

Growth and yield response of spring wheat (*Triticum aestivum* L.) to inoculation with rhizobacteria

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ABSTRACT

The growth and yield response of spring wheat to inoculation with foreign and local rhizobacteria of Erzurum (Turkey) origin was studied. At the first stage of the research, a greenhouse experiment was carried out with wheat cv. Kirik using 75 local bacterial strains isolated from the soil with 6 foreign bacteria, and a control. According to results of the greenhouse experiment 9 local strains were identified. At the second stage, the response of wheat cv. Kirik to 20 treatments (9 local strains, 6 foreign bacteria, 4 levels of N, and a control) was investigated in Erzurum field conditions. Seventeen strains had significant positive effects on tiller number per plant, 47 strains on plant height, one strain on dry matter yield, and 28 strains on plant protein content in the greenhouse experiment. Inoculation with certain rhizobacteria clearly benefited growth and increased the grain and N-yield of field grown wheat. The effects of local strains were observed to be in general superior to those of foreign strains. Inoculation with the local Strain No. 19, 73, and 82 increased total biomass by 18.7, 18.1, and 19.9%; grain yield by 18.6, 17.7, and 18.0%; total N-yield by 27.5, 24.3 and 26.0%, respectively, as compared to control. In conclusion, Strain No. 19, 73, and 82 can be a suitable biofertilizer for spring wheat cultivation in areas with similar conditions as in Erzurum. Inoculation with these strains may lead both to increases in wheat yield and savings of nitrogen fertilizer.

Keywords: inoculation; rhizobacteria; yield response; wheat

There has been an increased interest in biological nitrogen fixation in the context of sustainable agriculture as a result of cost of mineral fertilizers and their possible harms to the environment. Non-symbiotic nitrogen fixing bacteria that live in the rhizosphere (Döbereiner 1997) and/or endophytically (Hecht-Buchholz 1998) often increase yields of cereals and other crops. Many bacterial species were identified to have nitrogen fixing properties including *Azospirillum* sp., *Azotobacter* sp., *Bacillus* sp., *Beijerinckia* sp., *Clostridium* sp., *Enterobacter* sp., *Pseudomonas* sp., etc. (Kennedy and Tchan 1992, Döbereiner 1997). Enhancement of crop yields of cereals by inoculation with nitrogen fixing bacteria was observed in many experiments (Bhattarai and Hess 1993, Cakmakci et al. 2001, Ozturk et al. 2003). Yield increases obtained in inoculated plants were attributed to the production of plant growth substances by the root-colonizing bacteria (Kennedy and Tchan 1992). Promotion of root growth resulted in enhanced nutrient and water uptake from the soil (Okon 1984, Bhattarai and

Hess 1993). Inoculation of wheat with *Azospirillum* sp. increased root dry weight and 1000-kernel weight (Jagnow 1990), raised number of spikes per plant, number of grains per spike and grain and straw yield (Darmwall and Gaur 1988, Ozturk et al. 2003), N-uptake and N-yield (Bhattarai and Hess 1993) in wheat. Inoculation of *Bacillus* sp. increased the yields of wheat (Rodriguez et al. 1996), the mass of soil adhering to the roots (Gouzou et al. 1993), enhanced the stability of soil aggregates (Bethlenfalvay et al. 1997), and stimulated plant growth (Ryder et al. 1999). Similarly, inoculation with *Azotobacter*, *Enterobacter* or *Klebsiella* increased the number of root hairs, tillering ratio, dry matter contents, N-uptake or yields of wheat (Haahtela et al. 1988, Rai and Gaur 1988). Inoculation of corn with *Azotobacter*, *Beijerinckia*, *Escherichia*, *Derxia*, and *Klebsiella* increased the concentration of nitrogen in the aboveground plant parts (Saric et al. 1987).

