Wheat yield in the Cerrado as affected by nitrogen fertilization and inoculation with *Azospirillum brasilense*

Fernando Shintate Galindo[(1)], Marcelo Carvalho Minhoto Teixeira Filho[(1)], Salatiér Buzetti[(1)], José Mateus Kondo Santini[(1)], Cleiton José Alves[(1)] and Mariana Gaioto Ziolkowski Ludkiewicz[(1)]

[(1)]Universidade Estadual Paulista Júlio de Mesquita Filho, Faculdade de Engenharia, Avenida Brasil, nº 56, Centro, CEP 15385-000 Ilha Solteira, SP, Brazil. E-mail: fs.galindo@bol.com.br, mcmteixeirafilho@agr.feis.unesp.br, sbuzetti@agr.feis.unesp.br, santinijmk@gmail.com, cleiton.agr.feis@gmail.com, mariana.gaioto@gmail.com

Abstract – The objective of this work was to evaluate the effect of inoculation with *Azospirillum brasilense*, associated with N rates and sources, in soil of the Cerrado (Brazilian savanna), on the grain yield of irrigated wheat (*Triticum aestivum*). The experiment was carried out under a no-tillage system in a Typic Haplustox. The experimental design was randomized complete blocks with four replicates, in a 2x5x2 factorial arrangement: two N sources (urea and urea with NBPT urease inhibitor); five N rates applied as topdressing (0, 50, 100, 150, and 200 kg ha^{-1}); and with or without seed inoculation with *A. brasilense*. The increase in the N rates positively affected spike length, number of spikelets and of grains per spike, number of spikes per meter, N accumulation in the straw, leaf chlorophyll content, and grain yield of irrigated wheat, regardless of the use of NBPT urease inhibitor with conventional urea. Singly, inoculation with *A. brasilense* does not affect production components and grain yield, despite the increase in N content in wheat straw. The inoculation with *A. brasilense*, associated with the application of 140 kg ha^{-1} N, provides the highest grain yield of irrigated wheat cropped after corn in low-altitude Cerrado.

Index terms: *Triticum aestivum*, biological nitrogen fixation, nitrogen sources, no-tillage system, urease inhibitor.

Produtividade de trigo no Cerrado em função da adubação nitrogenada e da inoculação com *Azospirillum brasilense*

Resumo – O objetivo deste trabalho foi avaliar o efeito da inoculação com *Azospirillum brasilense*, associada a doses e fontes de N, em solo de Cerrado, sobre a produtividade de grãos de trigo (*Triticum aestivum*) irrigado. O experimento foi desenvolvido em sistema plantio direto, em Latossolo Vermelho distrófico. Utilizou-se o delineamento experimental de blocos ao acaso, com quatro repetições, em arranjo fatorial 2x5x2: duas fontes de N (ureia e ureia com inibidor da urease NBPT); cinco doses de N em cobertura (0, 50, 100, 150 e 200 kg ha^{-1}); e com ou sem inoculação das sementes com *A. brasilense*. O aumento nas doses de N apresentou efeito positivo no comprimento de espiga, número de espiguetas e de grãos por espiga, número de espigas por metro, acúmulo de N na palha, conteúdo de clorofila na folha e produtividade de grãos de trigo irrigado, independentemente do uso de inibidor de urease NBPT com ureia. Isoladamente, a inoculação com *A. brasilense* não afeta os componentes de produção e a produtividade de grãos, apesar de incrementar o N na palhada de trigo. A inoculação com *A. brasilense*, associada à aplicação de 140 kg ha^{-1} de N, propicia as maiores produtividades de grãos de trigo irrigado, cultivado após milho em Cerrado de baixa altitude.

Termos para indexação: *Triticum aestivum*, fixação biológica de nitrogênio, fontes de nitrogênio, sistema plantio direto, inibidor da urease.

Introduction

In the 2015 harvest season in Brazil, wheat (*Triticum aestivum* L.) was grown in 2.5 million hectares of land, where 6.7 million tons of grain were produced, with an average yield of 2.7 Mg ha^{-1}. Although the Southern region of the country is responsible for approximately 90% of the national production of wheat, this cereal is being gradually introduced into the Cerrado region (Brazilian savanna) using either irrigated or dry farming (Acompanhamento..., 2015).

Nitrogen fertilization is used to obtain high grain yields for this cereal. However, according to Lara

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Cabezas et al. (2000), there is a reduction of about 10 kg ha^{-1} in corn grain yield for each 1% volatilized N. In this context, the efficiency of N fertilization can be increased with the inhibitor N-(n-butyl) thiophosphoric triamide (NBPT), which can delay the hydrolysis of urea and significantly reduce the loss of ammonia in regions with a predominance of high temperatures, including the low-altitude Cerrado region (Valderrama et al., 2014).

Most studies conducted in Brazil showed that urea combined with a urease inhibitor as well as conventional urea have a similar efficiency in N nutrition and grain yield (Cantarella et al., 2008): the inhibitory activity of urease does not effectively reduce the losses by ammonia volatilization, which occurs when urea is applied to the soil surface. This can be explained by the fact that the action of NBPT depends on: environmental conditions, such as temperature and rainfall; physical and chemical characteristics of acid and weathered soils; and cultivation system, which is the no-tillage system with straw (a source of urease) on soil surface in most of the Cerrado region.

