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ORIGINAL PAPER

Pseudomonas bacteria and phosphorous fertilization, affecting wheat (*Triticum aestivum* L.) yield and P uptake under greenhouse and field conditions

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Abstract We have recently indicated the plant growth promoting activities of *Pseudomonas* sp. as well as their alleviating effects on some soil stressors such as salinity. This is because in recent years, biological fertilizers have received special attention by scientists in sustainable agriculture. Accordingly, it is pertinent to specify the beneficiary level of such soil bacteria on plant growth including phosphorous (P) uptake. Hence, the objectives were to determine: (1) the plant growth promoting effects of the tested *Pseudomonas* sp., and (2) its combined effects with different P fertilization rates on the nutrient uptake (N, P, and K) and yield of wheat (*Triticum aestivum* L.) under greenhouse and field conditions. The experiments were factorially arranged on the basis of a completely randomized block design with three replicates and were conducted at the Research Farm of Agriculture and Natural Resources Research Center of Khorasan, Mashhad, Iran. P was fertilized at three levels including 0, 25 and 50 kg/ha P_2O_5. *Pseudomonas* sp. including *Pseudomonas fluorescens* 153, *P. fluorescens* 169, *P. putida* 4, and *P. putida* 108 were tested. Activities such as production of ACC deaminase and IAA-like products, as well as P solubilization were among the most important activities of the tested *Pseudomonas* sp. Such bacterial effects greatly enhanced wheat growth and yield under greenhouse and field conditions. The results also showed that the effects of *Pseudomonas* sp. on wheat nutrient uptake and the effects of bacteria as well as P fertilization on wheat yield were significant. *P. putida* 108 was the most effective strain enhancing wheat P uptake and grain yield under greenhouse (96 and 58%) and field (80 and 37%) conditions, respectively. Hence, although *Pseudomonas* sp. could be a suitable replacement for high P fertilization, however, the optimum wheat yield resulted when the bioinoculants are combined with 50% (25 kg/ha P_2O_5) P fertilization. This finding has great agricultural and environmental implications.

Keywords ACC deaminase · P fertilization · Plant growth promoting rhizobacteria (PGPR) · P solubilization · Wheat (*Triticum aestivum* L.) yield

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Introduction

Production of wheat (*Triticum aestivum* L.) typically requires intensive use of chemical fertilizers. In addition to nitrogen (N) and potassium (K), phosphorous (P) is also one of the essential macronutrients, required for the growth and development of wheat. P is usually applied to the soil in the form of phosphatic fertilizers. However, a large portion of soluble inorganic phosphate applied to the soil as chemical fertilizer is immobilized rapidly and becomes

unavailable to plants. Hence, P availability for plant growth, especially in areas with high fixation and immobilization capacity, is of particular significance. Up to 39 kg/ha of P fertilization significantly increased wheat grain yield (Vig and Singh 1983; Manske et al. 2001; Salimpour et al. 2010).

Soil microorganisms can contribute to the enhanced availability of soil P through mineralization of organic P (Abd-Alla 1994; Bishop et al. 1994) or solubilization of inorganic P (Kucey et al. 1989; Salimpour et al. 2010) resulting in the reduction of P fertilization (Illmer and Schinner 1992; Fankem et al. 2008). Beneficial free-living rhizobacteria, which have been shown to improve plant health or increase yield, are usually referred to as plant growth-promoting rhizobacteria (PGPR) (Khalid et al. 2004; Jalili et al. 2009).

A collection of PGPR strains including *Azotobacter*, *Acetobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas*, and *Burkholderia* have been identified (Kloepper et al. 1980, 1988, 1989). Their activities including enhanced nutrients mobility, phytohormone production, controlling pathogens and stress alleviation can enhance plant growth (Glick et al. 2007; Jalili et al. 2009; Abbas-Zadeh et al. 2010). For example, bacterial potential of phosphate solubilization and hence increased P availability in the soil solution for plant growth is considered to be an important attribute of plant growth promoting rhizobacteria (PGPR) (Afzal et al. 2005).