Yield responses of cereals to inoculation with rhizobacteria depend on plant genotype (Murty and

Ladha 1988), bacterial strain and soil type (Baldani et al. 1987) as well as environmental conditions (Bhattarai and Hess 1993). As diazotrophic bacteria are widely distributed, it is possible that many diazotrophs, possibly including the most efficient one, have not been identified yet (Döbereiner 1988). Local isolates may be preferred in the selection of bacteria for inoculation of crop plants, as they are adapted in the environment and can be more competitive than the foreign bacteria (Bhattarai and Hess 1993). Considering this, free living nitrogen fixing bacteria were isolated from fields of Erzurum Plain and Pasinler Plain in Turkey, and growth and yield response of spring wheat to inoculation with these bacteria and foreign bacteria were tested under greenhouse and field conditions.

MATERIAL AND METHODS

Soil samples, isolation and identification of strains

The 145 soil samples were collected from fields of Erzurum Plain (29°55'N and 41°16'E) and Pasinler Plain (39°55'N and 41°42'E) in Turkey from the altitudes from 1650 m to 2000 m, where the farmers either do not use or use only very low amounts of chemical fertilizers. Samples (about 0.5 kg) were taken from rhizosphere soil of vigorously grown grasses and cereals at the depth of 20 cm, by randomly sampling method and were transported to Microbiology Laboratory, Department of Biology, Ataturk University, Erzurum, in June 1999. Samples were kept at 4°C until use.

Azotobacter medium was used for the isolation of bacterial strains. About 1.0 g of soil from each sample was placed in 250 ml flask including 99 ml sterile water. The samples were maintained on orbital shaker (100 rpm) for overnight, a serial dilution was prepared from each sample, and plated on *Azotobacter* medium containing glucose 5 g, mannitol 5 g, $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$ 0.1 g, $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ 0.1 g, $\text{Na}_2\text{MoO}_4 \cdot 2 \text{H}_2\text{O}$ 5 mg, K_2HPO_4 0.9 g, KH_2PO_4 0.1 g, $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ 0.01 g, CaCO_3 5 g, agar 15 g, distilled water 950 ml, pH 7.3. The plates were incubated at 26°C for 3 days. Then single colonies grown on *Azotobacter* medium were selected and sub-cultured for purification. The purified strains were maintained for a long-term storage in nutrient broth with 15% glycerol at -80°C for further test (Knowles 1982).

Total 9 out of 75 local bacterial strains tested in the greenhouse experiment were found to have

a potential to be used as plant promoting rhizobacteria (except Strain No. 51 that was selected for its negative effect on germination). All of these bacterial strains were identified based on FAME (Fatty Acid Methyl Ester) analysis using Sherlock Microbial Identification System (MIS Sherlock 4.0 MIDI, Inc., Newark, DE) at Ataturk University, Biotechnology Application and Research Centre (Miller and Berger 1985), as shown in Table 1.

In addition, six of foreign bacterial strains tested in the study were listed in Table 1. These bacterial strains including four of *Bacillus* sp. BA-140, BA-142, M-13 and M-58, and a strain of *Burkholderia* (BA-7) and *Azospirillum* (*A. brasilense* Sp246) were previously isolated from various sources and tested for their plant growth and antimicrobial effects in a number of studies (Cuppels et al. 1999, Cakmakci et al. 2001).

All of the bacterial strains listed in Table 1 were tested for nitrate reduction activities and growth on N-free basal medium, solubilization of inorganic phosphate on National Botanical Research Institute's phosphate growth medium (Nautiyal et al. 2000), siderophore production (Duijff et al. 1994), and IAA production (Zaffari et al. 1998) as described previously.

Greenhouse and field experiments

Pure cultures were grown in nutrient agar for experiments. A single colony from each strain was transferred to a 50-ml flask, containing nutrient broth (beef extract 1 g/l; yeast extract 2 g/l; peptone 5 g/l; sodium chloride 5 g/l), and grown aerobically in flasks overnight on a rotating shaker (200 rpm) at 25°C. Bacteria grown on nutrient broth were then diluted with sterile distilled water, containing 0.025% Tween 20 to a final concentration of 10^8 CFU/ml. For treatments, seeds were placed in bacterial suspensions of 10^8 CFU/ml for 30 min before sowing.

