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Research Article

Effects on *Glomus mosseae* Root Colonization by *Paenibacillus polymyxa* and *Paenibacillus brasilensis* Strains as Related to Soil P-Availability in Winter Wheat

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Greenhouse experiments were conducted to assess the effects of inoculating winter wheat (*Triticum aestivum*) with plant growth promoting rhizobacteria (PGPR) of the genus *Paenibacillus* under phosphate P-limited soil conditions in the presence or absence of the arbuscular mycorrhizal fungus (AMF) *Glomus mosseae*. Four *P. polymyxa* strains and one *P. brasilensis* strain were compared at two cell concentrations (10^6 and 10^8 cells g^{-1} seeds) of inoculation, and surface sterilized AMF spores were added to pots. Mycorrhizal root colonization, plant growth, and plant uptake of phosphorus were analyzed. Bacterial phosphate solubilization was examined separately *in vitro*. Most *P. polymyxa* strains, isolated from wheat, had dramatic effects *per se* on root growth and root P-content. No treatment gave significant effect on shoot growth. AMF root colonization levels and total plant uptake of P were much stimulated by the addition of most *P. polymyxa* strains. The AM fungus alone and the *P. brasilensis*, alone or in combination with the fungus, did not affect total plant P-levels. Our results indicate that practical application of inoculation with plant host-specific rhizobacteria (i.e., *P. polymyxa*) could positively influence uptake of phosphorus in P-deficient soils by wheat plants, provided that suitable AM fungi (e.g., *G. mosseae*) are present.

1. Introduction

Increased environmental awareness is progressively leading to a shift from conventional intensive agriculture to low-input sustainable agricultural cropping systems relying on biological processes rather than agrochemicals to maintain crop health and productivity. This shift has resulted in greater interest in naturally occurring soil microorganisms that facilitate improvement of soil fertility and/or stimulate plant nutrition and health, either alone or via specific interactions. One example of beneficial microbial interactions is the association between arbuscular mycorrhizal (AM) fungi and bacteria [1–3].

AM fungi and bacteria interact synergistically to stimulate plant growth via a range of mechanisms. For instance, certain bacterial species directly affect AM fungal germination and growth rate [4–7], thereby stimulating plant root colonization [8]. Earlier studies by our group showed that *Glomus mosseae* colonization of clover and wheat roots in pot cultures containing sterilized soil was higher upon co-inoculation with *Paenibacillus brasilensis* PB177, compared to controls without bacterial inoculates [8, 9]. Consistently, similar results regarding beneficial impact of *Paenibacillus* spp. on AM fungi have been reported also by other groups [10, 11].

In addition to direct interactions, specific bacteria, together with AM fungi, create a more indirect synergism that also supports plant growth [12], including nutrient acquisition [13], inhibition of plant pathogenic fungi [8], and enhancement of root branching [14]. Moreover, other bacteria collaborate with AM fungi to promote plant host growth by increasing phosphate uptake. Phosphate-solubilizing rhizobacteria may contribute to the soil phosphate pool available for extraradical AM fungal hyphae to

pass on to the plant, especially in soils with low phosphorus bioavailability [15].

Interactions between bacteria and AM fungi typically occur in the mycorrhizal hyphosphere [16] in the part of the soil surrounding individual fungal hyphae [17]. It has been shown that hyphal exudates of AMF might stimulate bacterial growth [18] and also change the bacterial community structure [19]. Moreover, certain bacterial groups seem to be stronger associated with AMF hyphae than others, and the composition of bacterial communities that attach to AMF hyphae has been shown to be different from the communities that are not attached [17]. For example, bacteria of the genus *Paenibacillus* have been related to AMF hyphae and mycorrhizal formation in several studies [10, 20], and the *Paenibacillus brasilensis* strain PB177 has been shown to display a higher degree of physical attachment to vital AM fungal hyphae, irrespective of the fungal species involved (*Glomus* spp. MUCL 43205 and *Glomus intraradices*), compared to a number of bacterial control strains that do not colonize hyphae to the same extent [21]. On the other hand, Scheublin et al. [17] were not able to identify *Paenibacillus* spp. within the bacterial communities associated to AMF hyphae. Further evaluation of the "specificity" of associations between AM fungi and paenibacilli strains is crucial for optimizing the future application of these microorganisms, such as in the context of mixed microbial inoculates.

The aim of the present study was to evaluate the potential effects on growth and P-uptake in winter wheat of five strains of paenibacilli (four *P. polymyxa* and one *P. brasilensis*) alone or in association with the AM fungus, *G. mosseae*. Mycorrhizal root colonization, plant growth, and plant uptake of P were analyzed. The *P. brasilensis* strain, isolated from maize, interacts with several AM fungi as well as wheat plants [8]. The *P. polymyxa* strains B1-4, originally isolated from wheat roots [22], infect wheat roots [23] and stimulate nitrogen fixation in association with the plant host [24]. At least, one of these strains, B2, analyzed with respect to plant growth stimulation, produces cytokinins [25]. The results of this study provide further information on the associations between AM fungi, rhizobacteria, and wheat plants that could enhance P utilization of wheat under P-limited conditions.

