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Action specificity of chemical treatment and inoculation with *Azospirillum brasilense* in wheat seed on the crop initial growth[1]

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ABSTRACT

The agronomic efficiency of the nitrogen-fixing inoculants or growth promoters depends on the growing conditions of the crops. One of the factors that may influence this response is the interaction between the bacteria present in the inoculant with the chemical treatment applied to the seeds. The objective of this study was to evaluate the influence of inoculation of wheat seeds with *Azospirillum brasilense* and its interaction with seed treatment on germination, vigor and initial growth of wheat plants of four cultivars. So, an experiment was carried out at the Federal University of Santa Maria, Santa Maria-RS. The experimental design was a completely randomized in a three-factorial (4x4x2), represented by the combinations between cultivar (FUNDACEP Bravo, OR/TBIO Quartzo, TBIO Itaipu e BRS 331), chemical treatment (1: Insecticide + Fungicide; 2: Insecticide; 3:Fungicide; 4: Witness without treatment) and *Azospirillum brasilense* inoculation (with and without inoculant). The determinations which were carried out were the following: emergence in beds, first germination test counting (vigor), germination, seedling length (root, aerial part and total), dry mass (root and aerial part). The responses to inoculants with *Azospirillum brasilense* are closely related to the cultivars used in the study. The treatment of seeds with fungicide and insecticide presented a phytotoxic effect, damaging the initial growth of the seedlings.

Keywords: inoculation; diazotrophic; bacteria; triadimenol.

RESUMO

Especificidade da ação do tratamento químico e inoculação com *Azospirillum brasilense* em sementes de trigo no crescimento inicial da cultura

A eficiência agronômica dos inoculantes fixadores de nitrogênio ou promotores de crescimento é dependente das condições de cultivo das culturas. Um dos fatores que pode influenciar essa resposta é a interação da bactéria presente no inoculante com o tratamento químico das sementes. Dessa forma, o estudo teve por objetivo avaliar a influência da inoculação de sementes de trigo com *Azospirillum brasilense* e sua interação com o tratamento de sementes na germinação, vigor e crescimento inicial de plantas de trigo de quatro cultivares. Para tal conduziu-se um experimento no Laboratório Didático e de Pesquisa em Sementes do Departamento de Fitotecnia da Universidade Federal de Santa Maria (UFSM), Santa Maria, Rio Grande do Sul. O delineamento experimental utilizado foi o inteiramente casualizado, em um trifatorial (4x4x2), sendo representado pelas combinações dos fatores cultivar (FUNDACEP Bravo, OR/TBIO Quartzo, TBIO Itaipu e BRS 331), tratamento químico de sementes (1- Inseticida + Fungicida; 2- Inseticida; 3- Fungicida; 4- Testemunha sem tratamento) e inoculação de *Azospirillum brasilense* (com e sem inoculante). As determinações realizadas foram: emergência em canteiros, primeira contagem do teste de germinação (vigor),

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germinação, comprimento de plântulas (raiz, parte aérea e total), massa seca (raiz e parte aérea). As respostas aos inoculantes com *Azospirillum brasilense* estão intimamente relacionadas às cultivares utilizadas no estudo. O tratamento de sementes com fungicida e inseticida apresentou efeito fitotóxico prejudicando o crescimento inicial das plântulas.

Palavras-chave: inoculação; bactérias diazotróficas; triadimenol.

INTRODUCTION

Brazil has an extensive farmable land, however, it is a major importer of wheat (*Triticum aestivum*), so a greater competitiveness of national wheat crop is essential to achieve self-sufficiency in production. Plant nutrition is one of the most limiting factors for crop yields, and for wheat, the macro element nitrogen (N) is the most limiting. Nitrogen directly influence productivity components, such as number and size of the spikes and grain mass (Mundosck, 2005). However, its supply through nitrogen fertilization has disadvantages in the economic and environmental issue, it is high cost and its excessive application increases the concentration of nitrate in the soil causing pollution in the groundwater (Berenguer *et al*., 2009). Therefore, practical alternatives have been searched to raise productivity at low cost and with less damage to the environment (Pietro-Souza *et al*., 2013). One of them is the inoculation of seeds with the bacterium *Azospirillum brasilense*, which has presented satisfactory results in several studies (Dartora *et al*., 2013a; Hungria *et al*., 2010; Díaz-Zorita & Fernández-Canigia, 2009).

