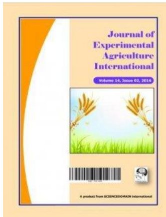

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Development of Wheat Plants Reinoculated with *Azospirillum brasilense*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CGC and LR were responsible for the idealization of the work and initial assembly of the experiment. Authors DKK, AGB and LFT participated in the methodological and bibliographical structuring process. Authors LGB and AKPS adapted the statistical methods and directed the adjustments of the analysis. Authors JD, IM and TSB participated in the final revision, adaptation of language and the theoretical material. Author VFG supervised and reviewed all stages of the work, directing the research.

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ABSTRACT

Aims: The objective of this study was to evaluate the effects of *Azospirillum brasilense* reinoculation in the initial development of wheat plants, from seeds submitted to inoculation with *A. brasilense* and different forms of nitrogen fertilization in the previous crop.
Study Design: The experimental design was completely randomized in a 2x2x4 factorial scheme with four replications.
Place and Duration of Study: The experiment was conducted in a greenhouse in the municipality of Marechal Candido Rondon – PR. The weather is classified by Koppen as a Cfa, subtropical with well distributed rains during the year, hot summers and annual average temperature between 22 an 23°C.
Methodology: The first factor was characterized by seeds of cultivar CD 108 (Corbélia/PR) and CD 150 (Marechal Cândido Rondon / PR). The second factor was absence and presence of reinoculation with *A. brasilense* in the seeds, harvested in the previous crop (F2). The third factor was the fertilization in the previous crop: absence of fertilization to the haul and absence of *A. brasilense* at sowing (control); 120 kg ha^{-1} of nitrogen (urea) associated with *A. brasilense*; absence of fertilizer to the haul and application of *A. brasilense* and 120kg ha^{-1} of nitrogen with fertilizer NET (Nitrogen Efficient Technology) associated with *A. brasilense*. The morphometric variables were: aerial and root length (AL, RL), stem diameter (SD), number of roots and volume (NR, RV) and aerial and root dry weight (ADW, RDW). The inoculation carried out initially increased the performance of the wheat plants, approaching the *Azospirillum* treatments with fertilization, specifically regarding the similarity to AL, ADW, RL, NR, RV.
Results: The fertilization with 120 kg ha^{-1} of NET associated with *A. brasilense* contributes to the development of the plants aerial part of the cultivar CD 150, according to results of AL and ADW, reducing stem diameter, increasing root length and volume in the absence of reinoculation. While reinoculating, 120 kg ha^{-1} of urea associated with *A. brasilense*, provided greater number of roots, root length and volume. On the other hand, the CD 108 showed little interference in the aerial part of the plants with respect to fertilization treatments. For the cultivar CD 150, reinoculation increased shoot length, regardless of fertilizer and inoculant treatment, besides increasing root dry weight. For the cultivar CD 108, the reinoculation reduced root length and volume, and increased number of roots, regardless of the treatment of fertilization carried out during the initial harvest.
Conclusion: Reinoculation proved to be a process that interfered in the development of wheat plants, differently to cultivate CD 108 and CD 150.

Keywords: Diazotrophic bacteria; Triticum aestivum L.; nitrogen biologic inoculation; seed inoculation; plant growth promoting bacteria.

1. INTRODUCTION

Since the 1990s it has been found that the association between grasses and diazotrophic bacteria of the genus *Azospirillum* results in positive results in the search for new alternatives that seek to reduce the application of nitrogen fertilizer and promote plant growth [1].

The benefits of this interaction are derived from a set of growth promotion mechanisms used by the bacteria, such as the increase in the absorption of nutrients from the soil [2], nitrogen supply (N) [3] and phytohormones synthesis [4]. In wheat, *A. brasilense* inoculation has provided increase in the grains N content as well as morphological changes in the roots and aerial part [3], as well as in rice cultivation [5].

