Chapter 6

Inoculation with *Azospirillum brasilense* Improves Nutrition and Increases Wheat Yield in Association with Nitrogen Fertilization

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Additional information is available at the end of the chapter

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Abstract

The management of nitrogen fertilization is performed in order to ensure adequate productivity, and depending on the N dynamics in the soil, large amount of N is added to the soil, raising production cost for the farmers. Considering the benefits attributed by seed inoculation with *Azospirillum brasilense* (diazotrophic bacteria), with emphasis on biological nitrogen fixation (BNF), greater development of the root system, and, consequently, greater absorption of water and nutrients, it infers that inoculation can improve crop performance allowing greater efficiency of nitrogen fertilization. Thus, the research that evaluates nutritional status and wheat yield, in terms of nitrogen rates in association with inoculation with *A. brasilense* is important. We found that increment of N rates in association with *A. brasilense* inoculation increases the wheat yield up to 139 kg ha^{-1} N, whereas without this inoculation linear increase occurred with lower maximum yield of wheat. That is, the inoculation afforded higher grain yield applying less nitrogen fertilizer in topdressing. This research demonstrated that inoculation with *A. brasilense* associated with nitrogen fertilization in topdressing is beneficial to nutrition and wheat yield. Therefore, inoculation is a low-cost technique, easy to apply and use, and nonpolluting, which fall within the desired sustainable context in actuality.

Keywords: diazotrophic bacteria, nitrogen, nutrient concentrations, bacterial promoters of plant growth, agronomic efficiency

1. Introduction

Wheat (*Triticum aestivum* L.) is an annual cycle plant, considered among the cool season cereal, one that has greater economic importance, with large grain yield capacity [1].

The final crop yield is defined according to the cultivar used, the amount of agricultural supplies, and management techniques employed. The increasing use of high-yield potential wheat has implicated in more frequent use of agricultural supplies, among which nitrogen fertilization shown to be important in defining the grain yield [2]. Therefore, there is a need to study wheat cultivars verifying their response to the uptake and utilization of nutrients in the soil and their performance and cultural practices in different environments [3].

Nitrogen fertilization is one of the highest costs of the production process of nonleguminous crops [4]. Wheat, corn, and rice crops utilize approximately 60% of the N fertilizer produced in the world [5]. The use of N fertilizer must be carefully controlled to ensure good yield and manage N in the soil; N fertilizer increases production costs for farmers [6].

Several authors reported a positive response of nitrogen fertilization on grain yield of wheat [3, 7–11]. Due to the high cost of fertilizers and awareness in support of sustainable agriculture and less polluting, in which the research is growing, one possibility would be to use inoculants containing bacteria that promote growth and increase the productivity of plants. Studies on biological nitrogen fixation (BNF) by *Azospirillum* in grass have been carried out in Brazil. Until recently, no commercial inoculants with these bacteria are available in the country [12].

Although the plant genotype performs an essential role in the colonization of bacteria, cultivars with high and low potential of association exist [13]. Several studies have been published confirming that *Azospirillum* produces phytohormones that stimulate root growth in many plant species. The components released by *Azospirillum brasilense* responsible for stimulating root growth are indoleacetic acid (IAA), gibberellins, and cytokinins [14]. Inoculation with *Azospirillum* can improve the leaf photosynthetic parameters, including chlorophyll content and stomata conductance, greater proline content in shoots and roots, improvement in water potential, an increase in water content in the apoplast, more elasticity of the cell wall, more biomass production, and greater plant size which were reported by Barassi et al. [15]. Increases in photosynthetic pigments such as chlorophyll a and b and auxiliary photoprotective pigments, such as violaxantine, zeaxantin, ateroxantine, lutein, neoxanthin, and beta-carotene, which result in greener plants without water-related stress, were verified by Bashan et al. [16].

In addition, the increase in root development caused by inoculation with *Azospirillum* is involved with several other effects. Increases in water and mineral uptake have been reported, as well as greater tolerance to stresses, such as salinity and drought, resulting in a more vigorous and productive plant [17, 18]. According to Dobbelaere et al. [19], positive responses to inoculation with *A. brasilense* are obtained even when the crops are grown in soils with high N content available, which indicates that the plant responses occur not only due to the N_2 fixed but also mainly depending on the production of phytohormone growth promoters such as cytokinin, gibberellin, and indoleacetic acid. Lemos et al. [20], studying five wheat cultivars, found a positive interaction of *A. brasilense* and nitrogen fertilization only for one wheat cultivar (CD 150). Increases in nitrogen fertilization efficiency associated with inoculation with *A. brasilense* were reported by Galindo et al. [21] but in the grain yields of corn in the Brazilian Cerrado.

Considering the benefits attributed to several crops by inoculation with *A. brasilense*, with emphasis on biological nitrogen fixation, greater development of the root system, and, consequently, greater absorption of water and nutrients, therefore, the inoculation can improve crop performance allowing greater efficiency of nitrogen fertilization. Thus, research that evaluates the nutritional status and wheat yield, in terms of nitrogen rates in association with inoculation with *A. brasilense*, is important.

2. Materials and methods

The wheat experiment was conducted in 2014, in an experimental area that belongs to the UNESP Engineering Faculty, located in Selvíria—MS/Brazil—with the following geographical coordinates 20°22′S and 51°22′W and an altitude of 335 m. Soil in this experimental area was classified as Distroferric Red Oxisol with clay texture (with values of particle size of 420, 50 kg^{-1}, and 530 g of sand, silt, and clay, respectively), according to Embrapa [22], which has been cultivated with annual cultures over 27 years and the last 11 years with no-tillage system. The area was under corn cultivation before sowing wheat. The annual average temperature was 23.5°C, annual average pluvial precipitation was 1370 mm, and annual average relative air humidity was between 70 and 80%.

