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Effect of Inoculation with *Azospirillum Brasilense* on Development and Yielding of Winter Wheat and Oat under Different Cultivation Conditions

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Abstract

Studies were carried out on the effect of inoculation with *Azospirillum brasilense* on development and yielding of winter wheat and oat. Plants were grown in field conditions. In the experiments conducted in randomized block design, three factors were applied: inoculation with an active strain of *Azospirillum brasilense,* two levels of nitrogen fertilizer, and seed treatment with fungicidal seed dressing. It was found that inoculation of cereals with active strain *of Azospirillum brasilense* may be a factor increasing their vigour and yield. Under climatic and soil conditions of Wielkopolska region and despite the fact that no dinitrogen fixation was observed, cereal inoculation with *Azospirillum brasilense* contributed to yield increases of up to 27% in wheat and 6% in oats.

Keywords! *Azospirillum brasilense,* diazotrophs, endophyts, oat, winter wheat.

Introduction

In the last few years much attention has been paid to non-symbiotic microorganisms fixing dinitrogen. Participation of these bacteria in the total balance of this process according to most authors constitute about 30% [9, 36] in soil of both tropical and temperate climate. There are many examples of studies pointing to possibilities of dinitrogen fixation by free-living bacteria under plants of different botanical families, which constitutes several, some tens [17, 22, 30, 39] and even 150 kg of nitrogen during a year [13].

Azospirillum brasilense and *Azospirillum lipoferum* are the most commonly studied organisms among the Azospirilla. They are a potential N2 fixer, which is associated closely with roots and rhizospheres of many economically important plants and grasses *[2,* 3, 4, 5, 24, 33, 44].

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Initially *Azospirillum* was isolated mainly from the roots of fodder grasses and cereals in tropical soils [2]. However, it soon become apparent that two previously discovered and the best known species *Azospirillum brasilense* and *Azospirillum lipoferum* were also found in soils of the temperate zone [15, 25, 27] and even under conditions of a cold climate of Finland [23]. The remaining, so-far described species have a restricted range. According to Reynders and Vlassak [37], *Azospirillum* occurs in about 90% of tropical soils and in about 60% of temperate climate soils.

Despite the fact that studies aimed at learning physiological and genetic properties of *Azospirillum* bacteria have recently greatly intensified, there is still relatively little known about relationships between these bacteria and plants and other microorganisms. The purpose of research is knowing the mechanisms of *Azospirillum* beneficial action on plant and also on other soil microorganisms. It is known that *Azospirillum* bacteria fix dinitrogen [14] also under mid-European conditions [8], that

they synthesize phytohormones - auxins, chiefly indoleacetic acid, gibberellins, cytokinins and other biologically active substances, which have not been identified yet [21, 45]. Some years ago it was revealed that some *Azospirillum* strains exudate part of fixed nitrogen in ammonium form directly into plant as it takes place in the case of rhizobia [10, 29].

In view of numerous signs indicating a beneficial action of *Azospirillum* bacteria on plants, since the beginning of studies on this genus species, attempts of their practical use in agriculture have been made through inoculation of crops with these bacteria. The effect of inoculation with *Azospirillum* strains on plant yield was shown many times; however, the studies concerned chiefly tropical climate.

The aim of the present study is to learn the influence of *Azospirillum* bacteria on development and yielding of winter wheat and oat plants under soil-climatic conditions of the Wielkopolska region with appplication of inoculation with *Azospirillum brasilense* strain actively fixing dinitrogen, nitrogen fertilization and plant protection with fungicidal seed dressings.

Material and Methods

Field experiments were carried out in 1996 and 1997 in Zlotniki on the fields of the Experimental and Didactic Station of August Cieszkowski Agriculture University of Poznari. The studies were conducted in winter wheat *(Triticum aestivum* L.) of the cv. Roma and oats *(Avena sativa* L.) of the cv. Santor. The basic criterion of plant selection for the experiments in the case of wheat was its ability to create associative relations with *Azospirillum brasilense* and its economical importance in our country. In the case of oats a criterion of plant selection for the experiments were lower soil requirements, also with regard to its nitrogen content, which may influence the ability of that plant to create associative relations with diazotrophs.

In the experiments conducted in randomized block design, three factors were applied: inoculation with an active strain of *Azospirillum brasilense* (strain 65B) originating from the Department of Microbiology, Institute of Soil Science and Plant Cultivation in Pulawy; two levels of nitrogen fertilizer, and seed treatment with fungicidal seed-dressing.

