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Rhizobacteria for reduced fertilizer inputs in wheat (*Triticum aestivum* spp. *vulgare*) and barley (*Hordeum vulgare*) on Aridisols in Turkey

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Abstract

The present study assessed the effect of seed inoculation with single or multiple plant growth-promoting rhizobacterial (PGPR) strains on yield in spring wheat (*Triticum aestivum* spp. *vulgare* var. Kırık) and barley (*Hordeum vulgare* cv. Tokak) plants in both greenhouse and field conditions during the years 2007 and 2008. The treatments of wheat and barley plants during the first year included: (1) Control (no inoculation and no fertilizer), (2) *Bacillus* OSU-142 (*B.*OSU-142), (3) *Bacillus megaterium* M3 (*B. megaterium* M3), (4) *Azospirillum brasilense* Sp.245 (*A. brasilense* Sp.245), (5) Mixed 1 (*B.*OSU-142 + *Bacillus* M3 + *Azospirillum* AB-245), (6) *Bacillus megaterium* RC07 (*B. megaterium* RC07), (7) *Paenibacillus polymyxa* RC05, (8) *Bacillus licheniformis* RC08, (9) mineral nitrogen N_1 (80 kg N ha^{-1}) and (10) N_2 (40 kg N ha^{-1} in the form of urea). In the second year treatments were: (1) *Raoutella terrigena* (*R. Terrigena*), (2) *Burkholderia cepacia* FS Tur (*B. cepacia* FS Tur), (3) *B.* OSU-142 ARM, (4) *B.* M3 ARM, (5) *A.* sp.245 ARM, (6) *P. polymyxa* RC14, (7) *B. megaterium* RC10, (8) Mixed 2 (*Bacillus* OSU-142+ *Bacillus* M3+ *Azospirillum brasilense* sp.245 + 40 kg N ha) in addition to the first year treatments. Greenhouse and the two years of field trials at two sites showed that seed inoculation with bacterial strains significantly affected yield, yield components and quality parameters both in spring wheat and barley. In greenhouse trials, single inoculations of seeds with PGPRs gave root and shoot weight increases by 11.0-16.5% and 14.4-30.4% in wheat and by 10.3-18.8% and 11.9-21.5% in barley. Combinations of three bacteria increased root and shoots weight by 18.3-31.5% in wheat and by 21.4-23.8 in barley and bacterial inoculations also increased grain yield by 4.3-18.5% in wheat and 8.3-19.1% in barley, respectively. In field

conditions wheat grain yields were increased by 25.6-40.4%, 17.4-25.2% and 31.4% while barley seed yield were increased by 16.2-33.7%, 4.2-14.4 and 16.8% with N fertilizer, single and combinations of PGPR bacteria inoculations compared to control. Plant-growth responses were variable and depended on the inoculants strain, plant species and growth parameters evaluated. In conclusion, seed inoculations with bacteria especially *B.* OSU-142, *A. brasilense* sp.245 and combinations of bacteria may satisfy nitrogen requirements of wheat and barley under green house and field conditions even in lowland and upland areas. The present results indicate that the selected bacterial isolates and multiple combinations did promote the growth and quality of wheat and barley in ways that could be harnessed to practical benefit for the farmer and consistent with sustainable and/or organic agricultural practices in Turkey.

Keywords: Aridisol; Biofertilizers; Plant growth-promoting bacteria (PGPR).

Introduction

Nitrogen and phosphorus fertilizers are essential components of modern agriculture, because they provide essential nutrients to plants. However, it is well known that much of the nutritional value of applied fertilizers is lost in agricultural soils through immobilisation, volatilisation and, in particular leaching when compared to controlled closed systems. Intensive agriculture entails the risk of excessive fertilization. Large quantities of chemical fertilizers are used to replenish soil N and P, resulting in high costs and severe environmental contamination. Conventional crop production methods have been linked to negative effects on the environment, human health and safety and long-term soil fertility.

Bio-fertilizers, including microorganisms may add nitrogen to the soil by symbiotic or asymbiotic N_2 fixation. On a worldwide basis it is estimated that about 175 million tonnes of nitrogen per year is added to soils through biological nitrogen fixation. Meanwhile super-phosphate fertilizers are expensive and in short supply, but bio-fertilizers can bridge the gap. There are several microorganisms which can also solubilize cheaper sources of phosphorus, such as rock phosphate. Bacteria such as Bacillus are widely used in plant production systems and are important phosphorus-solubilizing microorganisms, resulting in improved growth and yield of crops (Dobereiner, 1997)

Plant associated N_2-fixing and P-solubilizing bacteria have been considered as one of the possible alternatives to inorganic fertilizer for

promoting plant growth and yield. In general, beneficial free-living bacteria are usually referred to as plant-growth-promoting rhizobacteria (PGPR), which can affect plant growth directly or indirectly. One potential way is inoculation with PGPR to decrease negative environmental impacts resulting from continued use of chemical fertilizers. Apart from fixing N_2, PGPR can affect plant growth directly by the synthesis of phytohormones (auxins, cytokinins, gibberellins) and vitamins (Güneş et al., 2014), inhibition of plant ethylene synthesis, enhanced stress resistance and improved nutrient uptake, solubilization of inorganic phosphate and mineralization of organic phosphate. Indirectly, diazotrophs are able to decrease or prevent the deleterious effects of pathogenic microorganisms (Zahir et al., 2004; Bashan and de Bashan, 2005; Antoun and Prevost, 2006; Podile and Kishore, 2006).

Trials with rhizosphere-associated plant growth promoting N_2-fixing and P-solubilising *Bacillus* species indicated yield increases in many crops such as wheat (Rodriguez et al., 1996; Ozturk et al., 2003; Turan et al., 2012) barley (Cakmakci et al., 2001; Ozturk et al., 2003) sugar beet (Cakmakci et al., 2001), canola (de Freitas et al., 1997) and maize (Pal, 1998). Because of their spore-forming ability, plant growth promoting *Bacillus* strains are readily adaptable to commercial formulation and field application (Liu and Sinclair, 1993).

Wheat is one of the major cereal crops both in Turkey and the rest of world. It is grown both as a spring and winter crop; however, winter crops are more extensively grown than spring. Wheat and barley are two of the most important crops worldwide and sustainable agricultural systems for these crops are urgently required. Therefore, the sustainability of wheat and barley production systems based on high use of applied fertilizers needs to be reviewed in the light of their impact on the environment and use of non-renewable resources. For this purpose, the evaluation of supplementary or alternative nutrient sources and approaches for improving nutrient uptake efficiency is a main aim of wheat production systems.