2. Materials and Methods

2.1. Soil Sampling. The area for soil sampling represents a typical low-input agricultural region in Sweden. Soil (sandy loam) was collected from the upper 30 cm of a field recently harvested in early October at Krusenberg outside Uppsala, Sweden, and had a relatively low P-Al (plant-available phosphorus) content of 89 mg phosphate-P Kg^{-1} dw (dry weight). The soil was dried at room temperature, sieved through a 4 mm mesh, mixed, and autoclaved twice for 20 min at 121°C. Autoclaved soil was mixed with sterile sand (1 : 1), and 1 L pots were filled 1 cm from the top, covered with plastic, and positioned on a table in the greenhouse (18°C night, 6 h; 22°C day, 18 h, daylight tubes) without watering until the experiment was initiated 2 days later.

2.2. Arbuscular Mycorrhizal Fungus. The arbuscular mycorrhizal (AM) fungus, *Glomus mosseae* BEG12 (Nicol. and Gerd., Gerdemann and Trappe), was obtained from Biorize (Dijón, France) as sporocarps and divided into single spores with a very fine forcep under the microscope. Approximately 100 spores were surface-sterilized by incubating for 20 min on a rotating table (120 rpm) in a tube containing 1 mL sterilizing solution (500 mL water, Chloramin T (2%), and streptomycin (400 μg^{-1} mL)). The solution was decanted and spores rinsed five times with 1 mL of sterile deionized water. The sterilization procedure was confirmed as efficient by microscopic investigation of the treated spores. 10 spores suspended in 500 μL 0.1 M $MgSO_4 \cdot 7H_2O$ were transferred to soil into a 2 cm deep hole centrally located in each pot.

2.3. Coating of Wheat Seeds with Bacteria. Seeds of winter wheat (cultivar Tarso) were used in the present experiment. The seeds were surface sterilized [8] and, thereafter, separately coated with four different *Paenibacillus polymyxa* (B1–B4) strains [22–25] and one *Paenibacillus brasilensis* PB177 strain [26] pregrown in 100 mL of GB medium (1 L; 10 g glucose, 10 g peptone, 1 g yeast extract, 5 g sodium chloride) until an OD_{600} of 0.5 was reached. Cells were centrifuged for 15 min at 3500 rpm and the pellets dissolved in 5 mL of 0.1 M $MgSO_4 \cdot 7H_2O$. The cell concentrations were corrected to 10^6 and 10^8 per 10 g of wheat seeds, respectively, with 0.1 M $MgSO_4 \cdot 7H_2O$ (total volume of 3 mL). Bacterial cells and wheat seeds were mixed in tubes with slow agitation in the vertical position on a rotation table (120 rpm) for 5 min [27]. The bacterial suspension was discarded, and seeds dried on an absorbing paper for 10 min. As a control, seeds mixed with an equal amount of 0.1 M $MgSO_4 \cdot 7H_2O$ instead of bacterial suspensions were used.

2.4. Pot Cultures. The dry soil and sand mixture was presoaked by watering pots with tap water 6 hours before the addition of AM fungi and wheat seeds coated with bacterial suspension or 0.1 M $MgSO_4 \cdot 7H_2O$. There were three factors studied (bacterial strain inoculation with 6 levels (4+1 strains or none), AMF inoculation with 2 levels (with or without) and bacterial inoculation (quantity) with 2 levels). In total, there were 110 pots (50 with bacterial alone, 50 with bacterial plus AMF, 5 with only AMF added, and 5 untreated). The aim was to determine the impact of *G. mosseae* and *Paenibacillus* spp. alone or in combination on: (i) shoot and root growth, (ii) mycorrhizal colonization of roots, and (iii) phosphorus uptake in shoots and roots. Five different *Paenibacillus* strains (*P. polymyxa* B1–B4 and *P. brasilensis*) at two concentrations (10^6 and 10^8 per 10 g of wheat seeds) were used with only wheat or both wheat and *G. mosseae* spores. Each treatment was replicated in five pots planted with three seeds each making a total of 110 pots. The pots were arranged in randomized blocs with one replicate per treatment in each block. They were placed on a table in the greenhouse, and the entire blocs were, thereafter, rotated to new positions each day to compensate for minor variations in light intensity or temperature [28]. The pots were watered once a day with tap water with a pH of 8.2, conductivity of 42.2 mS/m, and dH

of 8.3 grades. The nutrient content was as follows: 11 mg/L nitrate, <0.04 mg/L ammonium, 14 mg/L magnesium, and 38 mg/L calcium. After three weeks, the emerging plants were counted and the tallest point of each plant measured (cm) from the soil surface. Seedlings were thinned to one plant per pot and the tallest retained. Fifteen weeks after sowing, roots and shoots were harvested in separate fractions and the dry weights measured after drying at 70°C for 3–5 days.

