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Azospirillum brasilense ASSOCIATION WITH STIMULATORS ON WHEAT CROP

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ABSTRACT

The objective of this research was to verify the association of stimulators and biofertilizers with the bacterium *Azospirillum brasilense* for wheat crop. Two experiments were carried out in which the mode and time of inoculation of *A. brasilense* bacteria were varied and different associations of growth stimulator and biofertilizer were tested. Number of plants per square meter, number of spikes per square meter, plant height, number of spikelets per plant, number of grains per spike, one thousand grain mass, hectoliter mass, and grain yield were evaluated. The association of *A. brasilense* with stimulators influenced grain yield. The use of stimulators in the seeds differed from the control in the variable “plant height”, where the inoculated plants presented 2 to 3 cm in relation to the plants without inoculation. The use of *A. brasilense* associated with biostimulator in foliar application increases the one thousand grain mass and grain yield.

Keywords: *Azospirillum* spp., inoculation, stimulators, *Triticum aestivum*

ASSOCIAÇÃO DE *Azospirillum brasilense* E ESTIMULADORES NA CULTURA DO TRIGO

RESUMO

Objetivou-se, com esta pesquisa, verificar a associação de estimuladores e biofertilizantes junto à bactéria *Azospirillum brasilense* para a cultura do trigo. Realizou-se dois experimentos, nos quais variou-se o modo e a época da inoculação da bactéria *A. brasilense* onde foram testadas diferentes associações do estimulador de crescimento e biofertilizante. Foram avaliados número de plantas por metro quadrado, número de espigas por metro quadrado, altura de plantas, número de espiguetas por planta, número de grãos por espiga, massa de mil grãos, massa de hectolitro e produtividade de grãos. A associação de *A. brasilense* com estimuladores influenciou a produtividade de grãos. O uso de estimuladores nas sementes diferiu da testemunha na variável altura de planta, onde as plantas inoculadas apresentaram de 2 a 3 cm em relação às plantas sem inoculação. A utilização de *A. brasilense* associado ao bioestimulador em aplicação foliar incrementa a massa de mil grãos e a produtividade de grãos.

Palavras-chave: *Azospirillum* spp., estimuladores, inoculação, *Triticum aestivum*

INTRODUCTION

In a global scale, wheat is the second most important source of calories for human feeding (LONG & ORT, 2010). In the last five decades, the world production in almost 450 million tons (CANZIANI & GUIMARÃES, 2009). In Brazil, an increase on the demand of this cereal exists (COSTA et al., 2013), leading to the culture being cultivated mainly in South, Southeast and Center-West regions of the country, being a viable economical option in the winter. Many technological innovations are verified nowadays in order to increase productivity and profitability of the culture. Also, many agricultural inputs were recently introduced in the Brazilian market, a lot of them destinated to triticulture. Among them, fertilizers and products based on microorganisms and growth-promoting substances stand out, with highlight to the nitrogen-fixing bacteria. However, the doubts upon the profitability and agricultural efficiency of these products are frequent and growing, due to the lack of scientific-accurate information.

The use of synthetic fertilizers represents the main form of nitrogen (N) supply needed by the wheat culture, presenting good solubility and quick availability to absorption. However, it has an elevated loss potential on environment (LEMOS et al., 2013), leading to a necessity of large quantities in order to supply the culture needs (TEIXEIRA FILHO et al., 2010), thus increasing production costs. The expenses with sowing inputs and during the culture conduction represents 60% of the variable costs for wheat production (CONAB, 2016), with nitrogen fertilization being the most significant part of them. Urea is the most used fertilizer in Brazil, both for its obtention easiness and for its economical viability. The use of N-fixing bacteria may be a viable alternative to the use of N in the mineral form (MILLÉO & CRISTÓFOLI, 2016), partially replacing the N application in the form of urea without damaging the grain productivity of the culture (FUKAMI et al., 2016). Among these bacteria, the ones from the *Azospirillum* genera are standouts. They associate with plants transforming atmospheric nitrogen into a form metabolically usable for them (FUKAMI et al., 2016). This group of associative diazotrophic bacteria, besides having elevated potential of fixing atmospheric nitrogen, also act on the synthesis of hormones such as auxins, gibberellins and cytokinins (CAVALLET et al., 2000), assisting the growth of the aerial part and

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root system of the plant (KAZI et al., 2016). The better development of the root system allows the plant to explore a higher volume of soil (CORASSA et al., 2013), enabling the absorption of a higher volume of water and nutrients present in the soil (UBERT & SOLIGO, 2015).