Experimental and field application of rhizobacteria has resulted in significant promotion of plant development, as observed in terms of emergence, vigour, biomass, development of root systems, nutrient uptake, plant height and root length in early growth stages and increased yield of different cereal crops including wheat (Çakmakçı et al., 2007a; Sahin et al., 2004), barley (Çakmakçı et al., 2001; Sahin et al., 2004) and the others (Rosas et al., 2009).

Nitrogen fixing and Phosphorus-solubilizing rhizobacteria are common in soils throughout the world and many research groups have isolated rhizobacteria strains from the wheat rhizosphere that are suspected to have plant growth promotion effects. Although the use of plant growth promoting bacteria has been advocated for a number of decades, the effective application of PGPR in cultivation of non-legumes is still very limited and remains an innovative crop management practice.

This study is a part of an international project with partners in Europe (UHOH, EWL, ENITIAA, KUL), UK (DAL), Brazil (Embrapa), Israel (AGRON), Chile (UACh) and Turkey (YUDGB), whose main goal is to develop an improved understanding of specific rhizobacteria, already identified and studied to some extent by the partners, with good potential as plant growth promoting "biofertilizers" (Spaepen, 2008; Baudoin et al., 2010; Gofer et al., 2011; Fan et al., 2012; Turan et al., 2012). RHIBAC (Rhizobacter) aims to reduce the high inputs of N and P chemical fertilizers commonly used in arable cultivation. The premise of RHIBAC is that wheat inoculates containing PGPR improved for enhanced plant growth promotion will enable reductions in inputs of chemical fertilisers, while maintaining yields and/or protein content of grain at levels acceptable for the relevant markets. Therefore PGPR can contribute to improving both local and global environments and reducing dependence on non-renewable resources, while still permitting cereal growers to supply grain with quality and price acceptable to consumers. PGPR inoculation can also help consolidate the competitiveness of the organic cereals sector, by boosting yield and protein content. Although the project focuses on wheat, several of the PGPR also interact with other cereals, including maize and barley. Also, RHIBAC includes the following aims: identification and modulation mechanisms of plant growth promotion in PGPR associated with wheat rhizosphere, including biological nitrogen fixation, auxin excretion and N and P mobilisation and obtaining reproducible, significant plant growth promotion in greenhouse studies and field trials in Europe, Israel, Turkey and South America, including larger scale demonstrations.

Materials and Methods

Greenhouse experiments

Two sets of trials were conducted to investigate the effects of plant growth promoting rhizobacteria alone and in combinations of three strains

on a spring wheat cultivar (*Triticum aestivum* spp. *vulgare* cv. Kırık) and barley (*Hordeum vulgare* cv. Tokak) growth and yields under greenhouse conditions. The treatments included: (1) Control (no inoculation and no fertilizer), (2) *Bacillus* OSU-142, (3) *Bacillus* M3, (4) *Azospirillum brasilense* sp.245 (AB-245), (5) OSU-142 + M3 + AB-245, (6) *Bacillus megaterium* RC07, (7) *Paenibacillus polymyxa* RC05, (8) *Bacillus licheniformis* RC08, (9) mineral nitrogen N_1 (80 kg N ha^{-1}) and (10) N_2 (40 kg N ha^{-1}). Pots were arranged on a bench in the greenhouse according to a randomized complete block design with four blocks (i.e. each block contained all 10 treatments × 2 harvests and totalling 80 pots per plant (10×2×4=80). The experiment was repeated twice. Wheat and barley seeds were surface-sterilised in 70% (v/v) ethanol for 2 min and rinsed ten-times in sterile tap water. For this application, pure cultures that were obtained from the culture collection of the Department of Genetics and Bioengineering, Faculty of Engineering and Architecture at Yeditepe University Istanbul, Turkey were grown in nutrient broth (NB) at 28 °C and diluted to a final concentration of 10^9 colony-forming units (cfu) ml^{-1} in sterile distilled water containing 0.025% (v/v) Tween-20. Surface-sterilised seeds were inoculated by immersion in the appropriate PGPR suspension (at 10^8 cfu ml^{-1}) for 2 h on a rotary shaker at 81 rpm, air-dried and sown immediately. For the greenhouse and field experiments, the cell densities in the PGPR suspensions were adjusted to a final density of approximately 10^8 cfu $seed^{-1}$. Pots were sterilised with 20% (v/v) sodium hypochlorite solution and filled with a loamy soil with an organic matter content of 1.9% (w/w), a pH of 7.1, an available Olsen-P content of 15.2 mg kg^{-1} and NH_4^+-N and NO_3^--N contents of 10.2 and 9.3 mg kg^{-1}, respectively. Cation exchange capacity and exchangeable K, Ca and Mg of soil were 26.2, 1.8, 15.1 and 4.3 cmol kg^{-1}, respectively. Available Fe, Mn, Zn and Cu contents were 4.3, 3.1, 2.6 and 1.5 mg kg^{-1}. Seeds were placed at the same depth (approximately 2.5 cm below the soil surface) in all pots. Per pot, 24 seeds were sown at twelve points (two seeds at each point) with the same distance apart and then thinned to twelve uniform plants per pot 10 d after sowing. The wheat and barley seedlings were grown in a greenhouse under a 15 h natural light photoperiod at 16-25 °C and 55% relative humidity. Pots were watered up to 60% of their maximum water-holding capacity and were maintained at this moisture content by watering to plant every 2-3 d. Sterile water was slowly added over the topsoil in each pot. Plants were harvested on day-30 (first sampling set I) and day-120 (second sampling set II), after emergence of the seedlings and separated into shoots and roots.

