Original Research Article

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

.

ABSTRACT

In the current Brazilian scenario, the production of wheat (*Triticum aestivum* L.) is insufficient, in which demand for cereal is higher than supply, highlighting the need to develop strategies to encourage the production. The use of inoculants based on bacteria that stimulate plant development, reducing economic and environmental costs is a technique used in diverse cultures. The micro-region of Curitibanos (SC) is one of main wheat producers at Santa Catarina state. Based on it, the objective was to evaluate the performance of inoculants containing the Ab-V5 and Ab-V6 strains of *Azospirillum brasilense* on growth and agronomic parameters of the crop at the region. Twelve treatments were tested, in which liquid inoculants containing the Ab-V5 single or with Ab-V6 strain were combined to two topdressing dosages. The parameters were dry shoot mass, plant height, ears size, grain yield, leaves and grains N content. There were significant differences for leaves (treatment HCA5) and grains (treatments HCB56 and CB56) N content. It indicated that strains of *A. brasilense* were effective at translocation of N.

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

1. INTRODUCTION

In 2017, wheat production reached 5.20 million tons[1] in Brazil, about 7% lower than in the previous harvest. This value corresponds to approximately 50% of the cereal consumption, around 10 tons[2]. The estimate for the crop projected reduction of planted 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, the main producers, with the state of Santa Catarina in third place. The decline in production and area planted in these states was approximately 26% and 10%, respectively[3].Santa Catarina recorded a fall of 14%[3], observed in all agricultural regions, including the micro-region of Curitibanos, one of the four most important. It is clear that the adoption of strategies to encourage cereal production is necessary, especially those that can reduce production costs and ensure the sustainability of agricultural systems.

Nitrogen plays a key role in wheat yield. The availability of this nutrient directly influences crop yield (Wiethölter, 2011). Depending on the content of SOM (Soil Organic Matter) and the previous crop, 60-80 kg N/ha is recommended (Wiethölter, 2011). In several crops, including wheat, urea is the most commonly used nitrogen fertilizer and represents about 40% of the production cost. In addition to considerably burdening production, it can lead to environmental disturbances (De Carvalho and Zabot, 2012). The biological fixation of N (BFN) has been an important and viable alternative for several crops (Araújo et al., 2014; Hungria et al., 2007; Mumbach et al., 2017). The technology of inoculation is quite widespread (Araújo et al., 2014; Zilli et al., 2010). Several bacteria, such as *Azospirillum brasilense*, have been studied because induce plant growth by mechanisms as BFN, phytohormone production, among others (Bashan and De-Bashan, 2010; Olanrewaju et al., 2017). Strains Ab-V5 and Ab-V6 from *A. brasilense* increased corn and wheat yields by 27% and 31%, respectively Hungria et al., 2010). Currently, the strains are part of several commercial inoculants, and it is necessary to establish differences at efficiency. In the micro-region of Curitibanos, inoculation is used for crops such as soybean, maize, but there is no information about its use to wheat. Based on it, the objective was to evaluate the effectiveness of *A. brasilense* inoculants on development and agronomic parameters to wheat at the region.

2. MATERIAL AND METHODS

The experiment was sowed at a farm in a Curitibanos county (SC). 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 in Curitibanos is 16°C and has average annual rainfall 1676 mm, and well distributed throughout the year. Soil of the area is classified as Cambisol (Embrapa, 2006).

Soil samples were collected from the depth of 0-20cm layer to fertility analysis (table 1). Liming and fertilization performed based on these.

Table 1 - Chemical characterization of the 0-20 cm layer of soil in the experimental area.

O.M	**P**	**pH**	**H+Al**	**Al^{+3}**	**K^{+}**	**Ca^{+2}**	**Mg^{+2}**	**CTC**	
g/dcm^3	**mg/dcm^3**	**$CaCl_2$**	$Cmol_c /dm^3$					%	**V**
35,61	11,30	5,30	4,28	0,00	0,23	5,54	3,26	13,31	67,84

The medium and methodology described in Estrada-de los santos et al. (2001) estimated soil diazotrophic bacteria. A soil fraction (10g) was subjected to serial dilution. 0.5 ml of each dilution (from 103 to 106) was inoculated in vials containing 4,5ml of the medium described in Estrada-de los Santos (2001), with three replicates for each. Checked the pellicle existence in the medium for diazotrophic bacteria presence.

