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Effect of different doses of *Azospirillum brasilense* and nitrogen fertilizer in wheat crop

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The objective of the present work is to evaluate the effect of different doses of nitrogen combined with different doses of *A. brasilense* in the agronomic characteristics of wheat. The experiment was conducted in Curuguaty, Paraguay in 2017 and the variety used was Canindé 11. The experimental design is a completely randomized block with factorial ordering and four repetitions. The treatments are a combination of: three doses of N, 0, 40, 80 kg ha^{-1} and 4 doses of *A. brasilense,* 0, 700, 1000 and 1300 mg kg^{-1} of wheat seeds. The variables analyzed were plant height, number of spikes per plant, thousand grain weight, hectoliter weight and yield. With the exception of plant height, significant interaction between treatments was observed in all variables. Inoculation with 1300 mg kg^{-1} of *A. brasilense* associated with the application of 130 kg N ha^{-1} promotes the highest yields of wheat grains.

Key words: *Triticum aestivum,* dystrophic bacterium, winter cereal.

INTRODUCTION

Wheat (*Triticum aestivum* L.) belongs to the family of gramineae whose center of origin is the Middle East (Garcia, 2005). It is the most important cereal in the world for its physical and chemical properties of gluten for manufacturing of bread and use in large quantities for pastry and other foods. The use of nitrogen fertilizers in the world increases year after year but has a very low assimilation rate for crops, losing more than 50% of the soil (up to 80%) by leaching, resulting in contamination (eutrophication of water mantles) and the increase in the cost of production. To appease this event there are microorganisms that have the ability to promote the growth of plants by providing nutrients such as nitrogen through biological fixation. In Paraguay, since 2003 the wheat crop reached a great growth in areas of sowing and export. In the year 2017, approximately 430000 ha was planted with an average yield of 1630 kg ha^{-1} and production of 700000 tons of grain (CAPECO, 2017). The sustainable production of the crop can be fulfilled with the combination of several factors of production such as adequate tillage, sowing, cultivation care, use of certified seeds, phytosanitary measures and good fertilization of the crop; the latter is essential and aims to replenish the nutrients to the soil that have been extracted by crops,

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with adequate amounts of nutrients you can get higher return, but without decreasing soil fertility (Mellado, 2008). Among the inputs used, nitrogen fertilization represents a large part of the productive costs and its application in cereals, such as wheat, significantly raises the cost of production. However, it is essential, because of nitrogen, to be the most limiting macro element in wheat productivity since it determines the number of tillers, being essential in the nodes formation phase and at the beginning of stem elongation (Sala et al., 2005). Thus, due to the growing search for sustainability in agricultural production systems, some authors have presented, as an alternative form of nitrogen fertilizer economics, the biological nitrogen fixation (BNF), which may supplement or even replace the utilization of these fertilizers (Baldani and Baldani, 2005; Bergamaschi et al., 2007, Hungria et al., 2010). The use of bacteria such as *A. brasilense* can reduce the fertilizer application level by 40-50%, which reduces the cost of production without a decrease in the yield of the crop, betting on a sustainable production of the wheat crop. These bacteria can directly stimulate the growth of plants, through various mechanisms such as the capacity of fixation of atmospheric nitrogen, production of phytohormones, solubilization of minerals and nutrients, increase in the volume of the root and induction of resistance to pathogens. The objective of this work was to evaluate the effect of inoculation with *A. brasilense*, associated with N rates and the grain yield of wheat.

