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Silicon as an acidity corrective associated with *Azospirillum brasilense* to improve nitrogen management and wheat profitability

Silício como corretivo de acidez associado ao *Azospirillum brasilense* para aumentar a eficiência da adubação nitrogenada e rentabilidade do trigo

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Highlights:
Inoculation with *A. brasilense* increases the profitability of wheat growing.
Ca and Mg silicate is relatively less profitable compared to dolomitc limestone.
Highest grain yield is obtained with139 kg ha^{-1} with limestone and inoculation.
Highest economic return is obtained with 100 kg ha^{-1} with limestone and inoculation.

Abstract

This research aimed to study a possible synergistic effect with inoculation with *A. brasilense* and N doses, associated with the application of silicon in acidity corrective form, evaluating the grain yield of irrigated wheat in Cerrado region. The experiment was conducted in Selvíria, MS, Brazil, under a no-till system, on a Latossolo Vermelho distrófico (Oxisol). The experiment was set up as a randomized block design with four replications, in a 2 × 5 × 2 factorial arrangement consisting of two soil corrective sources (dolomitic limestone and Ca and Mg silicate as source of Si); five N doses (0, 50, 100, 150, and 200 kg ha^{-1}) applied in topdressing; and with and without inoculation with *A. brasilense*. Inoculation makes wheat production more profitable, irrespective of N doses and corrective sources. The N dose of 139 kg ha^{-1} with dolomitic limestone associated with inoculation of *A. brasilense* provided the greatest grain yield. However, the highest economic return was obtained at the N dose of 100 kg ha^{-1}, with dolomitic limestone application and inoculation.

Key words: *Triticum aestivum* L. Biological nitrogen fixation in grasses. Plant growth-promoting bacteria. Ca and Mg silicate. Total operational cost.

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Resumo

O objetivo desse trabalho foi estudar um possível efeito sinérgico entre inoculação com *A. brasilense* e doses de N, associadas à aplicação de silício na forma de corretivo de acidez, avaliando-se economicamente a produtividade de grãos de trigo irrigado em região de Cerrado. O experimento foi conduzido em Selvíria, MS, Brasil, em sistema plantio direto, em um Latossolo Vermelho distrófico. O delineamento experimental utilizado foi em blocos casualizados com quatro repetições, dispostos em esquema fatorial 2 × 5 × 2, sendo duas fontes de corretivo de solo (calcário dolomítico e silicato de Ca e Mg como fonte de Si); cinco doses de N (0, 50, 100, 150 e 200 kg ha^{-1}) aplicados em cobertura; com e sem inoculação com *A. brasilense*. A inoculação propiciou maior lucratividade na produção de trigo, independentemente das doses de N e fontes de corretivo. A dose de N de 139 kg ha^{-1} com calcário dolomítico, associado à inoculação com *A. brasilense* propiciou maior produtividade de grãos. Entretanto, o maior retorno econômico foi obtido na dose de 100 kg ha^{-1}, com aplicação de calcário dolomítico e inoculação.

Palavras-chave: *Triticum aestivum* L. Fixação biológica de nitrogênio em gramíneas. Bactéria promotora de crescimento. Silicato de Ca e Mg. Custo operacional total.

Introduction

Wheat (*Triticum aestivum* L.) is an annual plant and is considered to have greater economic importance among the cool-season cereals, as it has large grain yield capacity. On average, wheat occupies over 17% of cultivable land in the world and represents approximately 30% of world grain production (Barlow, Christy, O´Leary, Riffkin, & Nuttal, 2015). In the 2018 harvest, the Brazilian area planted with wheat was around 2 million hectares, with a production of 5.4 million tons and a yield of 2.6 t ha^{-1}. In the same year, wheat consumption in Brazil was estimated to be 11.4 million tons of grains, of which about 60% had to be imported, making Brazil dependent on countries like Argentina, Canada, and the United States of America, and paying prices higher than for the indigenous product. Although the southern region is responsible for approximately 90% of the national production, the cereal has been introduced gradually in the Cerrado region, under both irrigation and on dry land (Companhia Nacional de Abastecimento [CONAB], 2018).

The Cerrado region has great potential for the expansion of wheat cultivation as it offers favorable climate and soil conditions, strategic market position, and industrialization capacity. However, many soils cultivated with wheat in the Cerrado region and the state of São Paulo are acidic and of low fertility, which limits grain yield (Galindo et al., 2017a). The demand for high wheat yield has resulted in the more frequent use of agricultural supplies, among which nitrogen fertilization is one of the most important (Teixeira et al., 2014; Galindo et al., 2017b). Nitrogen fertilization is one of the highest costs in the production of non-leguminous crops (Nunes et al., 2015). Wheat, corn, and rice crops utilize approximately 60% of the N fertilizer produced in the world (Espindula, Rocha, Souza, Campanharo, & Pimentel, 2014). Also, both nitrogen fertilizer production and application contribute to the emission of gases (CO_2 and NO_2) that contribute to the increase of the greenhouse effect on Earth (Xu, Fan, & Miller, 2012).

In this context, one possibility of increasing the efficiency of N fertilization due to the high cost of fertilizers and the awareness of sustainable and less polluting agriculture is the use of inoculants containing bacteria that promote growth and increase plant productivity. The technology of inoculation of non-legumes with non-symbiotic plant growth-promoting bacteria (PGPB), whose main representative is *Azospirillum* spp., is also being increasingly adopted in several countries, especially for crops such as corn and wheat (Díaz-Zorita & Fernandez-Canigia 2009; Hartmann & Bashan, 2009; Marks et al., 2015).

Another practice that exerts numerous benefits on grasses is the use of silicon (Si) in agriculture, especially when the plants are submitted to biotic and abiotic stresses, common in adverse edaphoclimatic conditions, such as in the Brazilian Cerrado. In addition, Ca and Mg silicate, besides correcting the acidity, raise the levels of soluble phosphorus, calcium, magnesium, and silicon, and, consequently, saturation by bases, reducing the toxic effect of iron, manganese, and aluminum (Reis, Arf, Silva, Sá, & Buzetti, 2008; Camargo, Korndörfer, & Wyler, 2014a; Camargo, Korndörfer, & Foltran, 2014b). Si can also stimulate plant growth and yield through the formation of upright leaves and better plant architecture, which increase the photosynthetic rate, with consequent reduction of bedding due to the greater structural rigidity of the tissues, and still presents another important benefit related to reduction in the transpiration rate (Reis et al., 2008; Camargo et al., 2014a, 2014b). When accumulating in the cells of the epidermal layer, the Si can act as a stable physical barrier against the penetration of some types of fungi, mainly in grasses.