The experimental design was a randomized block with twelve (12) treatments and five (5) replicates. Treatments are described in table 2. The plot size was 4m long by 6m wide and had thirty-two (32) rows, with 0.17 m. distance. The total plot area was 24m2. The two inoculants tested (A and B) were at liquid formulation, having about 108 CFU/mL of A. brasilense strains. Seeds were not treated with fungicides and insecticides. The inoculation performed according to the manufacturer's recommendation, and manual planting. 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). The experiment was carried out on july 30th 2016. The wheat cultivar (*Triticum aestivum* L.) was TRIO MESTRE, recommended for Santa Catarina state.

Table 2. Treatments tested for *A. brasilense* inoculants efficiency at wheat.

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
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

Samples were taken from the one square meter from the plot center. At 45 DAE flag leaves were taken to determine N content (Tedesco et al, 1995). At 115 DAE, it was performed the dry shoot weights, plant height, ear sizes, grains N contents and grain yield. The flag leaves and the grains were milled to perform the nitrogen content, as described by Tedesco et al. (1995). Plant heights were defined from plant bases to their apexes, using graduated tape, as well as the ear size. Dry shoot weights determined after 72h at 65°C. The grain weight moisture with 13% determined productivity. The results were submitted to variance analysis (ANOVA) and when significant, compared by Scott-Knot's test, at 5% significance at Sisvar 5.6 software.

3. RESULTS AND DISCUSSION

To the quantification of diazotrophic bacteria, no pellicle formation was observed even in the vials containing lesser-diluted aliquots (10^3), suggesting that the diazotrophic community was lower than 10^4 cells/g of soil. This result is consistent to those described by Soares et al. (2006) 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 to the seed, that community did not influenced significantly on the results.

There was no statistical difference for shoot dry weights, plant height, ears size and grain yield (figure 1). However, for the dry weight (Figure 1a), both inoculants A and B with the two strains, with either full or half dose had higher dry mass accumulation, highlighting CA56. The same could be observed for grain yield (figure 1b), which stood out HCA56. Half of topdressing N fertilization gave higher yield than in full dose (CA56). Regarding ear size, HCA56 and HCB56 had higher mean values (figure 1c). For plant height, HCA56 and CA56 treatments had higher individuals (figure 1d), especially at HCA56. These results are in agreement with those reported by several authors who did not observe a clear response to N fertilization and the inoculation with associative and / or endophytic diazotrophic bacteria (Barzotto et al., 2018; Spolaor et al., 2016; Dartora et al., 2013, Hungria et al., 2010). Spolaor et al. (2016) evaluated the association between topdressing N fertilization and two inoculants in IAC 125 popcorn cultivar, did not observe increase of productivity. Similar results were obtained in hybrid maize (Dartora et al., 2013) and barley (Barzotto et al., 2018). However, Mumbach et al. (2017) reported a positive relationship at inoculation and nitrogen fertilization for yield, number of ears/m2 and shoot dry matter to wheat. Araújo et al. (2014) also reported increases in the number of green corn ears when associated to increasing nitrogen fertilization, suggesting other growth promotion mechanisms, such as auxin production, already described (Olanrewaju et al., 2017, Bashan and Bashan, 2010). The results inconsistency found at different studies suggested high edaphoclimatic influence and even, plant genotype used in each analysis.