Inoculation was made just before sowing: cereal seeds were mixed with bacterium suspension and the field was sprayed with this mixture (soil in rows was mixed with grain drill coulters). After emergence, the field was additionally sprayed with *Azospirillum* suspension. The number of *Azospirillum* cells in suspension amounted to 10^8 - 10^9 c.f.u per ml. Such methods were applied many times in field experiments [1, 20, 31, 36, 37, 41].

The applied levels of nitrogen fertilizer were: Nl - no fertilization (control), N2 - half the full dose, N3 - full dose, which in terms of a pure ingredient amounted to 120 kg ha^{-1} for wheat and 100 kg ha^{-1} for oats. Nitrogen was applied in the form of ammonium nitrate.

The applied seed dressings were: Baytan Universal 19.5 DS (active ingredients: *2%* fuberidazole, 2.5% imazalil, 15% triadimenol) for wheat, and Oxafun T (active ingredients: 37.5% carboxine, 37.5% thiuram) for oats.

The experimental field was located on soils of classes IVa and IVb. These are loamy sands medium-rich in potassium, phosphorus and magnesium, with a slightly acid reaction. In the study period the vegetation conditions were favourable to cultivated plants.

The following aspects were subjected to analysis:
- dinitrogen fixation,
- cereal vigour at full vegetative development,
- cereal yielding (qualitative and quantitative analysis of cereal crop yield).

Dinitrogen fixation was determined three times in all plants during the vegetation season, i.e. at the shooting, heading and flowering stages. Dinitrogen fixation was determined on the basis of nitrogenase activity by acetylene to ethylene reduction method, modified by Sawicka [38]. Determinations were performed in three replications for each experimental combination. A mixture of gasses taken from under the bell glass was analyzed on gas chromatograph of the type CHROM 5.

Plant vigour was evaluated on the basis of chlorophyll dyes. Cereal crop vigour was determined at full vegetative development, i.e. before the heading stage. Chlorophyll content was measured in 100 leaf blades of the second joint from the top; therefore, in the youngest, but fully developed leaves. The analysis was carried out by the colorimetric method [34].

The final criterion for the analysis of particular combinations of the field experiment was the grain yield of grown cereals. Besides the yield size, the mass of a thousand caryopses was estimated as well as the content of protein and sugars - components, the amount of which is determined to a decisive degree by habitat conditions (if varietal differentiation is excluded). These analyses were carried out on the basis of the near infra-red method on Infralyzer 500 apparatus.

The obtained results were statistically evaluated using a multidirectional analysis of variance, while the means were compared by Tukey's test.

Results and Discussion

Analyses performed three times during the vegetation season showed no nitrogenase activity in the soil under cultivated plants in any experimental combination.

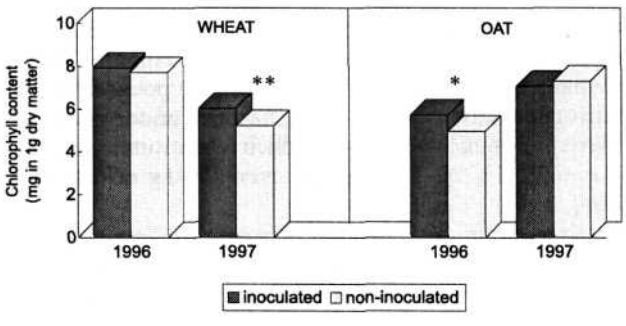
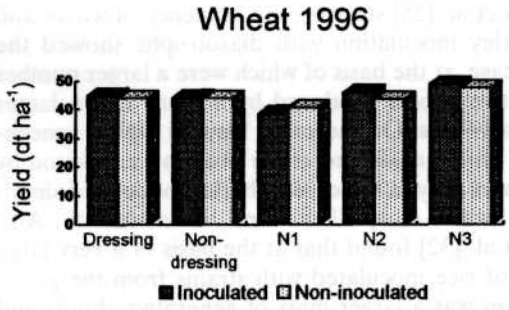
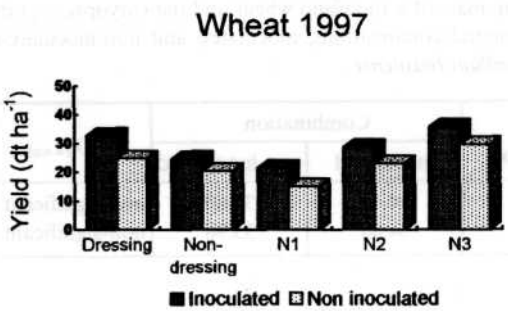