Such bacteria are able to enhance soil P solubilization through production of organic acids such as carboxylic, which reduces soil pH (Puente et al. 2004; Rodriguez et al. 2006; Fankem et al. 2008). Numerous investigations have documented the presence of phosphate solubilizing bacteria (PSB) and fungi in the rhizosphere of different crops (Ghosh et al. 2003). In the recent decades, increasing evidence indicates that besides increased nutrient uptake, the synthesis and export of phytohormones by microorganisms may also play an important role in plant growth promotion (Glick et al. 1994; Jalili et al. 2009).

Ethylene level in the plant can be used as the indicator of plant performance under different conditions. The synthesis of ethylene in plants is directly related to the concentration of 1-aminocyclopropane-1-carboxylic acid (ACC) (Li et al. 2000). It should also be mentioned that under stress the amount of ethylene produced by the plants increases (Glick et al. 1998; Jalili et al. 2009). Some specific microorganisms including PGPR produce an enzyme, called ACC deaminase, hydrolyzing ACC into ammonia and α-ketobutyrate (Glick et al. 1994, 1995). Hence, such bacteria are a sink for ACC, which has been stated to be very beneficial to the plant growth and increased yield under different conditions.

Using PGPR with the ability to solubilize phosphate (Abbas-Zadeh et al. 2010) can modify the amounts of P fertilizer necessary for crop production. It is also very important to determine as to which rate of bacterial inoculation results in the optimum rates of P fertilizer. This is affected by different parameters including soil, plant, climate, and bacterial species. Hence, the combined use of PGPR and P fertilization may indicate the appropriate rates at which the optimum yield is produced.

As previously mentioned, it has been indicated as to how and at what level chemical P can contribute to enhanced wheat grain yield. With regard to the significance of biological fertilization and the related benefits, especially when combined with P fertilization, this research was performed. There is little data related to PGPR characterization and their effects on plant growth, in the arid and semi-arid areas of the world under field conditions (Naiman et al. 2009). Hence, the objectives were to determine: (1) the most important PGPR activities affecting wheat nutrients uptake, growth and yield, and (2) the modifying effects of different PGPR strains on P fertilization under greenhouse and field conditions.

Materials and methods

Selected soil, its characteristics and the fertilizer use

The soil was a fine-loamy sand, mixed (calcareous) mesic xeric torriortents collected and sieved (using a 4-mm sieve) from the 0 to 30 cm depth of the uncultivated field of the Agricultural Research Center of Torgh, Mashhad, Iran. Soil physical and chemical properties are presented in Table 1 (Miransari et al. 2007, 2008). In pot experiments, N and K fertilizers were applied at 40 and 18 mg/kg soil in the form of urea, and potassium sulfate, respectively. All the K and half of the N were mixed with the soil at the time of sowing, while the remaining N was applied in a soluble form at the tillering stage. Pots measured 50 × 75 cm. In the field experiment N and K fertilizers were applied at 350 and 100 kg/ha in the form of urea, and potassium sulfate, respectively.

Table 1 Soil physical and chemical properties

pH	EC (dS m^{-1})	TNV (%)	OC (%)	Sand (%)	Silt (%)	Clay (%)	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
7.9	0.9	17	0.28	32	51	17	0.025	7.2	148	9.84	2.48	0.32	0.82

Microorganisms and inoculum preparation

The bacterial strains were obtained from the microbial bank of Soil Biology Division, Soil and Water Research Institute, Tehran, Iran. Perlite was used as the carrier for the inoculants (Khavazi et al. 2007). The populations of 1.3×10^9, 1.25×10^9, 1.2×10^9, 1.01×10^9, per gram of inoculum were used for the strains of P.f.153, P.f.169, P.p.4 and P.p.108, respectively (Glick et al. 2007; Jalili et al. 2009).