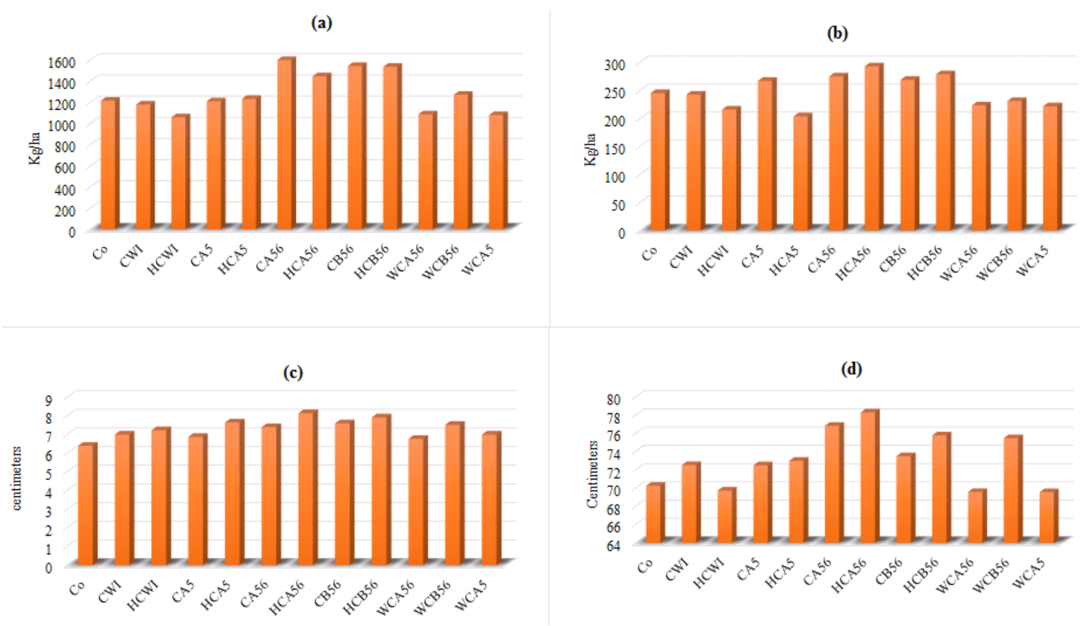

Figure 1 – Wheat growth and production parameters. (a) Shoot dry weight; (b) grain yield; (c) ear size; (d) Plant height.

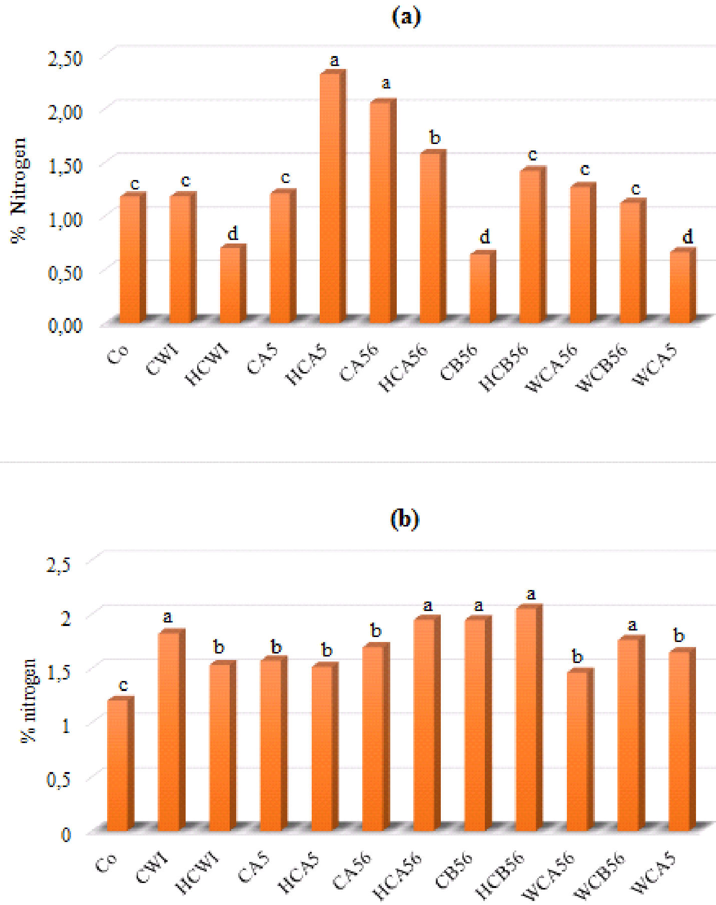

Figure 2 – Wheat N content. (a) – Leaves N content. (b) – Grain N content. (The letters on the bars refer to the Scott knott means test at 5%.).

Despite the lack of statistical significance, it seemed that inoculants A and B with the two strains showed the highest means compared to the treatment without inoculation and fertilization and those with topdressing, highlighting those with 60 kg N/ha (half topdressing) (figure 1). This may be due to the lower N amount available, especially as ammonium that, in high amount, can reduce or inhibit nitrogenase activity (Carvalho et al., 2014). 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 (Carvalho et al., 2014).

There were statistical differences for leaves and grain N contents (figure 2). The leaf N content was significantly higher at treatment with the inoculant A, strain Ab-V5 and with half of topdressing (HCA5), followed by treatments with inoculant A, Ab-V5 and Ab-V6 strains and full and half topdressing (CA56 and HCA56) (figure 2a). Other treatments did not differ from control without inoculation or those without fertilization, except HCWI, CB56 and WCA5 that accumulated less N in the leaves.

The N grain content was significantly higher at inoculant B, containing the two strains, with no, half and full topdressing (WC56, HCB56 and CB56, respectively), at inoculant A, both strains and half topdressing (HCA56) and full topdressing without inoculation (figure 2b). All treatments were significant different from Co (Control).