By considering the growth-promoting effect provided by *Azospirillum* and that substances produced by these bacteria improve root development, and subsequently increase the uptake of nutrients by wheat plants (Dobbelaere *et al*., 2001), inoculation is considered an alternative to promote a better establishment of seedlings. These improvements may be associated with the production of phytohormones by these bacteria, in particular the auxins, whose main hormone is the indoleacetic acid (IAA) (Tsavkelova *et al*., 2006). These phytohormones regulate plant growth, being essential for cell elongation, regulating apical dominance, promoting the formation of lateral and adventitious roots, delaying the onset of leaf abscission, coordinating phototropism and geotropism, among others (Taiz & Zeiger, 2013). However, the agronomic efficiency of the inoculants may vary as a function of wheat growing conditions (Brzezinski *et al*., 2014).

One of the factors that influence this response is the interaction between the bacterium with the chemical treatment of the seeds. However, their contact with these chemicals are harmful. However, the use of seed treatment has become an indispensable practice, since in addition to ensuring the establishment of the crop through the control of pathogens, it reduces or prevents their introduction and dissemination in the crop (Denardin, 2010).

Despite presenting good efficiency in controlling pathogens, triadimenol-based fungicides have shown harmful effects such as reduction of emergence speed and height of seedlings (Garcia Júnior *et al*., 2008; Souza *et al*., 2011), reduction in the length of the coleoptile and mesocotyl (Silva *et al*., 1993), indicating the existence of phytotoxicity in the initial growth of seeds of the treated seedlings (Goulart, 1988).

Therefore, the objective of this study was to evaluate the influence of inoculation of wheat seeds with *Azospirillum brasilense* and its interaction with seed treatment on germination, vigor and initial growth of wheat plants of four cultivars.

MATERIAL AND METHODS

The study was conducted at the Education and Studies on Seedsin the Plant Science Department at Universidade Federal de Santa Maria (UFSM), located in the city of Santa Maria, Rio Grande do Sul, Brazil. The experimental design was a completely randomized design with four replicates, in a trifactorial (4x4x2) design, represented by the combinations of wheat cultivar factors, seed treatment and inoculation of *Azospirillum brasilense*.

The study used the cultivars FUNDACEP Bravo, TBIO Quartzo, TBIO Itaipu and BRS 331. The different seed treatments were, as follows: 1) insecticide (imidacloprid + thiodicarb) + fungicide (triadimenol); 2) insecticide (imidacloprid + thiodicarb); 3) fungicide (triadimenol); 4) control with no treatment. The dose used for the insecticide was 2.5 mL kg^{-1} of seed and 2.7 mL kg^{-1} of seed for the fungicide. For the inoculation factor, liquid inoculant was added at a dose of 2.5 mL kg^{-1} of seed, which was composed of a culture of *Azospirillum brasilense* bacteria, strains AbV5 and AbV6, with a concentration of 2.0 x 10^8 CFU mL^{-1}. Sowing was performed on germitest paper and in beds, and the inoculation was carried out moments before sowing so that the bacterium did not lose its viability.

After the application of the treatments, the seeds were submitted to the emergency determinations, first count of germination (vigor), germination, length of seedlings (root, aerial part and total) and dry matter mass (root and aerial part), separated according to the Rules of Seed Analysis (Brasil, 2009).

The count of emerged seedlings at 14 days after sowing (DAS) was determined in beds with one meter in width and one meter in length. For each treatment, 50 seeds were distributed in each 1m furrow, each being one centimeter deep and in a row spacing of five centimeters.

For the first count and germination tests, the experimental units consisted of 50 seeds equidistantly distributed on three sheets of germitest paper moistened with distilled water at 2.5 times the mass of the dry paper (Brasil, 2009). After the seeds were deposited on the sheets of paper, they were wrapped in rolls, placed in plastic bags to reduce water loss and then were vertically packaged in biochemical oxygen demand (BOD) type germinator regulated at a temperature of 25 ºC and constant photoperiod (Brasil, 2009). The first count test was performed at four DAS and the germinated plants were removed from the paper and discarded. The rolls were placed back into the germinator and remained for another four days and then a new count was carried out. The germination was performed by adding the number of plants germinated on the fourth and eighth day after sowing and the results expressed as percentage of normal seedlings (Brasil, 2009).

