

# The effect of PGPR strain on wheat yield and quality parameters

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## Abstract

N<sub>2</sub>-fixing and P-solubilizing bacteria are important in plant nutrition increasing N and P uptake by the plants, and playing a significant role as plant growth-promoting rhizobacteria (PGPR) in the biofertilization of crops. In 2007 and 2008, a field study was conducted in two different locations (Erzurum and Ispir) in the Eastern part of Turkey for investigating the effects of two N<sub>2</sub>-fixing and P-solubilizing PGPR strains alone and in combinations on plant yield and nutrient content of wheat in comparison to control and optimum and half doses of N fertilizer application under field condition. All inoculations and fertilizer applications significantly increased grain, total biomass yields, and macro and micro nutrients of wheat over the control. Mixed PGPR inoculations with the strain of OSU-142 + M-13 + *Azospirillum* sp.245 has significantly increased grain yield of wheat as good as full doses of nitrogen. All bacterial inoculations especially mixed inoculation, significantly increased uptake of macro-nutrients (N, P, K, Ca, Mg and S) and micro-nutrients (Fe, Mn, Zn, and Cu) of grain, leaf, and straw part of the plant. The data suggested that seed inoculation with OSU-142 + M-13 + *Azospirillum* sp.245 may substitute N and P fertilizers in wheat production.

## Key words

Inoculation; grain yield, plant growth-promoting rhizobacteria; macro and micro element

## Introduction

Nitrogen and phosphorus are known to be essential nutrients for plant growth and development. Intensive farming practices that achieve high yield require chemical fertilizers, which are not only costly but may also create environmental problems. The extensive use of chemical fertilizers in agriculture is currently under debate due to environmental concern and fear for consumer health. Consequently, there has recently been a growing level of interest in environmental friendly sustainable agricultural practices. Bio-fertilizer is defined as a substance which contains living organisms which, when applied to seed, plant surface, or soil, colonize the rhizosphere or the interior of plant the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Vessey 2003). Biofertilizers are well recognized as an important component of integrated plant nutrient management for sustainable agriculture and hold a great promise to improve crop yield (Narula *et al.* 2005; Wu *et al.* 2005). A group of bio-fertilizers contain termed plant growth promoting rhizobacteria (PGPR) (Kloepper *et al.* 1980) and among them are strains from genera such as *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Rhizobium*, *Erwinia* and *Flavobacterium* (Rodriguez and Fraga 1999). Thus organisms are important for agriculture in order to promote the circulation of plant nutrients and reduce the need from chemical fertilizers. Most of the studies reporting beneficial effects of the above mentioned PGPR were carried out in warm and subtropical climates with favorable ambient temperatures. These bacteria may not be effective in cold temperature conditions. Therefore, a study was conducted in order to investigate the effects of alone and in combinations with N<sub>2</sub>-fixing and P-solubilising PGPR strains on nodulation, plant growth, nutrient uptake and grain yield of wheat in the cold highland (Erzurum) and low land ( Ispir) plateaus.

## Material and methods

### Site selection

In order to investigate the effects of seed inoculation with PGPR on yield and yield components of wheat (*Triticum aestivum* spp. *vulgare* var. Kirik) in the field experiments at two sites, 150 km apart from each other. The first (I) field is located in the Coruh valley in Erzurum in eastern Anatolia, 40° 28' N and 40° 58' E with at an altitude of 1120 m, and the second field is in the Erzurum Experimental Farm of the Atatürk University and in Erzurum in Eastern Anatolia, 29° 55' N and 41° 16' E with an altitude of 1950 m. The soils were classified as Entisol and Aridisols according to the USDA taxonomy (Soil Survey Staff 1992).

