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Yield response of a common spring wheat cultivar to inoculation with *Azospirillum brasilense* at various levels of nitrogen fertilization

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Summary The yield response of a common spring wheat cultivar, *Triticum aestivum*, to inoculation with *Azospirillum brasilense* was studied at four levels of N fertilization. Plant yield increased due to the inoculation treatment only at medium and high levels of N fertilization, with a maximum yield increase of about 8.0 per cent at the highest level (approximately 1.0 g of pure N per plant). Yield increase was mostly due to an increase in the number of grains per spike, and at the highest level of fertilization, also due to a higher number of spikes per plant. At all N levels, the inoculation caused an increase of 0.5–1.4 per cent in the number of fertile spikelets per main spike.

Grain protein percentage was unaffected by the inoculation, though significantly increased due to the fertilization treatments.

The occurrence of maximum yield response at the highest N level, the response by early-determined yield components, i.e., spikelet number, and the unaffected grain protein content are in accord with the suggestion that the contribution of *Azospirillum brasilense* to wheat yield is not through N_2-fixation.

Introduction

Various reports indicate that inoculation with the N_2-fixing bacteria *Azospirillum brasilense* may increase the yield of several forage grasses[4,10] as well as of summer cereals[6]. Recently, there has been an increased interest in the response of winter cereals, particularly wheat, to these bacteria[2,5,8,9]. It has been suggested that inoculation of summer cereals, such as corn (*Zea mays*) and sorghum (*Sorghum bicolor*), with these N_2-fixing bacteria may partially replace N fertilization[6]. Spring wheat, however, when grown during the winter season under Mediterranean or arid climate, is exposed to conditions unfavorable for N_2-fixation; low seasonal temperatures and high levels of available fixed N, applied to wheat under normal agricultural practice, are reported to suppress N_2 ase activity[1,3]. Nevertheless, wheat yield increase was reported under such conditions[2,7,8]. These findings greatly reduce the possibility that N_2-fixation was the main contribution of the bacteria for the wheat performance.

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Aiming at further evaluating this suggestion, the interaction between *Azospirillum brasilense* and an Israeli line of common wheat, previously found to respond to these bacteria, was studied at various levels of N fertilization. Yield and yield components, as well as grain protein content were determined at each N level and the implications of these findings as to the optimal N level for the bacterial effects and the possible mode of the bacterial action are discussed.

Materials and methods

Plants were grown outdoors in five-liter pots filled with a mixture of 50 per cent volcanic gravel, 33 per cent peat and 17 per cent vermiculite (v/v), and sufficiently irrigated. The peat was originally enriched with basic fertilizer containing 10 per cent pure N. This supplied about 150 mg pure N per pot. In addition, a slow-release fertilizer in the form of 'Osmocot' was added to the growth mixture at four rates of about 3.3, 6.6, 11.0 and 14.3 g per pot, calculated to release 450, 900, 1500 and 1950 mg of pure N per pot for the fertilization levels 1 to 4, respectively.

Seeds of the hexaploid common spring wheat cultivar Line 11 (small-seeded, very early, semi-dwarf spring variety of *T. aestivum*) were sown two per pot on 30.12.81. Inoculation was carried out by applying 3.6×10^9 bacteria per pot on the date of sowing and a similar treatment was applied on the fifth and the tenth day thereafter. The bacterial suspension was composed of an equal mixture of 20 h old cultures of *Azospirillum brasilense* (ATCC strains 29145 and 29729). Control plants were treated with sterilized medium.

The experiment was designed to include 16 split blocks; the inoculation treatments, consisting of four pots, served as main plots; each N level in a separate pot served as a secondary plot. Eighty cm wide spaces were kept between blocks or main plots in order to avoid cross inoculation. Pots were separated from the underlying soil by polyethelene coverage to prevent rooting of plants in the ground.

Yield and yield components were determined for each plant. Grain protein was determined in main spikes by measuring the NIR reflectance using a 'Neotec'* grain quality analyzer.

Results and discussion

The yield per plant was significantly decreased due to raising of the N level within the range tested (Table 1). This indicates that N was a limiting factor in obtaining the genetic yield potential in the rather good growth conditions, such as sufficient irrigation and plants spacing, prevailing in this experiment. Yet, inoculation brought about additional yield increase only at the medium (2nd and 3rd N levels) and the high (4th N level) of about 1.1, 1.0 and 1.5 g, respectively (Table 1). The lack of yield response to inoculation at the lowest N level and the maximum response at the highest N level, at which N_2-fixation is presumably suppressed[3], suggest that the bacteria did not contribute to the plants fixed N.