2.5. G. mosseae Root Colonization. The extent of root colonization by AM fungi was measured after harvest, and approximately 2 g of wheat fine roots (≤1 mm) from each plant were transferred to separate tubes. The extent of colonization was measured in all treatment. Three (out of five) randomly selected replicate pots per treatment with added AMF spores were sampled, and three 2-3 cm sections of wheat roots from each replicate stained. Staining and analysis of *G. mosseae* colonization was performed according to the method of Artursson and Jansson [29] with some minor changes, such as incubation times (30 min instead of 1 h in 10% KOH and 10 min instead of 30 min in 1% HCl) and destaining solution (acidified glycerol; 14 : 1 : 1 lactic acid : glycerol : water (vol/vol)). Fungal colonization was determined using the magnified intersection method of McGonigle et al. [30] .

2.6. Phosphorus Content in Root and Shoot. Triplicate roots and shoots (the same as for AMF colonization measurements) from all treatments were assessed for phosphorus content (%P g^{-1}). The phosphorus content in 1 g portions from dried root and shoot samples was analyzed after dry combustion (CNS 2000/IPC Optima 3000 DV) at the Department of Soil Science (Fertility and Plant Nutrition section, Swedish University of Agricultural Sciences, Sweden), according to standard procedures (protocol SS 02 83 11). 10 mL concentrated nitric acid (65%) was added to 1 g dry weight of plant material, and the samples were left over night to let the breakdown of organic matter start. The samples were subsequently boiled, with stepwise increasing temperature, for one hour at 60°C, one hour at 100°C and four hours at 125°C. After two hours at 125°C, an additional 5 mL concentrated nitric acid was added. The samples were diluted to 50 mL with distilled water after cooling, and the phosphorus content was determined by ICP-AES (Optima 7300 DV, Perkin Elmer, USA) [31].

2.7. Phosphorus Solubilization Capacity. Qualitative estimation of bacterial phosphate solubilization was conducted using a petridish assay on Pikovskaya's agar [16]. Spot inoculation of *P. polymyxa* B1–B4 and *P. brasilensis* was carried out using a sterile needle on petridishes incubated at 30°C for 12 days in triplicate. Solubilization of insoluble phosphate (tricalcium phosphate) was verified by formation of distinct clear zones around bacterial colonies (solubilisation index; SI) [32]. Clearing zones were measured, after 7 days, in size (ratio of total diameter (colony + halo zone) to colony diameter [33]) to discern differences between bacterial strains.

2.8. Statistical Analyses. The differences in phosphorus solubilization capacities (SI), shoot length, dry weight, and fungal colonization between treatments inoculated with solely bacteria, bacteria, and AMF, only AMF, respectively, or uninoculated, were tested for significance using one-way analysis of variance (ANOVA). Following ANOVA, Tukey's post-test was performed (GraphPad Software, Inc., San Diego, Calif, USA). Total P-uptake in each bacterial treatment was compared to the corresponding control without bacterial inoculation with the unpaired *t*-test (GraphPad Software, Inc.). Values were considered significantly different at $P < .05$. The D'Agostino and Pearson normality test (GraphPad Software, Inc.) indicated a Gaussian distribution among the values in the different data sets, hence justifying the use of ANOVA and *t*-test analyses. Interactions between the different factors among the treatments (e.g., cell concentrations, fungal, and bacterial species, resp.) were evaluated by factorial ANOVA (GraphPad Software, Inc.). The potential relationships between the level of AM fungal colonization and total P-uptake were investigated by linear and nonlinear regression (curve fit; GraphPad Software, Inc.) using the values of all single replicated samples.

3. Results

3.1. Plant Emergence and Early Development. Three weeks after sowing, the number of emerging plants (27–67%) in each pot and the lengths of the longest shoot of individual plants were estimated (Figure 1). Addition of bacteria, alone or in combination with *G. mosseae,* had no significant effect on plant emergence, with the exception of B1, whereby we observed a significantly lower number of emerging plants at a concentration of 10^8 bacteria per 10 g of seeds than in the treatment without bacterial addition ($P < .05$; data not shown). Application of bacterial strains B1, B4, and PB177 (at the higher cell concentration) resulted in significantly shorter plants, compared to the control without bacteria inoculated ($P < .01$; Figure 1), whereas no marked differences ($P > .05$) between bacterial treatments and control were observed at the lower inoculation concentration (10^6; Figure 1). Moreover, no effects were observed by *G. mosseae* alone or in combination with the bacteria except that the growth retardation of *P. polymyxa* strains B1 and B4 and *P. brasilensis* PB177 at the 10^8 level disappeared.