Although some reported benefits have been verified with inoculation with *Azospirillum brasilense* and application of Si as Ca and Mg silicate, positive responses in wheat grain yield were not always observed. Therefore, new research evaluating the effects of *Azospirillum* ssp. on plant development should be performed. Furthermore, there have been few investigations of how much mineral N associated with Si can be applied for successful FBN to increase productivity.

Given the above, and due to the lack of information about this interaction, this study hypothesized that there might be a synergistic effect between inoculation with *A. brasilense* and silicon application, thus allowing a higher efficiency of nitrogen fertilization and subsequent higher grain yield and profitability. Therefore, this research aimed to study the effect of inoculation with *A. brasilense* and N doses, associated with the application of silicon in the form of corrective acidity, evaluating the grain yield of irrigated wheat in economic terms in Cerrado region.

Materials and Methods

The experiment was conducted during 2016 and 2017, located in Selvíria, MS, Brazil (335 m above sea level). The soil of the experimental area was classified as a Latossolo Vermelho distrófico (Oxisol) of clayed texture, according to Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA] (2013), which had been cultivated with annual crops for over 28 years, with the last 11 years under the no-till system, and the crops prior to wheat were corn in both years, respectively. Precipitation rain, air relative humidity, and maximum, mean, and minimum temperatures recorded during the experimental period are shown in Figure 1.

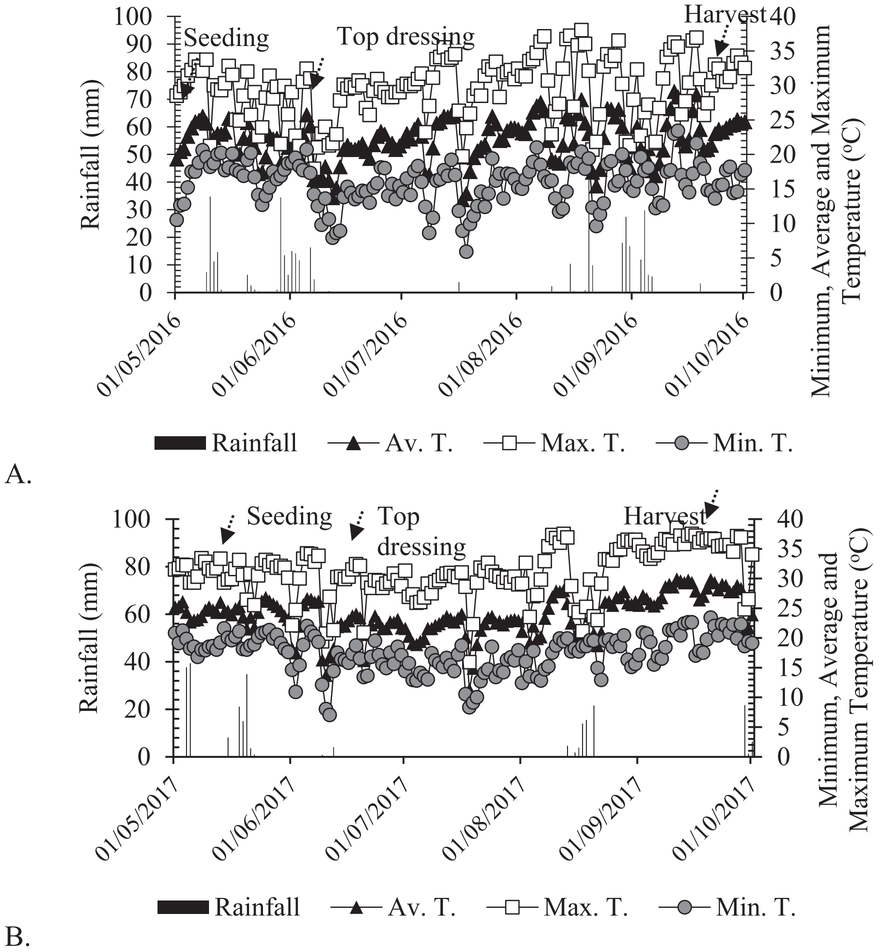

Figure 1. Rainfall, maximum, average and minimum temperatures obtained from the weather station located in the Education and Research Farm of FE / UNESP during the wheat cultivation in the period May 2016 to October 2016 (A) May 2017 to October 2017 (B).

In both crops, a randomized-block design with four replications was set up in a 2 × 5 × 2 factorial arrangement, consisting of two soil corrective sources (dolomitic limestone PRNT = 80%, CaO = 28%, and MgO = 20%; silicate of Ca and Mg as Si source PRNT = 88%, Ca = 25%, Mg = 6%, and Si total = 10%); five N doses (0, 50, 100, 150, and 200 kg ha^{-1}, in the form of urea) applied in topdressing; with and without inoculation of the seeds with *A. brasilense*. The plots of the wheat experiment were 5 m long with 12 lines spaced by 0.17 m, the plot area being the eight central rows, excluding 0.5 m from the extremities.

The herbicides glyphosate (1800 g ha^{-1} of the active ingredient [a.i.]) and 2,4-D (670 g ha^{-1} of the a.i.) were used for the desiccation of the agricultural area. The soil chemical attributes were determined before the implementation of the wheat experiment in 2016, following the methodology proposed by Raij, Andrade, Cantarella and Quaggio (2001). The following results were obtained: in the 0-0.20 m layer: 9.4 mg dm^{-3} Si, 19 mg dm^{-3} P (resin); 10 mg dm^{-3} of S-SO$_4$; 21 g dm^{-3} organic matter; 5.0 pH (CaCl$_2$); K$^+$, Ca^{2+}, Mg^{2+}, H+Al, and Al = 2.1, 19.0, 13.0, 28.0, and 1.0 mmol$_c$ dm^{-3}, respectively; Cu, Fe, Mn, and Zn (DTPA) = 3.1, 20.0, 27.2, and 0.8

mg dm^{-3}, respectively; 0.17 mg dm^{-3} B (hot water) and 55% base saturation; and in the 0.20-0.40 m layer: 10.2 mg dm^{-3} Si, 17 mg dm^{-3} P (resin); 30 mg dm^{-3} of S-SO$_4$; 16 g dm^{-3} organic matter; 4.8 pH (CaCl$_2$); K$^+$, Ca^{2+}, Mg^{2+}, H+Al, and Al = 1.2, 11.0, 8.0, 28.0, and 2.0 mmol$_c$ dm^{-3}, respectively; Cu, Fe, Mn, Zn (DTPA) = 2.1, 10.0, 10.7, and 0.2 mg dm^{-3}, respectively; 0.11 mg dm^{-3} B (hot water) and 42% base saturation.

