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Agronomic Efficiency of *Azospirillum brasilense* in Physiological Parameters and Yield Components in Wheat Crop

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Abstract: Inoculation of seeds with bacteria of the genus *Azospirillum* have been held in some grasses but with results still not widespread in the wheat crop in Brazil and worldwide. Given the above, the purpose of this study was to evaluate the agronomic efficiency of *Azospirillum brasilense* in physiological parameters and yield components of wheat under reduced nitrogen fertilization. In this context, we conducted two experiments, experiment 1: Maringa, PR and experiment 2: Alto Piquiri, PR, in randomized block design with four replicates using a wheat cultivar CD 150. The treatments consisted in the use of nitrogen fertilizer (control, 50 and 100 kg ha^{-1}) associated with inoculation of seeds with bacteria of the species *Azospirillum brasilense* in liquid form, in different doses (50, 100, 150 and 200 mL of *Azospirillum*). The results obtained in two experiments showed that the use of half level of nitrogen associated with the inoculation of seeds with *Azospirillum brasilense* promoted positive results on the agronomic performance and productivity of wheat.

Key words: *Triticum aestivum* L., inoculation, nitrogen, productivity, *Azospirillum brasilense*

INTRODUCTION

It is known that the majority of Brazilian arable soils have low levels of available nitrogen, necessitating the application of nitrogen fertilizers. However, its use is limited by the dynamic character of this nutrient in the soil (Paul and Clark, 1989), to be its availability in the soil affected by several factors such as soil microbial activity, soil moisture, soil temperature, matter the soil, leaching, soil texture and soil management (Azam, 2002).

Another important process that influences the availability of nitrogen is the biological fixation of atmospheric nitrogen. According to Moreira and Siqueira (2002), the fixation of atmospheric nitrogen by microbes is the main route of adding nitrogen in the soil-plant system, contributing to about two times more than the application via mineral fertilizer. However, the contribution of biological fixation of atmospheric nitrogen is low for the crop, requiring supplementation with nitrogenous fertilizer to meet crop nitrogen needs.

The great interest in the biological fixation in grasses is due to the ease of use of water of the same in respect of pulses, the more effective because they are photosynthetic and cereals used as food by man. Grasses have fasciculate roots, having advantages over the system of pivoting pulses to extract water and nutrients from the soil. Therefore, even if only a part of the nitrogen was supplied by fixing bacteria associated with the economy as a nitrogen fertilizer would be equal to or higher than that with the legumes that can be self-sufficient in nitrogen (Dobereiner, 1992).

In the last 20 years was found out about the potential of micro-aerobic diazotrophs of the genus *Azospirillum* ssp., nitrogen-fixing, when in the wild (Boddey and Dobereiner, 1995) which, when combined with the rhizosphere of plants, can contribute with the nitrogen nutrition of these plants, making it the subject of study by researchers in biology and soil fertility. Thus, the proper handling of this possible association *Azospirillum* spp. with the wheat crop could result in increased productivity and lower costs of production, mainly from the acquisition of nitrogen fertilizers (Okon and Vanderleyden, 1997).

The purpose of this of this study was to evaluate the agronomic efficiency of *Azospirillum brasilense* in physiological parameters and yield components of wheat under reduced nitrogen fertilization.

MATERIALS AND METHODS

Field experiment: Were conducted two field experiments in the State of Parana, Brazil, in 2011. The experiment 1 was installed at the Experimental Farm of Maringa State University, located in Maringa, Parana State, Brazil, located at latitude 23°25' south and longitude 51°57', with

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an average altitude of 540 m. The experiment 2 was installed in the city of Alto Piquiri, Parana State, Brazil, located at latitude 23°59' south and longitude 53°29', with an average elevation of 350 m. The climate in the region is Cfa, humid mesothermal, according to Koppen classification (Agronomic Institute of Parana, 1987). Data on rainfall, occurring during the conduct of experiments Maringa and Alto Piquiri, are shown in Table 1.

In both experiments were collected soil samples from the topsoil from 0 to 0.20 m depth to perform the analysis of soil chemistry and physics, presented in Table 2.