Inoculation method

The wheat seeds (cv. Mahdavi) were surface sterilized by dipping in a 95% ethanol solution for 5 min and in a 0.2% $HgCl_2$ solution for 3 min, and then washed thoroughly with sterilized water (Jacobson et al. 1994). For the inoculation treatments, the seeds were dressed using the inoculated peat, mixed with 10 ml of a 40% gum Arabic solution. For the uninoculated control, the seeds were coated with the sterilized (autoclaved) peat treated with sterilized broth and 10 ml of a 40% gum Arabic solution.

Plant growth promoting activities of the tested *Pseudomonas* strains

The plant growth promoting activities of the tested *Pseudomonas* strains including the production of ACC deaminase (Penrose and Glick 2003; Jalili et al. 2009), auxin and auxin-like products (Benizri et al. 1998; Jalili et al. 2009), and siderophore (Alexander and Zuberer 1991; Abbas-Zadeh et al. 2010) as well as P solubilization (Jeon et al. 2003; Abbas-Zadeh et al. 2010) were determined.

Growing conditions

Sixteen inoculated wheat seeds were sown in each pot containing 25 kg/pot sandy clay loam soil. After germination, seedlings were thinned to 12 in each pot. The pots were arranged in a greenhouse with ambient light and temperature according to a completely randomized design. The pots were irrigated with good quality water. The average night and daily temperatures were 20 and 30°C, respectively. The daily period and light intensity ranged from 12 to 14 h and 12,000 to 14,000 lux, using natrium and helium lights, respectively. Plants were harvested at physiological maturity. Data regarding the root elongation, root weight, number of tillers, 1,000-grain weight, plus straw and grain yields were collected at harvesting. The dry weights were determined by using an oven at 70°C for 72 h. The N, P, and K contents in the wheat grain and straw were determined in the laboratory of Soil and Water Research Department, Mashhad, Iran using the standard methods (Miransari et al. 2009a, b).

A factorial field experiment using a randomized complete block design was also conducted during 2007–2008 to study the ability of the selected *Pseudomonas* strains to increase the growth and yield of wheat under different levels of P fertilization (0, 25, 50 kg P_2O_5). The seeds were inoculated as described above. After drying overnight, inoculated and uninoculated (treated with sterilized peat) seeds were sown in the field, keeping a row to row and plant to plant distance of 50 cm with a plot size of 2.5 m × 10 m. The treatments were replicated three times using a randomized complete block design. K fertilizer was applied at 70 kg/ha as potassium sulfate to all the plots at the time of sowing. Additionally, N fertilizer was applied at 150 kg/ha in the form of urea in three stages (1/3 at sowing, another 1/3 at tillering and the remaining at booting). Canal water was used to irrigate the field.

At physiological maturity, 3 months after seed germination (Calderini et al. 2000), 10 plants per treatment were harvested and data regarding the plant height, number of tillers/m, number of grains/spike, 1,000-grain weight, plus straw and grain yields were recorded. The grain and straw samples were oven dried at 70°C and ground in a Wiley grinding mill. N, P, and K contents in the wheat grain and straw were determined in the laboratory of Soil and Water Research Institute, Mashhad, Iran.

Statistical analysis

The collected data were statistically analyzed using a factorial design in the case of the pot experiment and a randomized complete block design for the field experiment. The means were compared using the least significant difference (LSD) test. All the statistical tests were performed at $p = 0.05$, using the software MSTAT-C (Freed 1988).

Results

The ACC-deaminase activity of the PGPR strains varied from 2.305 to 5.03 mmol α-ketobutyrate/g protein/h. Three strains, *P. fluorescens* 169, *P. putida* 4, and *P. putida* 108 indicated ACC-deaminase activity. All the strains also produced IAA. The three rhizobacterial isolates were also able to solubilize tricalcium phosphate (Table 2).

