Effectiveness of *Azospirillum brasilense* inoculants to wheat (*Triticum aestivum*) in the micro-region of Curitibanos (SC).

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ABSTRACT

Aims: This work aimed to evaluate the effectiveness of *A. brasilense* inoculants to the development of wheat at the southern part of Brazil.
Study design: The experimental design was randomized block with twelve treatments, containing two liquid inoculants with the *A. brasilense* strains Ab-V5 and Ab-V6, with or without nitrogen fertilization, and five replicates. The plot area was 4m per 6m and thirty-two rows. The seeds inoculation was performed according to the manufacturer's recommendation, and manual sowing. The top-dressing N fertilization was Urea (Super N- 45%N) at 20 days after emergence (DAE), at 120 kg/ha (full dosage) or 60 kg/ha (half dosage).
Place and Duration of Study: The experiment was performed in a farm in Curitibanos county in Santa Catarina state, Brazil. The experiment was carried out on july to november 2016.
Methodology: At 45 DAE, it was performed flag leaves N content (Tedesco et al, 1995). At 115 DAE, it was performed dry shoot weight, plant height, ear sizes, grain N contents and grain yield. The results were submitted to variance analysis (ANOVA) and media compared by Scott-Knot's test at 5% of significance.
Results: There was no statistical difference for shoot dry weight, plant height, ear size and yield. The N leaf content was greater with Ab-V5 inoculation and half N dosage (HC5 - 109% higher than the control). The N grain content was greater with the two strains (inoculant B) without, half and full N dosage (WC56 - 51%, HCB56 - 76% and CB56 - 65%, respectively).
Conclusion: *A. brasilense* strains had the ability to increase wheat N accumulation with lower N fertilizing, suggesting their potential as growth inducers, emphasizing the importance of further studies to confirm and understand the mechanisms involved.

Keywords: Inoculation, N content, Plant growth promotion, Rhizobacteria, Poaceae.

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1. **INTRODUCTION**

In 2019, wheat production reached 5.20 million tons in Brazil [1], about 7% lower than in the previous harvests. This value corresponds to approximately 50% of the cereal consumption, around 10 tons [2]. The estimate for the crop projected reduction of area and production, due to environmental factors, costing, price and production of the last harvest [2,3]. This trend was confirmed, especially in the southern states of the country (Rio Grande do Sul, Paraná and Santa Catarina states) that are the main producers. The decline in area and production of wheat in these states was approximately 10% and 26%, respectively [3]. Santa Catarina recorded a fall of 14% [3] in its agricultural regions, including Curitibanos county, one of the four most important. It is clear that the adoption of strategies to encourage cereal production is necessary, especially those that could reduce production costs and ensure the sustainability of agricultural systems.

Nitrogen plays an important key role in wheat yield. The availability of this nutrient directly influences crop yield [4]. Depending on the content of SOM (Soil Organic Matter) and the previous crop, 60-80 kg N/ha are recommended [4]. In several crops, including wheat, urea is the most commonly used nitrogen fertilizer and represents about 40% of the production cost. Besides burdening the budget, it can lead to environmental disturbances [5]. The biological N fixation (BNF) has been an important and viable alternative for several crops [6,7,8,9]. The technology of N-fixing bacteria inoculation is quite widespread [8,10]. Several bacteria, such as *Azospirillum brasilense*, have been studied because it induces plant growth by mechanisms as BNF, phytohormone production, among others [6,11,12]. Strains Ab-V5 and Ab-V6 from *A. brasilense* increased corn and wheat yields by 27% and 31%, respectively [13]. Currently, the strains are part of several commercial inoculants and it is necessary to comprehend their efficiency in different edaphoclimatic conditions. In Curitibanos county, located in Santa Catarina state, inoculation is used for crops, such as soybean and maize. However, there is no information about its application to wheat. Based on it, the objective was to evaluate the effectiveness of *A. brasilense* on the development of wheat at this region.

2. MATERIAL AND METHODS

2.1 Description of the experimental area

The experiment was sown at a farm in Curitibanos county, Santa Catarina, Brazil. This is located at latitude 27º16'58" south, longitude 50º35'04" west and altitude of 987m. According to the Köppen classification, the climate is Cfb featuring warm and temperate. The average temperature is 16°C and average annual rainfall is 1676 mm, distributed throughout the year. Soil area is classified as Cambisol [14].

Soil samples were collected from a depth of 20 cm to the analysis (table 1). Liming and fertilization were performed based on these.

Table 1 - Soil analysis from experimental area.