Glyphosate [1800 g ha^{-1} of active ingredient (a.i.) and 2,4-D (670 g ha^{-1} of a.i.)] herbicides were used for desiccation and applied in 2 weeks prior to sowing wheat. Chemical attributes of the soil in the tillable layer were determined before the wheat experiment began. The methods proposed by van Raij et al. [23] showed the following results: 13 mg dm^{-3} of P (resin), 6 mg dm^{-3} of $S{=}SO_4$, 23 g dm^{-3} of organic matter (OM), pH ($CaCl_2$) of 4.8, 2.6 mmol$_c$ dm^{-3} of K^+, 13.0 mmol$_c$ dm^{-3} of Ca^{2+}, 8.0 mmol$_c$ dm^{-3} of Mg^{2+}, 42.0 mmol$_c$ dm^{-3} of H + Al, 5.9 mg dm^{-3} of Cu, 30.0 mg dm^{-3} of Fe, 93.9 mg dm^{-3} of Mn, 1.0 mg dm^{-3} of Zn (DTPA), 0.24 mg dm^{-3} of B (hot water), and 36% base saturation. After soil chemical analysis, 2.5 t ha^{-1} of dolomitic limestone (with 88% relative total neutralizing power) was directly applied as topdressing 80 days before the wheat was sown in 2014 in order to elevate base saturation to 70%, as recommended by Cantarella et al. [24].

The experimental design was a randomized block with four replications, in a factorial scheme 5 × 2, with five N rates (0, 50, 100, 150, and 200 kg ha^{-1}, as urea) applied as topdressing at the growth stage 3.2 on Zadok's scale [25], with and without seed inoculation with *A. brasilense*. Wheat seeds were inoculated with 300 mL ha^{-1} of inoculant liquid of *A. brasilense* bacteria AbV5 and AbV6 strains (guaranteed minimum analysis of 2 × 10^8 UFC mL^{-1}). The inoculant was mixed with the seeds using a cement mixer, 1 h before planting and after the seed treatments with carbendazim + thiram fungicides (45 + 105 g a.i. per 100 kg of seed) and thiodicarb + imidacloprid insecticides (45 + 135 g a.i. per 100 kg of seed). Each plot consisted of 6 m in length with 12 lines and an inter-row spacing of 0.17 m. The usable area of the plot was eight center lines, excluding 0.5 m extremities. The plot size was 10.20 m^2.

Were applied 350 kg ha^{-1} of the 08-28-16 formulation in the forms of urea, triple superphosphate, and potassium chloride, respectively, at wheat sowing was applied. The experiments were conducted in a no-tillage system. The area in both crops was irrigated by a central pivot sprinkler system. The water coverage was 14 mm over a period of around 72 h. The cultivar used was the CD 116, and sowing was done with an experimental machine on 05/16/14, with 80 seeds being sown per meter. Metsulfuron-methyl (3.0 g a.i. ha^{-1}), a postemergence herbicide, was applied 20 days after emergence (DAE) to control weeds, like *Ipomoea grandifolia*, *Tridax procumbens*, and *Spermacoce latifolia*. The seedling emergence was 6 days after sowing. Topdressing with nitrogen fertilization was performed at 35 DAE, manually distributing the fertilizer on the soil surface (no incorporation) beside and approximately 8 cm of sowing lines in order to avoid the contact of the fertilizer with the plants. After this topdressing, the area was irrigated by sprinkling (depth 14 mm) at night to minimize N losses by volatilization of ammonia, a procedure common in the irrigated wheat crop. The plants were harvested 110 days after wheat emergence.

Concentrations of N, P, K, Ca, Mg, S, Cu, Fe, Mn, and Zn were measured in the grain and straw (above the soil) of wheat at harvest occasion (the end of the crop cycle), in 10 plants per useful area of plot. The determination of nutrients was carried out as described by Malavolta et al. [26]. The wheat was harvested from the plants in the useful area of each plot, and grain yield was calculated after mechanical threshing. Data were transformed into kg ha^{-1} and corrected for 13% moisture (wet basis). The agronomic efficiency of the treatments was determined:

$$AE = \frac{\text{grain yield with fertilizer} - \text{grain yield without fertilizer}}{\text{amount of N applied}}. \quad (1)$$

The results were subjected to analysis of variance and Tukey's test at 5% probability to compare the averages of plants that had been inoculated with *A. brasilense* with those that had not been inoculated. Regression equations were fitted for the effect of N rates using the Sisvar program [27]. For the Pearson correlation analysis, separated from inoculated and non-inoculated treatments, we used SAS program [28].

3. Results and discussion

The increase in nitrogen rates isolated did not influence the nutrients concentrations in irrigated wheat grains, inclusive of N (**Table 1**). However, it is worth noting that the nutrients N, P, and S concentrations presented in the diagnosis leaf (data not shown) were higher

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than average recommended by Cantarella et al. [24], whose ranges for these nutrients are 20–34, 1.5–3, and 2.1–3.3 g kg^{-1}, respectively. For average of Ca and Mg, leaf concentrations are within the recommendation by Cantarella et al. [24] as appropriate, whose ranges for such nutrients are 2.5–10.0 and 1.5–4.0 g kg^{-1}. The K leaf concentration was slightly below 15 (13.5 g kg^{-1}), being the critical level considered as appropriate. However, the average of leaf concentrations of Cu, Fe, Mn, and Zn were suitable, whose ranges for these nutrients are 5–25; 10–300, 25–150, and 20–70 mg kg^{-1} [24], respectively.

