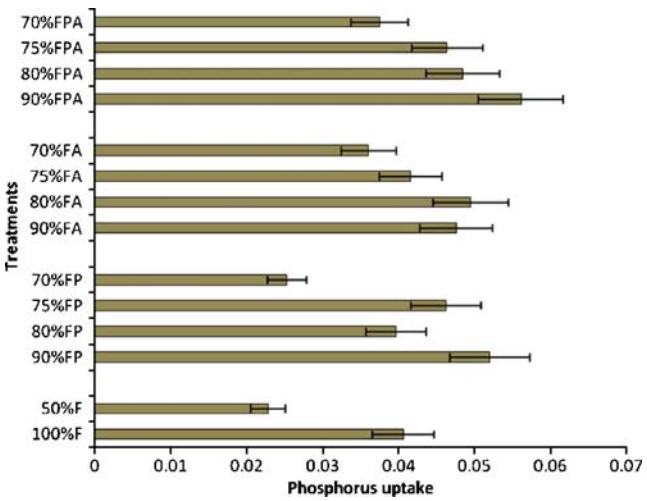

Figure 8 Phosphorus uptake on dry whole-plant basis with PGPR and AMF inoculation. Uptake was estimated by multiplying plant dry weight by % P per gram of tissue. *F* fertilizer, *P* plant growth-promoting rhizobacteria, *A* arbuscular mycorrhiza fungi

There were similarities in these results and those of Hernandez and Chailloux [19], who reported that the dry weight of tomato transplants grown in the greenhouse with 75% fertilizer plus two co-inoculated PGPR was significantly greater than those with full fertilizer rate without PGPR. At reduced fertilizer rates (down to 75%), inoculants consistently enhanced dry biomass (Fig. 4). Also, N uptake per gram of tissue and N uptake on a whole-plant basis were significantly better than the corresponding noninoculated controls (Figs. 5, 6, and 7). However, in the case of P, significant impacts resulted on a whole plant basis but not per gram of plant tissue (Fig. 8). Hence, enhanced N use efficiency in response to inoculation was greater overall than that of P.

Results indicate that the time of sampling tissue for nutrient analysis could be an essential factor to consider when making conclusions about the impact of inoculants on plant nutrient uptake. In the experiment with Hoagland solution, plants treated with 75% fertilizer plus inoculants consistently had comparable amounts of N at 4 weeks after planting to those with 100% fertilizer without inoculants. However, results were highly variable when samples were taken at 6 WAP. A possible explanation for this could be based on previous reports that the concentration of nutrients, particularly N, P, K, S, Cu, and Zn, decreases with age of plant tissues [28, 29].

Timing of sampling, microbial biomass/structure [21, 35], and nutrient content of the growth medium may account for some variability in results of different authors [37, 38]. Saubidet [37] reported that N content in wheat plants inoculated with *A. brasilense* decreased as N supply rate increased, and at the maximum N supply, the content of total N was the same between inoculated and noninoculated wheat plants. On the other hand, Shaharoona et al. [38] reported that N use efficiency increased in response to inoculation with *Pseudomonas fluorescens* at all fertilizer levels in wheat, causing 115%, 52%, 26%, and 27% increase over the noninoculated control at N, P, and K application rates of 25%, 50%, 75%, and 100% recommended doses, respectively. Also, other explanations for those differences in results could be the different effects of specific PGPR strains or other experimental factors.

Could there be a synergistic interaction between PGPR and AMF to improve the uptake of P and N? In this study, there appears to be some level of interaction with uptake of P, though little (Fig. 8). Co-inoculation of AMF and PGPR with 90% fertilizer resulted in plant uptake of P that was significantly higher than with full fertilizer rates, though its improvement over the inoculation of PGPR with 90% fertilizer was not significant. However, 70%

fertilizer plus AMF and PGPR resulted in more P uptake than the corresponding treatment with PGPR alone (Fig. 8). Aseri et al. [4] reported significant interaction of *Azotobacter chroococcum* and *Glomus mosseae* in pomegranate leading to better leaf area, shoot dry weight, and uptake of N, P, and K compared to either PGPR or AMF alone. This is different from a previous 3-year field study with corn [1], in which there was no consistent significant interaction between PGPR and AMF. Also, no detrimental interactions were observed in this study, which is in agreement with the results of Barea et al. [6].

One way that some previous studies have enhanced the performance of PGPR is co-inoculation of multiple PGPR strains [8, 15, 23, 32]. For example, Belimov et al. [8] reported significantly greater uptake of P in shoot of barley with co-inoculation of *Azospirillum lipoferum* 137 and *Arthrobacter mysorens* 7 or *A. lipoferum* 137 and *Agrobacterium radiobacter* 10 than single inoculation of any of the three organisms. A two-strain mixture was used in the current study, and it proved to be effective in both growth promotion and N and P uptake.