Field experiments (2007 and 2008)

First year field experiments were conducted with the same treatments as used in greenhouse trials. In order to investigate the effects of seed inoculation with PGPR on yield and yield components of spring wheat (*Triticum aestivum* spp. *vulgare* cv. Kirik) and barley (*Hordeum vulgare* cv. Tokak) field trials at two sites, 150 km apart from each other were conducted. Field I: Located in Coruh valley in Erzurum in eastern Anatolia, coordinates 40° 28' N and 40° 58' E with at an altitude of 1120 m and Field II at Atatürk University Experimantal farm located in Erzurum center in Eastern Anatolia, coordinates 29° 55' N and 41° 16' E with an altitude of 1950 m. The soils have been classified as Aridisols according to the USDA taxonomy (Soil Survey Staff, 2006). Average temperature and total rainfall were 10.2 and 5.3 °C and 622 and 473 mm in site I and site II, respectively: Plant growth in the region is restricted to the period between April-May and October. In the first year, the experimental design consisted of four randomized blocks each having 10 main treatments as (1) control (without inoculation and any fertilizer treatment), (2) N_1 (80 kg N ha^{-1}) (3) N_2 (40 kg N ha^{-1} in the form of urea), (4) *Bacillus* OSU-142, (5) B*acillus* M3, (6) *Azospirillum brasilense* sp.245, (7) (*Bacillus* OSU-142 + *Bacillus* M3+ *Azospirillum brasilense* sp.245), (8) *Bacillus megaterium* RC07, (9) *Bacillus polymyxa* RC05 and (10) *Bacillus licheniformis* RC08. In the second year of the experiment, 17 main treatments were used, including: (1) Control (no bacteria inoculation and no fertilizer), (2) N_1 (80 kg N ha^{-1}) (3) N_2 (40 kg N ha^{-1}), (4) *Bacillus* OSU-142, (5) *Bacillus* M3, (6) *Azospirillum brasilense* Sp.245, (7) *Bacillus megaterium* RC07, (8) *Bacillus polymyxa* RC05, (9) *Raoultella terrigena*, (10) *Burkholderia cepacia* FS Tur, (11) OSU-142 ARM (Ampicillin Resistant mutant), (12) M3 ARM, (13) *Azospirillum brasilense* Sp.245 ARM, (14) *Paenibacillus polymyxa* RC14, (15) *Bacillus megaterium* RC10, (16) combination of 1 (*Bacillus* OSU-142+ *Bacillus* M3+ *Azospirillum brasilense* sp.245) and (17) *Bacillus* OSU-142+ *Bacillus* M3+ *Azospirillum brasilense* sp.245 + 40 kg N ha^{-1}) used for wheat and barley.

Soil physical and chemical properties of experiment areas are given in Table 1. At first site, the soil was deeply ploughed in autumn and left until disk and rotary harrowing in spring. At the second site, the soil was deeply ploughed after cereal harvest (winter wheat) and left until disk- and rotary harrowing in spring. Nitrogen at the rate of 80 and 40 kg N ha^{-1} in a urea

form (%46) was applied during soil preparation in spring. In both years, wheat and barley were sown in 7 m × 4 m plots having 34 rows so as to respectively give 180 and 220 kg seeds ha^{-1} (430 and 350 seeds per m^2) on 10 and 17 and 4 and 2 May in 2007 and 2008 at site I and site II. Maximum care was taken not to contaminate and mix bacterial inoculations during sowing. The bacterial strains were kept in nutrient broth with 15% glycerol at –80 °C for long-term storage. To prepare inocula, a single colony was transferred to 500 mL flasks containing National Botanical Research Institute's phosphate (NBRIP) and grown aerobically on a rotating shaker (Merck, Darmstadt, Germany) for 48 h at 28 ± 2 °C and 150 rpm and diluted to a final concentration of 10^8 cells mL^{-1} using sterile distilled water containing 0.025% Tween 20. Seeds were sterilized in 70% ethanol for 2 min and in 1.2% sodium hypochlorite for 10 min and rinsed ten times with sterile distilled water. Seeds were then treated with the bacterial suspensions at a concentration of 10^8 CFU mL^{-1} for 30 min, under sterile conditions. Spring wheat and barley plants were irrigated twice at site I and three times at site II, at the beginning of stem elongation and booting stage in both years. 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 wheat, on 3 and 13th of September for barley at site I and site II. Second year harvesting was done on the 14 and 25th of September at site I and site II in both wheat and barley. Plants were cut by hand and approximately 5 cm above ground level to measure grain and stubble yields.

Soil Analysis

Before the experiment, soils were sampled from the fields and air-dried, crushed and passed through a 2-mm sieve prior to chemical analysis. Cation exchange capacity (CEC), total N, plant-available P, electrical conductivity (EC), soil pH, calcium carbonate, soil organic matter, exchangeable cations and micro elements in the soils were determined according to AOAC (2005).

Plant Analysis

Samples were oven-dried at 68 ^oC for 48 h and ground to pass 1 mm. The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N (Bremner, 1996).

Table 1. Physical and chemical properties of the soils used in the field experiments at site I and II in 2007-8.

Soil properties	Site I			Site II		
	2007	2008		2007	2008	
	Wheat-barley	Wheat	Barley	Wheat-barley	Wheat	Barley
Sand (%)	38.1	30.4	33.5	39.6	35.7	37.9
Silt (%)	37.4	32.8	38.1	35.1	38.4	40.9
Clay (%)	24.5	36.8	28.4	25.3	25.9	21.2
pH (1:2.5 W/V H_2O)	7.1	7.5	7.4	6.9	7.3	7.1
Organic matter (%)	2.2	1.7	2.9	1.8	2.8	2.2
NH_4^+–N (mg kg^{-1})	17	16	34	8.3	31	22
NO_3^-–N (mg kg^{-1})	18	20	37	7.6	35	28
Available P (mg kg^{-1})	15	5.8	6.1	11.7	4.12	3.4
$CaCO_3$ (%)	0.6	1.8	1.7	0.4	0.7	0.4
K, me/100 g	2.1	1.9	2.4	1.8	2	2.3
Ca, me/100 g	13	18	16	12.2	18.4	14
Mg, me/100 g	4	4.3	3.2	4.9	5.2	5.0
Na me/100 g	0.3	0.4	0.6	0.3	0.4	0.2
Fe, ppm	3.4	2.4	3.2	4.1	4.3	3.8
Zn, ppm	0.5	1.1	1.2	1.1	1.4	0.7
Cu ppm	0.9	0.8	1.5	1.0	1.3	1.2
Mn, ppm	6.8	5	8.5	7.1	7.0	8.0

Statistical analysis

The experiment was performed in a completely randomized design with four replicates. The greenhouse experiments were repeated twice. Field trials were conducted in field conditions at two sites in 2007 and 2008. Two years averaged data were first evaluated by a two-way ANOVA (SPSS 13.0 software, SPSS Inc., 2004) with a linear model component for treatment and time and treatment by time interactions were analyzed. When annual data were pooled, the "year × treatment interaction" term was insignificant for most of the evaluated parameters. The group means were compared by the LSD option at $P \leq 5\%$.

