# The effect of Plant Growth Promoting Rhizobacter strain on wheat yield and quality parameters

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**Abstract**

$N_2$-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 $N_2$-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 + M3 + *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 + M3 + *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 rhizsphere 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 called plant growth promoting rhizobacteria (PGPR) (Kloepper *et al.* 1980) contain strains from genera such as *Pseudomonas*, *Azospirillium*, *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 $N_2$-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 ( İspir) plateaus.

## Material and methods

*Plant Material*

Wheat *(Triticum aestivum spp. vulgare var.* Kirik*)*, described as a local bread wheat cultivar commonly grown in Erzurum province conditions, alternative- habit wheat genotype, suitable for rainfall or unirrigated conditions, white and 36 g 1000-seed weight, tall (115 cm), susceptible to lodging, cold, and stripe rust.

*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 Agricultural Research and Experimental Farm at the Atatürk University, Erzurum in Eastern Anatolia region of Turkey, 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 $ha^{-1}$), Nitrogen (40 kg N $ha^{-1}$), *Bacillus* OSU-142, B*acillus* M-3, *Azospirillum* sp. 245, Mixed (OSU-142 + M3+ *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 $ha^{-1}$. Wheat was sown in 7 m x 4 m plots having 34 rows so as to give 18 kg seeds $da^{-1}$ (430 seeds per $m^2$) on 10 and 17 May in 2007, 4 and 2 May in 2008 at site I and site II. Maximum care has been takennot to contaminate and mix bacterial inoculations during sowing. Soils samples (0-30 cm) were collected prior to the experiment sites in 2007 and 2008 (20 soil samples per plot) to determine baseline soil properties.Soil samples were air-dried, crushed, and passed through a 2-mm sieve prior to chemical analysis. Cation exchange capacity (CEC) was determined using sodium acetate (buffered at pH 8.2) and ammonium acetate (buffered at pH 7.0),. the Kjeldahl method was used to determine organic N while plant-available P was determined by using the sodium bicarbonate method),. electrical conductivity (EC) was measured in saturation extracts, soil pH was determined in 1:2 extracts, soil organic matter was determined using the Smith-Weldon method, exchangeable cations was determined ammonium acetate buffered at pH 7 and micro elements in the soils were determined by Diethylene Triamine Pentaacetic Acid (DTPA) extraction methods (AOAC 2005). Some soil physical and chemical properties are given in Table 1. Spring wheat was irrigated twice at site I and site II at the beginning of stem elongation and booting stage. Weeding was done by hand when required. No pesticide and/or herbicide were applied. Harvesting was performed excluding side rows and 1 m from each end of plots on 12 and 18th of September for 2007, and on 14 and 25th of September for 2008. Plants were cut by hand, approximately 5 cm above ground level to measure grain and stubble yields.

*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 $N_2$-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 2, 3). 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 2, 3). The highest GY (3.68-2.88 Mg $ha^{-1}$), SY (9.73-8.57 Mg $ha^{-1}$), and TY (13.41-11.45 Mg $ha^{-1}$) in both years average at two locations were obtained from 80 kg N $ha^{-1}$ , and followed by in combination (OSU-142 + M3+ *Azospirillum* sp. 245) treatment at 3.47-2.87 Mg $ha^{-1}$ for GY, 8.73-6.75 Mg $ha^{-1}$ for straw and 12.20-9.65 Mg $ha^{-1}$ for total yields, respectively. In other words, mixed PGPR inoculations with the strain of OSU-142 + M3 + *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 $ha^{-1}$ 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 $ha^{-1}$) treatments. While the lowest HI values were recorded in the control treatment, the highest value was obtained from the OSU-142 + M3+ *Azospirillum* sp. 245 and B*acillus* M3 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*

$N_2$-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 + M3 + *Azospirillum* sp.245 +40 kg N $ha^{-1}$, 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 + M3 + *Azospirillum* sp.245 and increasing rate were 64.9% for grain and 65.0% for straw. The highest P, K, Mn, Fe, and Zn contents were obtained from B*acillus* 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 (Table 4, 5, and 6). 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 M3 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.

**Conclusions**

Our results indicated that microbial inoculation of seeds with $N_2$-fixing and P-solubilising PGPR strains alone and in combination, may substitute costly N fertilizer in wheat production even in cold highland and low land areas and provide plant P requirement from soil P exchangeable and moderately available form reservoir via increasing the P solubility. 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 $N_2$-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 trough enhanced mineral concentration and accumulation in grain.