Based on the soil analysis and with the aim of increasing the saturation by bases to 80%, the dose of 1.94 t ha^{-1} of dolomitic limestone and 1.76 t ha^{-1} of calcium and magnesium silicate was applied 30 days before sowing of corn in the 2015/2015 crop (the predecessor crop) as top-dressing and without incorporation. During fertilization at planting, for both crop years of the experiment, 275 kg ha^{-1} of the 08-28-16 formulation was used, corresponding to 22 kg ha^{-1} N, 77 kg ha^{-1} P$_2$O$_5$, and 44 kg ha^{-1} K$_2$O, based on the soil analysis and the requirements of the wheat crop.

The inoculation of wheat seeds with the bacterium *Azospirillum brasilense* strains Ab-V5 and Ab-V6 [guarantee of 2×10^8 UFC/mL - Inoculants consisted of a mixture of strains CNPSo 2083 (=Ab-V5) and CNPSo 2084 (=Ab-V6) of *A. brasilense* from the Collection of Diazotrophic and Plant Growth-Promoting Bacteria of Embrapa Soja, WFCC # 1213, WDCM # 1054] was carried out at the dose of 300 mL of inoculant (liquid) per sack of 50 kg of planted seeds, with the aid of a clean mixer for incorporation in the seeds and was carried out 1 h before sowing the crop and after treatment of the seeds with insecticide and fungicide. For seed treatment, the fungicides pyraclostrobin + thiophanate-methyl (5 g + 45 g of a.i. per 100 kg of seed) and the insecticide fipronil (50 g of a.i. per 100 kg of seed) were used.

The mechanical sowing of the cultivar CD 1104 was carried out on 05/03/16 in the 2016 crop and 05/10/17 in the 2017 crop, being sown 70 seeds per meter and emergence of seedlings 5 days after sowing, on 05/08/2016 and 05/15/2017, respectively. The wheat crop was irrigated using a center pivot sprinkling system, with a mean water depth of 14 mm and an irrigation interval of approximately 72 h. The herbicide metsulfuron-methyl (3 g ha^{-1} of a.i.) was applied for the control of post-emergence weeds 20 days after emergence (DAE) of wheat in both seasons, on 05/28/2016 and 06/04/2017, respectively.

Nitrogen fertilization (treatments) in topdressing was applied on hauls and without soil incorporation, between the wheat lines, on 06/08/16, and 06/15/17 when the plants were in tillering. The application was made manually, distributing the fertilizer on the soil surface (without incorporation), to the side and approximately 8 cm of the rows, to avoid the contact of the fertilizer 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 harvest was carried out on 09/08/2016 and 09/12/2017, that is, at 120 and 117 days after wheat emergence, respectively.

Grain yield was determined by collecting the plants contained in the usable area of each plot. After mechanical harvesting, grains were quantified, and data were transformed into kg ha^{-1} and corrected for 13% moisture (wet basis) and transformed into 60-kg sacks. Results were subjected to analysis of variance and Tukey's test at 5% probability for comparison of means of acidity correctives sources and use or non-use of inoculation with *Azospirillum brasilense*, and adjusted to regression equations for the effect of N doses, on SISVAR software.

For the economic analysis, the structure based on the total operating production costs (TOC) used by the Institute of Agricultural Economics (IEA) was adopted, according to Matsunaga et al. (1976), consisting of the sum of operating expenses: operations performed, inputs (e.g., fertilizers, seeds, pesticides), labor, machinery, and irrigation, named effective operating costs (EOC). In this study, besides the TOC, other operating expenses and interests were included, considering 5% of the EOC (Matsunaga et al., 1976), thus resulting in the total operating cost (TOC), which was extrapolated to one hectare. This methodology has been already used in several studies on economic evaluation in crops such as Kaneko et al. (2010), Gitti, Arf, Melero, Rodrigues, & Tarsitano, (2012), Kaneko et al. (2015), and Galindo et al. (2017b).

To determine the profitability of the involved treatments, profitability analyses were carried out following Martin, Serra, Oliveira, Angelo and Okawa (1998). Here, the following variables were determined: gross revenue (GR) (in R$), as the product of the amount produced (in number of 60-kg sacks) by the average sale price (in R$); operating profit (OP), as the difference between the gross revenue and total operating cost; accumulated operating profit (AOP), as the sum of the OP obtained in the two years of study; profitability index (PI), understood as the ratio between operating profit (OP) and the net revenue (NR), in percent; equilibrium price, given a certain total operating production cost, as the minimum price calculated to cover this cost, considering the average productivity of the producer; and equilibrium yield, given a certain total operating production cost, as the minimum productivity to cover this cost, considering the average price paid to the producer.

The average prices were quoted in the region of Selvíria - MS, Brazil, in 2017 [average of 3 years (2015, 2016, and 2017), according to Instituto de Economia Agrícola [IEA], 2017]. In this study, simulations were performed as if each experimental treatment represented commercial crops. To facilitate the discussion, the values referring to the yields were transformed into 60-kg sacks, which was the basic unit of sale by local producers. The cost of the sack of wheat for the municipality of Selvíria (average of three years ago) was R$40.67 per unit produced. As regards the acidity correctives sources, the price paid by the farmer was R$85.00 and R$115.00 per ton for dolomitic limestone and Ca and Mg silicate in the region, respectively. For the inoculum with *Azospirillum brasilense*, the expenditure was around R$10.00 per dose, and three doses were used per sack of 50 kg sown in both wheat crops.

Results and Discussion

Table 1 provides the wheat grain yield data for the 2016 and 2017 crops as a function of the soil corrective sources and N doses, with or without inoculation with *A. brasilense*.

The interaction between N doses and inoculation was significant in both crops. There was an adjustment to the increasing linear function for the inoculated treatments and adjustment to the quadratic function for the non-inoculated treatments until the dose of 111 kg ha^{-1} of N in the 2016 crop. The increase in yield of the control N dose (0 kg ha^{-1}) up to the dose that provided the maximum yield obtained with and without inoculation of the seeds with *A. brasilense* was 9 and 8 sacks of 60 kg ha^{-1}, an increase equivalent to 15.6 and 14.4%, respectively (Figure 2).