### Field experiment

The experiments were conducted using a randomized complete block design in a factorial arrangement each having 10 main treatments as control (without inoculation and any fertilizer application), Nitrogen (80 kg N/ha), Nitrogen (40 kg N/ha), *Bacillus* OSU-142, *Bacillus* M-3, *Azospirillum* sp. 245, Mixed (OSU-142 + M-13+ *Azospirillum* sp. 245), *Bacillus megaterium* RC07, *Paenibacillus polymyxa* RC05 and *Raoultella terrigena* for 2007 and 7 treatment also added in 2008, FS Tur, OSU142 AMP Res, M-3 Amp Res, sp.245 Amp Res, *P. polymyxa* 2/2, *B. megaterium* T17, and Mixed + 40 kg N/ha. Wheat was sown in 7 m x 4 m plots having 34 rows so as to give 18 kg seeds da<sup>-1</sup> (430 seeds per m<sup>2</sup>) on 10 and 17 May in 2007, 4 and 2 May in 2008 at site I and site II. Maximum care has been taken not to contaminate and mix bacterial inoculations during sowing.

### Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SPSS 13.0 (SPSS Inc., 2004) statistical program. Since “year x treatment” interaction was not significant in many parameters evaluated, data were combined over years, and means were presented. Mean values were separated according to LSD test at  $P=0.05$ .

## Result and Discussion

### Yield and yield parameters

Bacterial inoculations improved the wheat growth and growth parameters. The performance of the plants was better in inoculated treatments in comparison to the control. The results showed that grain yield (GY), straw yield (SY), harvest index (HI) and total yield (TY) of wheat cultivars in each of two locations in both years significantly increased by N<sub>2</sub>-fixing and P-solubilizing PGPR strains application compared with the control. The lowest GY, SY, TY and HI were recorded in the control treatment and the bacterial inoculations increased GY by 8.6-43.7%, SY by 1.82-12.9%, TY by 1.93- 19.80% and HI by 1.33-45.7% over the control on 2-year average at two locations, respectively (Table 1, 2). The GY of plant in 2007 was higher than GY of 2008, but the highest TY of wheat was found in 2008 at each location (Table 1, 2). The highest GY (3.68-2.88 Mg/ha), SY (9.73-8.57 Mg/ha), and TY (13.41-11.45 Mg/ha) in both years average at two locations were obtained from 80 kg N/ha, and followed by in combination (OSU-142 + M-13+ *Azospirillum* sp. 245) treatment at 3.47-2.87 Mg/ha for GY, 8.73-6.75 Mg/ha for straw and 12.20-9.65 Mg/ha for total yields, respectively. In other words, mixed PGPR inoculations with the strain of OSU-142 + M-13 + *Azospirillum* sp.245 has significantly increased GY, SY, TY and HI of wheat as good as full doses of nitrogen. When it is compared to 40 kg N/ha treatment, bio-fertilizer applicants were more effective to increase the grain yield. On the other hand harvest index (HI) of two years average in two locations was better in inoculation treatments in comparison to the control and mineral fertilizer (80 and 40 kg N/ha) treatments. While the lowest HI values were recorded in the control treatment, the highest value was obtained from the OSU-142 + M-13+ *Azospirillum* sp. 245 and *Bacillus* M-3 treatments. In the current system, the results support reduced fertilizer rates down to 50% if PGPR was added because that is the minimum at which results were consistent. This is different from the observations of Canbolat *et al.* (2006) and Elkoca *et al.* (2008), 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.

### Effects of bio-fertilizer on plant nutrient element (PNE) contents of different parts of the plant

N<sub>2</sub>-fixing and P-solubilizing PGPR strains application promoted PNE contents of different parts of the plant. Although the highest leaf, grain, and straw N contents were obtained from mixed inoculation with the OSU-142 + M-13 + *Azospirillum* sp.245 +40 kg N/ha, which increased N contents of leaf, grain and straw of plant by 52.6%, 83.4%, and 83.0%, respectively, compared with the control treatment. While S contents were obtained from treatment OSU-142 + M-13 + *Azospirillum* sp.245 and increasing rate were 64.9% for grain and 65.0% for straw. P, K, Mn, Fe, and Zn contents were obtained from *Bacillus* M-3 treatment, and increase rate for leaf, grain and straw of plant were 42.7%, 50.6%, 82.5% for P, 26.3%, 112.0%, 110.0% for K, 49.1%, 121.2%, 120.0% for Mn, 90.1%, 102.6%, 105.2% for Fe, and 49.2%, 75.4%, 75.4% for Zn. Some of the previous studies with the same PGPR strains tested on chickpea, barley, raspberry, apricot and sweet cherry have been reported similar findings confirming our data in the present work. The use of the OSU-142 and M-3 in chickpea (Elkoca *et al.* 2008), barley (Cakmakci *et al.* 2007), raspberry (Orhan *et al.* 2006), apricot (Esitken *et al.* 2003), sweet cherry (Esitken *et al.* 2006) and strawberry (Güneş *et al.* 2009) stimulated macro- and micro-nutrient uptake such as N, P, K, Ca, Mg, Fe, Mn, Zn, Cu.