The highest levels of leaf N were at HCA5 and CA56, suggesting that the presence of the Ab-V5 strain in inoculant A was important for 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 to the grain N content (figure 2b). The N content in vegetative organs, such as leaves, is closely related to the translocation of sugars and nitrogen for grain (Taiz, Zieger, 2004). Lana et al., 2012 observed that the N leaf content was related to increase in plant development parameters and grains N accumulation. However, they observed reduction at N leaf content at inoculation associated to nitrogen fertilization. The different responses to N accumulation, for grasses vegetative and production parameters seem to be related to genotypes (Lana et al, 2012) and even species (Barzotto et al., 2018; Mumbach et al., 2017; Pereira et al., 2017). Pereira et al. (2017) observed that inoculation of Ab-V5 and Ab-V6 strains associated to 60 kg of N/ha increased grain yield and N content in the grain. However, there were no difference to the wheat shoot dry N content, comparing the inoculation methods and uninoculated treatments.

For grains N accumulation, CWI, HCA56 CB56, HCB56 and WCB56 stood out, suggesting that the two strains in the both inoculants were efficient to the translocation, as well as the 120 kg of N / ha fertilization. Vogel et al. (2013), reviewing results of A*. brasilense* inoculation on wheat, related positive results in productivity to the efficiency of translocation of biomass to the grains and increase at the photosynthetic activity. The authors also suggested detailed studies of those mechanisms. The results reinforce the need to expand these investigations, whereas those treatments that had highest N content in grain (HCB56 and CB56) showed no leaf nitrogen content differences to others, and especially CB56 had values below to the control. This may suggest 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 necessary for plant development through BNF, but to guarantee productivity indexes, it is necessary to associate with N fertilization (Barzotto et al., 2018; Mumbach et al., 2017; Pereira et al., 2017, Spolaor et al., 2016, Lana et al., 2012). Table headings should be placed above the table. Footnotes should be placed below the table with superscript lowercase letters. Sample table format is given below.

4. CONCLUSION

The inoculation increased leaf N content at strain Ab-V5 and half-topdressing (HCA5) treatment and strains Ab-V5 and Ab-V6 and full topdressing (CA56). Grains N content was higher at treatments with the two strains and no, half and full topdressing (WCB56, HCB56 and CB56, respectively). It suggested strains of *A. brasilense* were able to increase grains N accumulation as much as recommended N fertilization.

Reference:

1. Agricultural news. Bunge sees increase in wheat imports from Brazil in 2019 after 204 crop failure. Accessed 23 january. 2019. Available: 205 https://www.noticiasagricolas.com.br/noticias/trigo/224825-bunge-ve-a increase-da-206 importation of wheat-by-Brazil-in-2019-after-break- harvest.html # .XEjhBFVKjIU. 207
2. Conab (National Supply Company). 2017. Follow-up of the 208 Brazilian grain crop. Agricultural Observatory, v. 4 2016/17 Harvest - Tenth survey, Brasilia. 209 Accessed 26 october 2017. Available: 210 https://www.conab.gov.br/OlalaCMS/uploads/arquivos/17_07_12_11_17_01_boletim_graos_211 July_2017.pdf. 212
3. IBID IBID - LSPA (Systematic Survey of Agricultural Production). Table 1618- 213 Planted area, harvested area and production, by crop year and product. Accessed 26 october 214 2017. Available: https://sidra.ibge.gov.br/tabela/1618#resultado. 215
4. Araújo, R.M. et al. Green corn response to inoculation with Azospirillum brasilense and 216 nitrogen levels. Rural Science, v.44, n.9, p.1556-1560, 2014. 217
5. Barzotto, G.R. et al. Nitrogen fertilization and inoculation with Azospirillum brasilense in 218 barley. Native, v.6, n.1, p.01-08, 2018. 219
6.Bashan, Y .; De-Bashan, L. How the plant growth-promoting bacterium Azospirillum 220 promotes plant growth — a critical assessment. Advances in Agronomy, v.108, p.77 - 136, 221 2010. 222
7.CONAB (NATIONAL SUPPLY COMPANY). 2017. Follow-up of the 223 Brazilian grain crop. Agricultural Observatory, v. 4 2016/17 Harvest - Tenth survey, 224 Brasilia. 225 https://www.conab.gov.br/OlalaCMS/uploads/arquivos/17_07_12_11_17_01_boletim_graos_226 July_2017.pdf. Oct 26 2017. 227
8. Oak. T.L. et al. Nitrogen signalling in plant interactions with associative and endophytic 228 diazotrophic bacteria. Journal of Experimental Botany, v.65, n.19, p.5631–5642, 2014. 229
9.Dartora, J. et al. Nitrogen fertilization associated with inoculation with Azospirillum brasilense 230 and Herbaspirillum seropedicae in corn crop. Brazilian Journal of Agricultural Engineering 231 and Environmental, v.17, n.10, p.1023–1029, 2013. 232
10. By Carvalho, N.L.D .; Zabot, V. Nitrogen: nutrient or pollutant? Electronic Journal on 233 Environmental Management, Education, and Technology, v.6, p.960 - 974, 2012. 234
11. EMBRAPA. National Center for Soil Research (Rio de Janeiro, RJ). Brazilian soil classification system 235. 2. ed. - Rio de Janeiro: EMBRAPA-SPI, 2006. 306p.
12. Los Santos Road, P. et al. Burkolderia, a genus rich in plant-associated nitrogen 237 fixers with wide environmental and geographic distribution. Applied and Environmental 238 microbiology, v.67, n.6, p. 2790-2798, 2001. 239