Another possibility is the use of bacterial inoculants, which promote plant growth and increase productivity. Brazil has a tradition of research in biological N fixation (BNF) using *Azospirillum* spp. in graminaceous plants. However, until recently, no commercial inoculants with this bacterial strain were available in Brazil (Hungria, 2011). The observed effects after inoculation were increased uptake of water and minerals and increased tolerance to stress such as drought and salinity, which increased plant robustness and yield (Bashan et al., 2004). Barassi et al. (2008) reported an improvement in the photosynthetic parameters of leaves, including chlorophyll content and stomatal conductance, increased proline content in aerial parts and roots, improvement in water potential, increased water content of the apoplast, increased elasticity of the cellular wall, and greater plant biomass.

Dobbelaere et al. (2003) observed that positive responses to inoculation with *Azospirillum brasilense* were obtained even when cultures were grown in soils with high levels of available N, which indicates that plant responses might be due to the fixed N and the production of plant growth hormones, including cytokinin, gibberellin, and indoleacetic acid. Lemos et al. (2013) evaluated five wheat cultivars and observed a positive interaction between *A. brasilense* and N fertilization only for the CD 150 cultivar. Increases in the efficiency of N fertilization combined with inoculation with *A. brasilense* were reported by Galindo et al. (2016) for the productivity of corn grains in the Cerrado region.

Despite these benefits, increase in wheat grain productivity was not always observed after seed inoculation with *Azospirillum* ssp., which indicates the need for more research on this topic and for a better definition of the amount of mineral N that should be applied to achieve optimal BNF and grain yield (Galindo et al., 2016). It is also important to assess whether urea with a urease inhibitor (NBPT) is less harmful to BNF in graminaceous plants owing to the slower release of N.

The objective of this work was to evaluate the effect of inoculation with *A. brasilense*, associated with N rates and sources, in soil of the Cerrado, on the grain yield of irrigated wheat.

Materials and Methods

The study was conducted at the experimental station of Fazenda de Ensino, Pesquisa e Extensão of Faculdade de Engenharia, of Universidade Estadual Paulista Júlio de Mesquita Filho, located in the municipality of Selvíria, in the state of Mato Grosso do Sul, Brazil (20°22'S, 51°22'W, at an altitude of 335 m) in 2014 and 2015. The soil in the experimental area was classified as a clayey Latossolo Vermelho distrófico (Santos et al., 2013), i.e., a Typic Haplustox. The area has been cultivated with annual crops for more than 27 years, and the no-tillage system has been used for the past 10 years. Since the crop planted before wheat sowing was corn in both years of evaluation, only graminaceous plants were cultivated in the 2 years before the study. The average temperature was 23.5°C, the annual average precipitation was 1,370 mm, and the annual average relative air humidity was 70–80% (Figure 1). The climate type is Aw, according to Köppen's classifications, defined as humid tropical with rainy season in summer and dry in winter.

The experimental design was randomized complete blocks with four replicates, in a 2×5×2 factorial arrangement: two N sources – conventional urea, 45% N; and NBPT (Super N commercial fertilizer), conventional urea with NBPT urease inhibitor; five N rates – 0, 50, 100, 150, and 200 kg ha^{-1}; and N application using the broadcasting technique, with or without seed inoculation with *A. brasilense*. The experimental plots were composed of 12 lines with 6 m in length and spaced at a 0.17 m distance, and the useful area of the plot comprised the central eight lines, excluding 0.5 m from the ends.

The herbicides used in the experimental areas were glyphosate (1,800 g ha^{-1} a.i.) and 2,4-D (670 g ha^{-1} a.i.) for desiccation; these products were applied 2 weeks before wheat sowing in 2014 and 2015. The chemical attributes of the topsoil (depth of 0.00–0.20 m) were determined in 2014 before the initiation of the experiment, following the methodology proposed by Raij et al. (2001). The following results were obtained: 13 mg dm^{-3} P (resin); 6 mg dm^{-3} S-SO_4; 23 g dm^{-3} organic matter; pH 4.8 ($CaCl_2$); 2.6, 13.0, 8.0, and 42.0 $mmol_c$ dm^{-3} K, Ca, Mg, and H+Al, respectively; 5.9, 30.0, 93.9, and 1.0 mg dm^{-3} Cu, Fe, Mn, and Zn (DTPA), respectively; 0.24 mg dm^{-3} B (hot water); and base saturation of 36%.