Root, aerial part and total length tests were performed in experimental units consisting of twenty seeds equidistantly distributed over germitest paper moistened with 2.5 times the mass of the dry paper, and then rolled and placed a BOD-type germinator regulated at 25 °C and constant photoperiod. After seven days, the samples were taken from the germinator and, in ten normal seedlings, the root and shoot length were randomly measured (Brasil, 2009). After the seedlings were placed in paper bags and kept in a regulated oven at 60 ºC for 48 hours, after being taken from the oven, they were placed in a desiccator for fifteen minutes to stabilize the mass and then the dry plant mass was determined in a precision analytical balance.

The experimental data were submitted to the assumption of the mathematical model test. The analysis of variance was carried out by the F test ($p \leq 0.05$) and later, the variables that achieved significance were unfolded to the study of interaction or principal effects by the Skott-Knott test ($p \leq 0.05$). The software used for the analyzes was Sisvar (Ferreira, 2008).

RESULTS AND DISCUSSION

The analyzed variables showed significant triple or double interactions between the cultivar, chemical seed treatment and inoculation with *Azospirillum brasilense*, except for germination only where it was verified significance only for the sources of variation and seed treatment (Table 1).

For the variable emergence of seedlings in the beds, the inoculation factor had a significant difference in the cultivar TBIO Itaipu, and in the presence of the inoculant, a reduction was found in the emergence of fungicide + insecticide treatment and seed with no treatments (Table 2). These results are in disagreement with those obtained by Dartora *et al*. (2013b) and Rampim *et al*. (2012) who did not find significant differences on germination of seeds treated chemically or not and inoculated with *A. brasilense*.

It can be seen in Table 2 the contribution of the fungicide in the reduction of seedling emergence in the cultivars FUNDACEP Bravo and TBIO Itaipu without inoculant and in the cultivar FUNDACEP Bravo with inoculation of *Azospirillum*. The fungicide triadimenol presents toxicity to the seedlings, causing reduction in seedling emergence (Garcia Júnior *et al*., 2008), reduction in the number of stems, as well as coleoptile and mesocotyl length (Silva *et al*., 1993) due to the atrophy of this organ by the treatment with triadimenol (Forcelini, 1991), which can cause slower establishment of the crop and present negative responses in the final productivity.

The application of *A. brasilense* presented significant interaction only with the Itaipu cultivar for the first germination count, showing a reduction in vigor in the seed treatment made up of insecticide and fungicide mixture and in the control, without seed treatment (Table 3). This test is based on the principle that seeds with the highest germination speed are more vigorous (Oliveira *et al*., 2009) and provide a rapid and uniform establishment of the appropriate plant population in the field (Höfs *et al*., 2004). In a study by Souza *et al*. (2014) with sweet corn cultivation, a reduction was also observed in the vigor of inoculated seeds. However, Brzezinski *et al*. (2014) observed greater vigor of the wheat seeds that were inoculated with *Azospirillum*. This divergence of responses, mainly in relation to the other cultivars evaluated in the present study, may be related to the interaction between the bacterium with the genotype of the cultivar used or even with the strain selected for inoculation (Fibach-Paldi *et al*., 2012; Hungria, 2011).

The seeds treated with the fungicide presented a significant reduction in the percentage of germinated seedlings in the first count (Table 3). This may have occurred since triadimenol molecule display the reduction

Table 1: Summary of analysis of variance of the assessed variables of four wheat cultivars submitted to different chemical treatments of the seeds and inoculation on *Azospirillum* basis

SV	DF	Mean Square							
		SE	GF	GT	APC	RL	TL	APM	RM
CULT	3	1454.2*	1397.8*	732.3*	1.9*	60.7*	43.9*	6.4*	2.6*
ST	3	558.4*	10757.6*	572.7*	261.1*	165*	797.7*	52.3*	0.9^{ns}
INO	1	98.0^{ns}	520.0*	11.3^{ns}	1.3*	24.2*	36.9*	2.5*	0.1^{ns}
CULT*ST	9	100.6^{ns}	202.3^{ns}	36.3^{ns}	2.7*	2^{ns}	5.6*	3.2*	0.8*
CULT*INO	3	76.7^{ns}	226.1^{ns}	102.9^{ns}	1.8*	2.7^{ns}	4.7^{ns}	3.5*	0.6^{ns}
ST*INO	3	78.6^{ns}	82.6^{ns}	21.1^{ns}	0.4^{ns}	1.7^{ns}	2.9^{ns}	1^{ns}	0.2^{ns}
CULT*ST*INO	9	161.3*	314.2*	78.9^{ns}	1.2*	2.2*	2.8^{ns}	0.4^{ns}	2.0*
CV (%)		10.6	20.0	8.1	6.1	9.4	6.9	9.9	10.3
AVERAGE		74.6	52.9	78.5	8.2	11.2	19.4	6.4	5.9