Table 1

Wheat grain yield affected by sources of acidity correctives applied in soil, doses of nitrogen, with or without inoculation with *Azospirillum brasilense.* Selvíria MS, Brazil, 2016 and 2017

	Grain Yield	
	60 kg sack ha^{-1}	
Doses (D)	2016	2017
0	58.60	59.42
50	65.11	65.08
100	66.31	75.11
150	66.72	74.88
200	65.14	70.16
Acidity correctives (S)		
Dolomitic limestone	64.68 a	68.26
Ca and Mg Silicate	64.07 a	69.57
L.S.D.	3.58	4.06
Inoculation (I)		
With *A. brasilense*	62.51	66.42
Without *A. brasilense*	66.24	71.41
L.S.D.	3.58	4.06
Overall Mean	64.38	68.91
C.V. (5%)	12.41	13.16
F test		
DOSES	5.154*	13.547**
SOURCES	0.117ns	0.418ns
INOCULATION	4.379*	6.051*
D X S	2.256ns	3.932**
D X I	4.256*	5.588**
S X I	0.131ns	0.086ns
D X S X I	0.690ns	0.297ns

The letters correspond to a significant difference at 5% probability level ($p \leq 0.05$). ** and *: significant at $p<0.01$ and $0.01<p<0.05$, respectively.

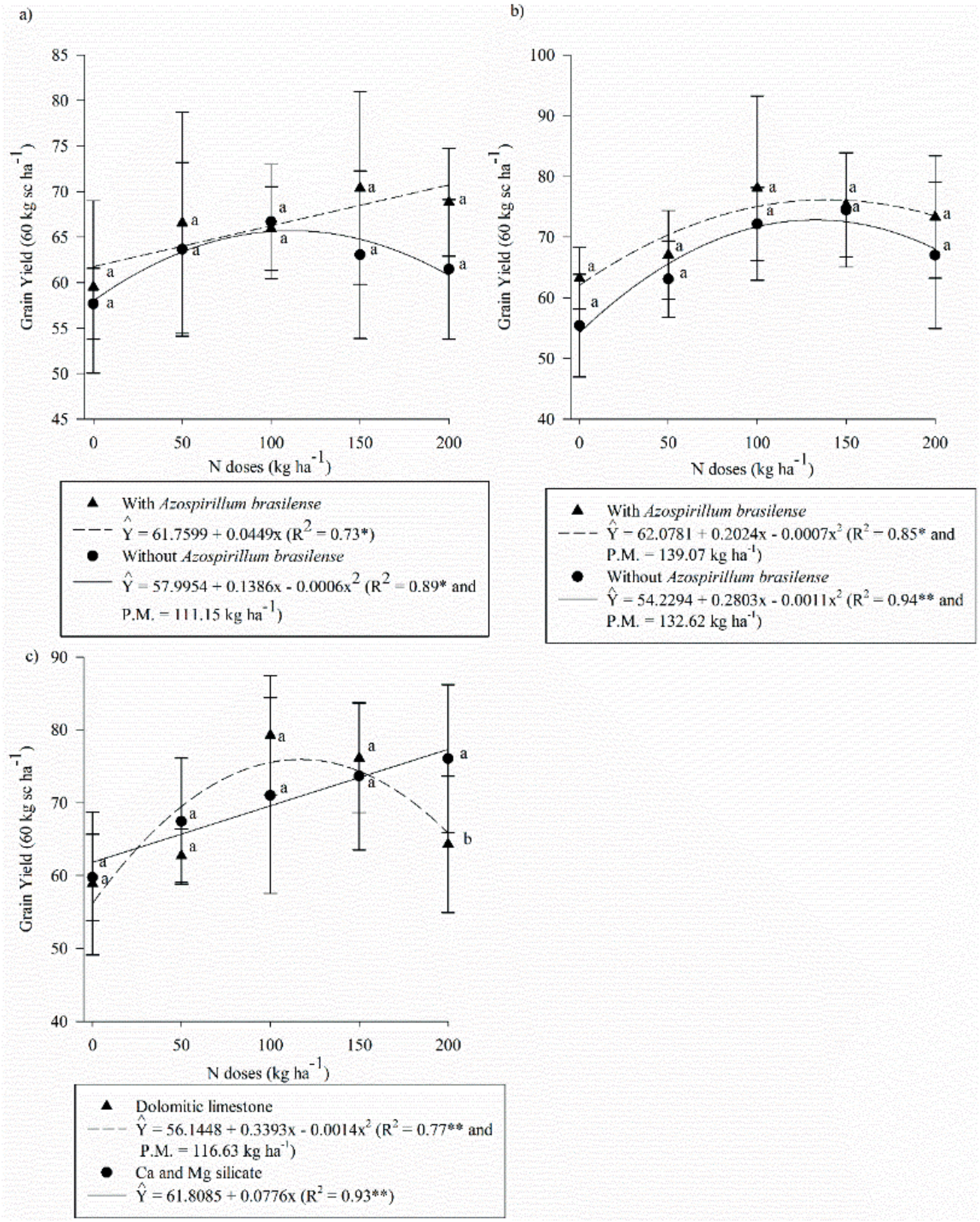

Figure 2. Interaction between nitrogen doses and inoculation with *Azospirillum brasilense* on wheat grain yield in (a) 2016 and (b) 2017, and interaction between nitrogen doses and sources of acidity correctives applied in soil on wheat grain yield in (c) 2017. Selvíria- MS, Brazil, 2016 and 2017. The letters correspond to a significant difference at 5% probability level ($p \leq 0.05$). ** and *: significant at $p<0.01$ and $0.01<p<0.05$, respectively. Error bars indicate the standard error of the mean ($n = 4$).

For the 2017 crop, there was an adjustment to the quadratic function for the treatments inoculated and not inoculated with *A. brasilense* until the dose of 139 and 133 kg ha^{-1} of N, respectively, with yield increase in absence of nitrogen fertilization until the dose that reached the maximum productivity in approximately 14 and 17 sacks of 60 kg ha^{-1}, equivalent to 21.8 and 29.9%, respectively, for treatments inoculated or not inoculated with *A. brasilense* (Figure 2).