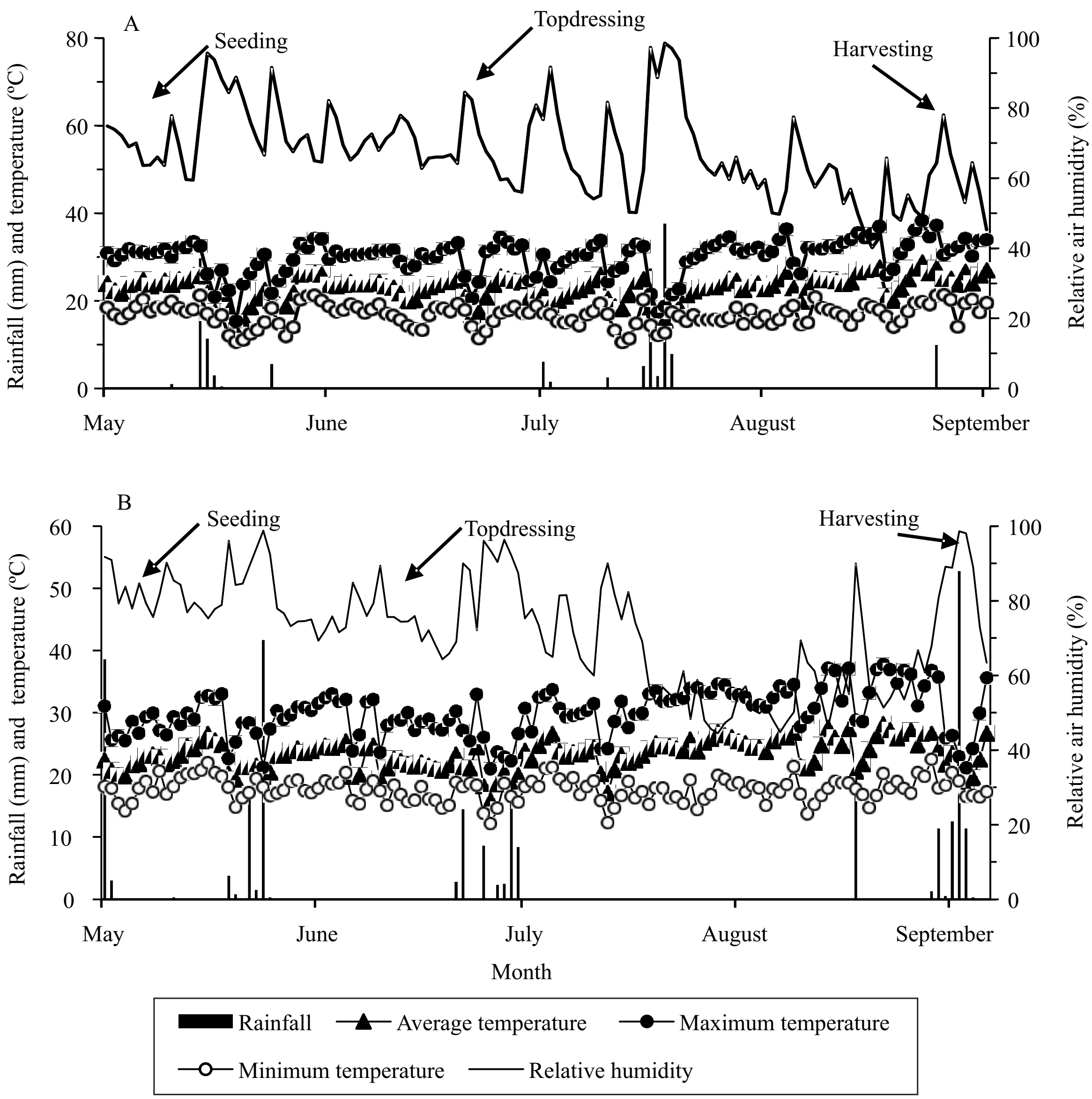

Figure 1. Rainfall, relative air humidity, and maximum, average and minimum temperatures during wheat cultivation from May to September 2014 (A) and May to September 2015 (B), obtained from the weather station located at Fazenda de Ensino, Pesquisa e Extensão of Universidade Estadual Paulista Júlio de Mesquita Filho, in the municipality of Selvíria, in the state of Mato Grosso do Sul, Brazil.

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%) were applied to the soil 65 days before sowing wheat in 2014. 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 in 2014 as in 2015 for sowing fertilization.

Inoculation of wheat seeds with strains AbV5 and AbV6 of *A. brasilense* (to achieve a density of 2×10^8 CFU mL^{-1}) was performed using 300 mL of the liquid inoculant Azototal (Total Biotecnologia, Curitiba, PR, Brazil) per hectare 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 area was irrigated using a center pivot sprinkler system, with a mean water depth of 14 mm and an irrigation interval of approximately 72 hours in both cultures. The cultivar used was 116 CD with mechanical seeding on 5/16/2014 and 5/15/2015 at a density of 80 seeds per meter. The seedlings emerged 6 and 5 days after sowing, on 5/22/2014 and 5/20/2015, respectively.

The growth of weeds was managed with the application of the herbicide metsulfuron-methyl (3 g ha^{-1} a.i.) 20 days after emergence (DAE) of wheat in both seasons. Nitrogen fertilization was performed manually on 6/26/2014 and 6/22/2015 (that is, 35 and 32 DAE respectively), and the fertilizer was spread on the soil surface without incorporation on the sides and at approximately 8 cm from the sowing lines to avoid contact with the plants. After cover fertilization, the area was irrigated by sprinkling (depth of 14 mm) at night to minimize losses by volatilization of ammonia, which is common in irrigated wheat. The plants were harvested manually in both seasons at 110 DAE, on 9/9/2014 and 9/8/2015 respectively.

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 spikes at harvest: spike length, measured from the apex to the base of the spike; number of spikelets, by counting all spikelets with grains; number of grains per spike, by counting the number of grains in each spike in each experimental unit; and number of defective grains, by calculating the number of undeveloped spikelets per spike. The N accumulation was determined in the wheat straw at the end of the production cycle. Nitrogen was measured after plant collection in three 0.5-m^2 experimental areas per plot after drying, weighing, grinding, and sulfuric digestion of the seeds following the methodology proposed by Malavolta et al. (1997). Subsequently, N accumulation was calculated in kg ha^{-1}. The other evaluated parameters were: the leaf chlorophyll index (LCI), which was indirectly measured in the middle third portion of the flag leaf in five plants per plot in the flowering period using a digital chlorophyll meter model CFL 1030 (Falker Automação Agrícola Ltda., Porto Alegre, RS, Brazil); number of spikes per meter, by counting the number of spikes at harvest on a 1-m plant row in the useful area of each parcel; 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 100 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).