Pot experiment

The results revealed that all the tested strains significantly increased root elongation at different P fertilization levels when compared with the uninoculated control, where *P. putida* 108 was the most efficient strain (86% increase),

Table 2 Production of ACC-deaminase enzyme, IAA and IAA-like products, phosphate solubilization and siderophore production by different bacterial strains

Parameter	Strains			
	P.p4	P.p108	P.f.169	P.f.153
ACC deaminase	2.305	5.030	3.508	–
IAA-like products (mg/l)	9.6	8.9	5.8	–
Solubilized P (mg/l)	38.75	57.32	53.50	–
Siderophore	+	+	+	+
IAA	+	+	+	+

ACC-deaminase activity determined based on the production of micromole α-keto butyrate/mg protein/h

followed by *P. putida* 4 and *P. fluorescens* 169 over the uninoculated control. The effect of inoculation with PGPR containing ACC-deaminase was more obvious on the root elongation, as all the strains, which significantly increased root elongation compared with the uninoculated control (Table 3).

Although different *Pseudomonas* strains significantly increased plant height at the second and third level of fertilization, however, the maximum increase (19%) was resulted by *P. putida* 108, at the first level of fertilization relative to the control treatment. The number of tillers/pot was also significantly increased after inoculation with *P. putida* 4, relative to the uninoculated control. Inoculation with the *Pseudomonas* strains also significantly increased the 1,000-grain weight, except for *P. fluorescens* 153, and the grain yield, as compared with the uninoculated control. Similarly, inoculation with *P. putida* 108 resulted in the maximum increase (59%) in grain yield compared with the other strains. A similar result was also observed for the straw yield, where inoculation with *P. putida* 108 resulted in the maximum increase (46%).

Field experiment

All the strains, researched in the pot experiment were also tested in the field with regard to their efficacy to increase wheat growth and yield. The results indicated that all the strains significantly increased the plant height as compared with the uninoculated control, with the maximum increase by *P. Putida* 108 (36% at the second level of P). Inoculation with all four *Pseudomonas* isolates also significantly increased the number of tillers/plant, where *P. Putida* 108 was the most promising strain, relative to the uninoculated control (Table 4). The data regarding the number of grains/spike revealed that only two strains, *P. putida* 108 and *P. fluorescens* 169, significantly increased the number of grains/spike.

Inoculation with all the *Pseudomonas* isolates, except for *P. fluorescens* 153, significantly increased the 1,000-grain weight as compared with the uninoculated control, where the maximum increase was recorded with *P. putida* 108 relative to the uninoculated control (21% increase at

Table 3 Effects of different *Pseudomonas* strains and P fertilization on root elongation and weight, tillers/plant, 1,000-grain weight and grain and straw yield of wheat in the pot experiment (average of three replicates)

P fertilization	PGPR	Root elongation (cm)	Height (cm)	Tillers/plant	1,000-grain weight (g)	Grain yield (g/pot)	Straw yield (g/pot)
0	Uninoculated control	325.9 h	49.67 j	2.00 c	33.67 i	12.99 g	16.49 g
0	*P. fluorescens* 153	404.3 g	54.33 hi	2.27 abc	38.00 def	18.79 def	22.27 bcdef
0	*P. fluorescens* 169	485.4 f	51.87 ij	2.17 bc	34.00 hi	14.19 fg	17.69 fg
0	*P. putida* 4	596.3 bcd	57.67 ef	2.27 abc	37.00 efg	19.88 cde	23.38 bcde
0	*P. putida* 108	607 bc	59.33 cde	2.33 abc	38.67 cde	20.62 cde	24.12 bcd
50%	Uninoculated control	582.7 cde	54.00 hi	2.33 abc	35.00 ghi	15.23 fg	18.71 efg
50%	*P. fluorescens* 153	584.5 cde	56.33 fgh	2.73 abc	38.33 cde	18.48 def	21.98 bcdef
50%	*P. fluorescens* 169	601.2 bcd	54.77 gh	2.17 bc	36.00 fgh	16.71 efg	20.20 defg
50%	*P. putida* 4	605.4 bc	63.07 b	2.57 abc	40.33 bc	22.19 bcd	25.69 abc
50%	*P. putida* 108	610.5 abc	57.90 ef	2.50 abc	39.67 bcd	21.96 bcd	25.45 abc
100%	Uninoculated control	575.2 de	57.13 efg	2.57 abc	36.67 efg	18.19 def	21.70 cdef
100%	*P. fluorescens* 153	562.5 e	61.80 bc	3.10 ab	40.33 bc	23.38 abc	26.88 ab
100%	*P. fluorescens* 169	622.2 ab	58.23 def	2.33 abc	36.67 efg	18.68 def	22.18 bcdef
100%	*P. putida* 4	598.5 bcd	66.00 a	3.23 a	44.33 a	26.73 a	30.21 a
100%	*P. putida* 108	635.8 a	60.67 bcd	3.17 ab	41.00 b	25.94 ab	29.42 a