CULT: Cultivar; ST: seed treatment; INO: inoculation, SV: source of variation; DF: degrees of freedom, SE: seedling emergence; GF: Germination test first count; GT: germination; APC: aerial part length; RL: root length; TL: total length; APM: aerial part mass; RM: root mass; ns: non-significant; *: Significant at 5% of error probability by the F test.

Table 2: Emergence of seedlings of the different wheat cultivars planted in seedbeds, with and without inoculant based on *Azospirillum*, in the different seed treatments (ST)

Inoculant	ST	FUNDACEPBravo	TBIOQuartzo	TBIOItaipu	BRS 331
With	Fung. + Insect.	α* 65.0 b B	α** 74.5 a A	β 52.0 b C	α 83.0 a A
	Insecticide	α 79.0 a A	α 78.0 a A	α 76.5 a A	α 88.0 a A
	Fungicide	α 62.5 b B	α 68.5 a B	α 77.0 a A	α 77.0 a A
	Without ST	α 67.5 b B	α 71.0 a B	β 69.0 a B	α 91.0 a A
Without	Fung. + Insect.	α 67.0 b A	α 74.0 a A	α 73.5 a A	α 80.5 a A
	Insecticide	α 73.0 a A	α 79.0 a A	α 76.5 a A	α 87.0 a A
	Fungicide	α 61.0 b B	α 67.0 a B	α 69.0 a B	α 87.0 a A
	Without ST	α 77.5 a A	α 73.5 a A	α 80.5 a A	α 81.5 a A

*Means not followed by the same letter are different at 5% probability of error by the test of Scott-Knott.

**Lower case letters in the column are the interaction between seed treatment within each level of inoculation and the analyzed cultivar. Uppercase letters in the row are the interaction between the cultivars within each level of seed treatment and inoculation. Greek letters in the column are the interaction between inoculation within each level of seed treatment and cultivar.

Table 3: Means for the first count and seedling germination variables of different wheat cultivars with and without inoculant on *Azospirillum* basis at different seed treatments (ST)

Inoculant	ST	Germination test first count (%)			
		FUNDACEPBravo	TBIOQuartzo	TBIOItaipu	BRS 331
With	Fung. + Insect.	α * 31.5 b A	α** 20.5 c B	β 9.00 c B	α 43.5 b A
	Insecticide	α 57.5 a A	α 54.5 b A	α 61.0 a A	α 72.5 a A
	Fungicide	α 41.0 b A	α 50.5 b A	α 39.0 b A	α 52.0 b A
	Without ST	α 71.0 a A	α 77.5 a A	β 54.5 a B	α 78.5 a A
Without	Fung. + Insect.	α 37.0 b A	α 36.0 c A	α 25.0 c A	α 35.5 c A
	Insecticide	α 60.5 a B	α 56.5 b B	α 50.5 b B	α 76.5 a A
	Fungicide	α 51.0 a A	α 41.5 c A	α 54.0 b A	α 56.5 b A
	Without ST	α 57.0 a B	α 75.5 a A	α 82.0 a A	α 83.5 a A
Germination (%)		74.31 C	78.87 B	75.69 C	85.06 A

Germination (%)	Seed Treatments			
	Fung. + Insect.	Insecticide	Fungicide	Without ST
	75.87 C	79.12 B	74.75 C	84.19 A

*Means followed by the same letter differ from each other at 5% of error probability by the test of Scott-Knott. **Lower case letters in the column represent the interaction of seed treatment within each level of assessed inoculation and cultivar. Upper-case letters in the row are the interaction of the cultivars within each level of seed treatment and inoculation, in the first count variable, and the principal effects of cultivar and ST on the variable germination. Greek letters in the column represent the interaction between inoculation within each level of seed treatment and the cultivar.

in the speed and emergence of the seedlings and inhibits the development of mesocotyl or subcoronal internodes as some of the consequences, presenting growth regulator characteristics (Souza *et al*., 2011; Garcia Júnior *et al*., 2008).