The results were subjected to the analysis of variance and to Tukey's test, at 5% probability, for the comparison of the average yields obtained with the different N sources and with or without inoculation with *A. brasilense*. The regression equations were adjusted to the effect of N rates using the Sisvar software (Ferreira, 2011).

Results and Discussion

The increase in the N rates significantly affected the number of spikelets per spike in the 2015 harvest and the spike length, number of grains per spikelet, and number of grains per spike in 2014 and 2015 (Figure 2). There were adjustments to increasing linear functions for spike length in 2014 and 2015 (Figure 2 A and B) and for the number of spikelets (Figure 2 C) and number of grains per spike in 2014 (Figure 2 D). The data fitted a quadratic function to the number of grains per spike in the 2015 harvest with a point of

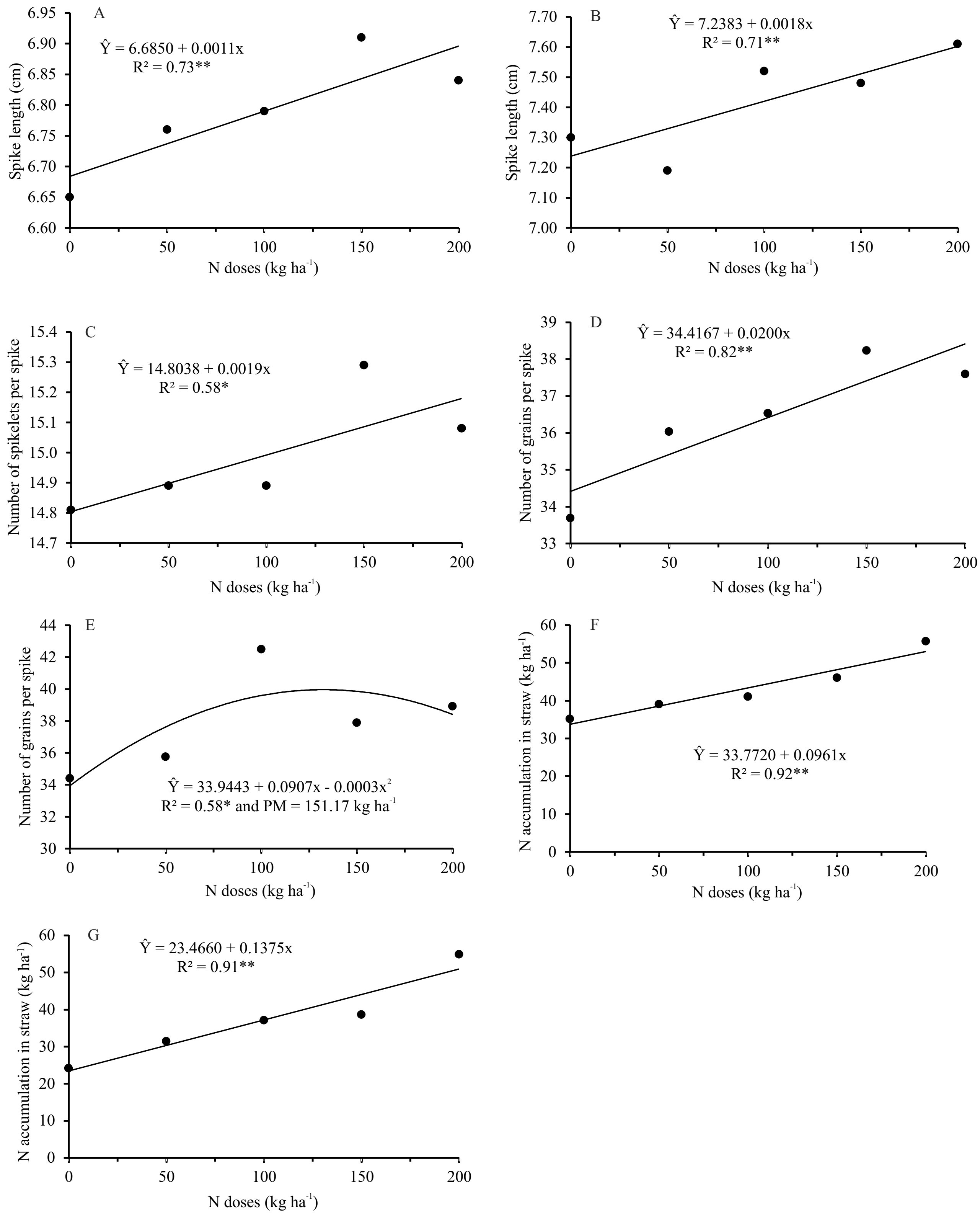

Figure 2. Spike length in 2014 (A) and 2015 (B), number of spikelets per spike in 2015 (C), number of grains per spike in 2014 (D) and 2015 (E), N accumulation in wheat straw in 2014 (F) and 2015 (G), in function of N rates. ** and *Significant at 1 and 5% probability, respectively.

maximum rate at 151 kg ha^{-1} N (Figure 2 E). However, plant height and the number of defective grains per spike were not affected by the increase in N rates (Table 1) regardless of the N source and inoculation with *A. brasilense*. Therefore, it was observed that the inoculation of diazotrophic bacteria associated with high N rates did not affect the growth of the aerial part of the plant, similarly to results reported in other studies (Hungria, 2010).