In spite of the positive results of this plant-bacteria interaction, the success of the inoculation depends on a series of factors, among which are: lineage of the used bacteria, genetic variation of the cultivar, environmental conditions and others [6].

In this sense, the reinoculation of diazotrophic bacteria, which consists in the seed inoculation of plants that had their seeds inoculated in previous cultivation, represents an alternative with the purpose of increase the inoculum concentration [7]. Economically, the use of reinoculation in crops increases yield with a modest increase in production costs, because of the low cost of the product which recommended doses vary around 100 ml per kg of seeds. Through higher supply of the inoculum, the reinoculation contributes to the success of the inoculation, maximizing the growth promotion parameters which can allow its use in seed production fields, increasing the use of seeds by producers who inoculate with *Azospirillum* that, in this context, would carry out reinoculation. Also, inoculants act in the biological characteristics of the soil by replacing soil natural microorganisms.

However, it is important to identify reflexes in the association of *Azospirillum* in areas managed with in different ways when doing nitrogen fertilization in coverage, with the finality to provide favorable responses. This fact is highlighted because the high availability of nitrogen can result in low quality seeds, due to favoring of pathogens during seeds development in the ears of the wheat crop. This situation can have a higher impact in seed fields, places with high investment in technology and fertilization, mainly related to the seeds value.

In view of the above the objective of this study was to evaluate the effects of *Azospirillum brasilense* reinoculation in the initial development of wheat plants, from seeds submitted to inoculation with *A. brasilense* and different forms of nitrogen fertilization in the previous crop.

2. MATERIALS AND METHODS

2.1 Field Experiment Conduction – Preliminary Phase

The cultivars of wheat used were CD 108 and CD 150, from grains harvested in field essays in the 2014 harvest, between April and August, in the municipalities of Cobelia – PR and Marechal Candido Rondon – PR, respectively, Brazil.

Wheat seeds were inoculated with *A. brasilense* strains AbV5 and AbV6 in the proportion of 2 ml of the inoculant to 1000 seeds of wheat, in the concentration of 10^8 CFU ml^{-1}. In the same way, 2 ml of water was applied in 1000 seeds in the control treatment. Seed treatment was applied by means of homogenization in polyethylene bags.

In the essays carried out in Corbelia and Marechal Candido Rondon, the plants were submitted to four treatments corresponding to the management of nitrogen fertilization: absence of fertilization at the haul and absence of *A. brasilense* in the sowing, characterizing the control (T1); 120 kg ha^{-1} of N with urea associated to *A. brasilense* application in the sowing (T2); absence of fertilizer to haul and application of *A. brasilense* ta sowing (T3); and 120 kg ha^{-1} of nitrogen fertilizer NET (Nitrogen Efficient Technology) associated with application of *A. brasilense* at sowing (T4).

Wheat grains were harvested separately by treatment, being posteriorly used in the reinoculation essay.

2.2 Reinoculation

The reinoculation essays were carried out in greenhouse in the municipality of Marechal Candido Rondon – PR. The weather is classified by Koppen as a Cfa, subtropical with well distributed rains during the year, hot summers and annual average temperature between 22 an 23°C.

The experimental design used was completely randomized with a 2x2x4 factorial scheme, with four replications, totalizing 64 experimental units, the first factor was characterized by two cultivars, CD 108 (Corbelia – PR) and CD 150 (Marechal Candido Rondon – PR). The second factor constituted in the absence and presence of seeds reinoculation with *A. brasilense* (same procedure of the inoculation described in the conduction of the experiment for seeds obtaining – Preliminary phase) and the third factor by the different nitrogen fertilization managements in field previous essay (mentioned in the preliminary phase).

The greenhouse used was covered with double film of low density polyethylene (LDP) added against UV (ultraviolet) rays. After reinoculation with *A. brasilense,* seeds remained in rest for 30 minutes and then placed in polyethylene trays containing sand substrate autoclaved and daily moisture with water until the evaluation.