3.2. Plant Biomass at Harvest. Most *P. polymyxa* strains, but not *P. brasilensis* PB177, dramatically promoted root biomass at a concentration of 10^6 cells per 10 g of seeds (Figure 2). The highest increase (2.6-fold) was induced by strain B4. Strain B3 additionally resulted in significantly larger root systems, compared to the control without bacterial inoculation ($P < .01$). At the higher cell concentration (10^8), the same *P. polymyxa* strains (B3 and B4) yielded roots with lower weights ($P < .05$ at 10^8), compared to lower bacterial numbers. No marked differences between any bacterial treatments and control without inoculated bacteria were, however, observed. Combinations of bacterial

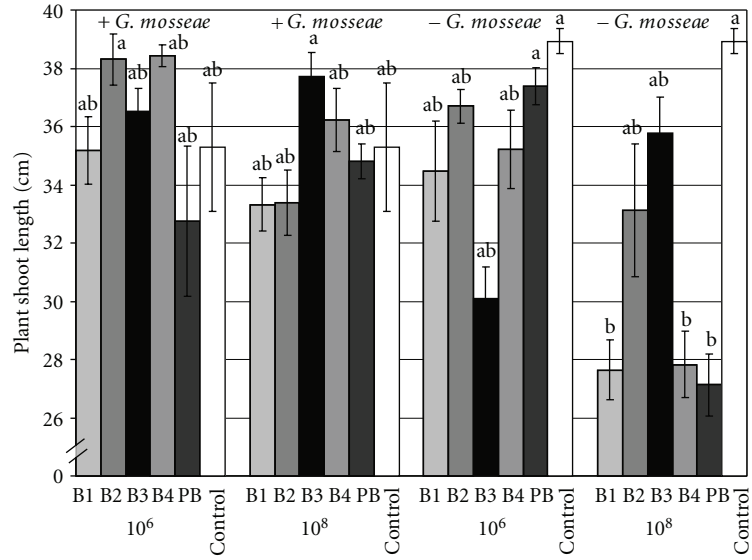

Figure 1: Average lengths of emerged wheat plant shoots, 3 weeks after sowing, inoculated with *G. mosseae* and/or different paenibacilli strains (B1–B4 and PB177) at concentrations of 10^6 or 10^8 bacterial cells 10 g^{-1} of seeds or left untreated. The same letters above the bars indicate treatments with no significant differences ($P > .05$). Error bars represent the standard error of the mean.

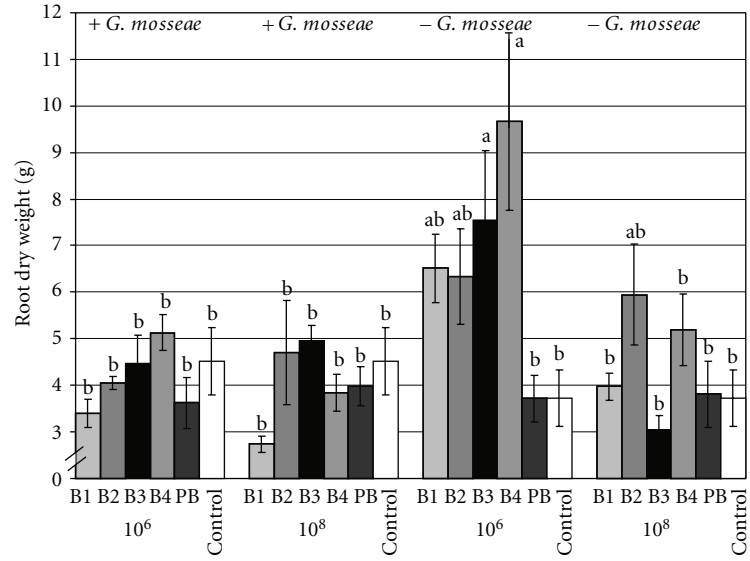

Figure 2: Average dry weight of roots at harvest (15 weeks) from wheat plants inoculated with *G. mosseae* and/or different paenibacilli strains (B1–B4 and PB177) at concentrations of 10^6 or 10^8 bacterial cells 10 g^{-1} of seeds or left untreated. The same letters above the bars indicate treatments with no significant differences ($P > .05$). Error bars represent the standard error of the mean.

treatments and *G. mosseae* induced no specific effects on root growth at either concentration (Figure 2).