With regard to spike length, Teixeira Filho et al. (2008) evaluated cultivars IAC 370 and IAC 24 at different plant densities and did not observe any effect of the N rates. Rodrigues et al. (2014) evaluated the agronomic characteristics of wheat grown in a greenhouse (presence of *A. brasilense*, humic acids, and N content) and observed a linear increase in spike length with an increase in N levels, which is consistent with results reported in the present work. There was no association between inoculation with *A. brasilense* and N rates. However, there was a positive correlation between the increases of N fertilization and the spike length.

Plant height was not affected by the N content (Table 1), as observed by Teixeira Filho et al. (2008) at the rates of 0, 30, 60, 90, 120, and 150 kg ha^{-1} N and by Teixeira Filho et al. (2010) at the rates of 0, 50, 100, 150, and 200 kg ha^{-1} N. By contrast, Pietro-Souza et al. (2013) found that the N rates affected plant height in the 'BRS Guamirim' wheat crop. Theago et al. (2014) analyzed the rates of 0, 50, 100, 150, and 200 kg ha^{-1} N and also observed that N affected plant height at a rate of up to 147 kg ha^{-1}.

The N rates also affected the number of grains per spike. Similar results were obtained by Theago et al. (2014), who reported that the increase in N levels (in topdressing) at a rate of up to 128 kg ha^{-1} produced the largest number of grains per spike and spikes per meter, irrespective of the N source used.

The N sources did not affect the plant height, spike length, number of spikelets per spike, number of grains per spike, and number of defective grains per spike in the harvests of 2014 and 2015 (Table 1). Megda et al. (2009), Teixeira Filho et al. (2010), and Theago et al. (2014) obtained similar results when using ammonium sulfonitrate, ammonium sulfate, and urea as N sources in irrigated wheat cultivated in the low-altitude Cerrado region.

Inoculation with *A. brasilense* did not affect the spike length, number of spikelets per spike, number of grains per spike, and number of defectives grains per spike in 2014 and 2015 and plant height in 2015 (Table 1). Similar results were found by Ferreira et al.

Table 1. Plant height, spike length, number of spikelets, number of grains per spike, number of defective grains per spike and N accumulation in straw of wheat (*Triticum aestivum*), affected by N rates and sources, with or without inoculation with *Azospirillum brasilense* (2014 and 2015)[1].

Treatment	Plant height (cm)		Spike length (cm)		No. of spikelets per spike		No. of grains per spike		No. of defective grains per spike		N accumulation in straw (kg ha^{-1})	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
N rates (kg ha^{-1})												
0	84.89	75.51	6.65	7.30	14.81	17.38	33.69	34.40	1.27	1.82	35.14	24.12
50	86.54	77.00	6.76	7.19	14.89	16.42	36.03	35.75	1.23	1.65	39.03	31.39
100	85.26	75.81	6.79	7.52	14.89	17.75	36.53	42.48	1.18	1.43	41.04	37.08
150	87.10	76.53	6.91	7.48	15.29	17.50	38.23	37.88	1.12	1.70	46.05	38.59
200	85.09	76.87	6.84	7.61	15.08	17.58	37.59	38.91	1.19	1.40	55.66	54.89
N sources												
Urea	86.34a	76.56a	6.81a	7.44a	14.93a	17.49a	36.08a	38.60a	1.21a	1.69a	43.22a	38.87a
NBPT	85.21a	76.13a	6.77a	7.39a	15.06a	17.17a	36.75a	37.17a	1.19a	1.51a	43.55a	35.56a
LSD (5%)	1.38	2.50	0.09	0.23	0.26	0.66	1.48	2.70	0.11	0.30	6.93	4.91
Inoculation												
With *Azospirillum*	85.03b	75.67a	6.81a	7.53a	14.93a	17.43a	36.43a	36.73a	1.18a	1.68a	48.54a	39.80a
Without *Azospirillum*	86.53a	77.02a	6.77a	7.31a	15.06a	17.22a	36.40a	39.04a	1.22a	1.52a	38.23b	34.82b
LSD (5%)	1.38	2.50	0.09	0.23	0.26	0.66	1.48	2.70	0.11	0.30	6.93	4.91
Overall mean	85.78	76.34	6.79	7.42	14.99	17.33	36.42	37.88	1.20	1.60	43.38	37.31
Coefficient of variation (%)	3.58	6.26	3.12	5.94	3.93	7.30	7.76	13.65	20.01	35.92	30.56	25.26

[1]Means followed by equal letters, in the columns, do not differ by Tukey's test, at 5% probability. LSD, lower significant difference.

(2014) and Galindo et al. (2015) with the inoculation of wheat leaves with *A. brasilense* in the Cerrado region. These authors found that bacterial inoculation did not affect the plant height, number of grains per spike, number of spikelets per spike, number of defective grains, and number of spikes per meter. Nunes et al. (2015) also found that the number of grains per spike was not affected by inoculation with *A. brasilense* in soils with low and high N availability. In the present work, however, the inoculated plants were shorter than non-inoculated plants in the 2014 harvest (Table 1).

Higher N rates increased N accumulation linearly in the wheat straw in the two crop years (Figure 2 F and G). However, there was no significant difference in N accumulation between conventional urea and conventional urea combined with NBPT urease inhibitor (Table 1). The inoculation with *A. brasilense* also caused a higher N accumulation in the wheat straw in both crop years, confirming the effect of associating this bacteria with graminaceous plants on BNF. Lemos et al. (2013) analyzed five wheat cultivars and found that inoculation with *A. brasilense* increased the N content of the aerial parts of the CD 108 cultivar.