Establishment of the experiment: The date of sowing experiment Maringa and Alto Piquiri was 27th April 2011 and 28th April 2011, respectively, using cultivar CD 150 with population density of 300 seeds m^{-2}. Plots consisted of 15 rows with five meters in length and spacing of 0.15 m.

The treatments and experimental design: The experimental design was randomized blocks with four repetitions and seven treatments: T1-control (without N and without *Azospirillum* spp); T2-100 kg N ha^{-1}; T3-50 kg N ha^{-1}; T4-50 kg N ha^{-1}+50 mL of *Azospirillum* spp.; T5-50 kg N ha^{-1}+100 mL of *Azospirillum* spp.; T6-50 kg N ha^{-1}+150 mL of *Azospirillum* spp.; T7-50 kg N ha^{-1}+200 mL of *Azospirillum* spp. Nitrogen levels were distributed 1/3 at sowing and 2/3 in coverage and levels of *Azospirillum* spp. were related to 50 kg of wheat seed.

Parameters measured: The dry biomass of plants was determined in roots and shoots after physiological maturity. Samples were taken from 10 plants per plot collected randomly in the experimental area. They were then placed in paper bags and taken to dry in an oven with forced air at 65°C until they reached constant weight after were weighed on a precision balance with two decimal places, resulting in dry biomass of shoot and roots.

After the stage of physiological maturity 10 plants were selected randomly within the usable area of the parcels for the following ratios: number of spikes m^{-2}, number of spikelets $spike^{-1}$ and grain number $spike^{-1}$.

At harvest, plants were harvested manually, five to eight days after the stage of maturation. The cobs were shelled in stationary threshing machine, cleaned with the aid of screens and digital impurities picker, model ME-06, brand MEDIZA, dried in natural conditions and stored in paper bags. The moisture content of seeds was determined by the method of oven at 105°C for 24 h (Brasil, 2009).

Starting with the seed yield in plots, the estimated yield in kg ha^{-1} for each treatment. Then the mass was determined by weighing a thousand seeds of eight subsamples of 100 seeds for each repetition of the field with the aid of an analytical balance with precision of a milligram, multiplying the result by 10 (Brasil, 2009). The results were expressed as grams of seeds (g). The Weight Hectoliter (WH) was determined in the balance Dalle Molle second method described in Brasil (2009), in which the results were standardized to 13% moisture (Puzzi, 1986).

Table 1: Climatic weather monthly rainfall, maximum and minimum temperatures, in Maringa (MA) and Alto Piquiri (AP), State of Parana, Brazil, in 2011

	Atmospheric temperature (°C)					
	MA		AP		Rainfall (mm)	
Months	Max. T.	Min. T.	Max. T.	Min. T.	MA	AP
March	28.1	19.3	27.3	18.5	129.5	159.5
April	27.4	17.8	26.6	16.9	111.1	124.1
May	25.1	13.7	24.3	12.9	7.5	11.8
June	22.3	10.4	21.5	9.6	136.8	158.8
July	23.6	14.1	22.8	13.3	137.8	166.1
August	25.9	14.3	25.1	13.5	32.8	47.5

Table 2: Results of chemical analysis and physical soil of the experimental area, the topsoil from 0-0.20 m depth, before the implementation of the wheat in Maringa (MA) and Alto Piquiri (AP), State of Parana, Brazil, in 2011

City	[1]pH	[2]Ca^{+2}	[2]Mg^{+2}	[3]K^{+}	Al^{+3}	CTC	[4]C g dm^{-3}	V (%)	[3]P mg dm^{-3}
		(cmol$_c$ dm^{-3})							
MA	4.7	2.68	0.99	0.36	0.0	7.45	9.12	54.09	7.90
AP	5.6	2.38	0.96	0.15	0.0	5.85	8.74	59.65	39.2

City	Thick sand	Fine sand	Clay	Silt
	(%)			
MA	51	16	28	5
AP	42	43	13	2

[1]$CaCl_2$ 0.01 mol L^{-1}; [2]KCl 1 mol L^{-1}; [3]Extrator Mehlich 1; [4]Matodo Walkley-Black

Statistical analysis: The results obtained in this study were analyzed, considering the recommendations of Gomes (2000), in which experiments of this nature, the most appropriate scheme of analysis is the joint analysis of experiments, it is necessary to verify the homogeneity of the mean squares waste; if the ratio between the highest $QM_{residue}$ and lowest $QM_{residue}$ is less than 7 (Banzatto and Kronka, 2008), we can adopt the joint analysis of experiments.