As to shoots, bacterial treatments alone (at either cell level) did not significantly reduce biomass, although allocation of C resources to roots (Figure 2) had in some cases (e.g., B3 and B4 at the 10^6 cell level) clearly taken place (Figure 3). A nonsignificant reduction in shoot biomass was generally observed in the treatments with low level of inoculated bacteria and no AMF compared to the corresponding treatments with added AMF. However, for the treatments containing inoculated *P. brasilensis* at the lower cell concentration, a significant increase in shoot biomass was seen when *G. mosseae* was inoculated (Figure 3). No significant effects were evident at the higher cell concentration (10^8).

3.3. Fungal Root Colonization. Pots with added spores of *G. mosseae* had 26% root colonization by typical arbuscular mycorrhizal fungi (Figure 4). No mycorrhizal fungal hyphae were observed in control treatments without added AMF. Coinoculation with a low concentration of strain B4 (10^6) with AMF led to significantly increased AM fungal root colonization ($P < .01$), compared to the control with

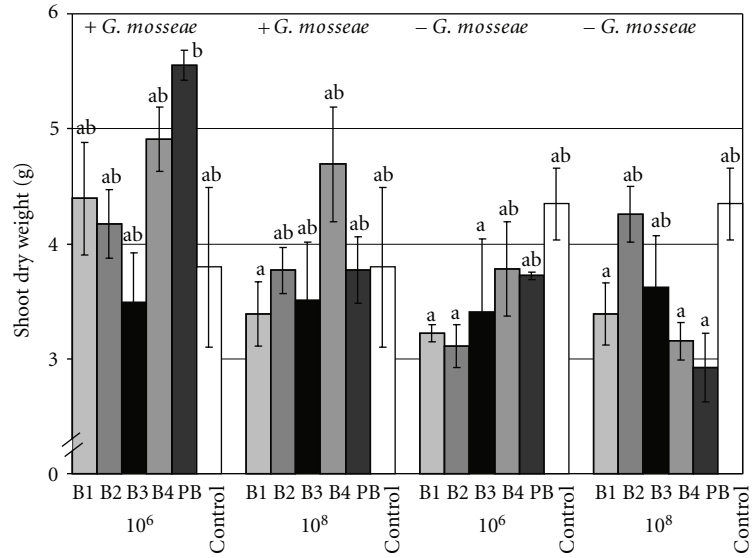

Figure 3: Average dry weight of shoots at harvest (15 weeks) from wheat plants inoculated with *G. mosseae* and/or different paenibacilli strains (B1–B4 and PB177) at concentrations of 10^6 or 10^8 bacterial cells 10 g^{-1} of seeds or left untreated. The same letters above the bars indicate treatments without significant differences ($P > .05$). Error bars represent the standard error of the mean.

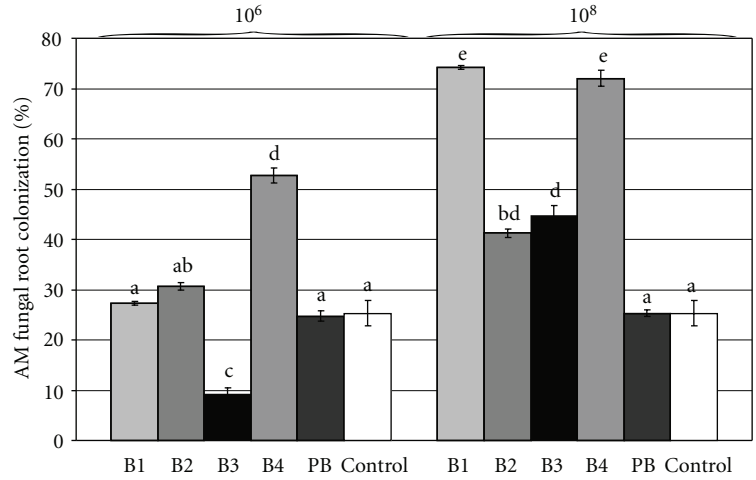

Figure 4: Percentage of AM fungal root colonization at harvest (15 weeks) in triplicate root samples with different inoculates of paenibacilli strains (B1–B4 and PB177) and *Glomus mosseae*. Two inoculation doses of 10^6 and 10^8 bacterial cells 10 g^{-1} of seeds were applied. The control was only inoculated with *G. mosseae*. The same letters above the bars indicate treatments without significant differences ($P > .05$). Error bars represent the standard error of the mean.

AMF but without bacterial inoculation. Inoculation with similar concentrations of strains B1, B2, and PB177 did not significantly affect fungal colonization, whereas B3-treated plants showed lower fungal colonization than the control without added bacteria ($P < .05$). At high concentrations (10^8), all *P. polymyxa* strains (B1–B4) significantly and remarkably enhanced the AM fungal root colonization levels ($P < .001$; up to 3-fold in the case of B1 and B4), while no effect was observed upon inoculation with *P. brasilensis* PB177. The increase in root colonization of *G. mosseae* was significantly higher at 10^8 cells 10 mL^{-1} than at the lower level (10^6) for the strains B1, B3, and B4.

