Polish Journal of Environmental Studies Vol. 9, No. 6 (2000), 505-509

Effect of Inoculation with *Azospirillum brasilense* on Development and Yielding of Maize *(Zea mays ssp. Saccharata* L.) under Different Cultivation Conditions

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Received: March 30,2000
Accepted- May 22, 2000

Abstract

The influence of inoculation with *Azospirillum brasilense* strain, two levels of nitrogen fertilizer, and seed treatment with fungicidal seed dressing on the development and yield of maize were determined in field experiments. In these studies nitrogenase activity, chlorophyll content in plants, yield size and quality were used as control parameters. It has been found that inoculation of maize crops with an active strain of *Azospirillum brasilense* has a beneficial effect on maize vigour and yield under the climatic and soil conditions of the Wielkopolska region.

Keywords: *Azospirillum brasilense,* diazotrophs, endophyts, inoculation, maize, fungicidal seed dressing.

Introduction

Among free-living microorganisms, which promise to be practically used in agriculture, bacteria from the *Azospirillum* genus as well as other endophyts are nowadays thought of as the most active component of associative dinitrogen fixation [2], particularly in tropical plant crops - sugar cane, oil-seed palm, rice, fodder grasses and cereals [2, 3, 4, 18, 25, 36].

Two species - *Azospirillum brasilense* and *Azospirillum lipoferum* were found in soils of a temperate zone [10, 19], and even in the cold climate of Finland [17]. According to Reyders and Vlassak [28], *Azospirillum* occurs in about 90% of tropical soils and in about 60% of soils in the temperate zone. *Azospirillum brasilense* is attributed to have affinity with plants with photosynthesis type C3 (wheat), whereas *Azospirillum lipoferum* - with plants of C4 type (maize). However, investigations of microorganisms isolated from under different crops and grasses in the area of Poland and other countries have shown that this relationship is not unique, since Krol [21] found the occurrence *of Azospirillum brasilennse* in maize rhizosphere, and Bashan et al. [7] even showed that different plant species can be inhabited by the same strain of *Azospirillum brasilense.*

In connection with numerous symptoms of a beneficial action of *Azospirillum* on plants, attempts have been made to use them practically in agriculture through inoculation of crops. The effect of inoculation with *Azospirillum* strains on the yield of cultivated plants has been demonstrated many times. However, the studies concerned mainly tropical climates.

The purpose of the present study is to study the effect of inoculation with *Azospirillum* strain, actively fixing dinitrogen, on maize crops. An analysis was performed against the background of varying nitrogen application and plant protection using fungicidal seed dressings. The effect of inoculation on dinitrogen fixation, chlorophyll content in leaf blades and on the maize cob yield were subjected to analysis.

Material and Methods

Field experiments were carried out in 1996 and 1997 in Zlotniki, in a field belonging to the Experimental and Didactic Station of August Cieszkowski Agricultural University of Poznań. The studies were performed on maize crops *(Zea mays ssp. saccharata* L.) of the cv. Gama. This species was selected on account of its ability to create associative relations with diazotrophs.

In the experiments performed in randomized block design the following three experimental factors were applied: inoculation with the 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 performed just before sowing: bacterium suspension was mixed with maize seeds and used for spraying the field. After that the field was harrowed to mix the applied suspension with soil. Additionally, after the emergences, at the developmental stage of 2-3 unfolded leaves, the suspension was individually applied as a strong stream under each plant in the vicinity of its roots. The number of *Azospirillum* cells per ml of the suspension amounted to 10^8-10^9 c.f.u. Such methods were used by different authors in field experiments many times [1, 16, 23, 33, 27, 28].

The applied numbers of nitrogen fertilizer: Nl - no nitrogen fertilizer applied (control), N2 - half the full dose (100 kg N ha^{-1}), N3 - full dose, which in terms of a pure ingredient amounted to 200 kg N ha^{-1} in the form of ammonium nitrate.

Maize kernels were treated with the fungicide Vitavax 200FS (active ingredients: 20.0% carboxine, 20.0% thiuram) before sowing. The experimental field was located on the soils of classes IVa and IVb. They presented loamy sands, medium-rich in potassium, phosphorus and magnesium, with a slightly acid reaction. In the study period the vegetation conditions were favourable to plants; however, unexpected showers which took place in the first year of the studies (1996) caused "washing out" of the upper soil layer on many maize plots, mainly in combinations where inoculations with *Azospirillum brasilense* were applied. That had a decisively negative effect on plant growth and development, leading to reduction of their yield, which could not be analyzed for that reason.