With regard to inoculation with *A. brasilense*, the concentrations of P, Ca, andMg were positively influenced by the use of bacteria, where inoculated treatments showed higher concentrations of these nutrients in wheat (**Table 1**). Increasing concentrations of P, Ca, and Mg in the grain raise the possibility of partial immobilization of nutrients in the plant by the bacteria and subsequent release of the same for plants. These bacteria can act on plant growth by producing substances promoting development (auxins, gibberellins, and cytokinins) which provide better root growth [29] and, therefore, have greater uptake of water and nutrients [30] resulting in a more vigorous and productive plant [12, 18], and to be free-living organisms with endophytic characteristics, it is possible to perform some of the metabolic and vital process use of nutrients in the plant, which would then be made available to reflect in increased concentrations in the grains.

Inoculation with *A. brasilense* provided higher concentrations of Mn and Zn in the grains compared to treatments not inoculated, probably due to the possibility of temporary immobilization and subsequent greater redistribution of nutrients to the grain filling (**Table 1**). This result is very interesting, because the increase of Zn in cereal grains such as wheat is the target of a series of research related to agronomic biofortification, since many people are deficient in zinc, especially in less developed countries of the world.

The interaction between nitrogen rates and inoculation with *A. brasilense* was significant for the Mg concentration in the grains. In the absence of N and at doses of 50 and 100 kg ha^{-1}, the treatments inoculated with *A. brasilense* via seed showed higher Mg concentration in the grains compared to treatment that was not inoculated (**Table 2**). There was linear increasing function adjusted for nitrogen rates where there was no inoculation (**Figure 1**), but these values always were lower compared to treatments with inoculation.

The increase in nitrogen rates did not influence the concentration of macronutrients and Cu, Mn, and Zn in wheat straw (**Table 1**). Only the Fe straw concentration was influenced by N rates, adjusting the increasing linear function (**Figure 2**).

Inoculation with *A. brasilense* influenced the concentrations of P and S in the wheat straw in distinct forms. For the P concentration, inoculation promoted lower concentrations of this nutrient in the straw, which is explained by the greater redistribution and accumulation of P in wheat grain filling, as previously reported. However, for the S concentration, in treatments that were performed, the inoculation showed higher concentration of nutrients in the straw (**Table 1**), which can be explained by the greater S uptake in the subsurface soil layers, due to further deepening of the system root of inoculated wheat.

N rates (kg ha^{-1})	N (g kg^{-1})		P (g kg^{-1})		K (g kg^{-1})		Ca (g kg^{-1})		Mg (g kg^{-1})	
	Grain	**Straw**	**Grain**	**Straw**	**Grain**	**Straw**	**Grain**	**Straw**	**Grain**	**Straw**
0	27.12^{ns}	5.32^{ns}	4.84^{ns}	0.62^{ns}	6.77^{ns}	23.22^{ns}	0.48^{ns}	1.36^{ns}	1.48^{ns}	0.76^{ns}
50	25.81	4.79	4.67	0.66	6.47	21.36	0.46	1.40	1.43	0.64
100	26.55	4.76	5.18	0.61	7.14	22.09	0.53	1.26	1.46	0.68
150	28.28	5.28	5.33	0.58	7.33	23.47	0.53	1.34	1.45	0.68
200	25.27	4.67	4.86	0.60	6.89	21.13	0.49	1.38	1.53	0.68
Inoculation										
With *A. brasilense*	26.92 a	5.23 a	5.23 a	0.35 b	7.08 a	22.09 a	0.56 a	1.30 a	1.65 a	0.70 a
Without *A. brasilense*	26.29 a	4.70 b	4.72 b	0.88 a	6.76 a	22.42a	0.43 b	1.40 a	1.30 b	0.67 a
LSD (5%)	0.99	0.45	0.33	0.11	0.39	1.21	0.05	0.14	0.14	0.05
Overall mean	26.61	4.96	4.98	0.61	6.92	22.25	0.50	1.35	1.47	0.69
CV (%)	7.09	17.38	12.79	23.77$^{\#}$	10.81	10.38	20.73	20.06	18.33	13.80

N rates (kg ha^{-1})	S (g kg^{-1})		Cu (mg kg^{-1})		Fe (mg kg^{-1})		Mn (mg kg^{-1})		Zn (mg kg^{-1})	
	Grain	**Straw**	**Grain**	**Straw**	**Grain**	**Straw**	**Grain**	**Straw**	**Grain**	**Straw**
0	2.59^{ns}	1.76^{ns}	7.25^{ns}	8.42^{ns}	45.08^{ns}	235.25**	63.83^{ns}	82.17^{ns}	39.92^{ns}	9.33^{ns}
50	2.58	1.67	6.25	10.33	39.67	275.67	59.50	84.42	37.08	9.42
100	2.55	1.65	7.25	6.50	44.58	232.25	76.58	78.42	41.83	8.33
150	2.67	1.72	7.83	8.33	50.67	263.08	79.58	78.83	47.83	9.42
200	2.62	1.74	7.67	9.17	42.17	321.33	67.58	88.42	39.50	8.75

N rates (kg ha^{-1})	S (g kg^{-1})		Cu (mg kg^{-1})		Fe (mg kg^{-1})		Mn (mg kg^{-1})		Zn (mg kg^{-1})	
	Grain	**Straw**	**Grain**	**Straw**	**Grain**	**Straw**	**Grain**	**Straw**	**Grain**	**Straw**
Inoculation										
With *A. brasilense*	2.57 a	1.92 a	6.87 a	7.87 a	46.33 a	270.30 a	76.30 a	82.47 a	44.53 a	9.40 a
Without *A. brasilense*	2.64 a	1.49 b	7.63 a	9.23 a	42.53 a	260.73 a	62.53 b	82.43 a	37.93 b	8.70 a
LSD (5%)	0.07	0.09	0.86	1.95	4.73	31.06	10.86	9.91	4.60	1.38
Overall mean	2.60	1.71	7.25	8.55	44.43	265.52	69.42	82.45	41.23	9.05
CV (%)	5.42	9.94	22.73	23.58#	20.39	22.38	29.92	23.00	21.34	29.22

Means followed by the same letter in the column do not differ by Tukey's test at 5%.