3.4. Phosphorus Content in Roots and Shoots. In plants inoculated with lower bacterial cell concentrations (10^6) without fungal inoculation, the phosphorus content in roots was significantly higher for strains B2, B3, and B4 at harvest, compared to the control without added bacteria ($P < .05$). The largest increase (about 2-fold) was observed with strains B3 and B4 (Figure 5(a)). No significant effects were, however, noted in roots at the higher (10^8) inoculation level (Figure 5(a)). The P-content patterns for roots and shoots were reversed in bacterial treatments combined with *G. mosseae* (Figure 5(b)). In most cases, combined treatment with bacteria and AM fungi decreased P-content in roots

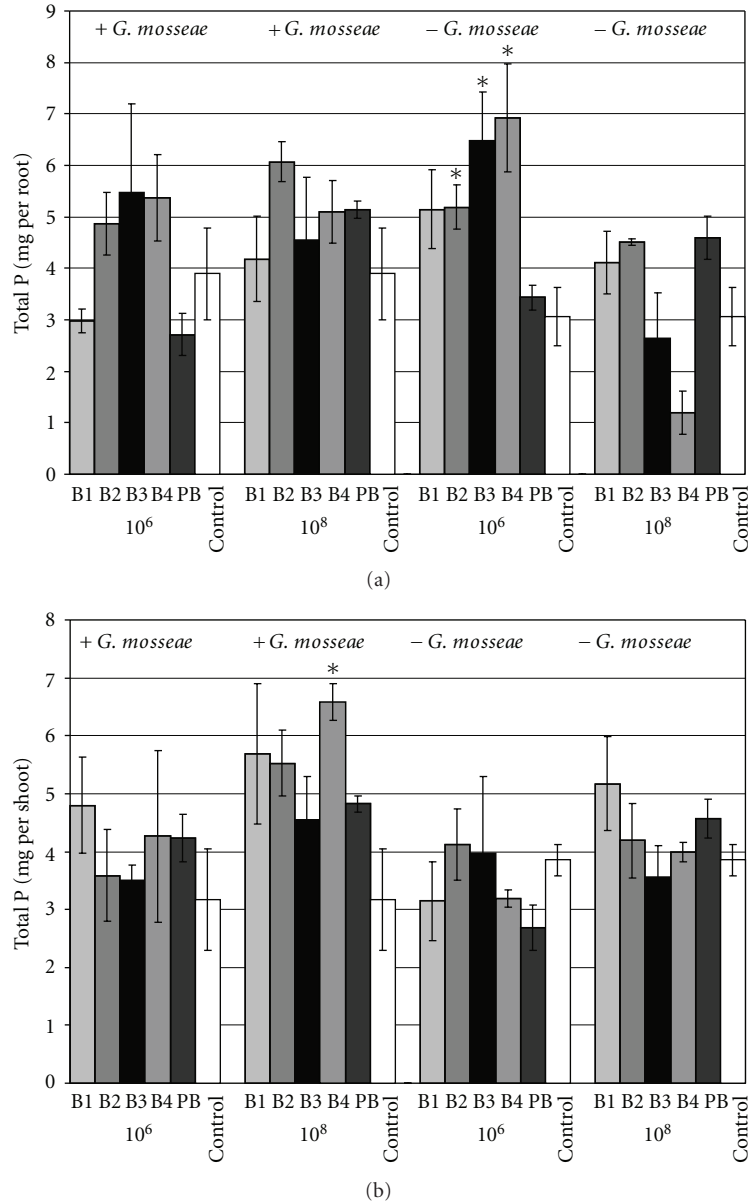

Figure 5: (a) Total phosphorus (milligram per plant) at harvest (15 weeks) in triplicate samples of wheat plant roots inoculated with or without *G. mosseae* or left untreated. Paenibacilli strains (B1–B4 and PB177) were added at a concentration of 10^6 or 10^8 bacterial cells $10\,g^{-1}$ of seeds. Treatments that were significantly altered from control pots without added bacteria, as assessed with the unpaired *t*-test, are indicated as $^*P < .05$. Error bars represent the standard error of the mean. (b) Total phosphorus (milligram per plant) at harvest (15 weeks) in triplicate samples of wheat plant shoots inoculated with or without *G. mosseae* or left untreated. Treatments and tests as in Figure 5(a).

compared with bacteria only, whereas the opposite occurred for shoots, with an observed increase in three out of five bacterial treatments. At higher bacterial levels (10^8), bacterial combinations with AM fungi mostly increased P-uptake both in roots and shoots. The P-content in shoots was clearly significantly higher for the strain B4 in comparison with the control with only AMF (Figure 5(b)). The AM fungus itself did not significantly alter the P-content in either roots or shoots, compared to untreated control.