O.M **g/dcm^3**	**P** **mg/dcm^3**	**pH** **$CaCl_2$**	**H+Al**	**Al^{+3}**	**K^+**	**Ca^{+2}**	**Mg^{+2}**	**CTC**	**V**
			Cmol$_c$ /dm^3						%
25,6	11,3	5,3	4,3	0,0	0,2	5,5	3,3	13,3	67,8

**O.M. -Organic Matter.*

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2.2 Estimation of diazotrophic bacteria community in soil

It was estimated soil diazotrophic bacteria community [15]. A soil amount (10g) was subjected to serial dilution. 0.5 ml of each dilution (from 10^3 to 10^6) was inoculated in vials containing 4.5 ml of the medium described in [15], with three replicates for each. It was checked the particle in the medium for diazotrophic bacteria presence.

2.3 Experimental design and parameters analysis

The experimental design was in randomized block with twelve treatments (table 2) and five replicates. The plot area was $24m^2$ (4m x 6m) with thirty-two rows spaced 0.17 m. The two inoculants tested (A and B) were a liquid formulation, containing 10^8 CFU (Colony Forming Units)/ml of *A. brasilense* strains, Ab-V5 and Ab-V6. The wheat cultivar was TBIO MESTRE, recommended for Santa Catarina state. Seeds were not treated with fungicides or insecticides. The seeds inoculation was performed according to the manufacturer's recommendation and manual planting. The top-dressing N fertilization was Urea (45%N) at 20 days after emergence (DAE), at 120 kg/ha (full dosage) or 60 kg/ha (half dosage). The experiment was carried out on july to november 2016.

Table 2. Treatments for analysis of *A. brasilense* inoculants efficiency to wheat development.

Treatments	Description
Co	Control - without inoculation and without Nitrogen topdressing fertilization.
CWI	Full Nitrogen topdressing fertilization without inoculation.
HCWI	Half of Nitrogen topdressing fertilization without inoculation.
CA5	Full Nitrogen topdressing fertilization with inoculant A containing Ab-V5.
HCA5	Half of Nitrogen topdressing fertilization with inoculant A containing Ab-V5.
CA56	Full Nitrogen topdressing fertilization with inoculant A containing Ab-V5 and Ab-V6
HCA56	Half of Nitrogen topdressing fertilization with inoculant A containing Ab-V5 and Ab-V6

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CB56	Full Nitrogen topdressing fertilization with inoculant B containing Ab-V and Ab-V6.
HCB56	Half of Nitrogen topdressing fertilization with inoculant B containing Ab-V5 and Ab-V6.
WCA56	Inoculant A containing Ab-V5 and Ab-V6 without Nitrogen topdressing fertilization
WCB56	Inoculant B containing Ab-V5 and Ab-V6 without Nitrogen topdressing fertilization
WCA5	Inoculant A containing Ab-V5 without Nitrogen topdressing fertilization

Plants analyzed were taken from the plot center ($1m^2$). At 45 DAE, flag leaves N content were quantified [16]. At 115 DAE, it was performed the dry shoot weight, plant height, ear sizes, grains N contents and grain yield. The flag leaves and the grains were milled to perform the nitrogen content [16]. Plant height was measured from the base to the top, using graduated tape, as well as the ear size. Dry shoot weight was determined after 72h at 55°C. The productivity was determined by the grain weight at 13% humidity. The results were submitted to variance analysis (ANOVA) and when significant, compared by Scott-Knott's test, at 5% significance at Sisvar 5.6 software.

3. RESULTS AND DISCUSSION

3.1 Diazotrophic bacteria community in soil.

No pellicle was observed in the medium, even in the vials containing vials greater concentrations of cells, suggesting that the diazotrophic community was lower than 10^4 cells/g of soil. This result is consistent to those described by [17], in which the diazotrophic community in non-rhizospheric soil was around 10^3 cells/g of soil, before the development of sorghum roots. As the amount of *A. brasilense* cells in the inoculants was 10^8 CFU (Colony Forming Unit)/ml, applied directly on the seeds, that soil community did not influenced significantly on the results.

3.2 Wheat development and productivity analysis

<u>3.2.1 Shoot dry weight, yield, ear size and plant height</u>

There was no statistical difference for shoot dry weight, plant height, ear size and grain yield (figure 1). However, it is possible to observe that for shoot dry weight (Figure 1a), both inoculants, A and B, with the two strains, either N full or half dosage had more dry mass accumulation, highlighting CA56 that increased it by 17% compared to the control. The same could be observed for grain yield (figure 1b), which stood out HCA56. The association of inoculants, A and B, to the N top-dressing reached greater yields, ranging from 8% for CB56

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to 17% for HCA56, respectively, when compared to the control. Among them, half N dosage reached more yields (12% for HCB56 and 17% for HCA56). The same effect was observed to ear size (figure 1c). For plant height, HCA56 and CA56 had higher individuals (figure 1d).