Fig. 1. Effect of cereal crop inoculations with *Azospirillum brasilense* on chlorophyll content in leaf blades. * significant differences at $\alpha = 0.05$ ** significant differences at $\alpha = 0.01$



Combination	Inoculated	Non-inoculated	LSD
dresssing	44.77	43.86	non sign.
non-dressing	44.10	43.89	non sign.
N fertilization:			
0kg ha $^{-1}$(N1)	38.60	40.61	non sign.
60kg ha $^{-1}$(N2)	45.99	43.52	non sign.
120kg ha $^{-1}$(N3)	48.78	47.50	non sign.



Combination	Inoculated	Non-inoculated	LSD
dresssing	32.69	24.77	5.99**
non-dressing	24.61	20.35	4.12**
N fertilization:			
0kg ha $^{-1}$(N1)	21.42	15.03	5.86*
60kg ha $^{-1}$(N2)	28.70	22.99	4.47*
120kg ha $^{-1}$(N3)	35.84	29.67	4.75*

* significant differences at α=0.05
** significant differences at α=0.01

Fig. 2. Effect of inoculation with *Azospirillum brasilense* strain on wheat yields.

The concentration of chlorophyll dyes in cereal leaf blades was taken as an index of plant vigour in the vegetation season. Results presented in Fig. 1 indicate a higher vigour of the studied cereals under inoculation conditions. Plants from combinations inoculated with *Azospirillum brasilense* almost always were characterized by a higher chlorophyll concentration. Inoculation of crops caused a statistically significant increase of chlorophyll content in the case of oats in 1996 (15%) and wheat in 1997 (15%). Chlorophyll appeared to be a sensitive indicator of inoculation effect, which was also supported by yield size (Table 1).

Table 1. Yielding of crops inoculated with *Azospirillum brasilense* strain.

Plant cultivation	Yield (dt ha^{-1})			
	average	inoculated	non--inoculated	different
1996				
Wheat	44.2	44.4	43.9	0.5
Oat	42.9	44.2	41.6	2.6
1997				
Wheat	25.7	28.7	22.6	**6.1****
Oat	43.2	43.5	42.8	0.7

** significant differences at α = 0.01

The literature concerning the influence of inoculation on cultivated plants most frequently gives no examples of research work monitoring of physiological plant conditions during the vegetation season. The primary criterion of the inoculation effect is the mass of entire plants or their selected organs after harvest. Few exceptions include, for instance, the work by Nguyen et al. [32], in which the authors determine young plants of rice as taller, more vigorous and with leaves more green than those of the control. In the case of their experiment carried out in a tropical climate, differences were very distinct. However, in general they are small. Concentration of chlorophyll dyes is a reliable indicator of physiological plant condition, though frequently it is not duly appreciated in agricultural sciences [19]. In this experiment, determination of chlorophyll concentration appeared to be a very helpful indicator of inoculation effect in the vegetation season, which is easy to examine under field conditions.

A beneficial effect of cereal crop inoculation with *Azospirillum* strain under different cultivation conditions was also noted with regard to yield size. The yields obtained in 1996 and 1997 are presented in Table 1 and in Figures 2 and 3. Inoculation of cereals with *Azospirillum brasilense* contributed to the increase of mean yields, which constituted from 1% to 27% in the case of wheat and from 2% to 6% in the case of oats.

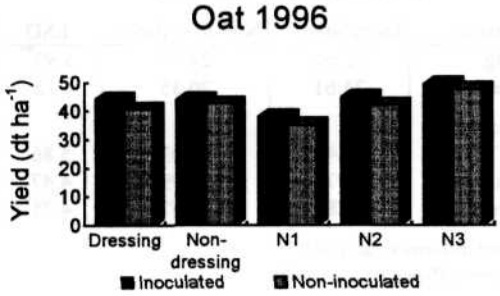
Higher yields from inoculated combinations were recorded in all cereals. However, statistically significant differences were noted only in 1997. The influence of inoculation with *Azospirillum* strains on the yield increase of cultivated plants was also shown by Bashan and Levanon [6]. However, the effect inoculation hastening caryopsis maturation reported by Nguyen et al. [32] in rice crops has not been observed in the present studies.

In the latest publication the authors find out a beneficial action on plant first of all in a combined action of different diazotrophs, VAM fungi and other rhizosphere microorganisms, the so-called PGPR ("plant growth promoting rhizobacteria") [7, 11, 12, 16, 18, 26, 40, 42, 43].

