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

5
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16
17 **ABSTRACT**
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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.

19
20 *Keywords: Inoculation, N content, Plant growth promotion, Rhizobacteria, Poaceae.*
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24 **1. INTRODUCTION**

25

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

37 Nitrogen plays an important key role in wheat yield. The availability of this nutrient directly
38 influences crop yield [4]. Depending on the content of SOM (Soil Organic Matter) and the
39 previous crop, 60-80 kg N/ha are recommended [4]. In several crops, including wheat, urea
40 is the most commonly used nitrogen fertilizer and represents about 40% of the production
41 cost. Besides burdening the budget, it can lead to environmental disturbances [5]. The
42 biological N fixation (BNF) has been an important and viable alternative for several crops
43 [6,7,8,9]. The technology of N-fixing bacteria inoculation is quite widespread [8,10]. Several
44 bacteria, such as *Azospirillum brasilense*, have been studied because it induces plant
45 growth by mechanisms as BNF, phytohormone production, among others [6,11,12]. Strains
46 Ab-V5 and Ab-V6 from *A. brasilense* increased corn and wheat yields by 27% and 31%,
47 respectively [13]. Currently, the strains are part of several commercial inoculants and it is
48 necessary to comprehend their efficiency in different edaphoclimatic conditions. In
49 Curitibaanos county, located in Santa Catarina state, inoculation is used for crops, such as
50 soybean and maize. However, there is no information about its application to wheat. Based
51 on it, the objective was to evaluate the effectiveness of *A. brasilense* on the development of
52 wheat at this region.

53

54 **2. MATERIAL AND METHODS**

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56 **2.1 Description of the experimental area**

57

58 The experiment was sown at a farm in Curitibaanos county, Santa Catarina, Brazil. This is
59 located at latitude 27°16'58" south, longitude 50°35'04" west and altitude of 987m. According
60 to the Köppen classification, the climate is Cfb featuring warm and temperate. The average
61 temperature is 16°C and average annual rainfall is 1676 mm, distributed throughout the
62 year. Soil area is classified as Cambisol [14].

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

65

66 **Table 1 - Soil analysis from experimental area.**

67

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						
35,6	11,3	5,3	4,3	0,0	0,2	5,5	3,3	13,3	67,8

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68 *O.M. -Organic Matter.

69 **2.2 Estimation of diazotrophic bacteria community in soil**

70

71 It was estimated soil diazotrophic bacteria community [15]. A soil amount (10g) was
72 subjected to serial dilution. 0.5 ml of each dilution (from 10^3 to 10^6) was inoculated in vials
73 containing 4,5ml of the medium described in [15], with three replicates for each. It was
74 checked the pellicle in the medium for diazotrophic bacteria presence.

75

76 **2.3 Experimental design and parameters analysis**

77

78 The experimental design was in randomized block with twelve treatments (table 2) and five
79 replicates. The plot area was 24m² (4m x 6m) with thirty-two rows spaced 0.17 m. The two
80 inoculants tested (A and B) were at liquid formulation, containing 10⁸ CFU (Colony Forming
81 Units)/ml of *A. brasilense* strains, Ab-V5 and Ab-V6. The wheat cultivar was TBIO MESTRE,
82 recommended for Santa Catarina state. Seeds were not treated with fungicides or
83 insecticides. The seeds inoculation was performed according to the manufacturer's
84 recommendation and manual planting. The top-dressing N fertilization was Urea (45%N) at
85 20 days after emergence (DAE), at 120 kg/ha (full dosage) or 60 kg/ha (half dosage). The
86 experiment was carried out on July to November 2016.

87

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

90

Treatments	Description
Co	Control - without inoculation and without Nitrogen topdressing fertilization.
CW1	Full Nitrogen topdressing fertilization without inoculation.
HCW1	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

91
92 Plants analyzed were taken from the plot center (1m²). At 45 DAE, flag leaves N content
93 were quantified [16]. At 115 DAE, it was performed the dry shoot weight, plant height, ear
94 sizes, grains N contents and grain yield. The flag leaves and the grains were milled to
95 perform the nitrogen content [16]. Plant height was measured from the base to the top, using
96 graduated tape, as well as the ear size. Dry shoot weight was determined after 72h at 55°C.
97 The productivity was determined by the grain weight at 13% humidity. The results were
98 submitted to variance analysis (ANOVA) and when significant, compared by Scott-Knott's
99 test, at 5% significance at Sisvar 5.6 software.