A pot experiment was carried out in the greenhouse during September 1999–March 2000 with the facultative bread wheat cultivar Kirik (awn less, white-grain, and the predominant cultivar in the region) in order to investigate the effects of seed inoculation with foreign and local bacteria. The greenhouse experiment consisted of 82 treatments and three replications, and the pots were distributed in a completely randomised design. There were 75 local strains (Strain No. 1, 2 ... and, 112), 6 foreign bacterial strains (BA-7, BA-140, BA-142, M-13, M-58, *Azospirillum brasilense* Sp246) and

a control treatment without inoculation and any fertilizer application. The soil was obtained from fallow field at the Experimental Farm of Ataturk University in Erzurum. It was a loam with an organic matter content of 1.56–1.61% and a pH of 7.58–7.66. Available P and K contents were 15.7–15.9 and 491.2–518.6 kg/ha, respectively. This soil was filled in eight-litre pots. Eight seeds were sown in each pot and thinned to five plants per pot after the full emergence of the first leaf. The pots were regularly irrigated to maintain a proper moisture level.

The field experiment with the wheat cultivar Kirik was carried out on the fallow field of the Experimental Farm of Ataturk University in Erzurum, Eastern Anatolia (29°55'N and 41°16'E with an altitude 1850 m), in 2000 and 2001. With an average temperature of 4.7°C and a total rainfall of 396 mm, plant growth in the region restricted to the period between May and October. Total rainfall and average temperatures in April, May, June, July, and August were 34.9, 42.0, 9.7, 4.0, 4.7 mm and 7.4, 9.8, 15.5, 22.3, and 19.3°C in 2000; 104.9, 68.7, 7.3, 36.6, 9.2 mm and 7.2, 9.3, 15.4, 20.6, and 19.9°C in 2001, respectively. The experimental soil was a loam with an organic matter content of 1.12–1.40% and a pH of 7.30–7.66.

Available P and K contents were 15.1–15.8 and 460.3–483.2 kg/ha, respectively. The experiment consisted of 20 treatments and three replications, and the plots were distributed in randomized complete block design. There were 15 N₂-fixing bacteria (BA-7, BA-140, BA-142, M-13, M-58, *Azospirillum brasilense* Sp246, Strain No. 19, 21, 39, 41, 43, 49, 51, 73, and 82), 4 levels of N fertilization (20, 40, 60 and 80 kg/ha) and a control treatment without inoculation and N fertilization. All plots were fertilized with 50 kg/ha P as triple super phosphate during tillage in spring. Nitrogen fertilizer was applied as ammonium sulphate in two equal amounts during soil preparation at sowing and at the beginning of stem elongation. Uninoculated and inoculated seeds with rhizobacteria were hand sown on 5.0 × 1.2 m plots (6 rows) so as to give 475 seeds per m². Maximum care was taken in order to avoid contamination and mixing of bacterial inoculations during sowing. Plants were irrigated at the beginning of stem elongation, heading and milky ripening stages. The following parameters were determined: flag leaf area, plant height, spike number per m², kernel number per spike, 1000-kernel weight, total biomass, grain yield, grain protein content, grain and total N-yield.

Table 1. Source and biochemical characteristics of the bacterial strains tested in field experiment