**significance at $p < 0.01$.

ns significance at not significant.

#data fitted by following equation $(x + 0.5)^{0.5}$.

Table 1. Means and Tukey's test concerning nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, cooper, iron, manganese, and zinc grain and straw concentrations of wheat affected by nitrogen rates, with or without inoculation by *A. brasilense*.

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Inoculation	N rates (kg ha^{-1})				
	0	**50**	**100**	**150**	**200**
With *A. brasilense*	1.77 a	1.65 a	1.68 a	1.47 a	1.62 a
Without *A. brasilense*	1.18 b	1.22 b	1.23 b	1.43 a	1.43 a
LSD (5%)	0.31				

Means followed by the same letter in the column do not differ by Tukey's test at 5%.

Table 2. Inoculation by *A. brasilense* and nitrogen rate interaction for magnesium grain concentration of wheat.

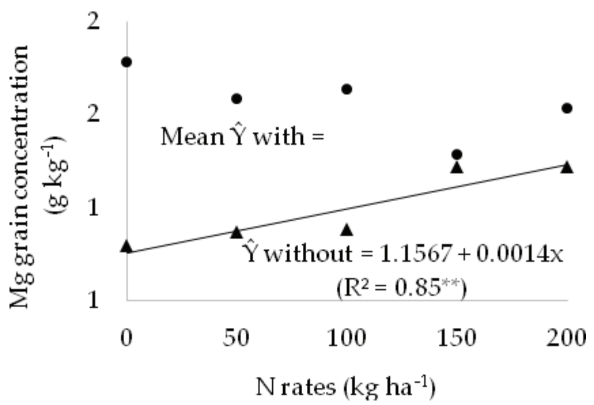

Figure 1. Magnesium grain concentration of wheat in regard to nitrogen rate interaction within inoculation.

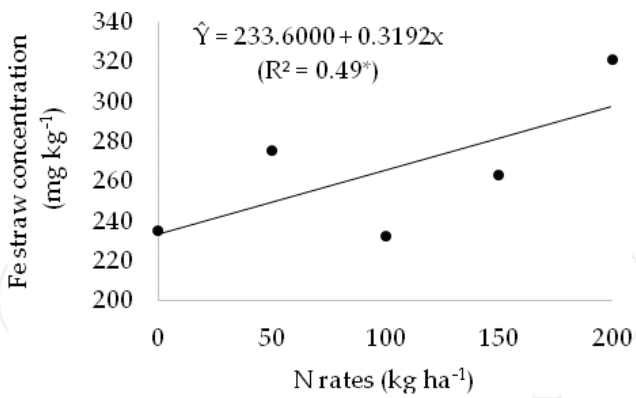

Figure 2. Iron straw concentration of wheat in regard to nitrogen rate.

The *Azospirillum* genus encompasses a group of bacteria that promote plant growth, free life that is found in almost all places of the earth; there are reports also that bacteria of this kind can be facultative endophytic [31]. *Azospirillum* genus of bacteria can act on plant growth by reducing nitrate to ammonia; this energy can be made available to other vital metabolic processes [32]. Nevertheless, this fixation process also requires energy in the form of adenosine triphosphate (ATP) to occur [33], which raises the possibility that these bacteria temporarily immobilize some plant nutrients such as K, Ca, Mg, S, Mn, Zn, and especially P for their metabolic processes and subsequently make available again to plants, reinforcing the results obtained in the nutritional assessment, which observed lower concentrations of P, K, and Ca in leaf tissue and a smaller concentration of P in the straw but increases in the concentrations of P, Ca, Mg, Mn, and Zn in the grains, being interesting from the point of view of human or animal nutrition.

The interaction between nitrogen rates and inoculation was significant for the N concentration in the straw. The treatments that were inoculated by seed with the bacteria *A. brasilense* at the rate of 100–150 kg ha^{-1} N showed higher N concentration in the straw compared to uninoculated treatments (**Table 3**) and, therefore, contributed more to the supply of this important nutrient for subsequent crops. There was linear decreasing function adjusted for nitrogen rates in treatments that were not inoculated, that is, the increase of N rates resulted in decreased concentration of N in the straw (**Figure 3**), indicating a greater need to redistribute N of the straw for grains. Thus, it appears that there was a greater contribution to the absorption of N due to the further development of the root system in relation to biological N_2 fixation when there was seed inoculation with *A. brasilense*. This bacterium can act on plant growth by producing substance promoters for development of (auxins, gibberellins, and cytokinins), which provide improved root growth [29] and consequently

Inoculation	**Doses de N (kg ha^{-1})**				
	0	**50**	**100**	**150**	**200**
With *A. brasilense*	5.23 a	4.62 a	5.32 a	5.88 a	5.08 a
Without *A. brasilense*	5.40 a	4.97 a	4.20 b	4.67 b	4.25 a
LSD (5%)	1.01				

Means followed by the same letter in the column do not differ by Tukey's test at 5%.