For seedling germination variable, no interaction was observed between the tested factors. The best germination mean among the cultivars was that of BRS 331, with 85.06% of germinated plants, which is greater than the germination test of the seed multiplier. The non-application of any product had the highest percentage of germination (84.19%) followed by the one that received only insecticide. Those treated with fungicide were the ones that presented the smallest number of emerged plants (Table 3), and germination was below the minimum standards required for seed commercialization (80%) (MAPA, 2005). According to some studies the application of systemic fungicides can result in the production of phenolic compounds that inhibit the synthesis of proteins inducing changes in the enzymatic system, ceasing the formation of ATP and NADP, which are necessary in the process of germination of seeds (Siddiqui *et al*., 1997). These data disagree with those of Ulguim *et al*. (2013) who did not observe any differences in germination when treated with triadimenol.

Nevertheless, the inoculation was not efficient in improving the germination, with the average of 78.19% and 78.78%, with and without inoculant, respectively. These data corroborate with those found by Dartora *et al*. (2013b). It is observed that the responses to inoculants are closely related to the cultivars used in the study, which exert a differential effect on the colonization by these bacteria, with a specificity between genotype and bacteria, which may be due to the chemical composition of exudates released by the plants (Bianchet *et al*., 2013; Bergamaschi *et al*., 2007).

Table 4: Means for lengths of aerial part, root and total of different assessed wheat cultivar seedlings with seed chemical treatment (ST) and *Azospirillum* inoculation

Inoculant	ST	FUNDACEP Bravo	TBIO Quartzo	TBIO Itaipu	BRS 331
		Aerial Part Length (cm)			
With	Fung. + Insect.	α 5.7 b A*	α 5.4 b A**	β 4.9 c A	α 5.8 c A
	Insecticide	α 9.8 a C	β 10.6 a B	α 11.3 a A	α 10.5 b B
	Fungicide	α 5.9 b A	α 5.2 b B	α 6.1 b A	α 5.8 c A
	Without ST	α 9.5 a B	β 10.1 a B	α 11.0 a A	α 11.2 a A
Without	Fung. + Insect.	α 6.0 b A	α 5.7 c A	α 5.8 b A	α 5.4 b A
	Insecticide	α 10.0 a B	α 11.5 b A	α 10.3 a B	α 10.4 a B
	Fungicide	α 6.2 b A	α 5.1 c B	α 6.3 b A	α 5.8 b A
	Without ST	α 9.3 a C	α 12.5 a A	α 10.8 a B	α 11.0 a B
		Root Length (cm)			
With	Fung. + Insect.	β 9.5 d A	β 7.7 c B	α 7.3 d B	α 9.4 c A
	Insecticide	α 12.8 b A	α 10.4 b B	α 10.6 b B	β 11.4 b B
	Fungicide	α 11.3 c A	α 8.0 c B	α 9.5 c B	α 10.7 b A
	Without TS	β 15.0 a A	α 13.3 a B	α 12.4 a B	β 13.8 a B
Without	Fung. + Insect.	α 11.8 c A	α 9.5 b B	α 8.2 c B	α 9.0 d B
	Insecticide	α 13.9 b A	α 11.8 a B	α 10.1 b C	α 13.1 b A
	Fungicide	α 11.7 c A	α 8.2 b B	α 9.4 b B	α 10.9 c A
	Without ST	α 17.4 a A	α 12.9 a B	α 12.7 a B	α 16.2 a A
		Total Length (cm)			
	Fung. + Insect.	16.5 c A	14.1 c B	14.8 d B	14.8 d B
	Insecticide	23.2 b A	22.2 b B	22.7 b A	22.7 b A
	Fungicide	17.5 c A	13.2 c C	16.6 c A	16.6 c A
	Without ST	25.7 a A	24.4 a B	26.1 a A	26.1 a A

Total Length(cm)	Inoculation	
	With Inoculante	**Without Inoculant**
	18.9 B	19.9 A

* Means not followed by the same letter are different at 5% of error probability by the test of Scott-Knott.

**Lower-case letter in the column are the interaction between seed treatment within each inoculation level and the assessed cultivars. Upper-Case letters in the row are the interaction between cultivars within each seed treatment level and inoculation at lengths of aerial part and root and the interaction of the cultivars within each ST and the principal effect of inoculation for the total length. Greek letters in the column are the interaction between inoculation within each treatment level of seeds and cultivar.