	Strain	Source	Growth in N-free <i>Azotobacter</i> medium	IAA biosynthesis	Siderophore production	Phosphate- solubilization	
Local bacteria	<i>Ralstonia</i> sp.	No. 19	bromegrass	+	–	+	–
	<i>Anthrobacter</i> sp.	No. 21	wheat	+	–	+	–
	<i>Stenotrophomonas</i> sp.	No. 39	bluegrass	+	–	+	–
	<i>Ralstonia</i> sp.	No. 41	wheat	+	–	+	–
	<i>Stenotrophomonas</i> sp.	No. 43	fescue	+	+	+	–
	<i>Ralstonia</i> sp.	No. 49	wheatgrass	+	+	+	–
	<i>Stenotrophomonas</i> sp.	No. 51	barley	+	–	+	–
	<i>Ralstonia</i> sp.	No. 73	wheat	+	–	+	–
<i>Stenotrophomonas</i> sp.	No. 82	bluegrass	+	–	–	–	
Foreign bacteria	<i>Burkholderia</i> sp.	BA-7	water	+	+	–	–
	<i>Bacillus</i> sp.	BA-140	tomato	+	–	–	–
	<i>Bacillus</i> sp.	BA-142	pepper	+	–	–	–
	<i>Bacillus</i> sp.	M-13	rice	+	–	–	+
	<i>Bacillus</i> sp.	M-58	soil	+	–	–	–
	<i>Azospirillum</i> sp.	Sp246	wheat	+	–	–	–

Table 2. Flag leaf area, plant height and yield components of wheat in response to inoculation with N₂-fixing bacteria and N fertilization (means of 2000 and 2001¹)

	Flag leaf area (cm ²)	Plant height (cm)	Spikes per m ²	Kernels per spike	1000-kernel weight (g)
Years (Y)					
2000	6.83 b	69.2 b	391.6 b	13.8 b	34.9 b
2001	8.26 a	75.6 a	445.3 a	15.3 a	36.4 a
Mean	7.54	72.4	423.7	14.5	35.2
Treatments (T)					
Control	6.99 fg	67.9 g	384.7 h	13.2 h	34.3 e
20 kg N/ha	7.37 cde	73.3 b-e	443.6 b-e	14.6 c-g	34.9 b-e
40 kg N/ha	7.55 bcd	75.9 ab	463.2 abc	15.2 bc	35.0 a-e
60 kg N/ha	7.78 ab	76.3 ab	477.6 ab	15.5 abc	34.5 cde
80 kg N/ha	8.12 a	77.8 a	490.3 a	16.1 ab	34.7 cde
BA-7	7.71 bc	72.4 b-f	419.8 d-h	13.5 fgh	35.6 abc
BA-140	7.61 bcd	69.7 efg	431.3 c-h	13.6 fgh	34.9 b-e
BA-142	7.71 bc	70.1 d-g	405.2 fgh	13.6 fgh	35.0 b-e
M-13	7.61 bcd	71.0 c-g	410.6 e-h	13.7 fgh	35.5 abc
M-58	7.86 ab	71.4 c-g	426.6 c-f	13.9 d-g	34.3 e
<i>A. brasilense</i>	7.72 bc	68.5 fg	453.5 a-d	13.9 d-g	35.7 abc
Strain No. 19	7.83 ab	74.1 a-d	453.3 bcd	14.9 cd	35.1 a-e
Strain No. 21	7.72 bc	74.0 a-e	435.6 c-f	13.7 fgh	35.6 abc
Strain No. 39	7.71 bc	74.9 abc	422.1 d-g	14.9 cd	34.6 cde
Strain No. 41	7.13 efg	74.9 abc	389.1 gh	14.6 c-f	36.1 a
Strain No. 43	7.06 efg	71.1 c-g	421.5 d-g	14.9 cd	35.4 a-d
Strain No. 49	6.93 g	71.4 c-g	416.3 e-h	14.8 cde	36.0 ab
Strain No. 51	7.29 def	68.7 fg	255.0 i	16.5 a	35.9 ab
Strain No. 73	7.80 ab	74.2 a-d	439.5 c-f	15.3 abc	35.6 abc
Strain No. 82	7.40 cde	70.6 c-g	443.9 b-e	14.6 c-f	35.0 b-e
CV (%)	2.65	3.83	5.49	3.39	2.58
F values					
Y	152.74**	157.70**	246.06**	279.06**	218.21**
T	13.56**	6.74**	27.72**	12.71**	2.06*
Y × T	12.28**	2.65**	8.29**	6.99**	1.50

¹means with the same letter within variables are not significantly different; *F*-values marked with * and ** are significant at 0.05 and 0.01 levels, respectively

Flag leaf area was measured at anthesis with an area meter (CID, Inc. model CI-202). Percentage of N was determined using the Kjeldahl method (A.A.C.C. 1983 method 46-12). Data were subject

to analysis of variance using MSTAT-C (1991) software package. Duncan's Multiple Range Test was used to determine the differences among the treatments (*P* = 0.01).