100

101 **3. RESULTS AND DISCUSSION**

102

103 **3.1 Diazotrophic bacteria community in soil.**

104

105 No pellicle was observed in the medium, even in the vials containing vials greater
106 concentrations of cells, suggesting that the diazotrophic community was lower than 10⁴
107 cells/g of soil. This result is consistent to those described by [17], in which the diazotrophic
108 community in non-rhizospheric soil was around 10³ cells/g of soil, before the development of
109 sorghum roots. As the amount of *A. brasilense* cells in the inoculants was 10⁸ CFU (Colony
110 Forming Unit)/ml, applied directly on the seeds, that soil community did not influenced
111 significantly on the results.

112

113 **3.2 Wheat development and productivity analysis**

114

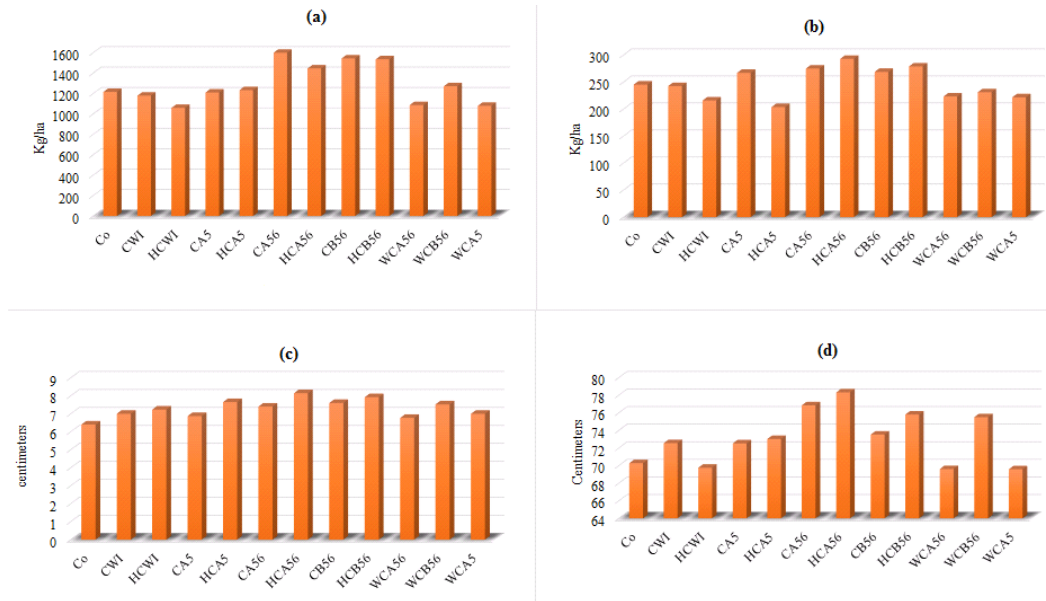
115 **3.2.1 Shoot dry weight, yield, ear size and plant height**

116

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

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123 to 17% for HCA56, respectively, when compared to the control. Among them, half N dosage
 124 reached more yields (12% for HCB56 and 17% for HCA56). The same effect was observed
 125 to ear size (figure 1c). For plant height, HCA56 and CA56 had higher individuals (figure 1d).
 126



127

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

130

131 The significance absence to these parameters could be related to data variation. This results
 132 inconsistency was found in different studies [14,18,19,20]. Several reports did not observe a
 133 clear response to N fertilization and the inoculation with associative and/or endophytic
 134 diazotrophic bacteria. It suggested high edaphoclimatic influence and even, plant genotype
 135 variability. Spolaor et al. [20] evaluated the association between topdressing N fertilization
 136 and two inoculants in IAC 125 popcorn cultivar and did not observe increase of productivity.
 137 Similar results were obtained in hybrid maize [21] and barley [19]. However, Mumbach et al.
 138 [7] reported a positive relationship at inoculation and nitrogen fertilization for yield, number of
 139 ears/m² and shoot dry matter to wheat. Araújo et al. [8] also reported increases in the
 140 number of green corn ears when bacteria were associated with gradual nitrogen dosage,
 141 suggesting other growth promotion mechanisms, such as auxin production, already
 142 described to rhizobacteria [11,12].

143

144 Despite the lack of statistical significance, the inoculants A and B, with the two strains,
 145 showed the highest means compared to treatments without inoculation or fertilization and
 146 that with 60 kg N/ha (half topdressing) (figure 1). This could be related to lower N amount
 147 available, especially as ammonium that, in high amounts, can reduce or inhibit nitrogenase
 148 activity [13]. Besides ammonium, glutamine, nitrate and nitrite can suppress the BFN and
 149 consequently many physiological processes, such as dry mass accumulation and yield. This
 150 indicates that the N amount in soil and organisms (plants or bacteria) regulates this process
 151 [13].