The N rates did not affect the mass of 100 grains and hectoliter mass (Table 2). Similar results were reported by Teixeira Filho et al. (2008) and Theago et al. (2014), who observed that the N rates did not affect these parameters, and by Nunes et al. (2015), who found that the N rates (in topdressing) did not affect the mass of 1,000 grains in areas with high N availability. Frank & Bauer (1996) observed that, in the period between the emergence of seedlings and the differentiation of panicles, N deficiency reduced the mass of 1,000 grains. In the present work, none of the evaluated crops presented N deficiency probably because of the irrigated wheat crop and because the number of grains per spike increased as affected by the increasing N rates, which enhanced the competition for photoassimilates in the spike (Teixeira Filho et al., 2010) but was not enough to reduce the grain mass.

There was a significant interaction between the N rates and bacterial inoculation for LCI in 2014 (Table 3). At the rate of 200 kg ha^{-1} N, the inoculated treatments showed the greatest LCI. The LCI was adjusted to an increasing linear function for N rates in the inoculated treatments and to a quadratic function in the non-inoculated treatments, with the point of maximum rate at 101 kg ha^{-1} N (Figure 3 A). In the 2015 harvest, the LCI increased linearly with increasing N levels (Figure 3 B). Other authors also reported an increase in the LCI

Table 2. Leaf chlorophyll index (LCI), number of spikes per meter, hectoliter mass, mass of 100 grains, and grain yield of wheat (*Triticum aestivum*) affected by N rates and sources, with or without inoculation with *Azospirillum brasilense* (2014 and 2015)[1].

Treatment	LCI		Number of spikes per meter		Hectoliter mass (kg 100 L^{-1})		Mass of 100 grains (g)		Grain yield (kg ha^{-1})	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
N rates (kg ha^{-1})										
0	49.89	50.92	81.00	69.17	84.86	87.23	3.83	4.20	2,269	1,906
50	55.41	52.31	85.94	76.50	84.74	86.83	3.84	4.10	3,004	3,027
100	56.17	54.50	95.00	84.42	84.73	87.42	3.96	4.19	3,132	3,363
150	58.77	55.23	97.88	73.75	84.78	86.14	3.93	4.13	3,266	3,167
200	57.91	56.16	92.13	74.50	84.77	85.96	3.88	4.12	3,161	3,263
N sources										
Urea	54.72a	52.66b	87.73a	75.50a	84.86a	87.14a	3.89a	4.17a	2,959a	2,930a
NBPT	56.53a	54.99a	93.05a	75.83a	84.70a	86.29a	3.88a	4.13a	2,974a	2,960a
LSD (5%)	2.08	1.31	5.67	5.90	0.43	1.40	0.09	0.06	227	212
Inoculation										
With *Azospirillum*	55.62	54.26a	88.38	76.00a	84.85a	86.61a	3.90a	4.14a	2,996	3,007
Without *Azospirillum*	55.63	53.39a	92.40	75.33a	84.70a	86.82a	3.88a	4.15a	2,937	2,883
LSD (5%)	2.08	1.31	5.67	5.90	0.43	1.40	0.09	0.06	227	212
Overall mean	55.63	53.82	90.39	75.67	84.78	86.71	3.89	4.15	2,966	2,945
Coefficient of variation (%)	7.15	4.64	14.02	14.92	1.15	3.61	5.00	3.36	17.12	16.08

[1]Means followed by equal letters, in the columns, do not differ by Tukey's test, at 5% probability. LSD, lower significant difference.

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in wheat according to the N rates: Theago et al. (2014) reported an increase with a rate up to 200 kg ha^{-1} N, and Teixeira Filho et al. (2010) verified an increase up to 147 kg ha^{-1} N. This characteristic is attributed to the increase in the chlorophyll concentration promoted by the increased availability of total N in the vegetative part of the wheat (Figure 2 F and G).

In the 2014 harvest, there was a significant interaction between the N rates and inoculation with *A. brasilense* in terms of the number of spikes per meter (Table 4). At rates of 150 and 200 kg ha^{-1} N, the non-inoculated treatments showed a higher number of spikes per meter. There was an adjustment to an increasing linear function for N rates in the non-inoculated treatments and an adjustment to a quadratic function in the inoculated treatments, with a point of maximum rate at 99.5 kg ha^{-1} N (Figure 3 C). However, the N rates individually affected the number of spikes per meter in 2015 (Table 2), with an adjustment to a quadratic function for levels of up to 110 kg ha^{-1} N (Figure 3 D). These results indicate that inoculation with *A. brasilense* alone or with high levels of N does not affect the number of spikes per meter. However, Teixeira Filho et al. (2007) observed that increasing N rates in the form of urea and no bacterial inoculation significantly affected the number of spikes per meter for wheat. The increased tillering, with the production of more spikes per meter due to the increase in N rates (Figure 3 C and D), explains in part the increase in crop yield.

Conventional urea and NBPT provided similar results for production components and grain yield of wheat (Tables 1 and 2) regardless of the inoculation with *A. brasilense*. Similarly, Megda et al. (2009), Teixeira Filho et al. (2010), and Theago et al. (2014) found no difference in the efficiency of N sources in the grain yields of irrigated wheat with satisfactory conditions of soil moisture in the Cerrado region. Prando et al. (2012) evaluated urea, urea + NBPT, and coated urea in a temperate climate and found no changes in the yield, grain mass, number of spikes per square meter, and plant height.