All statistical analysis was performed using the SAS Institute (1996) statistical software. The data, after being subjected to analysis of variance and statistical differences observed when the treatment means were tested using the Scott and Knott (1974) at 5% probability.

RESULTS AND DISCUSSION

Table 3 presents the data on the agronomic performance and yield components of wheat cultivar CD 150 in the city of Maringá and Alto Piquiri, Parana State,

Table 3: Results of the number of spikelets spike^{-1} (NSS), number of grains spike^{-1} (NGS), shoot dry mass (SDM), root dry mass (RDM), number of ears m^{-2} (NE), weight of 1000 grains (WG), weight hectoliter (WH) and grain yield (YIELD) cultivar CD 150 in the experiments of Maringa (MA) and Alto Piquiri (AP), State of Parana, Brazil, in 2011

	NSS		NGS		SDM (g)		RDM (g)	
TRAT	MA	AP	MA	AP	MA$^{n.s.}$	AP	MA$^{n.s.}$	AP
T1	11.0^{b}*	09.8^{b}	21.8^{b}	19.3^{b}	1.59	1.03^{c}	0.11	0.10^{b}
T2	12.3^{b}	12.0^{b}	29.5^{b}	27.0^{b}	2.43	2.43^{a}	0.22	0.24^{a}
T3	12.3^{b}	11.3^{b}	27.0^{b}	29.5^{b}	2.13	2.05^{a}	0.20	0.16^{b}
T4	12.8^{b}	11.8^{b}	26.5^{b}	24.3^{b}	2.30	1.63^{b}	0.15	0.16^{b}
T5	12.3^{b}	11.0^{b}	28.3^{b}	24.8^{b}	2.16	2.40^{a}	0.19	0.17^{b}
T6	12.3^{b}	11.8^{b}	27.5^{b}	21.5^{b}	2.06	2.25^{a}	0.22	0.22^{a}
T7	15.0^{a}	15.3^{a}	35.8^{a}	42.5^{a}	2.47	2.53^{a}	0.19	0.25^{a}
C.V.(%)	09.54		13.12		16.33		11.06	

	NE (m^{2})		WG (g)		WH (kg hL^{-1})		YIELD (kg ha^{-1})	
TRAT	MA$^{n.s.}$	AP$^{n.s.}$	MA$^{n.s.}$	AP $^{n.s.}$	MA	AP	MA	AP
T1	527	480	26,8	27,5	74.5^{b}	74.5^{b}	1245^{b}	1328^{d}
T2	575	600	32,8	34,0	79.0^{a}	79.0^{a}	2791^{a}	3212^{a}
T3	565	516	30,3	31,0	77.5^{a}	77.8^{a}	1834^{b}	2099^{c}
T4	510	598	32,0	32,3	78.5^{a}	78.5^{a}	2505^{a}	2533^{b}
T5	542	485	31,5	30,8	78.8^{a}	79.3^{a}	2182^{a}	2171^{c}
T6	555	614	31,5	31,8	78.5^{a}	78.0^{a}	2499^{a}	2623^{b}
T7	560	588	32,3	31,3	78.8^{a}	79.0^{a}	2462^{a}	2750^{b}
C.V.(%)	9.81		8.36		1.01		13.37	

*Mean followed by same letter in column do not differ at 5% probability by the test of Scott and Knott (1974). $^{n.s}$Not significant

Brazil. Analysis of variance of the data revealed that the characters number of spikes m^{-2} and 1000 grain weight were not influenced ($p>0.05$) by different levels of *Azospirillum brasilense* and neither the levels of nitrogen applied in both experiments.