Results and Discussion

Greenhouse experiments

Greenhouse conditions showed that fertilizer and seed inoculation with bacterial strains significantly affected plant growth both in wheat and barley. Seed inoculation with PGPR gave root weight increases by 11.0-16.5% in wheat and by 10.3-18.8% in barley (Table 2). Single inoculations with PGPR increased also wheat and barley shoot weight by 11.9-30.4% depending on the species while mineral fertilizers N_1 (80

kg N ha^{-1}) and N_2 (40 kg N ha^{-1}) gave weight increases by 16.2-37.0% compared to control. Mixed1 treatment (*A. brasilense* sp.245 + *B.* OSU-142 + *B.* M3) gave increases in shoot weight by 31.5% in wheat and by 23.8% in barley over control. Nitrogen fertilizer (40 and 80 kg N ha^{-1}) resulted in the highest root and shoot weight in both species, followed by the combination of three bacteria, *A. brasilense* sp.245, *B.* OSU-142 and *B.* M3 alone inoculation while the lowest weight was obtained in control pots (Table 2).

Table 2. Yield and quality component of wheat and barley under greenhouse conditions in response to fertilizer applications and seed inoculations with growth-promoting rhizobacteria (Values are the average from two separate experiments harvested on day-120).

Treatment	RW** (g plant^{-1})	SW g plant^{-1}	GWPS (g)	GYP (g pot^{-1})	Grain N (%)	Straw N (%)	HI**
			Wheat				
Control*	0.109^{b***}	0.181^{c}	0.66^{d}	9.2^{d}	1.52^{d}	0.32^{c}	26.9
N_1	0.132^{a}	0.248^{a}	0.78^{ab}	12.5^{a}	1.88^{a}	0.49^{a}	25.7
N_2	0.128^{a}	0.242^{a}	0.74^{bc}	11.1^{bc}	1.70^{bc}	0.40^{a-c}	25.2
OSU-142	0.124^{a}	0.235^{a}	0.73^{c}	10.9^{bc}	1.65^{b-d}	0.37^{bc}	28.2
M3	0.121^{ab}	0.218^{b}	0.75^{bc}	10.4^{c}	1.63^{b-d}	0.35^{bc}	27.1
AB-245	0.127^{a}	0.229^{a}	0.76^{bc}	10.6^{bc}	1.78^{ab}	0.42^{a-c}	26.2
Mixed1	0.129^{a}	0.238^{a}	0.80^{a}	11.2^{b}	1.75^{a-c}	0.44^{ab}	27.5
RC07	0.123^{ab}	0.213^{b}	0.74^{c}	9.6^{d}	1.76^{a-c}	0.39^{a-c}	27.0
RC05	0.126^{a}	0.236^{a}	0.75^{bc}	10.5^{bc}	1.68^{bc}	0.42^{a-c}	27.5
RC08	0.122^{ab}	0.207^{b}	0.74^{c}	9.6^{d}	1.61^{cd}	0.37^{bc}	27.8
			Barley				
Control	0.224^{d}	0.413^{d}	0.70^{b}	8.4^{e}	2.01^{c}	0.65^{c}	32.9
N_1	0.276^{a}	0.527^{a}	0.84^{a}	11.7^{a}	2.40^{a}	0.86^{a}	29.3
N_2	0.265^{a-c}	0.480^{bc}	0.85^{a}	11.1^{b}	2.29^{ab}	0.81^{ab}	31.3
OSU-142	0.266^{a-c}	0.479^{bc}	0.79^{ab}	9.6^{cd}	2.35^{ab}	0.74^{a-c}	29.5
M3	0.248^{c}	0.462^{c}	0.77^{ab}	9.3^{d}	2.19^{b}	0.68^{bc}	30.7
AB-245	0.259^{a-c}	0.502^{ab}	0.83^{a}	10.0^{c}	2.31^{ab}	0.78^{a-c}	30.3
Mixed	0.272^{ab}	0.511^{ab}	0.82^{a}	10.6^{b}	2.34^{ab}	0.77^{a-c}	30.4
RC07	0.256^{a-c}	0.471^{c}	0.77^{ab}	9.4^{d}	2.25^{ab}	0.69^{bc}	30.3
RC05	0.251^{bc}	0.489^{bc}	0.79^{ab}	9.5^{cd}	2.27^{ab}	0.71^{a-c}	30.2
RC08	0.247^{c}	0.469^{c}	0.75^{ab}	9.1^{d}	2.20^{b}	0.63^{c}	28.9

* Control (without bacteria inoculation or mineral fertilizers); N_1 and N_2 fertilizer (80 and 40 kg N ha^{-1} in the form of ammonium nitrate), *Bacillus* OSU-142; *Bacillus* M3; *Azospirillum brasilense* Sp.245; *Bacillus megaterium* RC07; *Paenibacillus polymyxa* RC05; *Bacillus licheniformis* RC08; Mixed1 (*Bacillus* OSU-142+*Bacillus* M3 + *Azospirillum brasilense* sp.245).

** RW, root weight (g plant^{-1}); SW, shoot weight (g plant^{-1}), GWPS, grain weight per spike (g); GYP, grain yield per pot (g/pot); HI, harvest index.

*** Averages of the same column values (each section separately) followed by same letter did not differ significantly from Duncan's multiple range tests at 0.05% significance. Bacterial strains are explained in Table 2.

Seed inoculation of wheat with OSU-142, M3, AB-245, RC07, RC05 and RC08 alone increased grain yield by 18.5, 13.0, 15.2, 4.3, 14.1 and 4.3% as compared to the control, respectively (Table 2). Combination of three bacteria (*A. brasilense* sp.245 + *Bacillus* OSU-142 + *Bacillus* M3) inoculations increased wheat grain yield by 21.7% and alone nitrogen application 80 and 40 kg N ha^{-1} gave yield increases by 38.8% and 20.6% compared to control, respectively. Similarly, single inoculations with PGPR increased wheat grain N and straw N content by 5.9-17.1 and 9.4-31.2%, but 15.1-37.5%, 23.6-11.8% and 53.1-25.1% increase ratios were determined for Mixed1, N_1 and N_2 applications compared to control, respectively.