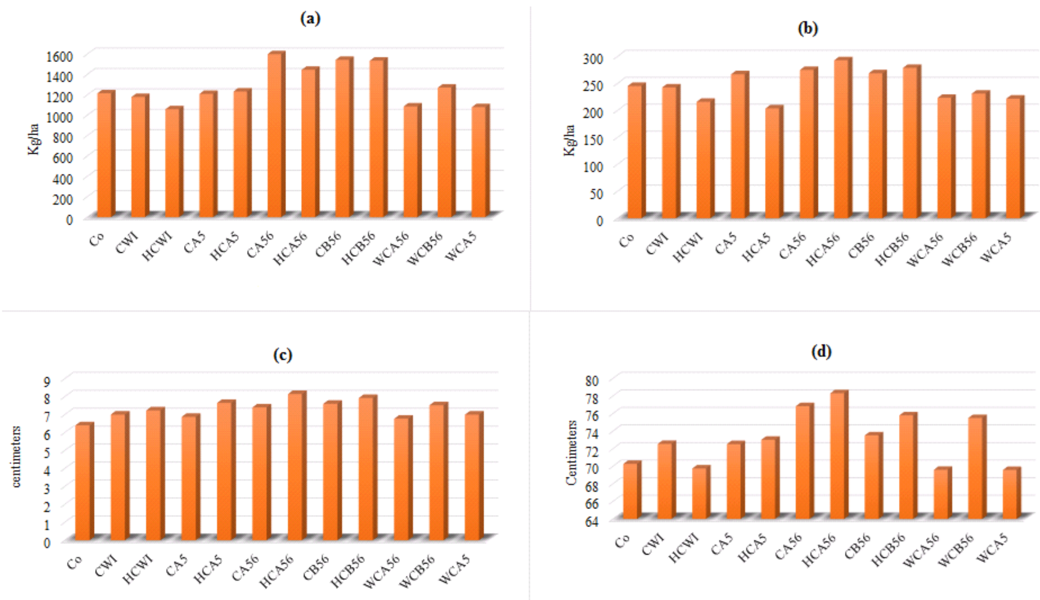

Figure 1 – Wheat growth and production parameters. (a) Shoot dry weight; (b) grain yield; (c) ear size; (d) Plant height.

The significance absence to these parameters could be related to data variation. This results inconsistency was found in different studies [14,18,19,20]. Several reports did not observe a clear response to N fertilization and the inoculation with associative and/or endophytic diazotrophic bacteria. It suggested high edaphoclimatic influence and even, plant genotype variability. Spolaor et al. [20] evaluated the association between topdressing N fertilization and two inoculants in IAC 125 popcorn cultivar and did not observe increase of productivity. Similar results were obtained in hybrid maize [21] and barley [19]. However, Mumbach et al. [7] reported a positive relationship at inoculation and nitrogen fertilization for yield, number of ears/m^2 and shoot dry matter to wheat. Araújo et al. [8] also reported increases in the number of green corn ears when bacteria were associated with gradual nitrogen dosage, suggesting other growth promotion mechanisms, such as auxin production, already described to rhizobacteria [11,12].
Despite the lack of statistical significance, the inoculants A and B, with the two strains, showed the highest means compared to treatments without inoculation or fertilization and that with 60 kg N/ha (half topdressing) (figure 1). This could be related to lower N amount available, especially as ammonium that, in high amounts, can reduce or inhibit nitrogenase activity [13]. Besides ammonium, glutamine, nitrate and nitrite can suppress the BFN and consequently many physiological processes, such as dry mass accumulation and yield. This indicates that the N amount in soil and organisms (plants or bacteria) regulates this process [13].

3.2.2 Nitrogen leaf and grain contents

There were statistical differences for leaves and grain N contents (figure 2). The leaf N amount was greater at the inoculant A, containing only strain Ab-V5, and half N dosage (HCA5), followed by the same inoculant A, with the two strains, and full top-dressing (CA56). The

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increases in N accumulation represented 109% and 87%, respectively, compared to the control (figure 2a). The treatment HCA56 increased N accumulation in leaves, but less extent (28%). The other treatments did not differ from the control. Although, HCWI, CB56 and WCA5 accumulated less N in the leaves than the control.