Using 10 normal seedlings were quantified the morphometric parameters: stem diameter (SD), aerial length (AL) and root length (RL), number of roots (NR) and root volume (RV). The vegetal material was dried in air forced circulation oven at 65±2°C for 72 hours until constant weight and then determined aerial dry weight (ADW) and root dry weight (RDW) using precision scale and also determined the relations AL/SD, RL/AL and RL/SD.

The statistical analysis was performed with the aid of the Sisvar 5.1 Build 72 program [8], so that the data were submitted to analysis of variance and, in the case of a significant effect, the Tukey test was used, both at 5% of probability of error for differentiation of means.

3. RESULTS AND DISCUSSION

There was triple interaction between factors studied for some variables evaluated (Table 1). However, the comparison between cultivars will not be performed due to the knowledge that each cultivar has intrinsic characteristic that define their different responses to the other factors.

Among the cultivar and fertilization factors there was interaction for stem diameter, aerial length and dry weight, being that for the CD 108 no difference was observed in relation to fertilization for these parameters (Tables 1 and 2).

For stem diameter of CD 150, the fertilization with 120 kg ha^{-1} of NET associated with *A. brasilense* presented inferior average to the control without differing from the other treatments (Table 2).

In the opposite way for aerial part length, fertilization with 120 kg ha^{-1} of NET associated with *A. brasilense* exceeded all other treatments to CD 150, while to aerial dry weight the treatment as well as fertilization with 120 kg ha^{-1} of NET associated with *A. brasilense* exceeded the other treatments (Table 2). Such results indicate positive influence of the inoculation associated with nitrogen fertilization in the aerial part development of wheat plants. Sabino et al. [9] also verified that the inoculation of *A. brasilense* associated with nitrogen fertilization (50 kg ha^{-1}) provided greater accumulation of biomass of rice plants, attributing the positive effects of the inoculation mainly to the indole compounds production capacity of the tested strain. Also highlight the importance of performing cover fertilization in seed production fields because the use of nitrogen makes it possible to obtain seeds that provide greater vigor when they are implanted by grain producers.

It is noteworthy that the aerial part length and dry weight, when using *A. brasilen*se in the absence of N to the haul presented an increase of 13.30% and 25% in relation to the absence of N and A. brasilense, respectively.

Cassán et al. [10], observed that both the isolated inoculation and combined inoculation of *A. brasilense* and *Bradyrhizobium japonicum* strains increased aerial length and dry weight of corn and soy plants. According to the authors, the positive effects of the inoculation in seed germination and initial plant development derive from the bacteria capacity to excrete plant growth promoting substances in sufficient concentration to make morphological and physiological changes in new plant tissues.

For Bashan and De-Basha [11] it is possible to consider a hormonal effect at very early stages of germination, since most strains of *Azospirillum* present in inoculants are capable of producing indoleacetic acid (IAA) and other growth regulators at a concentration sufficient to produce morphological and physiological changes in young seed tissues. The presence of living bacteria may contribute to the phytohormones production *in situ* during a long period, providing bacterial phytohormonal stimulus that is crucial in the initial development stages of plants, which will be complementary to other mechanisms that operate in more advanced stages of interaction of *Azospirillum* with the plants [10].

For the RL/AL ration for the cultivar CD 150 under effect of application of 120 kg ha^{-1} of urea associated with *A. brasilense* and 120 kg ha^{-1} of NET associated with *A. brasilense* it exceeded the other treatments tested, that is, the association of different nitrogen sources increased more the RL when compared to APL of wheat plants.

There was interaction between cultivars and reinoculation for root dry weight (Table 1). For CD 108 there was no difference regarding reinoculation whereas for CD 150 the presence of reinoculation provided higher root dry weight (Table 3). Lemos et al. [12], evaluating the inoculation with *A. brasilense* associated with nitrogen fertilization in different wheat cultivars verified that, when associated to the cultivar CD 150, inoculation and root dry weight had higher increase in the presence of only inoculation or seed in the presence of nitrogen.