Among ten treatments, the maximum grain weight per spike in barley was obtained in Mixed1 treatment, followed by N_1 fertilizer, *A. brasilense* sp.245 and *Bacillus* M3, respectively. The maximum grain yield was obtained with N_1 fertilizer followed by inoculation with *A. brasilense* sp.245 and Mixed1.

Field experiments

In the first year of the field experiment, considering average of two sites, wheat grain yields were increased by 6.6-17.2%, 19.7% and 21.7-26.9% while barley seed yields were increased by 0.4-10.8%, 12.7% and 17.1-21.1% respectively with single inoculations with PGPR, combination of three bacteria and fertilizer applications over control treatment. In wheat and barley as an average of both sites the highest biomass yields were obtained in N_1 and N_2 plots over control followed by the combination of three bacterial strains (19.6%) and alone AB-245 bacterial inoculations. In 2007, on the average of both sites, PGPR inoculation gave biomass yield increases by 0.7-14.8% in wheat and 1.2-12.4% in barley (Table 3).

In the second year, considering the average of both sites, single inoculations with PGPR increased wheat grain yields by 14.6-34.2% depending on the species while N fertilizers gave yield increases by 30.0-55.4% compared to control (Table 4). Mixed1 gave grain yield increases by 44.2% over control as compared with 36.7% yield increases by Mixed2. On the average of both sites, among the bacterial inoculations, the

best treatment was the combination of all three bacteria strains, followed by mixed inoculation of three bacteria combination plus half of N fertilizer, *A. brasilense* sp.245, *B.* M3, *B.* OSU-142 ARM and *B. megaterium* RC07 in terms of seed yield in wheat (Table 4).

Table 3. Grain and total biomass yield of wheat and barley in response to fertilizer applications and seed inoculations with single and multiple plant growth promoting bacteria species at two sites in the field as an average of 2007.

Treatments	Site I		Site II		Average	
	Grain yield (t ha^{-1})	Total biomass yield (t ha^{-1})	Grain yield (t ha^{-1})	Total biomass yield (t ha^{-1})	Grain yield (t ha^{-1})	Total biomass yield (t ha^{-1})
			Wheat			
Control*	3.34^{d**}	9.90^{d}	2.46^{d}	11.35^{c}	2.90^{e}	10.63^{ef}
N_1	4.02^{a}	12.83^{a}	3.34^{a}	14.00^{a}	3.68^{a}	13.41^{a}
N_2	3.79^{a-c}	11.66^{ab}	3.27^{ab}	13.33^{ab}	3.53^{ab}	12.50^{b}
OSU-142	3.69^{a-d}	11.15^{bc}	3.02^{a-c}	12.06^{bc}	3.35^{bc}	11.60^{a-d}
M3	3.49^{b-d}	10.18^{d}	2.85^{c}	10.83^{c}	3.17^{cd}	10.51^{f}
AB-245	3.76^{a-c}	11.83^{a}	3.04^{a-c}	12.22^{bc}	3.40^{bc}	12.03^{bc}
Mixed1	3.85^{ab}	11.89^{a}	3.10^{a-c}	12.51^{a-c}	3.47^{ab}	12.20^{bc}
RC07	3.43^{cd}	10.83^{c}	2.87^{c}	11.13^{c}	3.15^{cd}	10.98^{d-f}
RC05	3.72^{a-c}	11.23^{bc}	2.92^{bc}	11.61^{bc}	3.32^{b-d}	11.42^{c-e}
RC08	3.46^{cd}	10.11^{d}	2.72^{d}	11.28^{c}	3.09^{e}	10.70^{ef}
			Barley			
Control	2.64^{b}	8.31^{c}	2.39^{e}	7.86^{d}	2.51^{e}	8.08^{e}
N_1	3.12^{a}	9.96^{a}	2.95^{a}	10.08^{a}	3.04^{a}	10.03^{a}
N_2	3.08^{ab}	9.28^{ab}	2.81^{ab}	9.48^{a}	2.94^{ab}	9.38^{b}
OSU-142	2.93^{ab}	8.88^{bc}	2.60^{b-d}	8.37^{b-d}	2.77^{b-d}	8.62^{cd}
M3	2.78^{ab}	8.61^{bc}	2.32^{de}	7.99^{cd}	2.55^{de}	8.30^{de}
AB-245	2.88^{ab}	9.29^{ab}	2.67^{bc}	8.75^{bc}	2.78^{b-d}	9.02^{bc}
Mixed1	2.94^{ab}	9.29^{ab}	2.71^{ab}	8.87^{ab}	2.83^{a-c}	9.08^{bc}
RC07	2.80^{ab}	8.60^{bc}	2.41^{c-e}	8.10^{cd}	2.61^{c-e}	8.35^{de}
RC05	2.94^{ab}	8.88^{bc}	2.58^{b-d}	8.36^{b-d}	2.76^{b-e}	8.62^{cd}
RC08	2.75^{ab}	8.35^{c}	2.29^{e}	8.00^{cd}	2.52^{e}	8.18^{de}

* Control (without bacteria inoculation or mineral fertilizers); N_1 and N_2 fertilizer (80 and 40 kg N ha^{-1} in the form of ammonium nitrate), *Bacillus* OSU-142; *Bacillus* M3; *Azospirillum brasilense* Sp.245; *Bacillus megaterium* RC07; *Paenibacillus polymyxa* RC05; *Bacillus licheniformis* RC08; Mixed (*Bacillus* OSU-142+*Bacillus* M3+*Azospirillum brasilense* sp.245).

** Averages of the same column values (each section separately) followed by different lower-case letters in a column were significantly different ($P<0.01$) using Duncan's multiple range tests.

Table 4. Grain and total biomass yield of wheat and barley in response to fertilizer applications and seed inoculations with single and multiple plant growth promoting bacteria species at two sites in the field as an average of 2008.