We studied the differences between the total P-content of wheat plants (data from Figures 5(a) and 5(b)) and the mycorrhizal fungal root colonization level (Figure 4) in untreated controls and treatments with AM fungus plus various bacteria (10^6 and 10^8 cells per 10 g of seeds). Significant positive correlation ($r = 0.61$, $P < .01$) was observed between mycorrhizal fungal colonization with *P. polymyxa* strains B1–B4 and P-content (Figure 6). Addition of strain PB177 lowered the correlation coefficient and, thus, did not contribute significantly and was, therefore, excluded. *G. mosseae* alone (neither included in Figure 6) did not affect the total P-level of the plant host, compared with untreated control, and although root colonization was 26% it did not

differ from absolute uninoculated control in terms of total P-uptake. Accordingly, we conclude that most *P. polymyxa* strains, B4 in particular, enhance both mycorrhizal fungal colonization and total P-content of treated wheat plants.

3.5. Phosphorus Solubilizing Capacity of Bacterial Strains. All bacterial strains displayed good ability to solubilize inorganic phosphate, as evident from the formation of distinct clear zones around colonies on Pikovskaja's agar. Differences in capacity (SI at about 3) between strains were marginal (data not shown), and thus not statistically significant ($P > .05$).

3.6. Statistical Analyses. Statistical analyses of results at harvest showed no significant differences between blocs regarding P-uptake, plant growth, or AMF root colonization. Interactions between treatments at harvest neither reached statistically significant levels as evaluated by factorial ANOVA (results not shown).

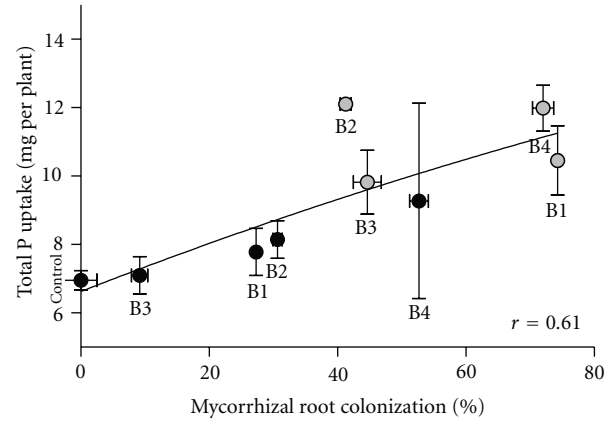

Figure 6: Non-linear regression analysis showing a significant positive correlation ($P < .01$) between total P-content in wheat plants and mycorrhizal fungal root colonization, in combination with all *P. polymyxa* strains (B1–B4) at harvest (15 weeks). The untreated control without added microorganisms (no AMF colonization) is included. Other treatments were omitted as they did not significantly affect total P-content. Black circles represent strains added at a concentration of 10^6 bacterial cells $10\,g^{-1}$ of seeds, whereas grey circles correspond to added amounts of 10^8 bacterial cells $10\,g^{-1}$ of seeds. Error bars represent the standard error of the mean.

4. Discussion

The harvest data, before flowering, significantly demonstrate that most *P. polymyxa* strains strongly stimulate root growth at low bacterial numbers (10^6), but not much at 10^8 level and not by the *P. brasilensis* strain (PB177) at either level. However, this was not evident when bacterial inoculations were combined with *G. mosseae*, indicating less C-allocation to roots. The direct influence of the *P. polymyxa* strains on root growth is possibly due to a combination of increased P-uptake and hormonal effects, for example, bacterial production of cytokinins [25]. The strong and intimate natural association between wheat plant roots and *P. polymyxa* was detected a few decades ago [22, 23, 34].

In the current study, most bacterial inoculations alone (10^6 and 10^8) suppressed shoot development. In contrast, dual inoculation of *Paenibacillus* with *G. mosseae* stimulated shoot growth by most strains, including *P. brasilensis,* at low (10^6) but not at high (10^8) bacterial levels. These results further emphasize the importance of the actual interactions between *Paenibacillus* and AMF for beneficial effects on plant growth. The finding is partly in keeping with our earlier results [8] showing no pronounced effects on shoot or root growth of winter wheat when *G. mosseae* and *G. intraradices* were combined with high levels of *P. brasilensis* PB177 (10^8). However, our group (Hjort, Arthurson and Granhall, unpubl.) found that this strain alone at high bacterial cell numbers could lead to complete inhibition of winter wheat seed germination if infested with snow mould (*Monographella nivalis* (Schaffnit) E. Müll), in contrast to *P. polymyxa*. This finding indicates that the plant host-bacterial associations studied are sensitive to strain specificity and the total numbers of bacteria inoculated. Inoculation levels above 10^6 per 10 g of seeds could thus sometimes have negative consequences. The presence of the AM fungus however, attenuated this negative effect in the current study.