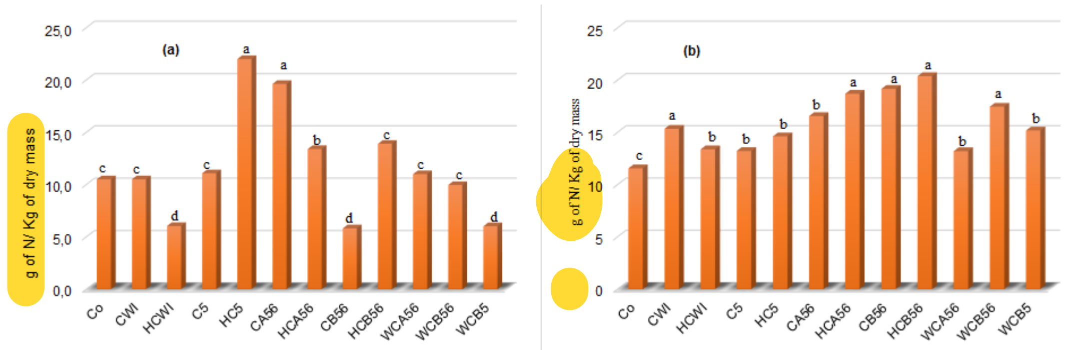

Figure 2 – Wheat N content in leaf (a) and in grain(b).
Same letters do not differ at average test Scott Knott (5%)

The N grain content was significantly greater at inoculant B, containing the two strains, with no, half and full topdressing (WC56 - 51%, HCB56 - 76% and CB56 - 65%, respectively) and at inoculant A with the two strains and half topdressing (HCA56 - 62%) (figure 2b). All treatments had more N in the grains than the Co (Control).
The HCA5 and CA56 reached the highest N leaf levels, suggesting that the Ab-V5 strain was relevant to the N assimilation (Figure 2a). However, despite the high N leaf content, the process of N translocation to the grains did not reflect the same efficiency (figure 2b). The N content in vegetative organs, such as leaves, is closely related to the translocation of sugars and nitrogen to grain [22]. Lana et al. [23] observed that the N leaf content was related to the increase in plant development parameters and N grain accumulation. However, they observed reduction at N leaf content for inoculation associated to nitrogen fertilization.These results were similar to those observed in this study. The N leaf accumulation was lower when complete nitrogen fertilization was associated to single Ab-V5 or to inoculant B, with the two strains (C5, HC5, CB56 and HB56 - Figure 2a). The different responses to N accumulation, for grasses vegetative and production parameters seem to be related to genotypes [23] and even species [7,19,23]. Pereira et al. [24] observed that inoculation of Ab-V5 and Ab-V6 strains associated to 60 kg of N/ha increased grain yield and N content in grain. However, there was no difference to the wheat shoot dry N content to the inoculated and uninoculated treatments. For grain N accumulation, CWI, HCA56 CB56, HCB56 and WCB56 stood out, suggesting that the two strains in both inoculants were efficient to the translocation, as well as the 120 kg of N/ha fertilization. Vogel et al. [25] reviewing results of *A. brasilense* inoculation on wheat, connected positive results of productivity to the efficiency of biomass translocation to grains and the increase of photosynthetic activity. The authors also suggested detailed studies of those mechanisms. Current results reinforced the need to expand these investigations. Treatments that had the highest N content in grain (HCB56 and CB56) showed no leaf nitrogen accumulation significance, especially CB56 that had quantity below to the control. This suggested that higher N dosages, besides inhibiting the BNF can influence physiological processes because the microorganism can act like a drain, competing to the plant. Nevertheless, in general, associative diazotrophic bacteria, such as *A. brasilense*, can supply part of the N needed to the plant development. However, to maintain productivity indexes, it is necessary to associate BNF with N fertilization [7,19,20,23,24].

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4. CONCLUSION

Inoculation increased N content in wheat. The N leaf content was raised by the strain Ab-V5 of *A. brasilense* plus 60Kg of N/ha (HCA5) and also by the strains Ab-V5 and Ab-V6 (inoculant A) plus 120Kg of N/ha (CA56). The N grain content was raised by the strains Ab-V5 and Ab-V6 (inoculant B), with or without N fertilization (WCB56, HCB56 and CB56). These diazotrophic bacteria inoculation seems to be involved in N translocation, especially with lower fertilizing supply, suggesting their potential as growth inducers, emphasizing the importance of further studies to confirm and understand the mechanisms involved.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS´ CONTRIBUTIONS

William Gilberto BALBINOT, André Luis GORDECHUK and Géssica Rogaleski EUTRÓPIO performed the experimental and statistical analysis. Cibele MEDEIROS and Glória Regina BOTELHO wrote the manuscript.

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