In similar climatic conditions for wheat cultivation as winter culture, Teixeira, Buzetti, Andreotti, Arf and Benett (2010a), Theago et al. (2014), and Galindo et al. (2017a) reported the maximum grain yield at 120 kg ha^{-1} of N (Teixeira et al., 2010a), 130 kg ha^{-1} of N (Theago et al., 2014), and 140 kg ha^{-1} of N (Galindo et al., 2017a). This small difference in the N doses that provide the maximum wheat grain yield is due to the differentiated N demand of the cultivars used, as well as the variation in the edaphoclimatic conditions of these surveys. Meira et al. (2009) observed that NH_3 volatilization losses of different N fertilizers, including urea, did not influence corn grain yield. Thus, the increase in N doses supplied provided greater grain yield, regardless of the N source used. There was N available in the soil solution in the period in which the plant requires higher amounts of the nutrient. An explanation would probably be because the N applied at sowing is already in the soil solution, and, when N is added, the plant has a larger amount of the element to be absorbed.

The results obtained demonstrate a positive effect of inoculation with *A. brasilense* on wheat in both crops, and it is important to note that considering only the effect of inoculation alone, independently of the applied N dose and corrective source, the increase in yield by the use of this bacterium via seed was approximately 4 and 5 sacks of 60 kg ha^{-1} in the 2016 and 2017 crops, corresponding to 6% and 7.5% increases in yield respectively. Similarly, Galindo et al. (2017a), studying N doses in topdressing (0, 50, 100, 150, and 200 kg ha^{-1}) with and without inoculation of *A. brasilense*, verified increase in grain yield as a function of inoculation compared to non-inoculated treatments in 2014 and 2015 crops, respectively, with increases of 59 and 124 kg ha^{-1}, equivalent to 2.0 and 4.3%.

Regarding inoculation with *A. brasilense*, positive and similar results were reported by Lemos, Guimarães, Vendruscolo, Santos and Offemann (2013), studying five cultivars of wheat (CD 104, CD 108, CD 119, CD 120, and CD 150) with and without inoculation and associated with N doses. According to these authors, the response to inoculation with *A. brasilense* in wheat culture occurs satisfactorily when carried out in conjunction with nitrogen fertilization, elucidating the issue that *A. brasilense* assists in the development of the plant with a reflection on yield and quality of the crop. Nevertheless, it does not replace nitrogen fertilization, but eventually decreases the N dose to be applied, as verified and mentioned in this research carried out in Cerrado of low altitude.

Positive results with the use of *Azospirillum* were also reported by Kappes et al. (2013), working with doses of N and inoculation with *A. brasilense* in corn in the first harvest. According to the authors, inoculation resulted in a 9.4% increase in grain yield. Similar results were obtained by Novakowiski et al. (2011), where corn grain yield was higher with inoculation of *A. brasilense* when compared to control, even with the increase of N applied. Hungria, Campo, Souza, & Pedrosa, (2010) also obtained increases in corn grain yield, and, depending on the strain of *A. brasilense* evaluated, the increase in productivity was of 24 to 30%, corresponding to 662 to 823 kg ha^{-1}. The analysis of results from a large number of field trials with various non-legume crops, conducted worldwide over 20 years under different soil and weather conditions, has demonstrated that yield increases of up to 30% could be obtained 70% of the time following inoculation with *Azospirillum* (Fukami, Nogueira, Araujo & Hungria, 2016; Fukami, Ollero, Megías, & Hungria, 2017).

These increases are commonly attributed to root growth promotion, accomplished by phytohormones produced by the bacterium, with an emphasis on indole acetic acid, gibberellins, and cytokinins (Tien, Gaskins, & Hubbell, 1979). Moreover, it is inferred that the application of *Azospirillum* is also responsible for higher rates of water and nutrients minerals uptake by the plant (Dardanelli et al., 2008) and higher tolerance to abiotic stresses, such as drought and salinity (Zawoznik, Ameneiros, Benavides, Vázquez, & Groppa, 2011). The relationship between different soil microorganisms and the role of metabolites secreted by them on growth of other surrounding microbial species and plants has been the subject of numerous studies (Marks et al., 2015; Fukami et al., 2016, 2017), and according to Bashan and De-Bashan (2010), due to the wide array of mechanisms proposed for stimulation of plant growth by *Azospirillum* spp., this bacterium probably possesses multiple mechanisms that might act either in a cumulative or sequential pattern.

There is still great divergence in the use of *A. brasilense* in wheat crop and even in other grasses, which is due to the greatly variable results achieved with inoculation. Also, the affinity of the cultivar with the strains of this diazotrophic bacteria might vary and determine whether or not the inoculation with *A. brasilense* is successful (Hungria et al., 2010). However, it is important to highlight the importance of research on the subject and the potential of using this technology, mainly because it is easy to apply and inexpensive, and with great potential to increase the efficiency of nitrogen fertilizers, since the N dose can be reduced when inoculated with *A. brasilense*. In this way, one can produce wheat more sustainably.

The interaction between doses and corrective sources was significant in the 2017 crop. At the dose of 200 kg ha^{-1} of N, the use of Ca and Mg silicate resulted in higher grain yields compared to dolomitic limestone (Figure 2). The increase in yield in this dose mentioned above with the use of silicate was approximately 12 sacks of 60 kg ha^{-1}, equivalent to an increase of 18.2%. This result is interesting and might be due to a higher photosynthetic rate, coming from a better leaf architecture of plants that received silicon. There was an adjustment to the increasing linear function for the treatments with Ca and Mg silicate application and adjustment to the quadratic function until the dose of 117 kg ha^{-1} of N for the treatments with dolomitic limestone application. The increase in yield of the control dose of N up to the dose that provided the maximum productivity obtained with limestone and silicate was 18 and 16 sacks of 60 kg ha^{-1}, an increase equivalent to 30.7 and 27.3%, respectively (Figure 2).

The use of silicon (Si) in agriculture exerts numerous benefits on grasses, especially when the plants are submitted to biotic and abiotic stresses, common in adverse edaphoclimatic conditions, such as in the Brazilian Cerrado (Reis et al., 2008). However, as proposed by Camargo et al. (2014a, 2014b), this element will provide benefits mainly to the plants considered as accumulators, which include many kinds of grass (e.g., rice and sugarcane) and contains a concentration of SiO_2 above 4% (Lima, Castro, Vidal, & Enéas, 2011), and that some factors will influence the uptake of Si by plants, such as genotype, type of soil, and plant species, which could explain the slight response of the use of Si in the form of Ca and Mg silicate in wheat crop in the present work. This finding further highlights the importance of new silicon studies in potentially accumulating crops, such as wheat in Brazilian Cerrado.