152

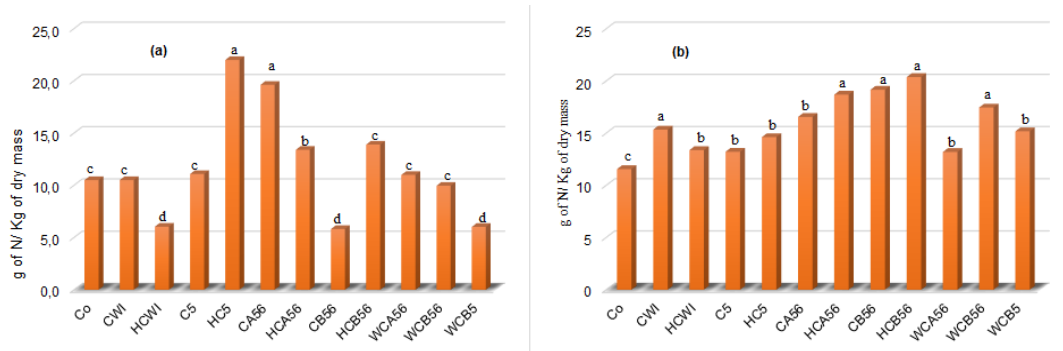
152 **3.2.2 Nitrogen leaf and grain contents**

153

154 There were statistical differences for leaves and grain N contents (figure 2). The leaf N
 155 amount was greater at the inoculant A, containing only strain Ab-V5, and half N dosage

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156 (HCA5), followed by the same inoculant A, with the two strains, and full top-dressing (CA56).
 157 The increases in N accumulation represented 109% and 87%, respectively, compared to the
 158 control (figure 2a). The treatment HCA56 increased N accumulation in leaves, but less
 159 extent (28%). The other treatments did not differ from the control. Although, HCWI, CB56
 160 and WCA5 accumulated less N in the leaves than the control.
 161
 162



163
 164
 165 **Figure 2 – Wheat N content in leaf (a) and in grain(b).**
 166 Same letters do not differ at average test Scott Knott (5%)
 167

168 The N grain content was significantly greater at inoculant B, containing the two strains, with
 169 no, half and full topdressing (WCB56 - 51%, HCB56 - 76% and CB56 - 65%, respectively) and
 170 at inoculant A with the two strains and half topdressing (HCA56 - 62%) (figure 2b). All
 171 treatments had more N in the grains than the Co (Control).

172 The HCA5 and CA56 reached the highest N leaf levels, suggesting that the Ab-V5 strain was
 173 relevant to the N assimilation (Figure 2a). However, despite the high N leaf content, the
 174 process of N translocation to the grains did not reflect the same efficiency (figure 2b). The N
 175 content in vegetative organs, such as leaves, is closely related to the translocation of sugars
 176 and nitrogen to grain [22]. Lana et al. [23] observed that the N leaf content was related to the
 177 increase in plant development parameters and N grain accumulation. However, they
 178 observed reduction at N leaf content for inoculation associated to nitrogen fertilization. These
 179 results were similar to those observed in this study. The N leaf accumulation was lower
 180 when complete nitrogen fertilization was associated to single Ab-V5 or to inoculant B, with
 181 the two strains (C5, HC5, CB56 and HB56 - Figure 2a). The different responses to N
 182 accumulation, for grasses vegetative and production parameters seem to be related to
 183 genotypes [23] and even species [7,19,23]. Pereira et al. [24] observed that inoculation of
 184 Ab-V5 and Ab-V6 strains associated to 60 kg of N/ha increased grain yield and N content in
 185 grain. However, there was no difference to the wheat shoot dry N content to the inoculated
 186 and uninoculated treatments.

187 For grain N accumulation, CWI, HCA56 CB56, HCB56 and WCB56 stood out, suggesting
 188 that the two strains in both inoculants were efficient to the translocation, as well as the 120
 189 kg of N/ha fertilization. Vogel et al. [25] reviewing results of *A. brasilense* inoculation on
 190 wheat, connected positive results of productivity to the efficiency of biomass translocation to
 191 grains and the increase of photosynthetic activity. The authors also suggested detailed
 192 studies of those mechanisms. Current results reinforced the need to expand these
 193 investigations. Treatments that had the highest N content in grain (HCB56 and CB56)
 194 showed no leaf nitrogen accumulation significance, especially CB56 that had quantity below
 195 to the control. This suggested that higher N dosages, besides inhibiting the BNF can
 196 influence physiological processes because the microorganism can act like a drain,
 197 competing to the plant. Nevertheless, in general, associative diazotrophic bacteria, such as
 198 *A. brasilense*, can supply part of the N needed to the plant development. However, to

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199 maintain productivity indexes, it is necessary to associate BNF with N fertilization
200 [7,19,20,23,24].

201

202 **4. CONCLUSION**

203

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

211

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217

218 **COMPETING INTERESTS**

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220 Authors have declared that no competing interests exist.

221

222 **AUTHORS´ CONTRIBUTIONS**

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

227

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