Table 3. Inoculation by *A. brasilense* and nitrogen rate interaction for nitrogen straw concentration of wheat.

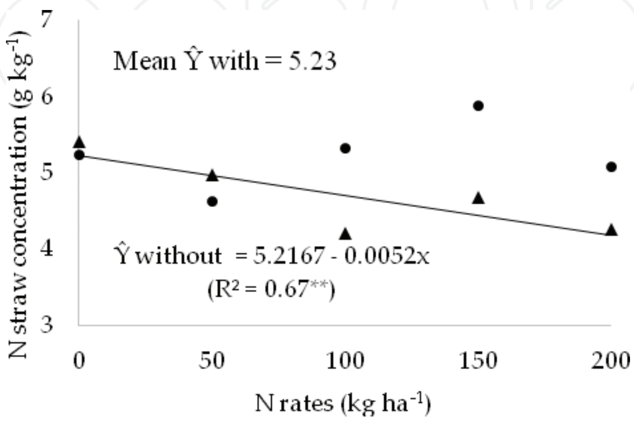

Figure 3. Nitrogen straw concentration of wheat in regard to nitrogen rate interaction within inoculation.

greater absorption of water and nutrients [30], resulting in more vigorous and productive plant [12, 18].

For treatments not inoculated, the Pearson correlation was significant between the concentration of N in the grains and K concentration in straw (0.5131*), Cu in straw (−0.5584*), and Zn in grains (0.4573*). For the treatments inoculated with *A. brasilense*, the correlation was significant between the concentration of N in grains and Fe concentrations in the straw (−0.4440*), K in grains (0.4547*), Ca in grains (0.4994*), and Mg in the grain (0.5087*).

The Pearson correlation was significant between the concentration of N in straw and concentrations in grains of N (1.0000**), of K (0.4547*), of Ca (0.4994*), and of Mg (0.5087*) in the treatments inoculated with *A. brasilense*. However, there was no correlation between the N concentration in straw and the other variables in the non-inoculated treatments.

For the agronomic efficiency of wheat, there was no significant difference between with or without inoculation by *A. brasilense* (**Table 4**), even though numerically in inoculated wheat, the efficiency of nitrogen fertilization has been higher. The interaction between N rates and inoculation was significant for the grain yield of wheat. Inoculated treatments at the rate of 150 kg ha^{-1} N were greater in grain yield of non-inoculated treatments (**Table 5**). There was linear increasing function adjusted for nitrogen rates in the treatments without inoculation and the quadratic function adjusted for the treatments inoculated with positive response up to the dose of 139 kg ha^{-1} N (**Figure 4**). However, *A. brasilense* alone is not effective enough to replace the entire nitrogen fertilization but, associated with N fertilization, makes it possible to achieve the highest yields of irrigated wheat grains in the Brazilian Cerrado.

N rates (kg ha^{-1})	**Grains yield (kg ha^{-1})**	**Agronomic efficiency (kg grain kg^{-1} N)**
0	2269	–
50	3004	13.92
100	3132	8.59
150	3266	6.75
200	3161	4.76
Inoculation		
With *A. brasilense*	2996	9.40 a
Without *A. brasilense*	2937	7.61 a
LSD (5%)	227	3.33
Overall mean	2966	8.51
CV (%)	17.12	24.78#

Means followed by the same letter in the column do not differ by Tukey's test at 5%.

#data fitted by following equation $(x + 0.5)^{0.5}$.

Table 4. Means and Tukey's test concerning grain yields and agronomic efficiency of wheat affected by nitrogen rates, with or without inoculation by *A. brasilense*.

Inoculation	N rates (kg ha^{-1})				
	0	**50**	**100**	**150**	**200**
With *A. brasilense*	2196 a	2916 a	3205 a	3544 a	3119 a
Without *A. brasilense*	2342 a	3092 a	3060 a	2989 b	3203 a
LSD (5%)	508				

Means followed by the same letter in the column do not differ by Tukey's test at 5%.

Table 5. Inoculation by *A. brasilense* and nitrogen rate interaction for grains yield of wheat.

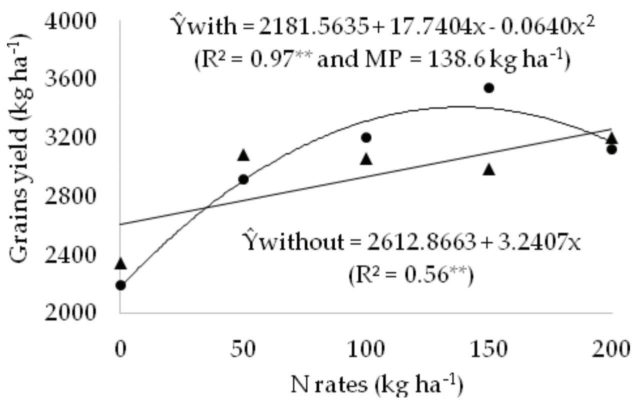

Figure 4. Grain yield of wheat in regard to nitrogen rate interaction within inoculation.

Regarding grain yield, several authors also reported a positive response to nitrogen fertilization on wheat [2, 3, 7–10]. In similar climatic conditions for the cultivation of wheat as a winter crop in the Cerrado region with low altitude, Cazetta et al. [7] and Teixeira Filho et al. [3, 8, 9] suggested maximum grain yield with N doses ranging from 78 kg ha^{-1} [7], 90 kg ha^{-1} [3, 8] to 120 kg ha^{-1} [9]. These differences in rates of N that provide maximum productivity of wheat are due to different requirements of N from the cultivars, as well as the variation in climate and soil conditions.