Table 2 shows the structure of the total operating costs (TOC) of the wheat crop in the municipality of Selvíria, describing the treatment with dolomitc limestone, nitrogen dose of 0 kg ha^{-1}, without inoculation. This TOC structure model was used in all treatments. As can be seen in Table 2, the expenses with mechanized operations, followed by fertilizers, were the highest, corresponding to 44.8 and 30.6% of the TOC, respectively. This outcome is in line with Kaneko et al. (2010), who studied the viability of the corn crop for the Selvíria- MS region (Brazilian Cerrado) in the 2007/2008 and 2008/2009 crops in a no-till system and N management up to the dose of 120 kg ha^{-1} and found higher expenditures with fertilizers and mechanized operations, corresponding to 32.74 and 30.91% TOC.

Table 2
Total operating costs structure model of wheat for the treatment control (zero kg ha^{-1} N in top-dressing), with limestone and without inoculation with *A. brasilense* per hectare. Selvíria - MS, 2016 and 2017

Description	Specification[1]	Times	Coefficient	Unitary Value (R$)	Total Value (R$)
A. OPERATIONS					
Soil acidity correctives application	HM	1.00	0.30	85.00	25.50
Desiccation	HM	1.00	0.50	85.00	42.50
Hoeing (triton)	HM	1.00	0.50	85.00	42.50
Seeding	HM	1.00	0.60	150.00	90.00
Pulverization	HM	1.00	0.60	85.00	51.00
Topdressing	HM	1.00	0.40	150.00	60.00
Harvest	HM	1.00	1.00	118.00	118.00
Irrigation (pivot)	mm	1.00	150.00	2.50	375.00
Subtotal A					804.50
B. AGRICULTURAL INPUTS					
Dolomitic limestone	t	1.00	0.49	85.00	41.23
Fertilizer 08-28-16	t	1.00	0.28	1,998.00	549.45
Urea	t	1.00	0.00	1,780.00	0.00
Inoculant (*A. brasilense*)	L	1.00	0.00	10.00	0.00
Wheat seed CD 1104	sc 50 kg	1.00	2.90	60.00	174.00
Glyphosate	L	1.00	4.00	14.51	58.04
2,4-D	L	1.00	1.00	13.24	13.24
Metsulfuron methyl	g ha^{-1}	1.00	3.00	1.02	3.05
Seed treatment pyraclostrobin + thiophanate-methyl + fipronil	L	1.00	0.04	350.00	14.00
Subtotal B					853.01
Effective operating costs (EOC)					1,657.51
Other expenses					82.88
Interest cost					53.87
Total operating cost (TOC)					1,794.25

HM = Hour machine; sc = sack
2016 and 2017 average exchange rate: R$2,97 = U$1,00.

Similar results have been reported by Galindo et al. (2017b) working with N doses and sources (0, 50, 100, 150, and 200 kg ha^{-1}, as urea or urea with NBPT), with and without inoculation with *Azospirillum brasilense* in corn crop under Brazilian Cerrado conditions. These authors found higher expenditures with fertilizers and mechanized operations, corresponding to 31.3 and 28.9% TOC. It should be pointed out that with the elevation of N doses and change of analyzed acidity corrective source, the percentage of expenses in relation to the TOC of the fertilizers tends to increase.

The costs with nitrogen top-dressing, as a function of the increasing N doses, ranged from 12.2 to 30.9% of the TOC. Working with N doses (0, 45, 90, 135, and 180 kg ha^{-1} in topdressing) with and without inoculation of *A. brasilense*, Kaneko et al. (2015) had costs with fertilization at planting in the first and second crops of 31.80 and 24.16% of the TOC, respectively. Galindo et al. (2017b), working with N doses (0, 50, 100, 150, and 200 kg ha^{-1}) with and without inoculation with *A. brasilense*, had costs with fertilization at planting that ranged from 7.6 to 24.8% of the TOC as a function of the increasing N doses. Gitti et al. (2012), studying the economic viability of the wheat crop under similar conditions to the present study, in low altitude Cerrado region, under no-tillage system with different green fertilizers and N doses (0, 25, 50, 75, and 125 kg ha^{-1}), reported that the expenditure with fertilization was 23.52% of the TOC, for yields ranging from 34.5 and 66.5 sacks ha^{-1}, and cost with N fertilization in topdressing, as a function of increasing doses, varied from 4.85 to 20.29% of TOC. For the costs with correctives application, the values ranged (in both crops) between 2.5 and 3.7% (dolomitc limestone) and 2.9 and 4.2% (Ca and Mg silicate).

Regarding the TOC and yield of the treatments (Table 3), the highest value for the corrective acidity was found in the treatment with Ca and Mg silicate and the N dose of 200 kg ha^{-1} plus inoculation with *A. brasilense*. The lowest TOC corresponded to the treatments with dolomitic limestone application, without N topdressing (0 kg ha^{-1}) or inoculation of *A. brasilense*. However, it is noteworthy that if N is not replenished in the soil, the soil reserves deplete as the nutrient is extracted, which compromises the productivity of crops over time. For grain yield, the highest values were found in the treatment with dolomitic limestone at the N dose of 150 kg ha^{-1} plus inoculation with *A. brasilense* in the 2016 crop, and with dolomitic limestone at the N dose of 100 kg ha^{-1} with inoculation with *A. brasilense* in the 2017 crop, with average yields of 72.15 and 82.76 sacks of 60 kg, respectively.

Table 3

Total operating cost (TOC), grain yield, gross revenue (GR), operating profit (OP), profitability index (PI), accumulated operating profit (AOP), equilibrium price (EP) and equilibrium yield (EY) of wheat affected by sources of acidity correctives applied in soil, doses of nitrogen, with or without inoculation with *Azospirillum brasilense*. Selvíria MS, 2016 and 2017