The interaction between the N rates and inoculation with *A. brasilense* was significant for wheat grain yield in 2014 and 2015 (Table 5). In 2014, at a rate of 150 kg ha^{-1} N, the grain yield of the inoculated treatment was higher than that of the non-inoculated treatment. The wheat grain yield in the 2014 harvest increased linearly with the N rates without inoculation and was adjusted to a quadratic function for the inoculated treatments, with a positive response for a rate of up to 139 kg ha^{-1} N (Figure 3 E). In 2015, the grain yield of the inoculated treatment was higher than that of the non-inoculated treatment for a rate of 100 kg ha^{-1} N (Table 5). The grain yield in the 2015 harvest was adjusted to a quadratic function for N rates in inoculated and non-inoculated treatments, with a positive response for rates of 142 and 134 kg ha^{-1} N,

Table 3. Interaction between inoculation and N rates (0, 50, 100, 150, and 200 kg ha^{-1}) determined by the analysis of variance of the leaf chlorophyll index of wheat (*Triticum aestivum*) in 2014[(1)].

Inoculation	Leaf chlorophyll index (LCI)				
	0	50	100	150	200
With *Azospirillum*	47.95a	52.96a	55.97a	59.79a	61.41a
Without *Azospirillum*	51.79a	56.86a	56.36a	57.74a	54.42b

[(1)]Means followed by equal letters, in the columns, do not differ by Tukey's test, at 5% probability. Lower significant difference (LSD), at 5% probability, was 4.65.

Table 4. Interaction between inoculation and N rates (0, 50, 100, 150, and 200 kg ha^{-1}) determined by the analysis of variance of the number of wheat (*Triticum aestivum*) spikes per meter in 2014[(1)].

Inoculation	Number of spikes per meter				
	0	50	100	150	200
With *Azospirillum*	82.75a	90.38a	94.25a	89.75b	84.75b
Without *Azospirillum*	79.25a	81.50a	95.75a	106.00a	99.50a

[(1)]Means followed by equal letters, in the columns, do not differ by Tukey's test, at 5% probability. Lower significant difference (LSD), at 5% probability, was 12.69 spikes per meter.

Table 5. Interaction between inoculation and N rates (0, 50, 100, 150, and 200 kg ha^{-1}) determined by the analysis of variance of the wheat (*Triticum aestivum*) grain yield in 2014 and 2015[(1)].

Inoculation	Grain yield (kg ha^{-1})				
	0	50	100	150	200
	2014 crop season				
With *Azospirillum*	2,196a	2,916a	3,205a	3,544a	3,119a
Without *Azospirillum*	2,342a	3,092a	3,060a	2,989b	3,203a
	2015 crop season				
With *Azospirillum*	1,671a	3,036a	3,663a	3,167a	3,497a
Without *Azospirillum*	2,141a	3,018a	3,063b	3,166a	3,029a

[(1)]Means followed by equal letters, in the columns, do not differ by Tukey's test, at 5% probability. Lower significant difference (LSD), at 5% probability, was 508 and 474 kg ha^{-1} in the 2014 and 2015 crop seasons, respectively.

respectively (Figure 3 F). Compared with the control (without N), the optimum N rate in the inoculated treatment provided higher grain yield (391 kg ha^{-1}) than the best N rate in the non-inoculated treatment, a 7% yield increase in the second crop year.

The inoculation with *A. brasilense* associated with N rates of 140 kg ha^{-1} provided the maximum yield in the evaluated crops. However, with no inoculation, the magnitude of the crop responses to the N rates was greater because the adjustment to the increasing linear function in 2014. Increases in the efficiency of N fertilization associated with inoculation with *A. brasilense* were also reported by Galindo et al. (2016) in corn crops in the Cerrado. These authors also found that inoculation increased the LCI and phosphorus concentration in the leaves. According to Dobbelaere