RESULTS

Analysis of variance showed that inoculation significantly affected all parameters investigated in the greenhouse experiment. Tiller number per plant of treatments ranged between 1.01 (Strain No. 69) and 2.42 (M-13). Seventeen strains caused significant increases in tiller number per plant, while 36 strains caused insignificant decreases, compared to the control (1.22). Inoculation with M-13, BA-142, Strain No. 19, *A. brasilense* Sp246, BA-140, and BA-7 increased tiller number per plant by 55–98%, compared to control. The highest plant heights were obtained from Strain No. 49 (56.8 cm), Strain No. 39 and 19 (55.8 cm), and BA-140 (54.6 cm). Forty-seven strains caused highly significant increases in plant height, while 9 strains caused insignificant decreases, compared to the control (45.9 cm). Dry matter yield of treatments ranged between 9.83 g (Strain No. 51) and 15.63 g (Strain No. 19). The only Strain No. 19 caused a significant increase in dry matter yield, compared to the control (11.20 g). Inoculation with Strain No. 73, 82, 41, 49, and 21 increased dry matter yield by 26.9, 24.8, 24.1, 23.8, and 22.6%, respectively. Twenty-eight strains significantly increased plant protein concentration, compared to the control (5.74%). The highest plant protein concentrations were obtained from *A. brasilense* Sp246 (6.97%), M-58 (6.94%), Strain No. 51 (6.94%), Strain No. 19 (6.92 %), Strain No. 43 and 73 (6.91%).

In the field experiment, analysis of variance showed statistically significant differences between years for the investigated characteristics. More favourable climatic conditions increased flag leaf area, plant height, spike number per m², kernel number per spike, 1000-kernel weight, total biomass, grain yield, grain N-yield, and total N-yield in 2001, but decreased grain protein concentration. As average of years, all the parameters were significantly influenced by treatments (Tables 2 and 3). Year vs. treatment interactions were significant for most parameters (except 1000-kernel weight and grain protein concentration) mainly due to different effects of Strain No. 51 in 2000 and 2001 (Tables 2 and 3).

Fifteen treatments caused significant increases in flag leaf area when compared to the control. The highest leaf area values were recorded for 80 kg N/ha, M-58, and Strain No. 19. Nitrogen applications (80 and 60 kg/ha) gave the highest plant height of wheat. Inoculation with Strain No. 21, 19, 73, 39, and 41 increased plant heights by 9–10% compared to the control. Spike number per m² significantly increased with N applications.

Inoculation with Strain No. 21, 73, 82, 19, and *A. brasilense* Sp246 increased spike number by 13.2, 14.2, 15.4, 17.8, and 17.9%, respectively, compared to the control. Kernel numbers per spike ranged between 13.2 (control) and 16.5 (Strain No. 51). Nitrogen applications as well as seed inoculations with N₂-fixing bacteria significantly increased kernel number per spike (Table 2). The highest 1000-kernel weights among the treatments were obtained by Strain No. 41 (36.1 g), Strain No. 49 (36.0 g), and Strain No. 51 (35.9 g).