Without *Azospirillum brasilense*										
	---------------------- Limestone ----------------------					----------------- Ca and Mg Silicate -----------------				
		2015/16	2016/17	2015/16	2016/17		2015/16	2016/17	2015/16	2016/17
Doses	TOC	YIELD		GR		TOC	YIELD		GR	
	R$	60 kg ha^{-1} sc		-------------(R$)-------------			60 kg ha^{-1} sc		R$	
0	1794.25	55.69	54.05	2264.91	2198.16	1804.4	59.66	56.78	2426.33	2309.41
50	2008.35	66.67	61.10	2711.33	2485.03	2018.5	60.64	65.05	2466.26	2645.72
100	2222.44	64.28	75.72	2614.28	3079.68	2232.59	69.09	68.59	2805.88	2789.35
150	2436.54	63.28	77.92	2573.77	3168.86	2446.69	62.83	70.95	2555.17	2885.37
200	2650.63	62.52	61.51	2542.58	2501.69	2660.78	60.41	72.52	2456.68	2949.19
Mean	2222.44	62.49	66.06	2541.37	2686.68	2232.59	62.53	66.78	2542.06	2715.81
With *Azospirillum brasilense*										
	---------------------- Limestone ----------------------					----------------- Ca and Mg Silicate -----------------				
		2015/16	2016/17	2015/16	2016/17		2015/16	2016/17	2015/16	2016/17

continue

continuation

Doses	TOC	YIELD		GR		TOC	YIELD		GR	
	R$	60 kg ha^{-1} sc		-------------(R$)-------------			sc 60 kg ha^{-1} sc		R$	
0	1888.43	53.34	63.77	2169.33	2593.33	1898.58	65.70	62.69	2672.08	2549.57
50	2102.53	72.14	64.32	2933.89	2615.95	2112.67	60.98	69.82	2480.23	2839.77
100	2316.62	67.86	82.76	2759.70	3365.88	2326.77	64.02	73.39	2603.61	2984.68
150	2530.71	72.15	74.33	2934.05	3023.01	2540.86	68.63	76.34	2790.99	3104.93
200	2744.81	68.90	67.08	2801.97	2728.28	2754.96	68.75	79.55	2796.06	3235.24
Mean	2316.62	66.88	70.45	2719.79	2865.29	2326.77	65.62	72.36	2668.59	2942.84

Without *Azospirillum brasilense*

	---------------- Limestone ----------------				-------------- Ca and Mg Silicate --------------			
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
Doses	OP		PI		OP		PI	
	R$		--------(%)--------		R$		-------- (%)--------	
0	470.66	403.91	20.78	18.37	621.93	505.01	25.63	21.87
50	702.98	476.68	25.93	19.18	447.76	627.22	18.16	23.71
100	391.84	857.24	14.99	27.84	577.29	556.76	20.55	19.96
150	137.23	732.32	5.33	23.11	108.48	438.68	4.25	15.20
200	-108.05	-148.94	-4.25	-5.95	-204.10	288.41	-8.31	9.78
Mean	318.93	464.24	12.56	16.51	310.27	483.22	12.06	18.10

With *Azospirillum brasilense*

	----------------- Limestone ----------------				------------- Ca and Mg Silicate -------------			
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
Doses	OP		PI		OP		PI	
	R$		--------(%)--------		R$		-------- (%)--------	
0	280.90	704.90	12.95	27.18	773.50	650.99	28.95	25.53
50	831.36	513.42	28.34	19.63	367.56	727.10	14.82	25.60
100	443.08	1042.26	16.06	31.17	276.84	657.91	10.63	22.04
150	403.34	492.30	13.75	16.29	250.13	564.07	8.96	18.17
200	57.16	-16.53	2.04	-0.61	41.10	480.28	1.47	14.85
Mean	403.17	547.27	14.63	18.73	341.83	616.07	12.97	21.24

Without *Azospirillum brasilense*

	--------------------- Limestone ---------------------				------------------- Ca and Mg Silicate -------------------			
		2015/16	2016/17			2015/16	2016/17	
Doses	AOP	EP		EY	AOP	EP		EY
	R$	R$ sc^{-1}		60 kg ha^{-1} sc	R$	R$ sc^{-1}		60 kg ha^{-1} sc
0	874.57	32.22	33.20	44.12	1126.94	30.25	31.78	44.37
50	1179.66	30.13	32.87	49.38	1074.98	33.29	31.03	49.63
100	1249.08	34.57	29.35	54.65	1134.05	32.31	32.55	54.90
150	869.55	38.50	31.27	59.91	547.16	38.94	34.49	60.16
200	-256.99	42.40	43.09	65.17	84.31	44.05	36.69	65.42
Mean	783.17	35.56	33.96	54.65	793.49	35.77	33.31	54.90

continue

continuation

With *Azospirillum brasilense*								
	---------------------- Limestone ----------------------				------------------ Ca and Mg Silicate ------------------			
		2015/16	2016/17			2015/16	2016/17	
Doses	AOP	EP		EY	AOP	EP		EY
	R$	R$ sc^{-1}		60 kg ha^{-1} sc	R$	R$ sc^{-1}		60 kg ha^{-1} sc
0	985.8	35.40	29.62	46.43	1424.49	28.90	30.29	46.68
50	1344.78	29.15	32.69	51.70	1094.66	34.64	30.26	51.95
100	1485.34	34.14	27.99	56.96	934.75	36.35	31.71	57.21
150	895.64	35.08	34.05	62.23	814.20	37.03	33.28	62.48
200	40.63	39.84	40.92	67.49	521.38	40.07	34.63	67.74
Mean	950.44	34.72	33.05	56.96	957.90	35.40	32.03	57.21

*Average wheat trading price R$ 40.67 per 60-kg sack according to IEA (2017).

Regarding the gross revenues per hectare obtained in the combination of treatments, in both studied harvests (Table 3), the price of wheat being constant, the gross revenues of the treatments followed the same trend as the yields (Table 3); that is, the accruals in revenue are due to the increases in grain yield. This result is in line with the findings of Duete, Muraoka, Silva, Trevelin and Ambrosano (2009), according to whom the yield is a principal factor to ensure good profitability to the producer. As asserted by Duete et al. (2009), even in regions where producers obtain good grain prices, if their productivity is low, profitability is compromised. Thus, investment in management practices, such as balanced N fertilization, elevates the grain yield and the gross margin of the crops, regardless of the location.

For the values referring to operating profit (Table 3), in both crops, the OP was positive for the majority studied treatments, irrespective of dose, source of acidity correctives, or inoculation, except at the dose of 200 kg ha^{-1} of N without inoculation and with dolomitic limestone in 2016 and 2017, with Ca and Mg silicate in 2016, and with inoculation and limestone in 2017, respectively, partially agreeing with the results obtained by Galindo et al. (2017b), who obtained, overall, a positive OP in 2013/2014 crop working with N doses and inoculation with *Azospirillum brasilense* in corn crop at Brazilian Cerrado.