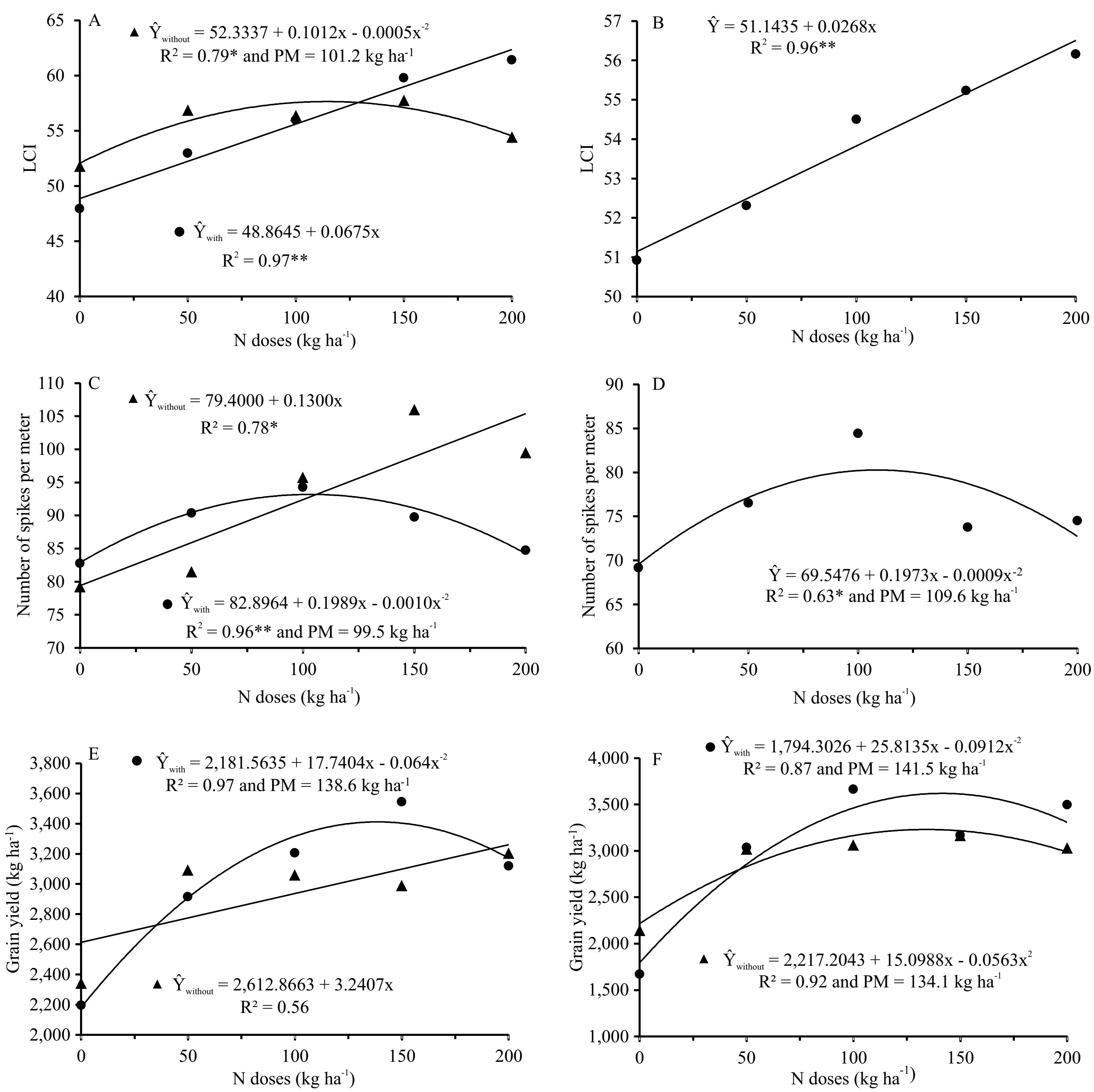

Figure 3. Interaction between N rates and inoculation with *Azospirillum brasilense* in terms of leaf chlorophyll index (LCI) in 2014 (A) and 2015 (B), as affected by N rates; number of spikes per meter in 2014 (C) and 2015 (D), as affected by N rates; and grain yield in 2014 (E) and 2015 (F). ** and *Significant at 1 and 5% probability, respectively.

et al. (2003), positive responses to inoculation with *A. brasilense* are obtained even when the crops are grown in soils with a high N content, which indicates that the plant responses do not occur only because of BNF, but also because of the production of plant growth hormones, including cytokinin, gibberellin, and indoleacetic acid.

With regard to grain yield, several authors reported a positive response to N fertilization in wheat (Cazetta et al., 2007; Teixeira Filho et al., 2007, 2008, 2010; Povh et al., 2008). The maximum grain yield was observed at N rates of 78 kg ha^{-1} (Cazetta et al., 2007), 90 kg ha^{-1} (Teixeira Filho et al. 2007, 2008), and 120 kg ha^{-1} (Teixeira Filho et al., 2010) in similar climate conditions for the cultivation of wheat as a winter crop in the low-altitude Cerrado region. These differences in the N rates that provide the maximum wheat grain yield are due to the distinct N requirement of each cultivar and the variation in environmental conditions.

Lemos et al. (2013) evaluated five wheat cultivars (CD 104, CD 108, CD 119, CD 120, and CD 150) with and without inoculation with *A. brasilense* associated with different N levels and observed that the response to inoculation was satisfactory in cases in which inoculation was associated with N fertilization. In the present work, a similar result was observed using N rates of 150 and 100 kg ha^{-1} in the first and second crop years, respectively (Table 5). Therefore, inoculation with *A. brasilense* in addition to the supply of N in the form of urea or NBPT increased the yield of irrigated wheat grains in the Cerrado region (Figure 3 E and F), although these treatments alone were not sufficiently effective to replace N fertilization. Conversely, Ferreira et al. (2014) inoculated *A. brasilense* in plant leaves and used N fertilization of wheat plantations in the Cerrado region and found that bacterial inoculation did not affect the plant height, number of spikes per square meter, number of grains per spike, hectoliter mass, mass of 1,000 grains, and grain yield. Similarly, Nunes et al. (2015) evaluated the inoculation of *A. brasilense* in soils with high and low availability of N, and Galindo et al. (2015) evaluated the periods of application of *A. brasilense* on plant leaves and found that bacterial inoculation did not affect production components and wheat grain yield in the Cerrado region with the application of 100 kg ha^{-1} of N in the form of urea. However, it is notable that the affinity between the cultivars and the strains of diazotrophic bacteria may vary and affect the outcome of inoculation.

Conclusions

1. The increase in the N rates positively affects spike length, number of spikelets per spike, number of grains per spike, number of spikes per meter, N accumulation in the straw, leaf chlorophyll content, and grain yield of irrigated wheat (*Triticum aestivum*), regardless of the use of NBPT urease inhibitor with conventional urea.

2. The inoculation with *Azospirillum brasilense* alone does not affect production components and grain yield, although it increases the N content in wheat straw.

3. The inoculation with *A. brasilense*, in addition to the application of 140 kg ha^{-1} N, provides the highest grain yield of irrigated wheat cropped after corn in low-altitude Cerrado.

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