Inoculation with Strain No. 82, 19, and 73 increased total biomass by 19.9, 18.7, and 18.1%, respectively as compared to the control. Nitrogen applications (80, 60, and 40 kg/ha) increased the total biomass by 34.2, 24.3, and 20.2%, respectively (Table 3). Bacterial inoculations and N applications significantly affected grain yield of wheat. When compared to the control 80, 60, and 40 kg N/ha applications increased grain yields by 31.2, 25.1, and 22.1%, respectively while inoculations with Strain No. 19, 82, and 73 increased grain yields by 18.6, 18.0, and 17.7 %, respectively. Treatments also affected grain protein concentration of wheat. Grain protein concentration was higher in 80, 60, and 40 kg N/ha (14.5, 13.9, and 13.6%, respectively), Strain No. 19 (13.7%), Strain No. 43 (13.6%), and Strain No. 82 (13.5%) treatments while protein concentration was lower especially in control (12.6%), BA-7 and M-13 (12.9%) inoculated plots (Table 3). The highest grain N-yields were obtained from 80, 60, and 40 kg N/ha applied plots, followed by Strain No. 82 inoculated plots while the lowest grain N-yields were recorded for Strain No. 51 and control (Table 3). As an average of years, total N-yield increased over control by 53.6, 40.8, and 33.6% with 80, 60, and 40 kg N/ha applications, respectively. Inoculations with Strain No. 19, 82, and 73 increased total N-yields, respectively by 27.5, 26.0, and 24.3% (Table 3).

DISCUSSION

The 81 tested strains displayed a large variability regarding their effects on the parameters investigated in the greenhouse conditions. Seventeen strains had significant positive effects on tiller number per plant, 47 strains on plant height, one strain on dry matter yield, and 28 strains on plant protein concentration. Negative but insignificant effects were also found. The response of wheat to inoculation with foreign strains was higher than that of local strains in terms of tiller number and

Table 3. Total biomass, grain yield, protein content and N-yields of wheat in response to inoculation with N₂-fixing bacteria and N fertilization (means of 2000 and 2001¹)

	Total biomass (kg/ha)	Grain yield (kg/ha)	Grain protein concentration (%)	Grain N-yield (kg/ha)	Total N-yield (kg/ha)
Years (Y)					
2000	6631 b	1690 b	13.6 a	40.0 b	54.1 b
2001	9263 a	2470 a	13.1 b	53.5 a	71.8 a
Mean	7939	2080	13.3	46.7	62.9
Treatments (T)					
Control	7234 f	1883 g	12.6 e	39.9 j	53.9 i
20 kg N/ha	8169 cde	2156 cde	13.3 bcd	49.8 cde	67.0 cde
40 kg N/ha	8694 bc	2300 bc	13.6 bcd	53.5 bc	72.0 bc
60 kg N/ha	8994 ab	2355 ab	13.9 ab	57.8 ab	75.9 b
80 kg N/ha	9711 a	2470 a	14.5 a	59.4 a	82.8 a
BA-7	7474 ef	1982 fg	12.9 cde	41.8 ij	56.8 hi
BA-140	7514 ef	1963 fg	13.3 bcd	44.7 e-j	59.5 f-i
BA-142	7461 ef	1969 fg	13.3 bcd	43.1 hij	57.8 ghi
M-13	7735 ef	2028 efg	12.9 de	44.0 f-j	59.3 f-i
M-58	7693 ef	1979 fg	13.0 cde	43.8 g-j	58.8 f-i
<i>A. brasilense</i>	7750 ef	2108 def	13.4 bcd	47.5 d-h	62.3 d-h
Strain No. 19	8584 bcd	2234 bcd	13.7 bc	49.3 cde	68.7 cd
Strain No. 21	7932 def	2046 efg	13.3 bcd	45.8 d-i	61.4 d-h
Strain No. 39	7664 ef	2038 efg	13.4 bcd	45.9 d-i	60.8 e-h
Strain No. 41	7603 ef	1966 fg	13.2 cde	43.6 g-j	58.7 f-i
Strain No. 43	7997 cde	2111 def	13.6 bcd	48.3 d-g	64.0 d-g
Strain No. 49	8146 cde	2151 cde	13.2 cde	48.2 d-h	64.6 def
Strain No. 51	5194 g	1419 h	13.1 cde	30.6 k	40.5 j
Strain No. 73	8546 bcd	2217 bcd	13.4 bcd	49.1 c-f	67.0 cde
Strain No. 82	8676 bcd	2222 bcd	13.5 bcd	50.7 bcd	67.9 cd
CV (%)	4.91	5.00	4.45	6.46	5.50
F values					
Y	12.49**	1686.09**	29.52**	605.17**	791.14**
T	23.32**	27.88**	5.17**	22.75**	28.64**
Y × T	12.49**	12.70**	0.43	8.70**	11.15**