It is known that the use of these bacteria on grasses interferes on the growth and productivity in function of the nitrogen biological fixation, production of phytohormones, siderophores and phosphorus solubilization on the soil (KAZI et al., 2016), besides increments on the concentrations of chlorophyll of the foliar limb (HUNGRIA, 2011). Researches indicate the possibility of reducing the use of nitrogen fertilizers on wheat and other grasses with the use of inoculation of *Azospirillum* spp. bacteria. It is verified that there are no negative effects on aerial part dry mass, root dry mass, height, cobs per square meter, and also on productivity (MUNARETO et al., 2019; KAPPES et al., 2013).

Biofertilizers are inputs which contain alive microorganisms that favors energy intakes to plants, assisting in moments of stress. They are also capable of regulating and triggering the hormonal production process, and accelerating the vegetal metabolism, leading to the production of phytohormones and growth promoters. On the other hand, stimulators are substances that enhance the root development, increasing the tolerance to hydric stresses and providing a quick plant growth in consequence of the higher soil volume that is explored by the roots. With the increase of use of these substances, researches in the area become more and more specific.

Thus, the objective of the research was to verify if the use of the bacteria *Azospirillum brasilense* in different application forms and associated with the use of stimulators favors the wheat agricultural performance compared to using only the recommended fertilization for the culture.

MATERIAL AND METHODS

The experiment was conducted in an experimental area of the Santa Maria Federal University (UFSM) Phytotechny Department in the state of Rio Grande do Sul, Brazil. The area soil is classified as a typical Dystrophic Red Argisoil (EMBRAPA, 2013), a sandy clay loam Acrisol according to the Food and Agriculture Organization of United Nations (FAO) classification. The soil contains the following chemical characteristics: water pH: 5.54; index: Shoemaker, Mac Lean and Pratt (SMP) 5.5; Bases saturation and Aluminum: 47.25 and 11.3%, respectively; effective cation exchange capacity (CTC): 7.9 (cmolc dm^{-3}); CTC pH7: 15.4 (cmolc dm^{-3}); potassium (K): 0.225 (cmolc dm^{-3}); phosphorus (P) – Mehlich: 35.55 (mg dm^{-3}); organic matter percentual (%MO): 2.6 (m/v); % Clay: 36 (m/v); calcium (Ca): 4.95 (cmolc dm^{-3}); magnesium (Mg): 2.05 (cmolc dm^{-3}); aluminum (Al): 0.7 (cmolc dm^{-3}); H + Al: 8.2 (cmolc dm^{-3}); and textural class 3. In accord with Köppen and Thornthwaite classification, the climate is classified as Cfa (PEEL, FINLAYSON & MCMAHON, 2007), with highest monthly temperature of 24.8ºC and lowest of 14.1ºC (HELDWEIN, BURIOL & STRECK, 2009).

There were conducted two experiments, where treatments from the first experiment were:

I- Complete mineral fertilization recommended for the culture (control);

II- *Azospirillum brasilense* via seeds (400 ml/ha), with inoculation conducted at the day of sowing;

III- *Azospirillum brasilense* in pulverization (400 ml/ha) + bioactivator in pulverization (300 ml/ha), applied at the beginning of tillering;

IV- *Azospirillum brasilense* in pulverization (400 ml/ha) + bioactivator in pulverization (300 ml/ha) + Spray (300ml/ha), with products applied at the beginning of tillering;

V- *Azospirillum brasilense* in pulverization (400 ml/ha) + bioactivator in pulverization (300 ml/ha), applied at beginning of tillering, and application of bioactivator (150 ml/ha) + Spray (150 ml/ha), applied at the second entrance with fungicide;

VI- *Azospirillum brasilense* in pulverization (400 ml/ha) + bioactivator (300 ml/ha), applied at the beginning of tillering and bioactivator (150 ml/ha) + Spray (150 ml/ha), applied at the first and second entrance with fungicide.

The second experiment contained the following treatments:

I- Complete mineral fertilization recommended for the culture (control);

II- *Azospirillum brasilense* in pulverization (400 ml/ha), applied at the beginning of tillering;

III- Root in application via seed (300 ml/ha);

IV- *Azospirillum brasilense* via seed (400 ml/ha);

V- *Azospirillum brasilense* via seed (400 ml/ha) + Root (300 ml/ha).

The inoculation of the three last treatments occurred moments before sowing. The bioactivator product is a fertilizer that favors the energy input on plants and also help them to overcome moments of stress. Spray is a fertilizer capable of regulating and triggering the hormonal production process on the plant and of accelerating the vegetal metabolism, inducing the process of hormones production. Root is a rooting, cellular protector, plant biostimulator product which also has nutrients that are essential for the initial phases of seedlings development.