Lemos et al. [20] studied five wheat cultivars (CD 104, CD 108, CD 119, CD 120, and CD 150), with and without inoculation and, associated with nitrogen rates, found that response to inoculation with *A. brasilense* in wheat crop occurs satisfactorily when held in conjunction with the nitrogen fertilization, as observed in this study at a dose of 150 kg ha^{-1} N (**Table 5**). On the other hand, Ferreira et al. [34], working with foliar application of *A. brasilense* and nitrogen rates in the wheat crop in the Brazilian Cerrado, observed that inoculation had no effect on grain yield. Similarly, Nunes et al. [4] studied inoculation with *A. brasilense* in soils with high and low availability of N, and Galindo et al. [35], in research with application times by leaf of *A. brasilense* with the application of 100 kg ha^{-1} N, found no effect of inoculation in the production components and grain yield of wheat in the Cerrado region.

Tarumoto et al. [36], analyzing inoculation with *A. brasilense* and seed treatment with pesticides on irrigated wheat yield in the Cerrado region and agreeing with the results obtained in this study, also did not verify influence of inoculation alone, on yield of irrigated wheat crop. However, Santa et al. [37] found significant effects on the wheat yield (average increase of 23.9% compared to the control) in the treatment inoculated with *A. brasilense*, both with and without the addition of nitrogen fertilization. While Piccinin et al. [38] suggested that the use of N can be reduced by up to 50% when inoculation with *A. brasilense* is performed. Zorita and Caniggia [39] also reported significant increases on grain yield after inoculation of *A. brasilense* on wheat seeds. Hungria [12] also observed a mean increase in grain yield of 31% for wheat. However, it is noteworthy that the affinity of cultivar with the strains of this bacterium diazotrophic may vary and determine the success or failure of inoculation with *A. brasilense*.

Agronomic efficiency was negatively affected by the increase of N rates (**Table 4**), with adjustment to decreasing linear function due to higher losses of N in the soil (**Figure 5**), as we know, the higher the dose, the greater will be the loss. Increases in the efficiency of nitrogen fertilization associated with inoculation with *A. brasilense* were reported by Galindo et al. [21] but in the corn crop in the Brazilian Cerrado. According to Dobbelaere et al. [19], positive responses to inoculation with *A. brasilense* are obtained even when the crops are grown in soils with high N content available, which indicates that the plant responses occur not only due to the N_2 biological fixation but also mainly depending on the production of phytohormones growth promoters such as the cytokinin, gibberellin, and indoleacetic acid. This fact may possibly has favored root development of wheat, which according to Novakowiski et al. [40] improved the utilization efficiency of the residual N, uptake of water, and other nutrients directly reflected in a higher agronomic efficiency of the plant with *A. brasilense* inoculation as found in the present study for grain yield.

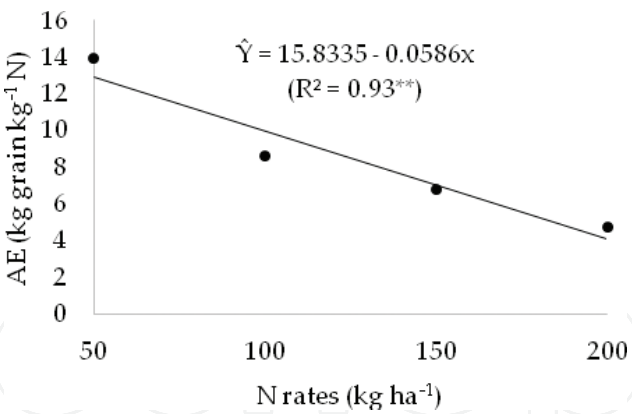

Figure 5. Agronomic efficiency (EA) of wheat in regard to nitrogen rate.

4. Conclusion

Inoculation with *A. brasilense* increased concentrations of Ca, Mg, Mn, and Zn in grain and concentrations of N and S in wheat straw. This bacterium decreases the straw concentration of P, but it increases grain concentration of P.

The straw concentration of N decreased linearly with the increase of N doses, only without inoculation with *A. brasilense*. That is, when inoculation with these bacteria occurred, there was a lower N redistribution from leaves and culms into the grain filling, without the reduction in the N grains concentration. So, it is a great interest for the supply of N to subsequent crops.

The increase in N rates in association with *A. brasilense* inoculation increases the wheat yield up to 139 kg ha^{-1} N, whereas without this inoculation, linear increase occurred with lower maximum yield of wheat. That is, the inoculation afforded higher grain yield applying less nitrogen fertilizer in topdressing.

This research demonstrated that inoculation with *A. brasilense* associated with nitrogen fertilization in topdressing is beneficial to nutrition and wheat yield. Therefore, inoculation is a low-cost technique, easy to apply and use, and nonpolluting, which fall within the desired sustainable context in actuality; the trend is that this technology be increasingly used in wheat crop.