Means, followed by different letters in the same column are not statistically different according to Duncan's multivariate test ($p < 0.05$)

Table 4 Effects of different *Pseudomonas* strains and P fertilization on plant height, tillers/m^2, number of grains/spike, 1,000-grain weight, grain and straw yield of wheat in the field experiment

P fertilization	PGPR	Spike length	Height (cm)	Tillers/plant	1,000-grain weight (g)	Grain yield (kg/ha)	Straw yield (kg/ha)
0	Uninoculated control	6.5 i	52.0 h	3.8 d	31.33 e	3,300 e	5,433 f
0	*P. fluorescens* 153	7.0 hi	53.40 h	4.2 cd	36.40 bcd	3,467 e	5,600 f
0	*P. fluorescens* 169	8.2 cdef	58.1 g	5.2 b	36.60 bcd	4,033 b	6,167 ef
0	*P. putida* 4	8.5 bcd	58.1 g	5.4 b	34.63 d	4,133 bc	6,200 ef
0	*P. putida* 108	8.7 bc	59.53 efg	5.1 bc	35.50 cd	4,533 bc	6,133 ef
50%	Uninoculated control	7.5 gh	58.3 fg	5 bc	35.97 cd	4,233 de	6,267 ef
50%	*P. fluorescens* 153	7.7 efg	59.4 efg	4.8 bc	39.67 b	4,767 cd	6,367 ef
50%	*P. fluorescens* 169	8.3 bcde	62.8 cdef	6.5 a	39.87 b	5,967 b	7,567 cd
50%	*P. putida* 4	8.6 bc	63.4 cde	6.6 a	39.50 b	5,700 bc	7,933 bc
50%	*P. putida* 108	8.8 bc	64.90 bcd	6.8 a	37.93 bcd	5,667 bc	7,000 cde
100%	Uninoculated control	7.6 fgh	60.4 defg	5.3 b	38.47 bc	4,800 cd	6,933 de
100%	*P. fluorescens* 153	7.9 defg	66.50 cdefg	5.4 b	38.80 bc	5,567 bc	8,700 b
100%	*P. fluorescens* 169	8.8 ab	66.3 abc	6.6 a	44.97 a	7,500 a	10,570 a
100%	*P. putida* 4	8.8 ab	67.80 ab	6.6 a	46.70 a	7,300 a	10,130 a
100%	*P. putida* 108	9.4 a	70.10 a	6.9 a	46.60 a	7,567 a	10,870 a

Means, followed by different letters in the same column are not statistically different according to Duncan's multivariate test ($p < 0.05$)

the highest level of P). Inoculation with all PGPR strains, apart from *P. fluorescens* 153, significantly increased the grain yield when compared with the uninoculated control (58% increase at the highest level of P, Table 4), where the most effective isolate was *P. putida* 108, compared with the uninoculated control, followed by *P. fluorescens* 169. Similarly, inoculation with all the strains, except *for P. fluorescens* 153, significantly increased the straw yield as compared with the uninoculated control. Similar to the other parameters, the maximum increase in straw yield resulted from inoculation with *P. putida* 108 relative to the uninoculated control (57% increase at the highest level of P).