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The bacteria concentration on the used *Azospirillum brasilense* based products is $2x10^8$ Ufc mL^{-1} and the stimulators used are based on bacterial metabolites, in other words products produced by a certain group of bacteria, for example auxin, which is produced by *Azospirillum brasilense*.

The base fertilization was applied on the sowing groove with 450 kg per hectare of the formula with 0% of nitrogen, 23% phosphorus and 30% potassium (00-23-30). In terms of nitrogen fertilization, there was used urea with 45% of nitrogen and one dose of 90 kg of nitrogen per hectare, divided in two times.

The experiment was conducted with random blocks design with nine treatments and seven repetitions, with parcels of 10 rows spaced in 0.2m, with length 3.87m, totaling $7.5m^2$ each parcel. The Quartzo cultivar was sowed on June 03, 2015 with density of 300 plants m^{-2}.

After the installation of the experiment, there was conducted counting of plants per linear meter, 16 days after sowing. At the beginning of the culture tillering, the first application of urea (45kg of N/ha; ½ dose) was conducted. The second application, by its turn, was conducted at the end of tillering, totaling the 90kg of N/ha. During the cycle there was conducted the evaluation regarding the number of cobs per liner meter and plant height, and the rest of evaluations were conducted after harvest.

For the evaluation of number of plants/m^2 (NP) and number of cobs/m^2 (NE), there was delimited in the field 1 linear meter on the second row of each parcel, where there were conducted the counting. The total height of plant (AP, cm) was measured and the collection of 10 plants was made in order to evaluate grain production, spikelets number (Nesp., plant^{-1}) and grains per cob (GE). The harvest was made with a motoharvester, in six central rows of each parcel, which were grinded and cleaned for the conduction of grain humidity correction (13% basis). After harvest the number of spikelets (Nesp., plant^{-1}) number of grains per cob (GE), one thousand grain mass (MMG, g), hectoliter mass (MH, kg 100L^{-1}), and grain productivity (PG, kg ha^{-1}) were estimated.

The results were submitted to variance analysis, using the SOC/NTIA software (EMBRAPA, 1997), and the F values significant to level 5% of probability were submitted to Duncan Test ($p<0.05$).

RESULTS AND DISCUSSION

The means of the variables that presented significant difference between treatments are: number of plants per m², number of cobs per m³, number of spikelets per plant, one thousand grains mass, and grain productivity. For experiment 2, only number of plants per square meter and plant height presented significant difference (Table 2). Table 1 shows the mean square (QM) of each variation source, or, the variance analysis regarding the variables number of plants, number of cobs, plants height, number of spikelets, grains per cob, one thousand grains mass, hectoliter mass, and grain productivity, in both experiments.

The results obtained in the experiments (Table 2) demonstrating significant effects ($p<0.05$) for the utilization of the diazotrophic bacteria *Azospirillum brasilense* on the variables number of cobs, number of spikelets, one thousand grains mass, and grains productivity on experiment 1, indicating that the use of these treatments enhances the development of the culture on the field. Number of plants per square meter, by its turn, was influenced only by the inoculation factor of the bacteria *Azospirillum brasilense*, because the application of the other treatments occurred after this evaluation. In both experiments, this treatment influenced negatively on the plants initial stands.

For the variable number of cobs per square meter, according to Duncan test, the treatments II and IV, which received the association of the inoculation via seed plus nitrogen fertilization and inoculation via pulverization plus nitrogen fertilization with stimulators, respectively, stood out in relation to the others, differing statistically from control, only with nitrogen fertilization. Mumbach et al. (2017) found similar results for number of cobs, indicating good effects to the plant when the association of nitrogen fertilization with inoculation and use of stimulators was conducted. In accord with results found by Munareto et al. (2019), the reduction of nitrogen fertilization is also possible, without prejudice to number of cobs when the inoculation with *A. brasilense* was conducted. Similarly, Mendes et al. (2011) did not found significant differences for the variables number of tillers, number of cobs, one thousand grains mass when reducing the nitrogen fertilization on in the treatments containing the *A. brasilense* bacteria. Different from experiment 2, where this variable did not present significant differences between the treatments with *A. brasilense*, biofertilizer and association of both products, supporting the results obtained by Silva & Pires (2017), in which there was no significant differences for this variable. Also, their results indicate that the rates of nutrients present in the soil, along with the fertilization provided on the

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base and on the coverage were enough to attend to the culture requirements, in a way that bacterium and growth promoting organisms did not express themselves in the absence of stress.

Table 1. Overview of the variance analysis with mean squares (QM), F value, variation sources (FV), variation coefficient for variables, number of plants (NP, m^{-2}), number of cobs (NE, m^{-2}), plants height (AP, cm), number of spikelets (Nesp., $plant^{-1}$), grains per cob (GE), one thousand grains mass (MMG, g), hectoliter mass (kg $100L^{-1}$), and grains productivity (PG, kg ha^{-1}). Santa Maria, Rio Grande do Sul State, Brazil, 2020.