Treatments	Wheat				Barley			
	Site I		Site II		Site I		Site II	
	Grain yield (t ha^{-1})	Total biomass yield (t ha^{-1})	Grain yield (t ha^{-1})	Total biomass yield (t ha^{-1})	Grain yield (t ha^{-1})	Total biomass yield (t ha^{-1})	Grain yield (t ha^{-1})	Total biomass yield (t ha^{-1})
Control*	2.8^{c**}	10.6^{c}	2.40^{g}	10.4^{a-c}	2.8^{b}	9.0^{b}	1.2^{e}	4.9^{c}
N_1	4.2^{a}	13.1^{a}	3.89^{a}	13.1^{a}	3.4^{a}	10.5^{a}	2.6^{a}	7.0^{a}
N_2	3.6^{a-c}	11.0^{bc}	3.16^{cd}	12.3^{ab}	2.9^{ab}	9.5^{ab}	1.7^{bc}	5.0^{bc}
OSU-142	3.4^{a-c}	11.7^{a-c}	3.07^{c-e}	9.6^{b-d}	2.9^{ab}	9.3^{b}	1.9^{b}	6.5^{ab}
M3	3.7^{a-c}	10.7^{c}	3.00^{c-e}	8.8^{cd}	2.6^{ab}	9.5^{ab}	1.9^{b}	6.1^{a-c}
AB-245	4.2^{a}	11.9^{a-c}	2.77^{ef}	10.4^{a-d}	3.0^{ab}	9.6^{ab}	1.5^{de}	5.3^{bc}
RC07	3.3^{a-c}	11.7^{a-c}	3.31^{bc}	7.6^{d}	2.8^{b}	9.4^{ab}	1.4^{de}	6.2^{a-c}
RC05	3.8^{a-c}	11.8^{a-c}	2.77^{ef}	9.6^{b-d}	3.0^{ab}	9.8^{ab}	1.4^{de}	6.0^{a-c}
R. TER	3.6^{a-c}	11.0^{bc}	2.77^{ef}	8.9^{cd}	2.9^{ab}	9.2^{b}	1.4^{de}	5.3^{bc}
FS Tur	3.0^{bc}	10.5^{c}	3.18^{c}	11.2^{a-c}	2.9^{ab}	9.4^{ab}	1.4^{de}	5.5^{bc}
OSU 142 ARM	3.7^{a-c}	11.9^{a-c}	2.99^{c-e}	9.7^{b-d}	2.7^{b}	9.5^{ab}	1.4^{de}	5.4^{bc}
M3 ARM	3.5^{a-c}	11.7^{a-c}	3.05^{c-e}	10.1^{b-d}	3.1^{ab}	9.1^{b}	1.5^{cd}	6.1^{a-c}
AB245 ARM	3.2^{a-c}	11.7^{a-c}	2.83^{d-f}	12.4^{ab}	2.9^{ab}	9.3^{b}	1.4^{de}	5.5^{bc}
RC14	3.2^{a-c}	12.1^{a-c}	2.76^{ef}	8.8^{cd}	2.9^{ab}	9.5^{ab}	1.4^{de}	5.9^{a-c}
RC10	3.4^{a-c}	11.8^{a-c}	2.60^{fg}	8.7^{cd}	2.9^{ab}	10.0^{ab}	1.4^{de}	5.6^{a-c}
Mixed 1	3.9^{ab}	12.6^{ab}	3.60^{b}	9.9^{b-d}	2.9^{ab}	9.3^{b}	2.0^{b}	6.5^{ab}
Mixed2	3.8^{a-c}	12.8^{a}	3.31^{bc}	9.6^{b-d}	3.0^{ab}	10.1^{ab}	1.8^{bc}	6.4^{ab}

* Control (without bacteria inoculation or mineral fertilizers); N_1 and N_2 fertilizer (80 and 40 kg N ha^{-1} in the form of urea), *Bacillus* OSU-142; *Bacillus* M3; *Azospirillum brasilense* sp.245; *Bacillus megaterium* RC07; *Paenibacillus polymyxa* RC05; *Raoutella terrigena, Burkholderia cepacia* FS Tur, *Bacillus* OSU-142 ARM; *Bacillus* M3 ARM; *Azospirillum* sp.245 ARM, *Paenibacillus polymyxa* RC14 (2/2); *Bacillus megaterium* RC10 (T17), Mixed 1 (*Bacillus* OSU-142+ *Bacillus* M3+ *Azospirillum brasilense* sp.245), Mixed 2 (*Bacillus* OSU-142+ *Bacillus* M3+ *Azospirillum brasilense* sp.245 + 40 kg N ha).

** Averages of the same column values (each section separately) followed by same letter did not differ significantly from Duncan's multiple range tests at 1% significance.

According to the 2-year average, all bacteria inoculations and fertilizer applications significantly increased grain and straw N content of wheat at site I and site II over the control (Table 5). Considering the average of both wheat growing sites, the highest grain and straw N content of wheat were obtained in N_1 (80 kg N ha^{-1}), Mixed 2 (*B.* OSU-142+ *B.* M3+ *A. brasilense* sp.245 + 40 kg N ha) and OSU-142 plots representing increases over control of 39.7%, 33.5% and 32.1% followed by RC07 (29.5%) and AZB inoculations (28.2%). On the average of both sites, all treatments increased

wheat grain and straw N content when compared to control. Similarly, all treatments significantly increased grain N content of barley at site I and site II over the control (Table 5). Except for RC08, all bacteria strains increased straw N content at average of both sites in barley. At first site, N_1 application and seed inoculations with AB-245 bacteria and/or combination of three bacteria increased straw N content. However, there was numerical but not statistical difference among other treatments in terms of straw N contents at site I in barley. In barley as an average of both growing sites, the highest N content were obtained in N_1 and N_2 and/or OSU-142 plots representing increases over control of 39.4%, 34.5% and 29.8% followed by the co-inoculation of *A. brasilense* sp.245 with *B.* OSU-142 and *B.* M3 (28.8%), RC05 (26.0%) and AZB inoculations (25%). On the average of both sites, all treatments increased barley grain N content when compared with control (Table 5).