The enhancement of N uptake by plants inoculated with the PGPR strains (*B. amyloliquefaciens* IN937a and *B. pumilus* T4) used in this study was not via associative N fixation based on the tests with JNFb medium and because there are no known N-fixing strains of *B. amyloliquefaciens* or *B. pumilus*. Therefore, the resulting enhancement of N uptake must be due to alternative bacterial effects. A combination of the activities of the plant and the inoculants [11, 23, 33, 37, 40] is being proposed as a model for PGPR-enhanced N uptake in plants, according to the following scenario. The PGPR promote the growth of the plant and increase the root surface area or the general root architecture [9, 25]. Plants growing better in turn release higher amounts of C in root exudates. The release of more C prompts increase in microbial activity, and this process continues in a cycle. The whole process makes more N available from the soil pool, influencing N flux into plant roots, and the plant is able to take up more available N. Overall, the results suggest that inoculants could be used to allow reductions in the current high rates of fertilizer and the resulting environmental problems [17, 27, 38] without compromising plant productivity. However, it should be noted that no microbial inoculant can be universal for all systems as the effectiveness may be affected by plant type, soil type, and some other factors. Further greenhouse and field studies should provide more definitive information about the movement and uptake of N and P to plants with the impacts of PGPR-based inoculants. Future studies should include ^{15}N isotope techniques to more clearly track uptake of N from applied fertilizers to plant tissues.

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References

1. Adesemoye AO, Torbert HA, Kloepper JW (2008) Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. Can J Microbiol 54:876–886
2. Altomare C, Norvell WA, Bjorkman T, Harman GE (1999) Solubilization of phosphates and micronutrients by the plant growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295–22. Appl Environ Microbiol 65:2926–2933
3. Amir HG, Shamsuddin ZH, Halimi MS, Marziah M, Ramlan MF (2005) Enhancement in nutrient accumulation and growth of oil palm seedlings caused by PGPR under field nursery conditions. Commun Soil Sci Plant Anal 36:2059–2066
4. Aseri GK, Jain N, Panwar J, Rao AV, Meghwal PR (2008) Biofertilizers improve plant growth, fruit yield, nutrition, metabolism and rhizosphere enzyme activities of pomegranate (*Punica granatum* L.) in Indian Thar Desert. Sci Hortic 117:130–135
5. Bakker PAHM, Raaijmakers JM, Bloemberg GV, Hofte M, Lemanceau P, Cooke M (2007) New perspectives and approaches in plant growth-promoting rhizobacteria research. Eur J Plant Pathol 119:241–242
6. Barea JM, Andrade G, Bianciotto V, Dowling D, Lohrke S, Bonfante P, O'Gara F, Azcon-Anguilar C (1998) Impact on arbuscular mycorrhiza formation of *Pseudomonas* strains used as inoculants for biocontrol of soil-borne fungal plant pathogens. Appl Environ Microbiol 64:2304–2307
7. Barea JM, Azcon R, Azcon-Aguilar C (2002) Mycorrhizosphere interactions to improve plant fitness and soil quality. Antonie van Leeuwenhoek 81:343–351
8. Belimov AA, Kojemiakov AP, Chuvarliyeva CV (1995) Interaction between barley and mixed cultures of nitrogen fixing and phosphate-solubilizing bacteria. Plant Soil 173:29–37
9. Biswas JC, Ladha JK, Dazzo FB (2000) Rhizobia inoculation improves nutrient uptake and growth of lowland rice. Soil Sci Soc Am J 64:1644–1650
10. Canbolat MY, Bilen S, Cakmakci R, Sahin F, Aydin A (2006) Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. Biol Fertil Soils 42:350–357
11. Clarholm M (1985) Possible roles for roots, bacteria, protozoa, and fungi in supplying nitrogen in plants. Ecol Interact Soil 4:355–365
12. de Freitas JR Germida JJ (1990) Plant growth-promoting rhizobacteria for winter wheat. Can J Microbiol 36:265–272
13. Dobbelaere S, Croonenborghs A, Thys A, Ptacek D, Vanderleyden J, Dutto P, Labandera-Gonzalez C, Caballero-Mellado J, Anguirre JF, Kapulnik Y, Brener S, Burdman S, Kadouri D, Sarig S, Okon Y (2001) Response of agronomically important crops to inoculation with *Azospirillum*. Aust J Plant Physiol 28:871–879
14. Egamberdiyeva D, Höflich G (2004) Effect of plant growth-promoting bacteria on growth and nutrient uptake of cotton and pea in a semi-arid region of Uzbekistan. J Arid Environ 56:293–301
15. Elkoca E, Kantar F, Sahin F (2008) Influence of nitrogen fixing and phosphorus solubilizing bacteria on the nodulation, plant growth, and yield of chickpea. J Plant Nutr 31:157–171
16. Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007) Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26:227–242