13.SIDERIAL FOUNDATION TO SUPPORT AGRICULTURAL RESEARCH. Conab increases 240 wheat production in Brazil by 9.2%. 241
http://www.fundacaomeridional.com.br/noticias/2018/03/12/conab-aumenta-producao-de-242 Brazil-wheat-in-92. Jan 23 2019,243
14. Hungary, M. et al. Inoculation with selected strains of Azospirillum brasilense and A. 244 lipoferum improves yields of maize and wheat in Brazil. Plant Soil, v.331, p.413–425, 2010. 245
15. Hungary, M. et al. The Importance of the Biological Nitrogen Fixation Process for 246 Soybean Crops: An Essential Component for the Competitiveness of Brazilian Produce 247 (Documents 283). Londrina: Embrapa Soy, 2007.80 p 248
16.Lana, M. et al. Inoculation with Azospirillum, associated with nitrogen fertilization in maize. 249 Ceres Magazine, v.59, n.3, p.399-405, 2012. 250
17. Mumbach, G.L. et al. Response of Azospirillum brasilense inoculation in 251 wheat and safrinha corn crops. Scientia Agraria, v.18, n.2, p. 97-103, 2017. 252
18. AGRICULTURAL NEWS. Bunge sees an increase in wheat imports from Brazil in 2019 253 after crop failure.
https://www.noticiasagricolas.com.br/noticias/trigo/224825-bunge-ve-254 increase-import-wheat-by-brazil-in-2019-after-255-crop failure. html # .XEjhBFVKjIU. Jan 23 2019,256
19. AGRICULTURAL NEWS. Wheat: USDA projects further increase in crop and stocks of 257 global crop 2017/18 and prices retreat at CBOT. 258
https://www.noticiasagricolas.com.br/noticias/usda/196866-trigo-usda-projeta-novo-259 increase-in-the-crop-and-ending-stocks-201718-and-prices- indent it-260 cbot.html # .WfIhD1tSzIU. Oct 26 2017. 261
20.Olanrewaju, O.S. et al. Mechanisms of action of plant growth promoting bacteria. World 262 Journal of Microbiology and Biotechnology, v.33, n.197, p.1-16, 2017. 263
21. Perira, L.C. et al. Wheat yield (Triticum aestivum) in response to different modes of inoculation with Azospirillum brasilense. Journal of Agricultural Sciences, v.240, n.1, 265 p.105-113, 2017. 266
22.SIDRA IBGE - LSPA (Systematic Survey of Agricultural Production). Table 1618-267 Planted area, harvested area and production, by crop year and product. 268
https://sidra.ibge.gov.br/tabela/1618#resultado. Oct 26 2017. 269
23.Spolaor, L.T. et al. Growth promoting bacteria associated with cover nitrogen fertilization on popcorn maize agronomic performance. Bragantia, v.75, no. 1, 271 p.33-40, 2016. 272
24.Taiz, L .; Zeiger, E. Plant Physiology. 3rd ed. Porto Alegre: Artmed, 2004. 719p. 273
25. Tedesco, M.J. et al. Soil, Plant and Other Material Analysis (Technical Bulletin 5). 2nd ed. 274 Porto Alegre: UFRGS - Department of Soils, 1995. 174p. 275
26. Vogel, G.F. et al. Agronomic performance of Azospirillum brasilense in rice crop: 276 a review. Journal on Agribusiness and the Environment, v.6, n.3, p.567-578, 2013. 277

27. Wiethölter, S. Soil fertility and wheat crop in Brazil. In: Pires, J.L.M. et al. (Eds.). Wheat in Brazil: bases for competitive and sustainable production. Passo Fundo: Embrapa Wheat, 2011. p.135-184.

Zilli, J.E. et al. Efficacy of Bradyrhizobium inoculation in soybean pre-sowing. Brazilian Agricultural Research, v.45, n.3, p.335-338, 2010.