T trials conducted under greenhouse conditions showed that most of PGPR in the absence of any fertilizer application achieved increases in root and shoot weight. Yield components after PGPR treatments were equal to the nitrogen treatment at the rate of 40 and 80 kg N ha^{-1} in wheat and barley. Among the bacteria tested, *B.* OSU-142, AB-245 and combined inoculation consistently gave equal or higher grain yield than in N applied pots. This was more profound in wheat than in barley. Although differences between various bacterial strains on grain yield were insignificant, yet, all the bacteria inoculants significantly improved root and shoot weight. In a second greenhouse experiment, grain yield, grain N content of wheat and barley increased with all treatments compared with control. Two set trials in the greenhouse indicated that growth promotion effects were mostly seen in early plant development and these subsequently translated into higher yields. PGPR inoculation strongly affected the weight of root and shoot in wheat and barley during the early growth stages. Similar results were reported in some of the previous studies showing that bacteria inoculation was found to affect early plant and root development, plant and root dry weight, grain yield and the N-uptake efficiency of plants (Çakmakçı et al., 2006; Çakmakçı et al., 2007a). Spaepen et al. (2008) also demonstrated that inoculation with IAA producing *Azospirillum brasilense* sp245 bacteria leads to a stimulation in early plant development and a significant increase in dry weight yield of plants and roots, the total root surface and root hair formation, the number of ears and the N-uptake efficiency in wheat.

Table 5. Effect of mineral fertilizer and inoculation of wheat and barley with plant growth-promoting rhizobacteria at two sites in the field on grain and straw N content.

Treatment***	Site I		Site II		Average	
	Grain N (%)	Straw N (%)	Grain N (%)	Straw N (%)	Grain N (%)	Straw N (%)
			Wheat			
Control*	1.48^{d**}	0.26^{d}	1.24^{e}	0.22^{d}	1.36^{e}	0.24^{d}
N_1	1.97^{a}	0.52^{a}	1.82^{a}	0.44^{a}	1.90^{a}	0.48^{a}
N_2	1.85^{b}	0.44^{a-c}	1.54^{cd}	0.27^{cd}	1.70^{cd}	0.35^{bc}
OSU142	1.83^{bc}	0.36^{cd}	1.76^{ab}	0.32^{a-d}	1.80^{b}	0.34^{c}
M3	1.72^{c}	0.38^{c}	1.54^{d}	0.30^{b-d}	1.63^{cd}	0.34^{c}
AB-245	1.82^{bc}	0.46^{a-c}	1.65^{b-d}	0.42^{ab}	1.73^{bc}	0.44^{ab}
Mixed1	1.85^{b}	0.51^{ab}	1.55^{cd}	0.43^{ab}	1.70^{cd}	0.47^{a}
RC07	1.82^{bc}	0.40^{a-c}	1.69^{a-c}	0.33^{a-c}	1.75^{bc}	0.36^{bc}
RC05	1.78^{bc}	0.45^{a-c}	1.58^{cd}	0.37^{a-c}	1.68^{cd}	0.41^{a-c}
RC08	1.71^{c}	0.39^{bc}	1.51^{d}	0.33^{a-c}	1.61^{d}	0.36^{bc}
Mixed2	1.90^{a}	0.55^{a}	1.79^{a}	0.43^{a}	1.85^{ab}	0.49^{a}
R,TER	1.70^{c}	0.34^{cd}	1.33^{d}	0.32^{a-c}	1.52^{d}	0.33^{c}
FS Tur	1.14^{c}	0.31^{cd}	1.38^{d}	0.37^{a-c}	1.26^{e}	0.34^{c}
OSU142 ARM	1.79^{bc}	0.38^{c}	1.40^{d}	0.41^{ab}	1.60^{d}	0.40^{c}
M3 ARM	1.74^{c}	0.40^{bc}	1.45^{d}	0.36^{a-c}	1.60^{d}	0.38^{c}
RC14	1.72^{c}	0.39^{c}	1.37^{d}	0.30^{a-d}	1.55^{d}	0.35^{c}
RC10	1.74^{c}	0.40^{c}	1.34^{d}	0.33^{a-c}	1.54^{d}	0.37^{c}
			Barley			
Control	1.89^{c}	0.70^{c}	1.72^{d}	0.53^{f}	1.80^{d}	0.61^{f}
N_1	2.56^{a}	0.92^{a}	2.49^{a}	0.73^{a}	2.53^{a}	0.82^{a}
N_2	2.46^{ab}	0.84^{a-c}	2.24^{b}	0.66^{bc}	2.35^{b}	0.75^{bc}
OSU142	2.46^{ab}	0.81^{a-c}	2.23^{b}	0.66^{bc}	2.34^{b}	0.73^{b-d}
M3	2.37^{b}	0.76^{bc}	1.99^{c}	0.59^{de}	2.18^{c}	0.67^{de}
AB-245	2.35^{b}	0.86^{ab}	2.17^{bc}	0.71^{ab}	2.26^{bc}	0.78^{ab}
Mixed	2.43^{ab}	0.85^{ab}	2.25^{b}	0.70^{ab}	2.34^{b}	0.78^{ab}
RC07	2.41^{b}	0.78^{a-c}	2.09^{bc}	0.60^{d}	2.25^{bc}	0.69^{ce}
RC05	2.43^{ab}	0.78^{a-c}	2.11^{bc}	0.64^{cd}	2.27^{bc}	0.71^{b-d}
RC08	2.40^{b}	0.72^{bc}	2.00^{c}	0.54^{ef}	2.20^{c}	0.63^{ef}
Mixed2	2.53^{a}	0.86^{a}	2.38^{ab}	0.72^{a}	2.45^{ab}	0.79^{b}
R.TER	2.26^{b}	0.71^{d}	1.77^{d}	0.58^{f}	2.01^{c}	0.65^{cd}
FS Tur	1.52^{c}	0.77^{d}	1.84^{d}	0.54^{f}	1.68^{e}	0.66^{cd}
OSU142 ARM	2.38^{b}	0.76^{d}	1.86^{d}	0.59^{f}	2.12^{c}	0.68^{cd}
M3 ARM	2.31^{b}	0.79^{d}	1.93^{d}	0.53^{f}	2.12^{c}	0.66^{cd}
RC14	2.29^{b}	0.77^{d}	1.82^{d}	0.56^{f}	2.05^{c}	0.67^{cd}
RC10	2.31^{b}	0.78^{d}	1.78^{d}	0.50^{f}	2.05^{c}	0.64^{cd}

* Control (without bacteria inoculation or mineral fertilizers); N_1 and N_2 fertilizer (80 and 40 kg N ha^{-1} in the form of urea), *Bacillus* OSU-142; *Bacillus* M3; *Azospirillum brasilense* sp.245; *Bacillus megaterium* RC07; *Paenibacillus polymyxa* RC05; *Raoutella terrigena, Burkholderia cepacia* FS Tur, *Bacillus* OSU-142 ARM; *Bacillus* M3 ARM; *Azospirillum* sp.245 ARM, *Paenibacillus polymyxa* RC14 (2/2); *Bacillus megaterium* RC10 (T17), Mixed 1 (*Bacillus* OSU-142+ *Bacillus* M3+ *Azospirillum brasilense* sp.245), Mixed 2 (*Bacillus* OSU-142+ *Bacillus* M3+ *Azospirillum brasilense* sp.245 + 40 kg N ha).