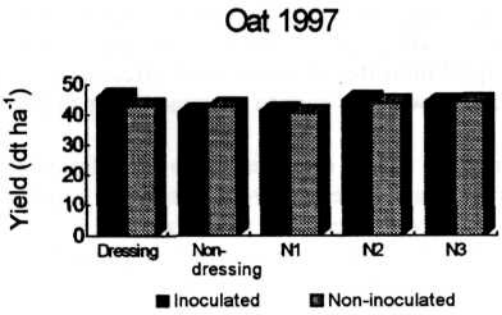
In connection with the discovery of a beneficial effect of inoculation with *Azospirillum brasilense* on the size of the studied cereal yield, qualitative yield studies were carried out. Table 2 presents a comparison of the mass of a thousand wheat and oat caryopses in inoculated and non-inoculated combinations in 1997.

Table 2. The mass of a thousand wheat and oat caryopses (g) in the experimental combinations, inoculated and non-inoculated with *Azospirillum brasilense.*

Plant cultivation	Combination		$LSD_{\alpha = 0.05}$
	inoculated	non-inoculated	
Wheat	34.44	32.60	non-significant
Oat	34.08	33.98	non-significant



Combination	Inoculated	Non-inoculated	LSD
dresssing	44.27	40.62	non sign.
non-dressing	44.05	42.55	non sign.
N fertilization:			
0kg ha $^{-1}$(N1)	38.10	35.08	non sign.
50kg ha $^{-1}$(N2)	44.90	41.96	non sign.
100kg ha $^{-1}$(N3)	49.49	47.70	non sign.



Combination	Inoculated	Non-inoculated	LSD
dresssing	46.09	42.80	3.18*
non-dressing	40.93	42.76	non sign.
N fertilization:			
0kg ha $^{-1}$(N1)	41.27	40.33	non sign.
50kg ha $^{-1}$(N2)	45.06	43.83	non sign.
100kg ha $^{-1}$(N3)	44.20	44.27	non sign.

* significant differences at α=0.01

Fig. 3. Effect of inoculation with *Azospirillum brasilense* strain on oat yields.

Caryopses from inoculated plots appeared to be larger in comparison to those from non-inoculated. However, that differentiation has not been statistically confirmed, and, therefore, it should be recognized that not only character, but first of all the number of generative shoots and the number of caryopses per ear were decisive factors which determined the increase of wheat yield under the effect of inoculation.

Lisowa et al. [28] studying the influence of wheat and spring barley inoculation with diazotrophs showed the yield increase, at the basis of which were a larger number of generative shoots produced by a plant and a larger mass of caryopses. On the other hand, a higher concentration of amino acids and crude protein were found by these authors only in the case of barley inoculated simultaneously with *Azospirillum* and *Enterobacter.* Also Nguyen et al. [32] found that at the basis of a very large yield rise of rice inoculated with strains from the genus *Azospirillum* was a larger mass of generative shoots and panicles and to a smaller degree - the mass of caryopses.

In our studies, such agrotechnical procedures as seed treatment with fungicides and application of nitrogen fertilizer were also noted to have a beneficial influence on wheat and oat yields. Cereal yields were higher and increased with an increase in application of nitrogen fertilizer (Fig. 2 and 3), which constitute an experimental regularity. Cereal seed treatment as well as nitrogen application to crops did not reduce the effect of inoculation.

Yield differences on a statistically significant level resulting from inoculation with *Azospirillum brasilense,* fungal seed-dressings and varying nitrogen application were noted only in wheat grown in 1997 (Fig. 2).

Results of chemical analyses of cereal grain for the content of crude protein and sugars have not been presented, since they were very even and the differences - statistically insignificant. That concerned the comparison between inoculated and non-inoculated combinations also on different levels of nitrogen application.

Conclusion

On the basis of obtained results it may be inferred that inoculation of cereals with *Azospirillum brasilense* can cause an increase in their yields. An expression of a beneficial action of *Azospirillum brasilense* during the vegetation season is a higher vigour of inoculated plants as compared to non-inoculated ones, manifesting itself in a higher chlorophyll concentration in the leaf blades.

Under soil-climatic conditions of our country, the effect of yield increase through inoculation with *Azospirillum brasilense* may appear to be more distinct and more unbiased when using new strains and inoculation technique ensuring the inoculated strains a larger chance in comptetiton with other soil microorganisms.

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