Nutrients uptake

The effects of inoculation with PGPR on the nutrient uptake in the pot and field experiments are summarized in Fig. 1. In the pot experiment inoculation with *Pseudomonas* strains (except *P. fluorescens* 153) significantly increased wheat N uptake, where *P. putida* 108 inoculation resulted in the highest increase, compared with the uninoculated control, followed by *P. fluorescens* 169. Among the four isolates, three significantly increased P uptake, where *P. putida* 108 caused the maximum increase, compared with the uninoculated control. The data also indicated that inoculation with all the *Pseudomonas* strains significantly increased K uptake relative to the uninoculated control. Inoculation with *P. putida* 108 increased K uptake at the highest, compared with the uninoculated control.

As shown by the results in the field experiment, inoculation with all the strains, except *P. fluorescens* 153, significantly increased N uptake compared with the uninoculated control, where *P. putida* 108 was the most

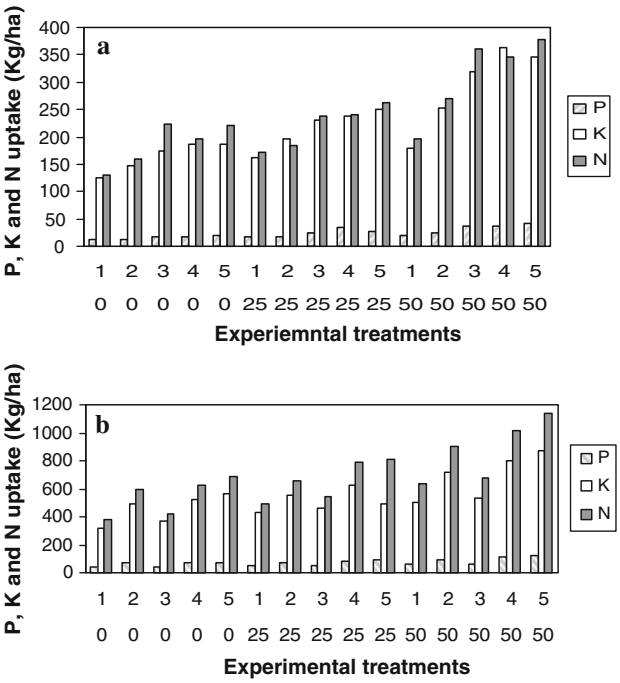

Fig. 1 Effects of different experimental treatments including bacterial strains (1: control, 2: *P. fluorescens* 153, 3: *P. fluorescens* 169, 4: *P. putida* 4 and 5: *P. putida* 108) and P fertilization (control 25 and 50 kg/ha) on wheat nutrient uptake (averaged for the three replications) under **a** field and **b** greenhouse conditions

effective. Inoculation with the three strains of *P. fluorescens* 169, *P. putida* 4, and *P. putida* 108 significantly increased wheat P uptake, and the maximum increase was related to *P. putida* 108 relative to the uninoculated control. The three strains of *P. fluorescens* 169, *P. putida* 4, and *P. putida* 108 significantly increased K uptake as compared with the uninoculated control, where *P. putida* 108 similarly resulted in the highest increase (Fig. 1).

Discussion

All the *Pseudomonas* strains isolated on the basis of ACC-deaminase activity had a notably positive effect on the wheat root growth in the pot experiment, and significantly increased root elongation as compared with the uninoculated control. This effect may have been due to a decrease in ethylene synthesis in the inoculated roots (Glick et al. 2007). According to the results, the three strains of *P. fluorescens* 169, *P. putida* 4, and *P. putida* 108 performed more efficiently, with the strain *P. putida* 108 being the most efficient one, relative to the strain *P. fluorescens* 153. According to Table 2 the latter strain just has the ability to produce siderophore and IAA. It did neither produce ACC deaminase and IAA-like products, nor solubilized P.

P. putida 108 produced the highest amount of ACC deaminase and solubilized P. This is very interesting indicating the great effects of ACC deaminase on plant growth as also indicated by the other researchers. For example they have already reported that root-increased growth was through the activity of ACC deaminase produced by the PGPR used to inoculate seed and/or root (Glick et al. 1994, 1995; Mayak et al. 1999; Arshad et al. 2007; Jalili et al. 2009). Yadegari et al. (2008) found that *Azospirillum* sp. is able to increase bean (*Phaseolus vulgaris* L.) yield when co-inoculated with *Rhizobium phaseoli*. The adverse effects of ethylene on plant growth have been previously elucidated. It has also been indicated that ACC deaminase is able to alleviate such unfavorable effects by changing the chemical structure of the prerequisite ACC necessary for ethylene production.