** Averages of the same column values (each section separately) followed by same letter did not differ significantly from Duncan's multiple range tests at 1% significance.

*** All of the values in the Table are 2-years average.

The two year field trials in all sites showed that inoculation with PGPR increased yields in both wheat and barley compared to control. Two years of trials under field conditions showed that treatments including bacterial seed inoculations and fertilizer applications significantly affected the parameters investigated compared to control in wheat and barley depending on the years, plants and soil types. In this study, both the site and plants tested responded differently to inoculation of different rhizobacterial isolates. It is suggested that plant-bacterial interactions may have played an important role in restricting the expression of growth promotion. As reported previously, the effect of PGPR is a complex process and depends on the bacterial strain and population, on the plant-bacterial strain combination, the plant genotype, the growth parameters evaluated and environmental conditions (Şahin et al., 2004; Çakmakçı et al., 2006; Çakmakçı et al., 2007a; Çakmakçı et al., 2009).

On the average of both sites, nitrogen fertilizers and inoculation with OSU-142, AB-245 and/or combination of three bacteria increased grain and total biomass yield at both sites in both wheat and barley plants. In 2007, all treatments significantly increased grain N content of barley at site I and II over the control. On the average of both sites, all treatments significantly increased grain and straw N content of wheat and barley plants compared to control. The first year trial indicated that single inoculations with PGPRs increased wheat grain protein content by 19.2-32.1% and barley protein content by 20.2-29.8% over control. Multiple inoculations of N_2-fixing bacteria plus P-solubilizing bacteria gave protein content increases by 25.6% in wheat and 28.8% in barley.

In the first year at the first site, the co-inoculation of *Azospirillum brasilense* sp.245 with *Bacillus* OSU-142 and *Bacillus* M3 showed statistically similar results with recommended chemical fertilizers alone and it increased grain and total biomass yield by 15.3 and 20.1% in wheat and by 11.4 and 11.8% in barley, respectively, compared to control. Similarly, in the second year at the first site, combined inoculation and combination of inoculation of three bacteria and 50% of recommended N fertilizers showed statistically similar results with recommended N fertilizers alone and increased grain and total biomass yield by 39.3-35.7 and 18.9-20.8% in wheat and by 3.6-7.1 and 3.3-12.2% in barley, respectively, over control. In field conditions, considering the average of both sites and years, wheat grain yields were increased by 19.6, 25.1, 31.3 and 40.4% whereas barley grain yields were increased by 14.2, 12.0, 17.3 and 34.2% respectively by OSU-142, AB-245, combination of three bacteria strains and N_1 applications over

control. These findings indicated that the combined inoculations of plant growth promoting rhizobacteria consistently increased the growth, yield and quality of wheat and barley grains in accordance with the findings from other studies (Şahin et al., 2004; Khan et al., 2007). A previous study showed that a combined bio-inoculation of PGPR strains improved growth, nutrient uptake and the nutritional quality of wheat grain (Selvakumar, 2008). In addition to this, some of the previous studies showed that combined bacteria inoculations increased grain and dry matter yields as compared with single inoculation of individual organisms in barley (Çakmakçı et al., 1999; Şahin et al., 2004), in pearl millet and blackgram (Poonguzhali, 2005) and in other species (Çakmakçı et al., 1999; Felici, 2008). Seed inoculation with *Azospirillum brasilense* (Madhaiyan, 2010), *Bacillus* OSU-142 and *Bacillus* M3 (Şahin et al., 2004) strains alone or under dual inoculation increased plant growth in terms of shoot or root length and increased nutrient uptake in different plant species. Inoculation of *Azospirillum* is the well-known and profitable PGPR treatment if combined with other microorganisms (Bashan, 2004) and *A. brasilense* Sp245 may be efficiently applied in multi-microbial formulations (Russo, 2005).

In general, microbial inoculation of seeds with effective *B.* OSU-142 and *A. brasilense* sp.245, alone or in combination with *B.* M3, may substitute costly mineral fertilizers and can be used in organic and sustainable agriculture in wheat and barley production. Bacteria like *Azospirillum* and *Bacillus* are widely used in organic production systems and they are also important N_2-fixing, P-solubilizing and phytohormone-producing microorganisms, resulting in improved growth and yield of crops (Spaepen et al., 2008). *B.* OSU-142 and M3 are commonly used bacteria in scientific studies in Turkey, which have N_2-fixation, P-solubilisation, IAA and cytokinin production and increased root and shoot growth and yield (Şahin et al., 2004; Çakmakçı et al., 2006; Çakmakçı et al., 2007a). Trials with these *Bacillus* species indicated yield increases in sugar beet, wheat and barley (Çakmakçı et al., 2001; Ozturk, 2003; Şahin et al. 2004; Çakmakçı et al., 2006; Çakmakçı et al., 2007a), raspberry (Orhan et al., 2006), sweet cherry (Eşitken et al., 2006), spinach (Çakmakçı et al., 2009), tomato (Turan et al., 2007) and strawberry (Güneş et al., 2009; Eşitken et al., 2010).

In the present study, P-solubilizing N_2-fixing and phytohormone-producing PGPR strains stimulated plant growth, yield and/or quality parameters both in wheat and barley. Although some parameters evaluated were still behind those obtained with N fertilization, bacterial inoculation especially alone with OSU-142 and AB-245 and in combined applications

resulted in considerable increases in growth and yield, nutrient concentration and quality of wheat and barley. Microbial fertilization could be an alternative to N fertilization in both greenhouse and field conditions. This study showed that inoculation of seeds with PGPR under greenhouse and field conditions may help to reduce or replace the total amount of mineral fertilizer which is necessary to obtain maximum yield and quality parameters for wheat and barley production in Aridisol. Further studies may be important to investigate the effect of different application methods of PGPR strains on wheat and barley yield and quality parameters on location as well as other crops grown in different geographical locations.

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