There was interaction between fertilization and reinoculation for aerial length (Table 1). It is observed that the fertilization with 120 kg ha^{-1} N

(NET) associated with *A. brasilense* provided superior aerial length in relation to the control and as well as in the absence (24%) and presence (38%) of reinoculation without differing from the other treatments. Considering the reinoculation, there was difference only for the control and that the absence of reinoculation provided higher aerial length in relation to the presence (Table 4). In study performed by Corassa et al. [13], it was verified that the highest plant lengths were present in the presence of inoculation associated with nitrogen fertilization.

These results are consistent, as it was also verified lower root length and lower root volume when performing reinoculation. Only was observed increase in the root number in the cultivar CD 108 when performing reinoculation (Table 1). This fact may be related to the possibility of intense action of *Azospirillum* and even, higher hormones availability in the seeds when performing reinoculation added to inoculation performed in previous crop, providing a higher number of roots.

When studying specifically the variables length, number and volume of roots, interaction between reinoculation, cultivar and fertilization was also observed (Table 1).

According to previous comments, for root length, the cultivar CD 108 without reinoculation did not present difference to fertilization, although in the presence of reinoculation the fertilization of 120 kg ha^{-1} N (Urea) associated with *A. brasilense* was superior to control without differing from the other treatments. For this cultivar, the reinoculation reduced root length for all fertilization treatments (Table 5).

For the cultivar CD 150, in the absence of reinoculation the fertilization with 120 kg ha^{-1} N ($NET^{(2)}$) associated with *A. brasilense* exceeded all treatments while in the presence of reinoculation the fertilization of 120 kg ha^{-1} N (Urea) associated with *A. brasilense* was superior to the other treatments. For this cultivar, the presence of reinoculation provided higher root length for the fertilization with 20 kg ha^{-1} N (Urea) associated to *A. brasilense* and decrease of this parameter to the fertilization with 120 kg ha^{-1} N (NET) associated with *A. brasilense.* Didonet and Magalhães [14] verified the induction of root lengthening and root branching in wheat plants inoculated with *Azospirillum* and associated with the bacteria capacity to produce IAA and nitrite, substances capable to promote plant growth. Probably there is an alteration in the level of hormones liberated by bacteria when the inoculation is carried out, differing from when the reinoculation is carried out.

It is possible to verify that reinoculation for the cultivar CD 150 reinforced the positive effect of fertilization with 120 kg ha^{-1} N (Urea) associated with *A. brasilense* in the root development of plants, according to the results obtained for root length and dry weight. The bacterial inoculum provided by reinoculation may had reinforced the plant-bacteria association, which defines the location of the bacteria inside the plant in function of the specificity of the interaction. Once inside the plant, endophytic diazotrophic bacteria are mainly intercell allocated, but they can colonize the plants intracellularly [15].

For number of roots, CD 108 did not present difference regarding fertilization and reinoculation. For CD 150, without reinoculation there was no difference for fertilization while in the presence of reinoculation the fertilization with 120 kg ha^{-1} N (Urea) associated with *A. brasilense* exceeded the other treatments. For this cultivar, the reinoculation provided lower NR for the control and higher NR for fertilization with 120 kg ha^{-1} N (NET) associated with *A. brasilense* under reinoculation (Table 5).

For root volume, there was no difference in relation to fertilization in the absence or presence of reinoculation for the cultivar CD 108, for which the reinoculation reduced root volume for all fertilization treatments, except for the control (Table 6).

For CD 150, without reinoculation the fertilization with 120 kg ha^{-1} N ($NET^{(2)}$) associated with *A. brasilense* provided a higher root volume in relation to the treatment without N to the haul associated with *A. brasilense* while with reinoculation, the fertilization with 120 kg ha^{-1} N (Urea) associated to *A. brasilense* surpassed the control. The reinoculation promoted a higher VR for 120 kg ha^{-1} N (Urea) associated to *A. br*asilense but did not affect the other fertilization treatments (Table 6).