Also according to Table 2 the effective strains were able to solubilize insoluble P, which is another great enhancing effect of the *Pseudomonas* strains. The favorable effects of plant hormones such as IAA and siderophore (through enhancing the availability of different nutrients) on plant growth have been previously indicated. These all indicate that the higher the plant growth promoting activities of rhizobacteria the higher the chance of increased plant growth under different conditions. Hence, isolation and characterization of strains with the higher plant growth promoting abilities, adapted under certain climates, can be very useful for the production of effective biofertilizers (Glick et al. 1994, 1995; Mayak et al. 1999; Jalili et al. 2009; Abbas-Zadeh et al. 2010).

It is also interesting to mention that different scientists have specified the great effects of P on root growth and development, especially when it became available by microorganisms such as arbuscular mycorrhiza (Miransari et al. 2007, 2008, 2009a, b; Miransari 2010a, b). This indicates that in addition to the inhibitory effects of ACC deaminase on the ethylene activity in the plant roots, the enhanced availability of P by *Pseudomonas* strains through solubilizing insoluble P products is also a very important process enhancing plant growth and yield.

In the case of field experiments, inoculation with *Pseudomonas* strains also enhanced wheat growth and yield and *P. putida* 108 was identified as the most effective contributor. It is highly probable that the greater effectiveness of this strain may be related to its high auxin production and P solubilization, and its ACC-deaminase activity, as identified the most effective in the pot experiment. Other factors not determined in this study such as the ability of the strains to inhibit pathogens, etc., may also have been important in determining the ability of the strains to survive and increase the wheat growth under the field conditions, as compared with the pot experiments. The production of antifungal metabolites by fluorescent pseudomonads has also been previously reported to suppress soil borne fungal pathogens (Glick et al. 1994, 1995; Mayak et al. 1999; Jalili et al. 2009).

P. fluorescens 153 did not prove as effective as the other strains, in both the pot and field experiments, possibly because of no ACC-deaminase activity, and P solubilization. This suggests that ACC-deaminase activity is a good tool for the efficient selection of promising PGPR; nonetheless, other growth-promoting attributes of PGPR should also be considered for the selection/screening of effective PGPR strains. Thus, strains with a good combination of these traits (ACC-deaminase activity, auxin production, root colonization, P solubilization, chitinase activity, siderophore production, and antibiotic production) are likely to perform better as inocula for biofertilizer formulations.

Accordingly, it may be stated that the significance of this research work with regard to the experimental objectives is that the use of the most efficient *Pseudomonas* strains with their above-mentioned abilities can be very effective to enhance the efficiency of soil production. However, it does not mean that P fertilizer must not be used for crop production, as according to the results the highest efficiency of P fertilizer is achieved when it is used at 50% in combination with *Pseudomonas* inoculum. The addition of P fertilizer to the soil results in its accumulation in the soil, and hence its combination with methods, which biologically provide P is of great agricultural and biological significance.

Conclusion

The results indicate that different *Pseudomonas* strains differ in their ability to enhance plant growth and yield, and the strains with the higher PGPR activities (for example *P. putida* 108) are more effective on the growth and yield of wheat. It is also interesting to mention that although using the tested *Pseudomonas* strains as bioinoculants can be a good method of providing the adequate amount of P for crop production, however, the amount of crop production is optimum when such bioinoculants are used in combination with P fertilization. In some cases the highest *Pseudomonas* activity was observed at the control level of P fertilization and with increasing the level of P fertilization such activities decreased. These results verified the experimental suggested objectives. *P. putida* 108 is among the most efficient strains that can be efficiently used for bioinoculant production. It should also be mentioned that there are very little data regarding such effects.

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