MATERIALS AND METHODS

The study was conducted in the municipality of Curuguaty, in the state of Canindeyú, Paraguay (24°28'18'' S and 55°41'32'' W, at an altitude of 335 m). The soil in the experimental area was classified as a Rhodic paleudult, Ultisol order. The area has been cultivated with annual crops for more than 12 years, and the no-tillage system has been used for the past 8 years. Since the crop planted before wheat sowing was corn, only soybean and corn were cultivated in the 2 years before the study. The average temperature was 23°C, and the annual average precipitation was 1,300 mm (Figure 1). The climate type is Cfa, according to Köppen's classifications, subtropical humid mesothermal dry winter with rainfall well distributed throughout the year and hot summers. The experimental design was randomized complete blocks with four replicates, in a 3×4 factorial arrangement: three N doses: 0, 40, 80 kg ha^{-1}; four *A. brasilense* rates: 0, 700, 1000, and 1300 mg kg^{-1} of wheat seed. The experimental plots were composed of 10 lines with 5 m length and spaced at a 0.20 m distance, and the useful area of the plot comprised the central eight lines, excluding 0.5 m from the ends. The herbicide used in the experimental areas was glyphosate (1,600 g a.i. ha^{-1}) for desiccation; this product was applied 2 weeks before wheat sowing.

The chemical attributes of the topsoil (depth of 0.00–0.20 m) were determined in 2017 before the initiation of the experiment, following the methodology proposed by Raij et al. (2001). The following results were obtained: 11.45 mg dm^{-3} P (resin); 7.37 mg dm^{-3} S-SO_4; 19.38 g dm^{-3} organic matter; pH 5.40 ($CaCl_2$); 0.27 3.45, 0.61, and 3.18 Cmol$_c$ dm^{-3} K, Ca, Mg, and H+Al, respectively; 0.9, 2.5, 78.8, and 5.2 mg dm^{-3} Cu, Fe, Mn, and Zn, respectively and base saturation of 57.63%. Based on the results of soil analysis and the need to increase base saturation to 70%, as recommended by Cantarella et al. (1997), 2.5 Mg ha^{-1} of dolomitic limestone (ECCE 88%) was applied to the soil 50 days before sowing wheat. Furthermore, based on the results of soil analysis and culture requirements, 28, 98, and 56 kg ha^{-1} N, P_2O_5, and K_2O, respectively, were supplied for sowing fertilization. Inoculation of wheat seeds with *A. brasilense* (to achieve a density of 2×10^8 CFU mL^{-1}) was performed using 0, 700, 1000 and 1300 mL of the liquid inoculant Azototal per kilogram of wheat seed. The inoculant was mixed with the seeds in the shade using a clean mixer 1 h before sowing after treatment of the seeds with insecticide and fungicide. For seed treatment, the fungicides carbendazim + thiram (45 g + 105 g a.i. per 100 kg of seed) and the insecticides imidacloprid + thiodicarb (45 g + 135 g a.i. per 100 kg of seed) were used. The experimental area was managed under a no-tillage system. The cultivar used was Caninde 11 with mechanical seeding on 5/10/2016 and density of 76 seeds per meter. The growth of weeds was managed with the application of the herbicide metsulfuron-methyl (3 g a.i. ha^{-1}) 30 days after emergence (DAE) of wheat. Nitrogen fertilization was performed manually 35 DAE, and the fertilizer was spread on the soil surface without incorporation on the sides and at approximately 10 cm from the sowing lines to avoid contact with the plants. The plants were harvested manually at 108 DAE. Plant height at maturity (defined as the distance (m) from the ground level to the apex of the spike) was determined. The following characteristics were also evaluated in ten plants at harvest: number of spikelets, by counting all spikelets with grains; hectoliter mass, corresponding to the mass of wheat grains in a 100-L container determined on a 1/4 scale after adjusting the water content of the grains to 13% (wet basis); mass of 1000 grains, measured in a 0.01-g precision scale at 13% (wet basis); and yield, determined by counting the spikes of plants present in the four useful lines of each plot. After mechanical tracking, the grains were quantified, and the data were converted into kg ha^{-1} at 13% (wet basis). All statistical analyses were performed using the SISVAR statistical program (Ferreira, 2003). The data obtained were submitted to variance of analysis and when statistical differences were verified, their averages were obtained using Tukey test at 5% probability.