The following aspects were subjected to analysis:

- dinitrogen fixation,
- maize vigour at full vegetation development,
- cob yield and size.

During the vegetation season, dinitrogen fixation was determined three times at the following developmental stages of plants: 6-8 leaves, beginning of tassel emergence, flowering. Dinitrogen fixation was analyzed on the basis of nitrogenase activity by acetylene to ethylene reduction method modified by Sawicka [29]. Determinations for each experimental combination were made in three replications. Gas analysis was carried out on a gas chromatograph, type: CHROM 5.

Plant vigour was estimated on the basis of chlorophyll concentratration. Vigour was determined at full vegetative development, that is, at the beginning of tassel emergence. Chlorophyll content was estimated in 30 leaf blades counting them from the top, i.e. in the youngest, but already fully developed leaves. The analysis was performed using the colorimetric method [26].

The final criterion for the analysis of particular combinations of the field experiment was the yield of cobs devoid of outer leaves. Besides yield size, the mass of developed cobs was also measured.

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

Results and Discussion

Analyses carried out three times in the vegetation season showed no nitrogenase activity in the soil under grown plants in any experimental combination. However, taking into consideration the yield increase and chlorophyll concentration in maize leaf blades under the influence of inoculation presented in this paper, it cannot be excluded that dinitrogen fixation took place. Many field experiments have been described, including those conducted under conditions of temperate climate, in which dinitrogen fixation was found in maize crops [10, 30, 31]. The sum of fixed dinitrogen consists of the activity of various diazotrophic microorganisms; nevertheless, Chalk [9] found that bacteria from the genus *Azospirillum* can annualy fix 30-40 kg of dinitrogen in association with grasses and up to 200 kg in association with sugar cane.

For an index of plant vigour in the vegetation season, the concentration of chlorophyll dyes in maize leaf blades was taken. Concentration of chlorophyll dyes is a reliable index of physiological plant condition [15], though frequently it is not duly appreciated in agricultural sciences. Results presented in Fig. 1 point to its higher vigour under inoculation conditions. Plants from combinations inoculated with *Azospirillum brasilense* were characterized by a higher chlorophyll concentration. The chlorophyll concentration increase as a result of inoculation in 1997 was 25% higher and appeared to be statistically highly significant. Differences in chlorophyll concentration were in agreement with size of the obtained yields (Fig. 2).

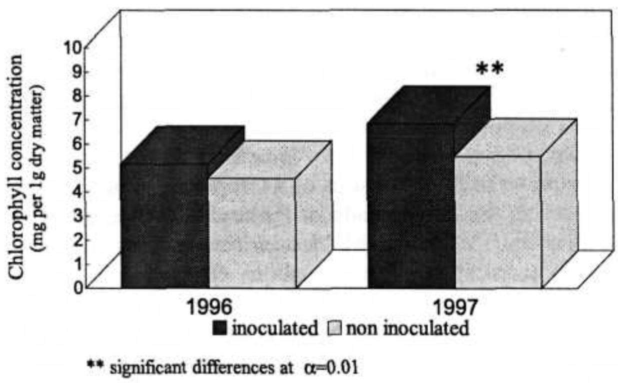
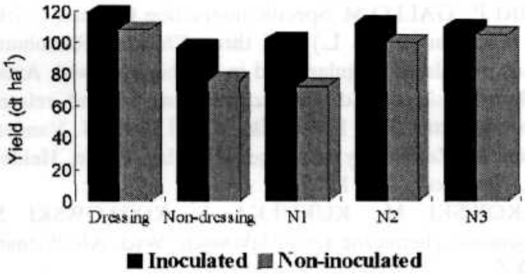


Fig. 1. Effect of inoculaion with *Azospirillum brasilense* on chlorophyll content in maize leaf blades.