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Table 1.Some physical and chemical properties of soils in the experimental fields

| | 2007 | | 2008 | |
|---|---|---|---|---|
| **Soil properties** | Site 1 | Site 2 | Site 1 | Site 2 |
| pH (1:2,5 s/w) | 7.1 | 6.9 | 7.40 | 7.3 |
| Organic Matter % | 2.2 | 1.8 | 1.55 | 2.8 |
| $CaCO_3$, % | 0.6 | 0.4 | 0.3 | 0.7 |
| P, kg $P_2O_5$ $da^{-1}$ | 3.0 | 3.4 | 3.7 | 4.12 |
| K, $cmol_c$ $kg^{-1}$ | 2.1 | 1.8 | 2.1 | 2.0 |
| Ca, $cmol_c$ $kg^{-1}$ | 13.0 | 12.2 | 13.0 | 18.4 |
| Mg, $cmol_c$ $kg^{-1}$ | 4.0 | 4.9 | 4.0 | 5.2 |

| | | | | |
|---|---|---|---|---|
| Na, $cmol_c$ $kg^{-1}$ | 0.2 | 0.3 | 0.2 | 0.4 |
| CEC, $cmol_c$ $kg^{-1}$ | 25.3 | 24.9 | 21.3 | 30.1 |
| N, % | 0.025 | 0.012 | 0.02 | 0.019 |
| $NH_4$, mg $kg^{-1}$ | 17.0 | 8.3 | 11.0 | 31.0 |
| $NO_3$, mg $kg^{-1}$ | 18.0 | 7.6 | 21.0 | 35.0 |
| Fe, mg $kg^{-1}$ | 3.4 | 4.1 | 2.9 | 4.3 |
| Zn, mg $kg^{-1}$ | 0.5 | 1.1 | 0.3 | 1.4 |
| Cu, mg $kg^{-1}$ | 0.9 | 1.0 | 0.4 | 1.3 |
| Mn, mg $kg^{-1}$ | 6.8 | 7.1 | 4.6 | 7.0 |
| Total salt,% | 0.10 | 0.15 | 0.10 | 0.12 |
| Sand, % | 38.0 | 40.0 | 31.0 | 36.0 |
| Silt, % | 37.0 | 35.0 | 42.0 | 38.0 |
| Clay, % | 24.0 | 25.0 | 27.0 | 26.0 |

Table 2. Yield and yield components of wheat plant growth at two locations in 2007 (t $ha^{-1}$)

| 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 $ha^{-1}$) | 4.02 a | 8.82a | 12.83 a | 3.34 a | 10.66a | 14.00 a |
| Nitrogen ( 40 kg N $ha^{-1}$) | 3.79 a-c | 7.87b | 11.66 ab | 2.27 ab | 11.07a | 13.33 ab |
| *Bacillus* OSU-142 | 3.69 a-d | 7.47 bc | 11.15 bc | 3.02 a-c | 9.04 b | 12.06 bc |
| B*acillus* M3 | 3.50 b-d | 6.69e | 10.18 d | 2.85 c | 7.98 d | 10.83 c |
| *Azospirillum* 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 |
| *B. megaterium* RC07 | 3.43 cd | 7.39 cd | 10.83 c | 2.87 c | 8.26 c | 11.13 c |
| *P. polymyxa* RC05 | 3.72 a-c | 7.52 bc | 11.24 bc | 2.93 bc | 8.69 c | 11.61 bc |
| *B. licheniformis RC08* | 3.46 cd | 6.65e | 10.11 d | 2.72 d | 8.56 c | 11.28 c |

Table 3. Yield and yield components of wheat plant growth of two locations in 2008 (t $ha^{-1}$)

| 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 $ha^{-1}$) | 4.18 a | 8.90 a | 13.08a | 1.95 a | 8.51a | 10.46 a |
| Nitrogen ( 40 kg N $ha^{-1}$) | 3.57 a-c | 7.46d | 11.03bc | 1.58 cd | 8.25a | 9.83 ab |
| *Bacillus* OSU-142 | 3.35 a-c | 8.37 a-c | 11.72 a-c | 1.53 cde | 6.18bc | 7.72 bcd |
| *Bacillus* M3 | 3.65 a-c | 7.03d | 10.68 c | 1.50 cde | 5.51 d | 7.01 cd |
| *Azospirillum* sp.245 | 4.17 a | 7.78d | 11.95 a-c | 1.39 ef | 4.70 e | 6.09 d |
| OSU-142 + M3+ *Azospirillum* sp.245 | 3.94 ab | 8.68 ab | 12.62ab | 1.80 b | 4.83 e | 6.63 cd |
| *Bacillus megaterium RC07* | 3.28 a-c | 8.41 bc | 11.69a-c | 1.66 bc | 6.65 bc | 8.31 abcd |
| *Paenibacillus polymyxa RC05* | 3.81a-c | 8.02 bc | 11.83a-c | 1.39 ef | 6.39 bc | 7.77 bcd |
| *Raoultella terrigena* | 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 |
| M3 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 |
| *P. polymyxa* 2/2 | 3.20 a-c | 8.92ab | 12.12 a-c | 1.39 ef | 6.25bc | 7.64 bcd |
| *B. megaterium* T17 | 3.41 a-c | 8.42 a-c | 11.83 a-c | 1.30 fg | 6.63bc | 7.93 bcd |

| | | | | | | |
|---|---|---|---|---|---|---|
| OSU-142 + M3 + *Azospirillum* sp.245 +40 kg N $ha^{-1}$ | 3.83 a-c | 8.92a | 12.75a | 1.66 bc | 4.47 e | 6.12 d |