The length of the aerial part was influenced by the inoculation, where only in TBIO Quartzo (without seed treatment and insecticide) and TBIO Itaipu (insecticide + fungicide) cultivars had a reduction in the length of the aerial part. Root length was also reduced in some treatments by the presence of the inoculant, but not the same ones where the aerial part reduction was observed. For the total length, the main effect of the inoculation was also detrimental to the plants (Table 4). This effect was also observed in a study by Kuss *et al*. (2008) in a rice crop. According to Taiz & Zeiger (2013), the indolacetic acid (IAA) is essential in several plant growth processes, such as cell elongation, regulation of apical dominance, formation of lateral and adventitious roots, coordination of phototropism and geotropism, among others. In small doses of IAA, root length is increased, but as the concentration increases, a reduction in root length occurs (EL-Khawas & Adachi, 1999). Bianchet *et al*. (2013) and Taiz & Zieger (2013) report that there is a high sensitivity of plant roots to the high concentration of auxins, which can cause an opposite effect to the target one, since the hormone released in large amounts can cause negative effects on plant growth, which contributed to this reduction also in the total length of the seedlings.

The treatment of seeds has a great influence on the length of the aerial part, root and total length of the seedlings. Moreover, the presence of the fungicide is the promoter of the smallest measures (Table 4). Fungicides with triadimenol active principle present a negative effect on the height of the seedlings (Moraes *et al*., 1997; Garcia Júnior *et al*., 2008) and on the length of the hipocotile (Rampim *et al*., 2012), indicating that a phytotoxic effect of the seed treatment may have occurred in wheat seedlings (Goulart, 1988). This effect can be caused by the chemical stress caused by the fungicide that triggers the production of phenolic compounds that limit the growth, photosynthesis, respiration and protein synthesis for plant (Macias *et al*., 1992). On the other hand, in a work by Rampim *et al*. (2012) when evaluating the hypocotyl diameter, treatments containing triadimenol provided the greatest values, being greater than the control in all cultivars, therefore providing positive effects to reduce the effects of lodging (Zagonel & Fernandes, 2007).

Although insecticide application is carried out at a lower scale than that of fungicide, it also reduced root and total length (Table 4). This may have occurred in response to the exogenous stress produced by insecticides, which is caused by the formation of free radicals (Soares & Machado, 2007). Free radicals damage DNA, membrane lipid peroxidation, and oxidative protein modification, which may affect seedling initial development (Dan *et al*., 2012).

Dry mass production of the aerial part displayed an interaction between cultivars and seed treatment and between cultivars and inoculant. It is verified that the BRS 331 cultivar showed the greatest accumulation of dry matter mass. Each cultivar has different characteristics, but in this case, it is possible to associate this result with the growth speed since among the four cultivars, BRS 331 presents the shortest cycle (125 to 130 days), and consequently the fastest biomass accumulation in comparison to the studied genotypes (Table 5).

Seed treatment presented the same behavior displayed by the aerial part, root and total length variables, where the treatment did not interfere with triadimenol, and a consequent reduction in the dry mass of the aerial part by the phytotoxic effect of the product (Table 4 and Table 5). Inoculation was favorable for this variable, as it showed increases in dry matter mass in the cultivars FUNDACEP Bravo, TBIO Itaipu and BRS 331 (Table 5). Nozaki *et al*. (2013) and Rampim *et al*. (2012) also observed increases in the dry matter mass of aerial part of wheat. In addition, according to Rampim *et al*. (2012) this effect happens

Table 5: Means for aerial part dry mass of seedlings for different assessed wheat cultivars with or without seed chemical treatment and inoculation on *Azospirillum* basis

	FUNDACEP Bravo	**TBIO Quartzo**	**TBIO Itaipu**	**BRS 331**	**Average**
ST			**Aerial Part Mass (g)**		
Fung. + Insect.	5.2 b A *	4.8 c A **	4.9 b A	5.1 c A	5.0
Insecticide	6.6 a B	7.1 b B	7.7 a A	8.1 a A	7.3
Fungicide	5.2 b A	4.7 c B	5.5 b A	6.1 b A	5.4
Without ST	6.2 a C	7.8 a B	7.8 a B	8.7 a A	7.6
			Aerial Part Mass (g)		
With Inoculant	6.4 a B	5.8 b C	7.0 a A	7.2 a A	6.6
Without Inoculant	5.7 b B	6.4 a A	6.3 b A	6.7 b A	6.3

*Means not followed by the same letter are different from each other at 5% of error probability by the test of Scott-Knott. **Lowercase letters in the column are the interaction of the seed treatment within each cultivar analyzed and the interaction of the inoculant within each cultivar. Uppercase letters presented in the row are the interaction of the cultivars within each level of seed treatment or inoculation.