17. Gyaneshwar P, Kumar GN, Parekh LJ, Poole PS (2002) Role of soil microorganisms in improving P nutrition of plants. Plant Soil 245:83–93
18. Han HS, Lee KD (2005) Phosphate and potassium solubilizing bacteria effect on mineral uptake, soil availability, and growth of egg plant. Res J Agric Biol Sci 1:176–180
19. Hernandez MI, Chailloux M (2004) Las micorrizas arbusculares y las bacterias rizosfericas como alternativa a la nutricion mineral del tomate. Cult Trop 25(2):5–12
20. Hershley DR (1994) Solution culture hydroponics: history & inexpensive equipment. Am Biol Teach 56:111–118
21. Horwath WR, Paul EA (1994) Microbial biomass. In: Weaver RW, Angle S, Bottomley P, Bezdicek D, Smith S, Tabatabai A, Wollum A (eds) Methods of soil analysis, part 2, microbiological and biochemical properties-sssa book series, no. 5. Soil Science Society of America Inc, Madison, Wisconsin, USA, pp 753–773
22. Kennedy IR, Pereg-Gerk LL, Wood C, Deaker R, Gilchrist K, Katupitiya S (1997) Biological nitrogen fixation in non-leguminous field crops: facilitating the evolution of an effective association between *Azospirillum* and wheat. Plant Soil 194:65–79
23. Kloepper JW, Gutierrez-Estrada A, McInroy JA (2007) Photoperiod regulates elicitation of growth promotion but not induced resistance by plant growth-promoting rhizobacteria. Can J Microbiol 53:159–167
24. Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O (2006) SAS® for mixed models, 2nd edn. SAS Institute Inc, Cary, North Carolina, USA, pp 21–41
25. Lucy M, Reed E, Glick BR (2004) Application of free living plant growth-promoting rhizobacteria. Antonie van Leeuwenhoek 86:1–25
26. Mahaffee WF, Kloepper JW (1997) Temporal changes in the bacterial communities of soil, rhizosphere, and endorhiza associated with field-grown cucumber (*Cucumis sativus* L.). Microb Ecol 34:210–223
27. Malakoff D (1998) Coastal ecology: death by suffocation in the Gulf of Mexico. Science 281:190–192
28. Maynard DN, Hochmuth GJ (2007) Knott's handbook for vegetable growers. 5th edn Wiley, Hoboken, New Jersey, pp. 65-68, 92-101, 170-213.
29. Mills HA, Jones JB (1996) Plant analysis handbook II: a practical sampling, preparation, analysis, and interpretation guide. Micro-macro Publishing, Athens, Georgia, USA, pp. 6-18, 69, 81.
30. Olivares FL, Baldani VLD, Reis VM, Baldani JI, Döbereiner J (1996) Occurrence of the endophytic diazotroph *Herbaspirillum* spp. in roots, stems, and leaves predominantly of Gramineae. Biol Fertil Soils 2:197–200
31. Probanza A, Mateos JL, Luca Garcia JA, Ramos B, de Felipe MR, Guiterrez Manero FJ (2001) Effects of inoculation with PGPR *Bacillus* and *Pisolithus tinctorius* on *Pinus pinea* L. growth, bacterial rhizosphere colonization and mycorrhizal infection. Microb Ecol 41:140–148
32. Raupach GS, Kloepper JW (2000) Biocontrol of cucumber diseases in the field by plant growth-promoting rhizobacteria with and without methyl bromide fumigation. Plant Dis 84:1073–1075
33. Raynaud X, Lata JC, Leadley PW (2006) Soil microbial loop and nutrient uptake by plants: a test using a coupled C:N model of plant-microbial interactions. Plant Soil 287:95–116
34. Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339
35. Runion GB, Prior SA, Reeves DW, Rogers HH, Reicosky DC, Peacock AD, White DC (2004) Microbial responses to wheel-traffic in conventional and no-tillage systems. Commun Soil Sci Plant Anal 35:2891–2903
36. Ryu C-M, Murphy JF, Reddy MS, Kloepper JW (2007) A two-strain mixture of rhizobacteria elicits induction of systemic resistance against *Pseudomonas syringae* and *Cucumber mosaic virus* coupled to promotion of plant growth on *Arabidopsis thaliana*. J Microbiol Biotechnol 17:280–286
37. Saubidet MI, Fatta N, Barneix AJ (2002) The effect of inoculation with *Azospirillum brasilense* on growth and nitrogen utilization by wheat plants. Plant Soil 245:215–222
38. Shaharooma B, Naveed M, Arshad M, Zahir ZA (2008) Fertilizer-dependent efficiency of Pseudomonads for improving growth, yield, and nutrient use efficiency of wheat (*Triticum aestivum* L.). Appl Microbiol Biotechnol 79:147–155
39. Tahmatsiodu V, O'Sullivan J, Cassells AC, Voyiatzis D, Paroussi G (2006) Comparison of AMF and PGPR inoculants for the suppression of *Verticillium* wilt of strawberry (*Fragaria* × *ananassa* cv. Selva). Appl Soil Ecol 32:316–324
40. Vassey JK, Buss TJ (2002) *Bacillus cereus* UW85 inoculation effects on growth, nodulation, and N accumulation in grain legumes: controlled-environment studies. Can J Plant Sci 82:283–290
41. Wu SC, Cao ZH, Li ZG, Cheung KC, Wong MH (2005) Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125:155–166
42. Zhang S, Reddy MS, Kokalis-Burelle N, Wells LW, Nightengale SP, Kloepper JW (2001) Lack of induced systemic resistance in peanut to late leaf spot disease by plant growth-promoting rhizobacteria and chemical elicitors. Plant Dis 85:879–884