Combination	Yield of cultivars (dt ha^{-1})		LSD
	inoculated	non-inoculated	
	Azospirillum brasilense		
Dressing	118.62	108.23	non sign.
Non-dressing	96.74	76.15	20.06*
N - fertilization			
N1 (0kg ha^{-1})	101.68	72.26	26.12**
N2 (100kg ha^{-1})	111.68	99.86	non sign.
N3 (200kg ha^{-1})	109.69	104.45	non sign.
Average	107.68	92.19	12.45*

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

Fig. 2. Effect of inoculation with *Azospirillum brasilense* on maize yields under different cultivation conditions

Literature concerning the influence of inoculation on cultivated plants frequently gives no examples of studies monitoring plant physiological conditions in the vegetation season. The primary criterion of inoculation effect is the mass of entire plants or their selected organs after harvest. Few exceptions include, for instance, the paper by Nguyen et al. [24] in which the authors define inoculated rice plants as taller, more vigorous, with the leaves more green than non-inoculated control plants. In the case of their experiment, conducted in a tropical climate, differences were very distinct. However, in general they are small and it is indispensable to find such a sensitive estimation without unreasonable interference in plant or experiment. In connection with the above, chlorophyll concentration appears to be a very suitable index of inoculation effect in the vegetation season, which is easy to examine under field conditions due to the present-day optico-electronic indicating devices.

The basic determinant of the effect of maize inoculation with *Azospirillum brasilense* under different cultivation conditions was yield size. In 1996, yield size was not analyzed because of water erosion of soil on part of the experimental field (described in Material and Methods).Yields obtained in 1997 are presented in Fig. 2. Inoculation with *Azospirillum brasilense* significantly contributed to the increase of maize average yield by 17%. That increase was independent of the experimental combination, though differences were not always statistically significant. The highest, 27% yield increase under the effect of inoculation was found in combinations not treated with a fungicide as well as in combinations without the application of nitrogen fertilizer. In the second case the yields from the inoculated plots were 41% higher.

As the application of nitrogen fertilizer increased, the effect of inoculation with *Azospirillum brasilense* on maize yield was less and less distinct. When the application of nitrogen fertilizer was on the level of 100 kg ha"^{1} (N2), the yield from inoculated plots was only about 12% higher, whereas at the application of nitrogen fertilizer at the rate of 200 kg ha^{-1} (N3) the yield was only 5% higher. These results indicate that inoculation with *Azospirillum brasilense* may contribute to maize yield increase, particularly under the most diffficult cultivation conditions, when plant access to mineral nitrogen in soil is resricted.

Maize (non-inoculated) yields were higher in treated combinations and increased with an increase of nitrogen application, which constituted an experimental regularity and was not subjected to further analysis (Fig. 2).

The effect of inoculation with *Azospirillum* strains on the yield increase of cultivated plants was also shown by Bashan and Levanon [6]. However, the inoculation effect on hastening caryopsis maturation in rice crop, which was reported by Nguyen et al. [24], was not observed in the present studies. In the latest publications the authors see a beneficial action on plant first of all in a combined action of different diazothrops, VAM fungi and other rhizosphere microorganisms, the so-called PGPR - "plant growth promoting rhizobacteria" [8, 11, 12, 13, 14, 20, 32, 34, 35].

In connection with the revealed beneficial effect of inoculation with *Azospirillum brasilense* on the size of cob yield it was checked whether that increase was determined by an increase in mean cob mass. Table 1 presents a comparison of the corn cob mass in inoculated and non-inoculated combinations in 1997.

Table 1. Corn cob mass in experimental combinations inoculated and non-inoculated with *Azospirillum*

Combination	Cob mass (g)
Inoculated	201.00
Non-inoculated	192.00
$LSD_{\alpha = 0,05}$	8.14

It was found that cobs produced on the inoculated plots were larger in comparison to those from the non-inoculated plots. The increase in cob size significantly determined a higher yield for the inoculated crops. In the case of other cereals the authors saw yield increase chiefly in a larger number of generative shoots and in larger inflorescences [22, 24].

Conclusions

On the basis of the obtained results the following conclusions may be given:

1. Inoculation of maize with *Azospirillum brasilense* bacteria contributed to an increase of that plant vigour and yield.

2. The chlorophyll content in maize leaf blades is a suitable indicator of plant vigour during the vegetation season.

Acknowledgements

These studies were financed by the Committee for Scientific Research (project No. 5PO6B 081 10).

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