**Table 1. Yield and yield components of wheat plant growth at two locations in 2007 (t/ha)**

Inoculant	I. Field			II. Field		
	Grain	Straw	Total biomass	Grain	Straw	Total biomass
Control	3.34 d	6.57 e	9.90 d	2.46 d	8.90 c	11.35 c
Nitrogen (80 kg N/ha)	4.02 a	8.82a	12.83 a	3.34 a	10.66a	14.00 a
Nitrogen ( 40 kg N/ha)	3.79 a-c	7.87b	11.66 ab	2.27 ab	11.07a	13.33 ab
<i>Bacillus</i> OSU-142	3.69 a-d	7.47 bc	11.15 bc	3.02 a-c	9.04 b	12.06 bc
<i>Bacillus</i> M-3	3.50 b-d	6.69e	10.18 d	2.85 c	7.98 d	10.83 c
<i>Azospirillum</i> sp.245	3.76 a-c	8.07a	11.83 a	3.04 a-c	9.18 b	12.22 bc
OSU-142 + M3+ Az.245	3.85 ab	8.04a	11.89 a	3.10 a-c	9.41 b	12.51a-c
<i>B. megaterium</i> RC07	3.43 cd	7.39 cd	10.83 c	2.87 c	8.26 c	11.13 c
<i>P. polymyxa</i> RC05	3.72 a-c	7.52 bc	11.24 bc	2.93 bc	8.69 c	11.61 bc
<i>B. licheniformis</i> RC08	3.46 cd	6.65e	10.11 d	2.72 d	8.56 c	11.28 c

**Table 2. Yield and yield components of wheat plant growth of two locations in 2008 (t/ha)**

Inoculants	I. Field			II. Field		
	Grain	Straw	Total biomass	Grain	Straw	Total biomass
Control (without inoculation and fertilizer)	2.79 c	7.79d	10.58 c	1.20 g	7.11b	8.31abc
Nitrogen (80 kg N/ha)	4.18 a	8.90 a	13.08a	1.95 a	8.51a	10.46 a
Nitrogen ( 40 kg N/ha)	3.57 a-c	7.46d	11.03bc	1.58 cd	8.25a	9.83 ab
<i>Bacillus</i> OSU-142	3.35 a-c	8.37 a-c	11.72 a-c	1.53 cde	6.18bc	7.72 bcd
<i>Bacillus</i> M-3	3.65 a-c	7.03d	10.68 c	1.50 cde	5.51 d	7.01 cd
<i>Azospirillum</i> sp.245	4.17 a	7.78d	11.95 a-c	1.39 ef	4.70 e	6.09 d
OSU-142 + M-13+ <i>Azospirillum</i> sp.245	3.94 ab	8.68 ab	12.62ab	1.80 b	4.83 e	6.63 cd
<i>Bacillus megaterium</i> RC07	3.28 a-c	8.41 bc	11.69a-c	1.66 bc	6.65 bc	8.31 abcd
<i>Paenibacillus polymyxa</i> RC05	3.81a-c	8.02 bc	11.83a-c	1.39 ef	6.39 bc	7.77 bcd
<i>Raoultella terrigena</i>	3.59 a-c	7.37d	10.96bc	1.39 ef	5.71 d	7.09 cd
FS Tur	2.99 bc	7.47d	10.46 c	1.59 c	7.35b	8.94 abc
OSU142 AMP Res	3.67 a-c	8.23 a-c	11.90a-c	1.49 cde	6.27bc	7.77 bcd
M-3 Amp Res	3.54 a-c	8.19 a-c	11.73 a-c	1.53 cde	6.53 bc	8.06bcd
Sp.245 Amp Res	3.20 a-c	8.52 a-c	11.72 a-c	1.42 def	8.49a	9.90 ab
<i>P. polymyxa</i> 2/2	3.20 a-c	8.92ab	12.12 a-c	1.39 ef	6.25bc	7.64 bcd
<i>B. megaterium</i> T17	3.41 a-c	8.42 a-c	11.83 a-c	1.30 fg	6.63bc	7.93 bcd
OSU-142 + M-13 + <i>Azospirillum</i> sp.245 +40 kg N/ha	3.83 a-c	8.92a	12.75a	1.66 bc	4.47 e	6.12 d