RESULTS AND DISCUSSION

Of the analyzed variables, all presented a significant interaction among the factors using doses of *A. brasilense* associated with nitrogen doses, with the exception of plant height, in which significantly different variances were observed for N doses (Table 1).

The different doses of *A. brasilense* did not significantly affect the height of wheat plant (Table 2). Similar results were found by Barbieri et al. (2012) that concluded there was no influence of inoculation with *A. brasilense* in plant height with irrigation. Also, Ferreira et al. (2014) and Galindo et al. (2015) observed no influence with the inoculation of wheat leaves with *A. brasilense* in the Cerrado Region. These results disagree with Bashan et al. (2004), who demonstrated that *Azospirillum* spp. stimulates the growth and productivity of plants like wheat. There is evidence literature that supports the beneficial root bacteria inoculated in wheat; also in rice, oats, maize, sorghum and other grasses (Dos Reis et al., 2000; Pan et al., 2002) exert an effect growth, because the BBR uses the organic compounds excreted by the

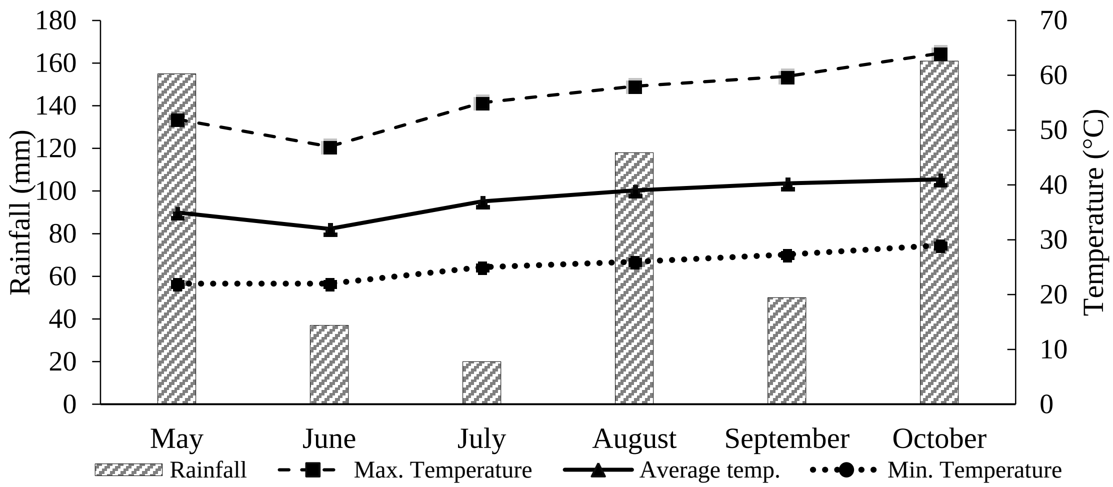

Figure 1. Rainfall, average, maximum and minimum temperature during wheat cultivation from May to October 2017 obtained from the weather station located in the municipality of Curuguaty, in the state of Canindeyú, Paraguay.

Table 1. Summary of analysis of variances for plant height (PHEIG), number of spikes per plant (NESP), thousand grains weight (TGRW), hectoliter weight (HECW) and yield (YIELD) of wheat using different doses of *A brasilense* and N.

Source of variation	DF	Mean sum of squares				
		PHEIG	NESP	TGRW	HECW	YIELD
A. brasilense rates	3	20.13^{ns}	0.33**	0.61^{ns}	38.30**	775060.68**
N rates	2	181.15**	0.31**	9.11**	11.40**	2903617.04**
Interaction	6	6.82^{ns}	0.08**	3.99*	2.64**	369520.22**
Error	33	10.82	0.02	1.41	0.47	24362.74
CV (%)		5.95	9	3.25	0.81	7.74
Average		55.27	1.74	36.55	84.95	2016.41

Table 2. Height of wheat plants submitted to inoculation of different doses of *A. brasilense* and N under field conditions.