Table 1. Results of variance analysis and stem diameter (SD), aerial length (AL), root length (RL), number of roots (NR), root volume (RV), aerial dry weight (ADW) and RDW/ADW ratio, AL/SD ratio, RL/SD ratio and RL/AL ratio with absence and presence of *A. brasilense* reinoculation of cultivars CD 108 and CD 150 managed with seeds harvested in previous test in the presence and absence of fertilization at the haul and absence of *A. brasilense* at sowing (control), 120 kg ha^{-1} of N with nitrogen fertilizer urea associated to *A. brasilense* at sowing, absence of fertilizer at the haul, and application of *A. brasilense* at sowing and 120 kg ha^{-1} of NET associated with *A. brasilense* at sowing. Marechal Cândido Rondon, PR, 2014

Source of variation	SD	AL	RL	NR	RV	ADW	RDW	RDW/ADW	AL/SD	RL/SD	RL/AL
	---- mm ----	------------- cm -------------			----cm^3----	------------- g ---------------					
Factor Cultivar											
CD 108 – Corbélia	1,18 a	12,24 a	17,57 a	4,16 b	1,19 a	0,11 a	0,10 a	0,10 a	0,62 a	6,55 a	10,56 a
CD 150 – Marechal	1,14 a	11,03 b	9,87 b	4,44 a	0,98 b	0,10 a	0,11 a	0,11 a	0,60 a	5,68 a	9,87 a
Factor reinoculation											
Absence *A. brasilense*	1,22 a	12,08 a	16,97 a	4,09 b	1,23 a	0,10 a	0,10 a	0,09 b	0,64 a	6,33 a	10,04 a
Presence *A. brasilense*	1,10 b	11,18 b	10,47 b	4,51 a	0,94 b	0,10 a	0,11 a	0,11 a	0,58 a	5,90 a	10,39 a
Factor kinds of fertilization											
Control	1,22 a	9,92 c	10,91 b	4,00 b	0,91 b	0,08 c	0,11 a	0,09 a	0,64 a	5,55 a	8,21 b
120 kg ha^{-1} N (Ureia) + *A. brasilense*	1,14 a	12,42 ab	14,39 a	4,55 a	1,24 a	0,12 a	0,11 a	0,11 a	0,60 a	6,38 a	9,79 ab
Absence of N at the haul+ *A. brasilense*	1,16 a	11,24 bc	13,62 ab	4,27 ab	1,01 ab	0,10 bc	0,09 a	0,10 a	0,60 a	5,80 a	11,15 a
120 kg ha^{-1} N (NET) + *A. brasilense*	1,13 a	12,96 a	15,95 a	4,38 a	1,20 a	0,11 ab	0,12 a	0,10 a	0,59 a	6,74 a	11,70 a
General Average	1,16	11,63	13,72	4,30	1,09	0,10	0,11	0,10	0,61	6,12	10,22
F Value											
F cultivar	1,50 ns	7,57 **	77,90 **	7,88 **	7,12 *	0,14 ns	1,41 ns	3,04 ns	0,06 ns	0,67 ns	10,21 ns
F fertilization	1,76 ns	9,50 **	5,84 **	5,44 **	4,17 *	8,82 **	0,92 ns	1,16 ns	0,05 ns	0,26 ns	9,08 **
F reinoculation	15,09 **	4,23 *	55,35 **	18,39 **	14,45 **	0,01 ns	1,36 ns	12,55 **	0,35 ns	0,16 ns	0,48 ns
F cultivar x fertilization	3,29 *	9,47 **	4,95 **	0,37 ns	2,67 *	2,67 *	2,19 ns	0,55 ns	0,09 ns	0,57 ns	10,31 **
F cultivar x reinoculation	0,03 ns	1,28 ns	36,53 **	21,38 **	15,07 **	0,57 ns	8,56 **	12,74 **	0,00 ns	0,00 ns	0,97 ns
F fertilization x reinoculation	0,51 ns	2,92 *	7,63 **	6,57 **	2,37 ns	2,25 ns	0,89 ns	4,62 **	0,06 ns	0,07 ns	1,33 ns
F cultivar x fertilization x reinoculation	0,87 ns	1,07 ns	2,96 *	4,00 *	3,43 *	2,22 ns	0,74 ns	2,84 *	0,04 ns	0,07 ns	1,68 ns
C.V. (%)	10,38	15,04	25,44	9,23	28,42	23,74	40,83	22,91	66,69	70,04	20,25