## Conclusions

Our results indicated that microbial inoculation of seeds with N<sub>2</sub>-fixing and P-solubilizing PGPR strains alone and in combination, may substitute costly NP fertilizer in wheat production even in cold highland and low land areas. In view of environmental pollution in case of excessive use of fertilizers and due to high costs in the production of N and P fertilizers, bacteria tested in our study may well be suited alone or in combination to achieve sustainable and ecological agricultural production in the region. An important nutritional problem of developing countries is micro-nutrient malnutrition, also called hidden hunger. Our results also indicated that alone or in combination inoculations with N<sub>2</sub>-fixing and P-solubilising PGPR strains could increase mineral concentration and accumulations in the grain. This paper supports the view that inoculations with PGPR have some potential to serve as a means to reduce hidden hunger through enhanced mineral concentration and accumulation in grain.

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## References

- Cakmakci R, Donmez MF, Erdogan U (2007) The effect of plant growth promoting rhizobacteria on barley seedling growth, nutrient uptake, some soil properties, and bacterial counts. *Turkish Journal of Agriculture and Forestry* **31**, 189-199.
- 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
- Dobbelaere S, Vanderleyden J, Okon Y (2003) Plant growth-promoting effects of diazotrophs in the rhizosphere. *Crit. Rev. Plant Sci.* **22**, 107–149.
- Elkoca E, Kantar F, Sahin F (2008) Influence of Nitrogen Fixing and Phosphorus Solubilizing Bacteria on the Nodulation, Plant Growth, and Yield of Chickpea. *Journal of Plant Nutrition* **31**, 157–171.
- Esitken A, Karlidag H, Ercisli S, Turan M, Sahin F (2003) The effect of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot (*Prunus armeniaca* L. cv. Hacihaliloglu). *Australian Journal of Agricultural Research* **54**, 377-380.
- Esitken A, Pirlak L, Turan M, Sahin F (2006) Effects of floral and foliar application of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrition of sweet cherry. *Scientia Horticulturae* **110**, 324-327.
- Güneş A, Ataoğlu N, Turan M, Eşitken A, Ketterings QM (2009) Effects of phosphate-solubilizing microorganisms on strawberry yield and nutrient concentrations. *Journal of Plant Nutrition and Soil Science* **172**, 385–392
- Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* **286**,885-886.
- Narula N, Kumar V, Singh B, Bhatia R, Lakshminarayana K (2005) Impact of biofertilizers on grain yield in spring wheat under varying fertility conditions and wheat-cotton rotation. *Archives of Agronomy and Soil Science* **51**, 79-89.
- Orhan E, Esitken A, Ercisli S, Turan M, Sahin F (2006) Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. *Scientia Horticulturae* **111**, 38-43.
- Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances* **17**,319-339.
- Soil Survey Staff (1992) Keys to Soil Taxonomy. 5<sup>th</sup> edition. SMSS Technical monograph No:19 Blacksburg Pocahontas Press Inc.
- SPSS Inc. (2004) SPSS Inc. SPSS® 13.0 Base User's Guide, Prentice Hall.
- 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.
- Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil* **255**,571-586.