One of the *P. polymyxa* strains, B4, investigated exerted strong stimulatory effects on AM colonization rates at both cell densities. At high inoculation levels, all other *P. polymyxa* strains (but not *P. brasilensis*) also promoted mycorrhizal root colonization. The total levels of AMF root colonization were thus highest (over 70% of roots) at high inoculation levels of *P. polymyxa*. In another experiment with slightly different conditions [8], also *P. brasilensis* was shown to stimulate the mycorrhizal root colonization (by *G. mosseae* and *G. intraradices*) which was not the case in the present study.

However, plant shoot growth in the present study was not significantly related to root colonization level or P-uptake. The reason was possibly that the experiment ended before flowering since much transfer of nutrients (both N and P) occur during maturing stages. Inoculation with low levels of all *P. polymyxa* strains in the absence of AM fungi enhanced the total P-content in roots, but not in shoots. At high levels, the results were quite variable. However, as previously remarked, in combination with *G.mosseae*, P-levels displayed an opposite trend, that is, lower content in roots but mainly increased in shoots. This is probably a reflection of the influence of the hormonal effects of the AM fungus on P-allocation within the plant [35]. The changes in total plant P-levels were highly correlated with the AMF colonization level of *P. polymyxa* strains (at both levels). This finding indicates that most *P. polymyxa* strains not only act as "mycorrhiza helper bacteria" (MHB) [36, 37], for example, through increased spore germination and mycelial growth [36], but also that specific combinations (e.g., *P. polymyxa* strain B4 plus *G. mosseae*) significantly enhance P-uptake by the host wheat plant. In consistency, other groups have shown a similar increase in plant P-uptake resulting from phosphate-solubilizing bacteria and AMF for other plant hosts [38, 39]. Notably, these effects were not observed with *P. brasilensis* PB177 in the present study.

The apparent difference between the two bacterial species may be attributed to a higher plant specificity of bacteria isolated from the actual plant host (all *P. polymyxa* strains were isolated from wheat whereas *brasilensis* PB177 was isolated from maize). Interestingly, the AMF alone did not affect the total P-levels of the plants, compared with the treatment without AMF in contrast to many data obtained from studies under natural nonsterile conditions [38, 40, 41]. Based on these results, we propose that only the bacteria, which were all efficient phosphosolubilizers, and not the AM fungus released P from actual P-deficient soil. In contrast, others claim that AMF are themselves involved in release of P from insoluble sources [42, 43]. In cases where only bacteria (*P. polymyxa* strains) were present, root growth was highly stimulated and the total P-content increased in the roots, whereas little transfer to the shoots occurred. On the other hand, the dual presence of *G. mosseae* and *P. polymyxa* appeared to have initiated a change in the P-allocation patterns from roots to shoots [35]. A longer duration of the experiment (up to grain filling) would have been needed to verify this. Practical field applications of some of the *P. polymyxa* strains used in this study have been previously shown to stimulate final grain harvest and N-contents in both wheat and barley [44]. The mechanism of P-solubilization of the bacteria in the present study was lowering of the pH by production of organic acids (not analysed in detail).

In conclusion, we demonstrate the following:

(i) Inoculation levels and specificities of bacterial strains are crucial in determining effective combinations for associations between AM fungi, MHB/PGPR bacteria, and plant hosts, such as wheat. Certain bacterial strains (e.g., *P. polymyxa* strain B4) exerted its highest effect on total P-uptake and AM fungal root colonization at high (10^8) levels, demonstrating that optimal combinations of PGPR (strain specificity and concentration) and normally present (e.g., *G. mosseae*) or added AMF can be found and further developed for practical application. Optimal parameters still need to be identified and further investigation of the specific interactions of certain *Paenibacillus* strains with different AMF is therefore warranted.

(ii) All *P. polymyxa* strains isolated from the particular plant host (wheat) stimulated root growth and P-content in roots and increased mycorrhizal root colonization by *G. mosseae*, thus acting as MHB. The confirmed release of P from insoluble sources (*in vitro*) and P-deficient soils (this study) enhances total P-uptake in wheat plants either directly (root association) [23] or through interactions with the AM fungus [9]. In this study, the AM fungus mainly acted in transport and allocation of P released by the bacteria. It should be interesting to monitor the fate of the *Paenibacillus* bacteria during the plant growth, possibly contributing to an increased understanding of these tripartite interactions.

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