Table 6: Means for root dry matter mass of seedlings of different assessed wheat cultivars with or without seed chemical treatment (ST) and inoculation on *Azospirillum* basis

Inoculant	ST	FUNDACEP Bravo	TBIO Quartzo	TBIO Itaipu	BRS 331	Average
		Root Mass (g)				
With	Fung. + Insect.	α 5.8 a A*	α 5.6 a A*	β 4.6 b B	α 6.5 a A	5.6
	Insecticide	α 5.8 a B	α 5.1 a B	α 6.6 a A	α 6.2 a A	5.9
	Fungicide	α 6.7 a A	α 5.7 a A	α 6.2 a A	α 6.3 a A	4.7
	Without TS	α 5.8 a A	β 5.1 a B	α 6.1 a A	α 6.3 a A	5.8
Without	Fung. + Insect.	α 6.1 a A	α 5.2 b A	α 5.9 a A	α 6.2 a A	5.8
	Insecticide	α 6.2 a A	α 5.4 b B	β 5.2 a B	α 6.4 a A	5.8
	Fungicide	α 6.3 a A	α 5.6 b A	α 6.3 a A	α 6.3 a A	6.1
	Without ST	α 5.4 a B	α 7.1 a A	α 5.6 a B	α 5.8 a B	6.0

*Means not followed by the same letter differ from each other at 5% of the probability error by the test of Scott-Knott. **Lower case letter in the column are the interaction between seed treatment within each inoculation level e assessed cultivar. Upper-case letter in the row are the interaction between cultivars within each seed treatment level and interaction. Greek letters in the column are the interaction between inoculation within each seed treatment level and cultivar.

because of the ability of the bacteria to stimulate root development, increasing the absorption of water and nutrients by the roots, and it may also favor the hormonal balance of the plant. In addition, *A. brasilense* produces indoleacetic acid, cytokinins, gibberellins and ethylene, which affect plant growth and may increase their green mass (Gray & Smith, 2005). In order to cultivate TBIO Quartzo, the inoculation reduced the dry matter mass of the seedlings. This effect was also observed by Kuss *et al*. (2008) who observed a positive effect of inoculation on the dry matter mass of rice seedlings in only one cultivar, attributing this effect to the efficiency of the plant-bacteria association.

Table 6 shows the triple interactions for the root dry matter mass of wheat seedlings. The highest averages were observed for cultivar BRS 331, following the same behavior of shoot dry matter mass. The inoculation had an effect only on the cultivars TBIO Quartzo and TBIO Itaipu, not proving efficient to increase the root dry mass, because the differences were very punctual, and are not sufficient to explain the behavior of the bacteria for this variable. However, the inoculation of *A. brasilense* provides a positive effect on the initial growth of wheat seedlings (Pereyra *et al*., 2009), which needs to be further studied and understood.

Root dry matter mass was not influenced by seed treatment, except for the cultivar TBIO Quartzo without inoculant. It was verified a reduction in the dry matter mass in this cultivar for both chemical treatments (Table 6). Contradictory results were observed by Dartora *et al*. (2013b), where the chemical treatment of the seeds showed significant increases of the root dry matter mass in the wheat crop.

Although the root length showed reductions in the treatments that received the triadimenol fungicide, a trend not observed for the dry matter mass of these roots. Perhaps the product could have caused physiological interference and triggered an increase in the diameter or mass accumulation of these roots.

CONCLUSIONS

The responses to inoculants with *Azospirillum brasilense* are closely related to the cultivars used in the study, which exert a differential effect on the colonization by these bacteria.

Seeds treated with the fungicide triadimenol is harmful, since emergence, first count, germination, aerial part length, root length, total length and dry matter mass of the aerial part of the wheat seedlings are all reduced.

The insecticide proved to be detrimental to the variables root length and total length of the seedlings.

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