** and **: significant at a level of 5% and 1%, respectively, by the F test*
ns: not significant at 5% probability level of error by the F test

Table 2. Stem diameter (SD), aerial length (AL), aerial dry weight (ADW) and RL/AL ratio of the cultivars CD 108 and CD 150 in function of nitrogen fertilization management in previous crop. Marechal Candido Rondon – PR, 2014

Fertilization [1]	SD				AL				ADW				RL/AL			
	CD 108		CD 150		CD 108		CD 150		CD 108		CD 150		CD 108		CD 150	
	mm				cm				g							
C	1,23	aA	1,20	aA	12,02	aA	7,82	cB	0,09	aA	0,07	bA	9,94	aA	6,48	cB
U + AZO	1,11	aA	1,17	abA	12,99	aA	11,85	bcA	0,12	aA	0,13	aA	12,06	aA	10,25	aA
A/N + AZO	1,15	aA	1,16	abA	12,13	aA	10,13	bcB	0,11	aA	0,09	bA	10,59	aA	8,99	bcA
NET + AZO	1,23	aA	1,03	bB	11,80	aB	14,11	aA	0,10	aA	0,13	aA	9,64	aB	13,77	aA
MSD A	0,16				2,32				0,03				2,75			
MSD C	0,12				1,76				0,02				2,08			

C-Control; U + AZO - 120 kg ha^{-1} of N (Urea) + A. brasilense; A/N + AZO – absence of N + A. brasilense; NET + AZO - 120 kg ha^{-1} of N (NET) + A. brasilense. MSD A – Fertilization; MSD C - Cultivars
Means followed by the same lowercase letter in the column and upper case in the row do not differ statistically from each other at the 5% probability level by the Tukey Test

Table 3. Root dry weight of wheat plants, cultivars CD 108 and CD 150, in the absence and presence of reinoculation with *A. brasilense.* Marechal Candido Rondon – PR, 2014

Reinoculation	Root dry weight			
	CD 108		CD 150	
	g			
Absence	0,11	aA	0,09	bA
Presence (*A. brasilense*)	0,09	aB	0,14	aA
MSD Inoculation	0,03			
MSD Cultivars	0,03			

Means followed by the same lowercase letter in the column and upper case in the row do not differ statistically from each other at the 5% probability level by the Tukey Test

Table 4. Aerial part length of wheat plants in the presence and abscence of reinoculation with *A. brasilense* 150 under different management of nitrogen fertilization in previous cultivation. Marechal Candido Rondon – PR, 2014

Fertilization [1]	Aerial part length			
	Absence of reinoculation [3]		Presence of reinoculation	
	cm			
C	11,03	bA	8,82	bB
U + AZO	11,81	abA	13,03	aA
A/N + AZO	11,75	abA	10,73	abA
NET [2] + AZO	13,75	aA	12,16	aA
MSD Inoculation	1,76			
MSD Fertilization	2,33			

[1]C-Control; U + AZO - 120 kg ha^{-1} of N (Urea) + A. brasilense; A/N + AZO – absence of N + A. brasilense; NET + AZO - 120 kg ha^{-1} of N (NET) + A. brasilense.
Means followed by the same lowercase letter in the column and upper case in the row do not differ statistically from each other at the 5% probability level by the Tukey Test