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References

[1] Marini N, Tunes LM, Silva JI, Moraes DM, Cantos FAA. Carboxim Tiram fungicide effect in wheat seeds physiological quality (*Triticum aestivum* L.). Revista Brasileira de Ciência Agrárias. 2011; 6:17-22. doi:10.5039/agraria.v6i1a737 (in Portuguese with abstract in English)

[2] Zagonel J, Venancio WS, Kunz RP, Tanamati H. Nitrogen doses and plant densities with and without a growth regulator affecting wheat, cultivar OR-1. Ciência Rural. 2002; 32:25-29. doi:10.1590/S0103-84782002000100005 (in Portuguese with abstract in English)

[3] Teixeira Filho MCM, Buzetti S, Alvarez RCF, Freitas JG, Arf O, Sá ME. Response of wheat cultivars irrigated by sprinkler to side dressing nitrogen under savannah soil. Acta Scientiarum:Agronomy. 2007; 29:421-425. doi:10.4025/actasciagron.v29i3.471 (in Portuguese with abstract in English)

[4] Nunes PHMP, Aquino LA, dos Santos LPD, Xavier FO, Dezordi LR, Assunção NS. Yield of the irrigated wheat crop subjected to nitrogen application and to inoculation with

Azospirillum brasilense. Revista Brasileira de Ciência do Solo. 2015; 39:174-182. doi:10.1590/01000683rbcs20150354 (in Portuguese with abstract in English)

[5] Espindula MC, Rocha VS, Souza MA, Campanharo M, Pimentel AJB. Urease inhibitor (NBPT) and efficiency of single or split application of urea in wheat crop. Revista Ceres. 2014; 61:273-279. doi:10.1590/S0034-737X2014000200016

[6] Teixeira Filho MCM, Buzetti S, Andreotti M, Benett CGS, Arf O, Sá ME. Wheat nitrogen fertilization under no till on the low altitude Brazilian Cerrado. Journal of Plant Nutrition. 2014; 37:1732-1748. doi:10.1080/01904167.2014.889150

[7] Cazetta DA, Fornasieri Filho D, Arf O. Response of cultivars of wheat and triticale to nitrogen in a no-till system. Científica. 2007; 35:155-165. doi:10.15361/1984-5529.2007v35n2p155+-+165 (in Portuguese with abstract in English)

[8] Teixeira Filho MCM, Buzetti S, Alvarez RCF, Freitas JG, Arf O, Sá ME. Response of wheat cultivars to plant population and nitrogen fertilization in a cerrado region. Científica. 2008; 36:97-106. doi:10.15361/1984-5529.2008v36n2p97+-+106 (in Portuguese with abstract in English)

[9] Teixeira Filho MCM, Buzetti S, Andreotti M, Arf O, Benett CGS. Doses, sources and time of nitrogen application on irrigated wheat under no-tillage. Pesquisa Agropecuária Brasileira. 2010; 45:797-804. doi:10.1590/S0100-204X2010000800004 (in Portuguese with abstract in English)

[10] Povh FP, Molin JP, Gimenez LM, Pauletti V, Molin R, Salvi JV. Behavior of NDVI obtained from an active optical sensor in cereals. Pesquisa Agropecuária Brasileira. 2008; 43:1075-1083. doi:10.1590/S0100-204X2008000800018 (in Portuguese with abstract in English)

[11] Theago EQ, Buzetti S, Teixeira Filho MCM, Andreotti M, Megda MM, Benett CGS. Nitrogen application rates, sources, and times affecting chlorophyll content and wheat yield. Revista Brasileira de Ciência do Solo. 2014; 38:1826-1835. doi:10.1590/S0100-06832014000600017

[12] Hungria M. Inoculation with *Azospirillum brasilense*: innovation in performance at low cost. Documents, 325. Londrina: Embrapa Soja; 2011. 37 p. (in Portuguese)

[13] Quadros PD. Inoculation of *Azospirillum* spp. in seeds of maize genotypes grown in Rio Grande do Sul. [Dissertation]. Porto Alegre: Federal University of Rio Grande do Sul, Brazil; 2009. 74 p. (in Portuguese)

[14] Tien TM, Gaskins MH, Hubbell DH. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). Applied and Environmental Microbiology. 1979; 37:1016-1029.

[15] Barassi CA, Sueldo RJ, Creus CM, Carrozzi L, Casanovas EM, Pereyra MA. *Azospirillum* potential to optimize plant growth under adverse conditions. In: Cassán FD, Garcia de Salomone I, editors. *Azospirillum* ssp.: cell physiology, plant interactions and agronomic research in Argentina. Argentina: Asociación Argentina de Microbiologia; 2008. pp. 49-59. (in Spanish)

[16] Bashan Y, Bustillos JJ, Leyva LA, Hernandez JP, Bacilio M. Increase in auxiliary photoprotective photosynthetic pigments in wheat seedlings induced by *Azospirillum brasilense*. Biology and Fertility of Soils. 2006; 42:279-285. doi:10.1007/s00374-005-0025-x

[17] Dobbelaere S, Croonenborghs A, Thys A, Ptacek D, Vanderleyden J, Dutto P, Labandera-Gonzalez C, Caballero-Mellado J, Aguirre JF, Kapulnik Y, Brener S, Burdman S, Kadouri D, Sarig S, Okon Y. Response of agronomically important crops to inoculation with *Azospirillum*. Australian Journal of Plant Physiology. 2001; 28:871-879. doi:10.1071/PP01074

[18] Bashan Y, Holguin G, De-Bashan LE. *Azospirillum*-plant relations physiological, molecular, agricultural, and environmental advances (1997–2003). Canadian Journal of Microbiology. 2004; 50:521-577. doi:10.1139/w04-035

[19] Dobbelaere S, Vanderleyden J, Okon Y. Plant growth-promoting effects of diazotrophs in the rhizosphere. Critical Reviews in Plant Sciences. 2003; 22:107-149. doi:10.1080/713610853

[20] Lemos JM, Guimarães VF, Vendruscolo ECG, Santos MF, Offemann LC. Response of wheat cultivars to inoculation of seeds with *Azospirillum brasilense* and to nitrogenous fertilizer side dressed to the plants. Científica. 2013; 41:189-198. doi:10.15361/1984-5529.2013v41n2p189-198 (in Portuguese with abstract in English)