In the 2016 crop, the highest OP was obtained using dolomitic limestone at the N dose of 50 kg ha^{-1} plus inoculation with *A. brasilense* (R$831.36). For 2017, the best result (R$1,042.26) was obtained by using dolomitic limestone at the N dose of 100 kg ha^{-1} plus inoculation with *A. brasilense* (Table 3), and despite being the best outcome obtained in this harvest, it is still much lower than that found in the previous harvest. Even with nitrogen fertilization as topdressing in high doses up to 150 kg ha^{-1} (which would increase costs, possibly reducing the OP), wheat-growing would be viable because of the high yield obtained; this reinforces the importance of nitrogen fertilization management to achieve high yields and consequently high financial return. Aguiar, Silveira, Moreira and Wander (2008) verified a positive effect of corn, grown in a no-tillage system, without N fertilization, partially differing from what we obtained in this study. The same was reported by Kaneko et al. (2015), who obtained a profitability index without N fertilization, with and without inoculation, of 42.36 and 57.84%, respectively.

Evaluating the OP balance of both harvests, in the absence of inoculation, the treatments that provided the highest OP were those without an N dose of 100 kg ha^{-1} with dolomitic limestone application (Table 3), generating R$1,249.08. When the treatments were inoculated with *A. brasilense*, the highest OP was also obtained at the N dose of 100 kg ha^{-1}, using dolomitic limestone, generating R$1,485.34. For the acidity correctives sources, the highest OP was obtained for the dolomitic limestone when the N dose of 100 kg ha^{-1} was applied and with inoculation with *A. brasilense*, providing R$1,485.34, and for the Ca and Mg silicate, the highest OP was obtained without application of N and with seed inoculation with *A. brasilense*, providing R$1,424.49 (Table 3). An expressive increase was observed in OP at the end of 2 years of cultivations, with inoculation with *A. brasilense*, making the wheat crop more profitable, with an 18.9% increase from the highest OP without inoculation to the treatment that provided the highest OP with inoculation. In turn, Galindo et al. (2017b) verified that the inoculation with *A. brasilense* associated with the N dose of 100 kg ha^{-1} in topdressing promotes higher profitability with irrigated corn crop in the Brazilian Cerrado.

Concerning the profitability index, the highest value was obtained for the 2016 crop, with the treatment with Ca and Mg silicate without N application with *A. brasilense* providing a PI of 28.95. For the 2017 crop, application of dolomitic limestone with 100 kg ha^{-1} of N plus inoculation provided a PI of 31.17. Kaneko et al. (2015) obtained average PIs of 49.92-37.58 and 48.30-36.56 with a urea source, with and without inoculation of *A. brasilense*, respectively, as a function of the N doses of 0, 45, 90, 135, and 180 kg ha^{-1}. Teixeira et al. (2010b), studying N doses (0, 50, 100, 150, and 200 kg ha^{-1}), sources (ammonium sulfonate, ammonium sulfate, and urea), and wheat cultivars (E21 and IAC 370) in the low altitude Cerrado in irrigated cultivation, obtained higher gross margin of gain with 50 kg ha^{-1} dose in topdressing, presenting values close to R$ 500.00 with urea source. Gitti et al. (2012) obtained a higher cost-benefit ratio; in doses between 50 and 75 kg ha^{-1}, values that were in agreement with the doses that provided higher PI in the two crops evaluated in the present study (50 and 100 kg ha^{-1}).

In the analysis of equilibrium yield (minimum yield to cover the costs), for the price of R$40.67 per sack of 60 kg of wheat (Table 3), when Ca and Mg silicate was used at the N dose of 200 kg ha^{-1} plus inoculation with *A. brasilense*, the equilibrium yield was higher (67.44 sacks ha^{-1}), while the lowest equilibrium yields were observed without application of N fertilizer: 44.12 and 46.43 sacks ha^{-1}, to cover the costs of production without and with inoculation of *A. brasilense*, respectively. Although the lowest equilibrium yield was obtained in the absence of N fertilization, it is noteworthy that a lack of N supply in cultivated soil will reduce its concentration in the soil, thus compromising the success of the activity over time, in case the nutrient is not replenished.

Little variation was observed between non-inoculated and inoculated treatments with regard to equilibrium yield, at the same doses and soil acidity correctives sources applied, which is due to the low cost of the inoculation (R$ 10.00 per dose), which is one of the main advantages of inoculation.

The values (in R$) of the 60-kg wheat sack for the equilibrium price (minimum price to cover the TOC) are shown in Table 3. The grains produced with the use of dolomitic limestone had a lower equilibrium price compared with that produced using Ca and Mg silicate in 2016 crop (average of R$35.14 for limestone and R$35.59 for silicate, respectively), but in 2017 the use of Ca and Mg silicate had a lower equilibrium price (average of R$33.51 for silicate and R$32.67 for limestone, respectively). Concerning the N doses, without N application provided lower equilibrium prices when associated with inoculation with *A. brasilense*: R$28.90 in 2016, with Ca and Mg silicate, and in 2017 lower equilibrium price was obtained with 100 kg ha^{-1} of N with dolomitic limestone and inoculation with *A. brasilense*: R$27.99. By contrast, when the treatments with Ca and Mg silicate and dolomitic limestone at the N dose of 200 kg ha^{-1} N were used, both without inoculation, in 2016 and 2017, respectively, the highest equilibrium prices were found: R$44.05 and R$43.09, respectively.

The results obtained demonstrate benefits in wheat grain yield, elucidating the need for new research related to the beneficial effects of inoculation with *A. brasilense* associated with N fertilization and calling attention to the possibility of wide use of this technology in the field due to low economic cost, non-toxic, and with high potential of response of wheat crop, even with the application of N doses considered high for BNF. For this reason, this technique is likely increasingly adopted by rural farmers.

Brazil is a wheat importing country, becoming dependent on countries like Argentina, Canada, and the United States, selling the cereal in dollars, in this way, buying wheat over R$ 50.00 per sack of 60 kg, which is much higher than that paid to wheat produced in the country, which is around R$ 40.00 per sack. Thus, the need for the Government to provide an incentive to the national wheat producers is evident, not only to stimulate production, but also to invest more in technology and in the reduction of the use of foreign currency for the acquisition of the product, especially given that the value of the dollar is increasing.

Conclusions

Inoculation with *A. brasilense* increases the profitability of wheat growing, irrespective of the N dose and source of corrective. Using Ca and Mg silicate is relatively less profitable. Therefore, we recommended using dolomitic limestone, given its easy acquisition and greater economic return.

The N dose of 139 kg ha^{-1} with dolomitic limestone, associated inoculation with *A. brasilense*, provides the greatest grain yields, but the highest economic return is obtained at the N dose of 100 kg ha^{-1}, with limestone and inoculation, ensuring profitability from production of irrigated wheat in the Cerrado.

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