Table 5. Root length (RL) and number of roots (NR) in the absence (A/AZO) and presence (P/AZO) of reinoculation with *A. brasilense* of the cultivars CD 108 and CD 150 under different management of nitrogen fertilization in previous cultivation. Marechal Candido Rondon – PR, 2014

Fertilization (1)	Root length								Number of roots							
	CD 108				CD 150				CD 108				CD 150			
	Reinoculation															
	A/AZO (3)		P/AZO		A/AZO		P/AZO		A/AZO		P/AZO		A/AZO		P/AZO	
	------------------- cm -------------------															
C	29,95	aA	8,54	bB	5,60	bA	4,59	bA	3,63	aB	4,28	aA	4,12	aA	3,43	bB
U + AZO	22,45	aA	15,17	aB	6,73	bB	6,73	aA	3,88	aB	4,91	aA	4,28	aA	4,67	aA
A/N + AZO	23,35	aA	12,10	bB	9,54	bA	9,47	bA	3,60	aB	4,58	aA	4,68	aA	4,63	bA
NET (2)+ AZO	23,33	aA	10,93	bB	20,02	aA	9,54	bB	3,78	aB	4,65	aA	4,75	aB	4,98	bA
MSD Inoculation	4,96								0,56							
MSD Fertilization	6,57								0,75							

C-Control; U + AZO - 120 kg ha^{-1} of N (Urea) + A. brasilense; A/N + AZO – absence of N + A. brasilense; NET + AZO - 120 kg ha^{-1} of N (NET) + A. brasilense. Means followed by the same lowercase letter in the column and upper case in the row do not differ statistically from each other at the 5% probability level by the Tukey Test

Table 6. Root volume and RDW/ADW ration in the absence (A/AZO) and presence (P/AZO) of reinoculation with *A. brasilense* of cultivars CD 108 and CD 150 under different management of nitrogen fertilization in previous cultivation. Marechal Candido Rondon – PR, 2014

Fertilization (1)	Root volume								RDW/ADW							
	CD 108				CD 150				CD 108				CD 150			
	Reinoculation															
	A/AZO (3)		P/AZO		A/AZO		P/AZO		A/AZO		P/AZO		A/AZO		P/AZO	
	-- mL --															
C	1,13	aA	1,00	aA	0,88	bA	0,63	bA	0,08	aA	0,09	bA	0,12	aA	0,08	bA
U + AZO	1,63	aA	0,88	aB	1,00	bB	1,45	aA	0,07	aB	0,15	aA	0,12	aA	0,10	abA
A/N + AZO	1,58	aA	0,95	aB	0,63	bA	0,88	bA	0,07	aB	0,11	bA	0,11	aA	0,11	abA
NET (2)+ AZO	1,63	aA	0,75	aB	1,43	aA	1,00	bA	0,08	aB	0,12	abA	0,09	aB	0,13	aA
MSD Inoculation	0,44								0,03							
MSD Fertilization	0,58								0,04							

C-Control; U + AZO - 120 kg ha^{-1} of N (Urea) + A. brasilense; A/N + AZO – absence of N + A. brasilense; NET + AZO - 120 kg ha^{-1} of N (NET) + A. brasilense.
Means followed by the same lowercase letter in the column and upper case in the row do not differ statistically from each other at the 5% probability level by the Tukey Test

4. CONCLUSION

The first inoculation increased wheat plants development, being similar to the *Azospirillum* treatments with fertilization, specifically regarding the similarity to AL, ADW, RL, NR, RV.

Reinoculation of the cultivar CD 150 increased aerial length, regardless of fertilization and inoculation, besides increasing root dry weight.

Reinoculation of cultivar CD 108 increased root volume, root length and number of roots, regardless the fertilization used during the obtainment of the seeds initially.

Reinoculation proved to be a process that interferes wheat plants development, differently for cultivars CD 108 and CD 150.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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