[21] Galindo FS, Teixeira Filho MCM, Buzetti S, Santini JMK, Alves CJ, Nogueira LM, Ludkiewicz MGZ, Andreotti M, Bellotte, JLM. Corn yield and foliar diagnosis affected by nitrogen fertilization and inoculation with *Azospirillum brasilense*. Revista Brasileira de Ciência do Solo. 2016; 40:e0150364. doi:10.1590/18069657rbcs20150364

[22] Embrapa – Empresa Brasileira de Pesquisa Agropecuária, National Center for Soil Research. Brazilian system of soil classification. 3rd ed. Brasília: Embrapa; 2013. 353 p. (in Portuguese)

[23] van Raij B, Andrade JC, Cantarella H, Quaggio JA. Chemical analysis to evaluate the fertility of tropical soils. 2nd ed. Campinas: IAC; 2001. 285 p. (in Portuguese)

[24] Cantarella H, van Raij B, Camargo CEO. Cereals. In: van Raij B, Cantarella H, Quaggio JA, Furlani AMC. Recommendations liming and fertilization for the state of São Paulo. 2nd ed. Campinas: IAC; 1997. 285 p. (in Portuguese)

[25] Zadocks JC, Ghang TT, Konzak CF. A decimal code for the growth stages of cereals. Weed Research. 1974; 14:415-421.

[26] Malavolta E, Vitti GC, Oliveira SA. Evaluation of the nutritional status of plants: principles and applications. 2nd ed. Piracicaba: Brazilian Association for Research of Potash and Phosphate; 1997. 319 p. (in Portuguese)

[27] Ferreira DF. Sisvar: A computer statistical analysis system. Ciência e Agrotecnologia, 2011; 35:1039-1042. doi:10.1590/S1413-70542011000600001

[28] Sas Institute INC. SAS/STAT® User's Guide, Version 9.1, v.1-7. Cary, NC: SAS Institute Inc.; 2004.

[29] Okon Y, Vanderleyden J. Root-associated *Azospirillum* species can stimulate plants. Applied and Environment Microbiology. 1997; 6:366-370. citeulike:6806747

[30] Correa OS, Romero AM, Soria MA, Estrada, M. *Azospirillum brasilense*-plant genotype interactions modify tomato response to bacterial diseases, and root and foliar microbial communities. In: Cassán FD, Garcia SI, editors. *Azospirillum* ssp.: cell physiology, plant interactions and agronomic research in Argentina. Argentina: Asociación Argentina de Microbiologia; 2008. pp. 87-95.

[31] Döbereiner J, Pedrosa FO. Nitrogen-fixing bacteria in non-leguminous crop plants. Science Tech. Madison. 1987; 155 p.

[32] Ferreira MCB, Fernandes MS, Döbereiner J. Role of *Azospirillum brasilense* nitrate reductase in nitrate assimilation by wheat plants. Biology and Fertility of Soils. 1987; 4:47-53. doi:10.1007/BF00280350

[33] Hoffmann LV. Molecular biology of biological nitrogen fixation. In: Silveira APD, Freitas SS, editors. Microbiota soil and environmental quality. Campinas: IAC; 2007. cap. 9, pp. 153-164. (in Portuguese)

[34] Ferreira JP, Andreotti M, Arf O, Kaneko FH, Nascimento V, Sabundjian MT. Inoculation with *Azospirillum brasilense* and nitrogen in topdressing in wheat in Cerrado region. Tecnologia e Ciência Agropecuária. 2014; 8:27-32. (in Portuguese with abstract in English)

[35] Galindo FS, Ludkiewicz MGZ, .Bellote JLM, Santini JMK, Teixeira Filho MCM, Buzetti S. Leaf application times of *Azospirillum brasilense* in yield of wheat irrigated. Tecnologia e Ciência Agropecuária. 2015; 9:43-48. (in Portuguese with abstract in English)

[36] Tarumoto MB, Vazquez GH, Arf O, Rodrigues RAF, Silva PHF. Inoculation with *Azospirillum brasilense* and seed treatment with pesticides in productivity irrigated wheat in Cerrado region. In: Proceedings of the Reunião da Comissão Brasileira de Pesquisa de Trigo e Triticale; 2–6 August 2012; Londrina: IAPAR; 2012. pp. 1-5. (CD ROM)

[37] Santa ORD, Santa HSD, Fernández R, Michela G, Ronzelli P, Soccol CR. Influence of *Azospirillum* sp. inoculation in wheat, barley and oats. Ambiência. 2008; 4:197-207. (in Portuguese with abstract in English)

[38] Piccinin GG, Braccini AL, Dan LGM, Bazo GL, Hossa KR, Ponce RM Yield and agronomic performance of wheat in management with *Azospirillum brasilense*. Revista Agrarian. 2013; 6:393-401. (in Portuguese with abstract in English)

[39] Zorita MD, Caniggia MVG. Field performance of a liquid formulation of *Azospirillum brasilense* on dryland wheat productivity. European Journal of Soil Biology. 2009; 45:3-11. doi:10.1016/j.ejsobi.2008.07.001

[40] Novakowiski JH, Sandini IE, Falbo MK, Moraes A, Novakowiski JH, Cheng NC. Residual effect of nitrogen fertilization and *Azospirillum brasilense* inoculation in the maize culture. Semina: Ciências Agrárias. 2011; 32:1687-1698. doi:10.5433/1679-0359.2011v32n4Sup1p1687 (in Portuguese with abstract in English)