A. brasilense **doses (mg kg^{-1} seed)**	**Plant height (cm)**
0	54.48
700	53.87
1000	56.15
1300	56.57
DMS	3.63
N doses (kg ha^{-1})	
0	51.40
40	56.86
80	57.54
DMS	2.85
CV (%) = 5.95	

Source: *Curuguaty (*2017*)*.

roots of wheat as a source of carbon and energy into substances that can stimulate greater radical absorption of the N. However, plant height was affected by the different doses of N where the highest height was

Table 3. Number of spikes per wheat plant submitted to inoculation of different doses of *A. brasilense* and N under field conditions.

N doses (kg ha^{-1})	*A. brasilense* doses (mg kg^{-1} seed)			
	0	700	1000	1300
0	1.37^{bB}	$1.62^{b\,AB}$	$1.60^{b\,AB}$	$1.77^{b\,A}$
40	1.75^{aB}	$1.70^{b\,B}$	$1.47^{b\,B}$	$2.10^{a\,A}$
80	$1.62^{ab\,B}$	1.97a^{A}	$1.87^{a\,AB}$	$2.02^{b\,A}$
CV (%) = 9.00				

Means followed by the same letter, lowercase in the columns and upper case in the lines, do not differ by the LSD test (t test) at the 5% probability level.
Source: Curuguaty (2017).

Table 4. Hectoliter weight in grams of wheat submitted to inoculation of different doses of *A. brasilense* and N under field conditions.

N doses (kg ha^{-1})	*A. brasilense* doses (mg kg^{-1} seed)			
	0	700	1000	1300
0	80.43^{cC}	84.10^{bB}	85.60^{aA}	86.40^{aA}
40	82.82^{bC}	84.58^{abB}	85.63^{aBC}	86.74^{aA}
80	84.32^{aB}	85.70^{aA}	86.48^{aA}	86.72^{aA}
CV (%) = 0.81				

Means followed by the same letter, lowercase in the columns and upper case in the lines, do not differ by the LSD test (t test) at the 5% probability level.
Source: Curuguaty (2017).

observed with the dose of 80 kg N ha^{-1}. These results coincide with those of Zagonel et al. (2002), who verified that with the increase of the dose of N increase of the height of plants of wheat occurs. However, Zagonel and Fernandes (2007) found varied responses of cultivars to increase the dose of N for plant height which also suggests the influence of genetic factors. For Castro et al. (2008), plant height is influenced by the availability of nitrogen in the soil, since this nutrient participates directly in cell division and expansion and the photosynthetic process, which would explain the positive response of the N doses applied in the corn crop height growth; it corroborates with Gross et al. (2006), who recommend that nitrogen fertilization done in coverage, in one application or two, influences plant height. However, this increment is not favorable, since the greater length of the plants is usually accompanied by a greater predisposition to lodging.

Significant interaction between doses of N and *A. brasilense* was observed for the number of ears per plant (Table 3). The best results were 2.10 ears per plant with the combination of 40 kg ha $^{-1}$ of N and 1300 mg of *A. brasilense* per kg of seeds (Table 3). According to Lopes et al. (2007), the number of ears per area is dependent on the genotype, where fertilization does not become a preponderant factor in the final result, corroborating with the results obtained in this study. On the other hand, Espindula et al. (2010) observed a linear increase in the number of ears of wheat at doses of N of 0, 40, 60, 80, 100 and 120 kg N ha^{-1}. This is probably because the higher doses of N promote greater vegetative vigor, especially in the phases of tillering and differentiation of the reproductive meristem, which results in higher values for these production components (Espindula et al., 2010)

The variable hectoliter weight was influenced by the interaction between nitrogen and *A. brasilense* (Table 4), and the combination of 40 kg N ha^{-1} and 1300 mg kg^{-1} seeds provided higher hectoliter weight, but did not differ statistically with the other doses of N. However, Trindade et al. (2006), when testing doses of urea, found values of decreasing hectoliter mass, when they increased the dose of N of 0 to 200 kg ha^{-1}. Similarly, Feldmann (2014) observed that increasing N fertilization adversely affected the hectoliter weight of three wheat varieties. This response may be related to the increase in the incidence of foliar diseases due to N application, reducing the production of photoassimilates during the period of grain filling (Feldmann, 2014).