*Neotec Instruments, Inc., 2431 Linden Lane, Silver Spring, MD, U.S.A.

Table. 1. Yield and yield components of inoculated and non-inoculated common wheat plants grown at four different N levels

Yield and yield component	N level[a] 1		2		3		4		S.E.1[†]	S.E.2[†]
	Non-inoc.	Inoc.	Non-inoc.	Inoc.	Non-inoc.	Inoc.	Non-inoc.	Inoc.		
Yield per plant (g)	13.43	13.30	15.49	16.59	16.84	17.86	18.71	20.23	0.96	0.91
Yield per main spike (g)	2.66	2.63	2.71	2.73	2.74	2.83	2.81	2.94	0.09	0.10
No. fertile spikelets per main spike	19.4	19.5	19.9	20.0	19.7	19.9	19.6	19.8	0.2	0.2
No. grains per main spike	62.9	64.3	65.4	67.2	66.1	68.6	67.7	70.7	2.0	2.1
No. spikes per plant	7.3	7.6	10.3	8.9	10.3	9.7	13.0	13.6	0.5	0.5
Mean yield of fertile tiller (g)	1.78	1.64	1.49	1.81*	1.56	1.79	1.39	1.44	0.10	0.10
Mean grain weight (mg)	39.7	38.7	38.0	39.2	38.2	38.4	37.9	37.7	0.8	0.8

[†]S.E.1 for the bacterial effect at each N level; S.E.2 for the N effect at each inoculation level.

* Significantly higher than the non-inoculated treatment ($P \leq 0.05$).

[a]N was applied by a slow release fertilizer at rates of 450, 900, 1500 and 1950 mg of pure N per pot for the 1–4 N levels, respectively.

Table 2. Grain protein percentage (dry basis) of inoculated and non-inoculated common wheat plants grown at four different N levels

Inoculation level	N level*			
	1	2	3	4
non-inoculated	15.7c**	16.8b	17.1b	20.4a
inoculated	15.1c	17.1b	17.2b	20.1a

*N was applied by a slow release fertilizer at rates of 450, 900, 1500 and 1950 mg of pure N per pot for the 1–4 N levels, respectively.
**For each inoculation level or for each N level values followed by a similar letter do not differ significantly ($P \leq 0.05$).
S.E. for the bacterial effect at each N level 0.38.
S.E. for the N effect at each inoculation level 0.32.

These findings are in agreement with those obtained in out winter 1982 experiment[7]: the same wheat cultivar inoculated with the same two races of *Azospirillum brasilense* at a relatively high N lvel (825 mg per plant) responded by a significant yield increase of 0.75 g per plant; inoculation at low N level (150 mg per plant) resulted in a non-significant yield increase of 0.14 g per plant.

Inoculation at the various N levels resulted in a differential response of yield components. At the medium N levels, the mostly responding yield component was the mean yield per spike, affected by an increase in the grain number but not in grain weight (Table 1); at the highest N level the main responding yield component was the number of spikes per plant. It is well known that relatively widely spaced plants under sufficient irrigation tend to produce many tillers. In such cases, late appearing spikes are usually very small and any further increase in their number results in a decrease in the average yield per tiller. Such a decrease was observed in the non-inoculated plants as the N level was increased. Inoculation, as mentioned, caused yield increase at the medium and high N levels, but the mode of this effect was different at the different N levels; at levels 2 and 3 the unexplained decrease in tiller number following inoculation was over compensated for by the increase in the mean yield of fertile tiller (significant at level 2). Yet, inoculation at the highest N level brought about an increase both in the number of fertile tillers as well as in the mean yield per tiller.

One of the most consistent contributions of the bacteria was a slight increase in the number of spikelets in the main spike and presumably in other spikes too (Table 1). The inoculation caused a significant average increase of 0.17 fertile spikelets per spike by increasing the total number of spikelets and decreasing the number of non-fertile spikelets at the base of the spike. The number of spikelets per spike is

determined at a very early stage in wheat development. At this stage N_2-fixation by Azospirillum, as measured by acetylene reduction, is almost null (Kapulnik, personal communication), supporting the suggestion that the contribution of the bacteria to the plant is other than reduced N.

Protein percentage in the grains of the main spike was significantly affected by the level of fertilization (Table 2). However, inoculation had no apparent effect on the protein percentage. This further supports the assumption that the contribution of these bacteria for wheat yield is not exerted necessarily through the supply of reduced N. Other studies carried out at our laboratory[5] suggest that plant growth substances produced by these bacteria[11] may promote the yield increase in wheat. The response of various wheat lines to inoculation with Azospirillum at various N levels is currently being studied under experimental field conditions.

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