¹ means with the same letter within variables are not significantly different; *F*-values marked with * and ** are significant at 0.05 and 0.01 levels, respectively

protein content, but local strains were superior to foreign strains for plant height and dry matter yield. These results show clearly that some N₂-fixing bacteria are capable of inducing positive effects on

plant growth. The enhancing effect of seed inoculation with N₂-fixing bacteria on the growth and yield of wheat were reported by many researchers (Bhattarai and Hess 1993, Ozturk et al. 2003). Such

an improvement might be attributed to the high nitrogen uptake of the inoculated plants as well as to the ability of both organisms to produce growth-promoting substances (Haahtela et al. 1988).

The two-year field experiments showed that there were significant differences in the effect of the treatments. In general, the effect of inoculants (except strain No. 51) was observed to be superior to that of the control. Inoculation with Strain No. 51 decreased grain and N-yields. This effect was related to possible decreases in spike number per m² due to its negative effect on germination. The effect of inoculation on germination may be negative for some strains (Bashan 1986). Inoculation with certain rhizobacteria clearly benefited growth and increased the grain and N-yield of wheat. The effects of local strains were observed to be superior to those of the foreign strains, in general. Of the local bacteria tested, Strain No. 19, 73, and 82 in the absence of any nitrogen application achieved flag leaf area, plant height, spike number per m², kernel number per spike, total biomass, grain yield, and grain protein concentrations equal to the 40 kg N/ha (Tables 2 and 3). Inoculation with the Strain No. 19, 73, and 82 increased total biomass by 18.7, 18.1, and 19.9%; grain yield by 18.6, 17.7, and 18.0%; total N-yield by 27.5, 24.3, and 26.0%, respectively as compared to the control. Similar increases in grain yield of wheat through inoculations with local bacteria were reported in previous studies (Bhattarai and Hess 1993). The increases in total biomass and grain yield were derived mainly from increases in the number of fertile tillers per m² and grain number per spike (Ozturk et al. 2003). Inoculation also increased the grain N-yield and total N-yield of wheat. The increases were derived from both enhancement of dry matter and N content in the plant parts. Similar increases were observed in corn inoculated with *Azospirillum*, *Azotobacter*, *Beijerinckia*, *Escherichia*, *Derxia*, and *Klebsiella* strains (Saric et al. 1987). Increases in total N-yield were higher than the total biomass and grain yield increases, stressing the effect of inoculation on N assimilation by the plants. The possible mechanisms of higher N-yield may be the transfer of atmospheric nitrogen to the plant through bacterial nitrogen fixation and/or by improved nitrogen uptake in the inoculated plants (Murty and Ladha 1988, Bhattarai and Hess 1993). Growth substances can enhance the root growth and in turn increase the soil volume explored by the roots (Okon 1984).

The results of the present study clearly showed the beneficial role of rhizobacteria. The higher

grain and N-yield responses to the local rhizobacteria (Strain No. 19, 73, and 82) compared to the foreign rhizobacteria indicate the possibility of improved associations using olden and predominant wheat cv. Kirik and local rhizobacteria. Yield improvement by inoculation with associative bacteria requires the most successful combinations between the plant genotype and a particular bacterial strain. Local isolates should be preferred in the selection of rhizobacteria for inoculation, as they are adapted in the environment and can be more competitive than the foreign rhizobacteria (Bhattarai and Hess 1993). In conclusion, Strain No. 19, 73, and 82 that showed the highest positive responses in inoculated wheat can be suitable inoculants for spring wheat cultivation in areas with similar conditions as in Erzurum. Further studies may be useful on combinations of these local bacteria.

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