The highest averages of thousand grains were observed with the application of 80 kg ha^{-1} of N and no inoculation with *A. brasilense* (Table 5). These results are similar to those obtained by Baribieri et al. (2012) where plants inoculated with *A. brazilian* bacteria did not influence the weight of a thousand grains. In contrast to

Table 5. Weight of a thousand grains in grams of wheat submitted to inoculation of different doses of *A. brasilense* and N under field conditions.

N doses (kg ha^{-1})	*A. brasilense* doses (mg kg^{-1} seed)			
	0	700	1000	1300
0	35.18^{bAB}	37.00^{aA}	34.70^{bB}	36.51^{aAB}
40	36.23^{bA}	35.82^{aA}	36.61^{abA}	37.21^{aA}
80	38.43^{aA}	36.84^{aA}	37.45^{aA}	36.67^{aA}
	CV (%)=3.25			

Means followed by the same letter, lowercase in the columns and upper case in the lines, do not differ by the LSD test (t test) at the 5% probability level.

Table 6. Yield of wheat in kg ha^{-1} submitted to inoculation of different doses of *A. brasilense* and N under field conditions.

N doses (kg ha^{-1})	*A. brasilense doses* (mg kg^{-1} seed)			
	0	700	1000	1300
0	1200^{cB}	1726^{aA}	1806^{bA}	1563^{bA}
40	1594^{bB}	1730$^{a B}$	2337^{aA}	2540^{aA}
80	2561^{aA}	1966^{aB}	2527^{aA}	2642^{aA}
	CV (%)=7.74			

Means followed by the same letter, lowercase in the columns and upper case in the lines, do not differ by the LSD test (t test) at the 5% probability level.
Source: Curuguaty (2017).

that observed in this study, Sala et al. (2007) found that, with different strains of plant growth promoting bacteria, all strains used resulted in a larger mass of thousand grains in wheat. In this way, it is known that the interaction between the bacterium and the plant occurs in the rhizosphere, which is stimulated by root exudates. The composition of these exudates is dependent on soil type, nutrient availability, genotypes and environmental conditions. All these factors influence the response of the plants to inoculation, and in general, the benefits of the plant bacterium interaction are more accentuated in soils with low natural fertility.

The interaction between the N doses and doses of *A. brasilense* was significant for wheat grain yield. The best wheat grain yield in the 2017 harvest was the combination of 80 kg N ha^{-1} and 1300 mg kg^{-1} seed of A. *brasilense* (Table 6). These results agree with Hungria et al. (2010), Mendes et al. (2011), Bashan et al. (2004) and Didonet et al. (2004): they observed an increase in the production of wheat grains when associated with the use of *A. brasilense*. On the other hand, the results observed in the present work are related by Barbieri et al. (2012), who concluded that inoculation of seeds with *A. brasilense* did not interfere in the irrigated wheat yield in the cerrado Region. However, Sala et al. (2008) found significant effects on wheat grain yield (mean of 23.9% in relation to the control) in inoculated *A. brasilense* treatment, with and without the addition of nitrogen fertilization (Table 6).

Conclusions

The different doses of N and *A. brasilense* did not affect the plant height. The increase of N and *A. brasilense* positively affects the number of spikes per plant and hectoliter weight. The highest doses of N increase the thousand-grain weight. The inoculation with 1300 mg kg^{-1} seed of *A. brasilense*, in addition to the application of 130 kg N ha^{-1}, provides the highest grain yield.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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