For the characteristics of the dry mass of shoot and root dry weight were not significant differences ($p>0.05$) for the experiment carried out in Maringá. However, for the experiment conducted in Alto Piquiri observed for the variable shoot dry mass than the T2, T3, T5, T6 and T7 were significantly superior to T4, while the worst result in this variable was observed in the control (T1), as was expected and, with respect to dry mass of root treatments T2, T6 and T7 were those showing the greatest accumulation of dry biomass of the root system.

Note, that for the Alto Piquiri experiment, the use of inoculant levels of 150 and 200 mL of *Azospirillum* spp., Associated with 50 kg N ha^{-1} (treatments T6 and T7, respectively) showed results comparable with the level of 100 kg N ha^{-1} (T2). Therefore, the results obtained allow us to infer that seed inoculation with *Azospirillum* spp. is a positive practice to increase the dry weight of roots, as well as economically feasible, to allow up to 50% reduction in nitrogen fertilization of wheat.

The variables number of spikelets spike^{-1}, number of grains spike^{-1}, weight hectoliter and yield significant differences in the 5% level of probability, in two experiments. It was observed for both experiments the number of spikelets spike^{-1} and number of grains spike^{-1} for the treatment T7 (50 kg N ha^{-1}+200 mL of *Azospirillum* spp.) Was significantly higher than others, including compared to the absolute control (T1).

For information regarding Table 3 shows that the component that most influenced ($p<0.05$) increase in grain yield of wheat was the weight hectoliter.

In the evaluation of grain yield, is observed for the experiment in Maringa the T1 and T3 (control and 50 kg N ha^{-1}, respectively) had the lowest average, with this, T2, T4, T5, T6 and T7 were those who had the best average earnings. A different result was obtained in the experiment Alto Piquiri, where he observed that treatment T2 (100 kg N ha^{-1}) was significantly higher than others and the treatments T4, T6 and T7 showed greater than average productivity T3 and T5.

Diaz-Zorita and Fernandez-Canigia (2009), after evaluating the efficiency of *Azospirillum brasilense* in wheat seeds 297 experiments in the region of the Pampas in Argentina, report that the inoculated plants showed more vigorous growth with further expansion of root surface area and greater accumulation of dry matter (12.9 and 22%, respectively). Inoculation also increased the number of harvested grain yield and 6.1 and 8.0%, respectively. According to Hungria (2011), eight trials evaluated strains Ab-V5 and Ab-V6 of *Azospirillum brasilense* in liquid and peat and observed a 26% average increase in productivity of corn and 31% in wheat crop.

The treatments T6 and T7, compared with T1 (absolute control), allowed yield increments of approximately 97.8 and 100.7% higher than the control (T1), respectively, for the experiment in Maringa. Regarding the experiment of Alto Piquiri this increase was 97.5 to 107.1% for T6 and T7, compared to T1 (absolute control). Similar results were obtained by Sharief *et al*. (2006) who observed an increase in rice yield of 33.2 and 32.8% with the interaction of *Azospirillum* and levels of 45 and 60 kg N ha^{-1}, respectively, over the level of 15 kg N ha^{-1} without *Azospirillum*.

From the data obtained in this study we can infer that the inoculation of wheat seeds with levels of 150 and 200 mL of *Azospirillum* spp., Associated with 50 kg N ha^{-1} (treatments T6 and T7), was better than the isolated application of 50 kg N ha^{-1} (T3). Therefore, these treatments were effective in increasing the yield of wheat cultivar CD 150, using the same level of nitrogen fertilization. This result is relevant, since the inoculation of seeds with *Azospirillum brasilense* ensures excellent levels of productivity, minimizing costs of production in the culture of wheat. Similar results were obtained by Hungria *et al*. (2010) for the cultivation of wheat and Cavalett *et al*. (2000) for maize.

CONCLUSIONS

The management of seed inoculation of wheat with *Azospirillum brasilense* can ensure a reduction in production costs with increased productivity of the crop. The application of half the level of nitrogen associated with the use of different levels of inoculum tested in different soil and climatic conditions, provides positive results on the agronomic performance and productivity of wheat.

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