Experiment 1		**NP**		**NE**		**AP**		**Nesp**	
FV	GL	**QM**	**PR>F**	**QM**	**PR>F**	**QM**	**PR>F**	**QM**	**PR>F**
Treatments	5	5,290.95	0.00	10,303.81	0.042	1.76	0.960	1.25	0.159
Block	6	1,089.48	0.287	3,089.48	0.579	5.35	0.721	2.07	0.025
Mean		175.95		394.05		82.10		18.40	
CV*		16.45		15.79		3.61		4.62	
		GE		**MMG**		**MH**		**PG**	
FV		**QM**	**PR>F**	**QM**	**PR>F**	**QM**	**PR>F**	**QM**	**PR>F**
Treatments	5	10.26	0.610	3.02	0.128	3.10	0.599	184,689.57	0.047
Block	6	17.51	0.315	3.30	0.089	7.42	0.138	76,103.91	0.407
Mean		39.53		31.64		60.77		2,269.01	
CV		9.52		4.01		3.36		11.80	
Experiment 2		**NP**		**NE**		**AP**		**Nesp**	
FV		**QM**	**PR>F**	**QM**	**PR>F**	**QM**	**PR>F**	**QM**	**PR>F**
Treatments	5	10.26	0.610	3.02	0.128	3.10	0.599	184,689.57	0.047
Block	6	17.51	0.315	3.30	0.089	7.42	0.138	76,103.91	0.407
Mean		39.53		31.64		60.77		2,269.01	
CV		9.52		4.01		3.36		11.80	
		GE		**MMG**		**MH**		**PG**	
FV		**QM**	**PR>F**	**QM**	**PR>F**	**QM**	**PR>F**	**QM**	**PR>F**
Treatments	4	9.68	0.480	2.49	0.195	3.52	0.379	43,115.50	0.723
Block	6	52.67	0.002	5.12	0.014	9.04	0.032	76,488.23	0.498
Mean		40.08		31.35		60.52		2,234.42	
CV		8.19		3.92		2.96		12.91	

*CV: Percentual variation coefficient.

Plants height was influenced by the treatments only in the experiment 2, which presented better results only with the inoculation of the *A. brasilense*, similar to the work of Silva et al. (2004), where, in the use of these stimulators on the seeds the plants that were inoculated with the bacteria obtained a better development. This can be explained by the bacteria ability of stimulating the production of growth promoting hormones (CAVALLET et al., 2000).

The treatment with *A. brasilense* via seeds also provided the best result for the variable number of spikelets per plant. However, differed statistically only from treatment IV in experiment 1, while in experiment 2 there was no differences between treatments for this variable, similar to the results of Silva & Pires (2017) again.

Number of grains per cobs did not show any influence from treatments in both experiments. The treatments means did not differ statistically between each other, as in the researches from Mumbach et al. (2017) and Nunes et al. (2015), who did not find significant difference for this variable even in soil with low availability of N. Pereira et al. (2016) observed that in all treatments in which the inoculation of the *A. brasilense* was conducted, they presented higher number of grains per cob when compared to the treatments without inoculation, correlating with the increase of grains productivity, similar to the results of Munareto et al. (2019).

For the variable one thousand grains mass, in experiment 1 all treatments containing the *A. brasilense* or association of the bacteria with biofertilizer and growth stimulator presented significant difference from control, only with nitrogen fertilization. These results suggest that, in the presence of the bacteria, the rhizosphere explored by the plant is bigger, allowing a higher nutrients extraction (CORASSA et al., 2013) and being more efficient in the photosynthesis process, in function of improving the chlorophyll levels of the leaf (HUNGRIA, 2011), which can direct to a higher volume of photo-assimilated for the grain. This occurs mainly because of the morphological and physiological changes that occur at the root system, which possibly occur by the production of growth hormone, that are produced and secreted by *Azospirillum brasilense* (BASHAN & HOLGUIN, 1997).

The hectoliter mass, in both experiments, was influenced negatively by the high precipitation rates that occurred at the periods next to harvest, which provided results that were below the expected ones. Also, the treatments did not have influence upon this variable, which did not present significant differences between the treatments means. Pereira et al. (2017), in the other side, observed the favoring of this variable on the presence of *A. brasilense*, where, independent

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from the mode of application and dose, the treatments presented the highest means with inoculation presence, differing only from the control and from the treatment with 50% of the dose of N.

The variable grains productivity presented results with significant differences between treatments in experiment 1, where the best results were found in treatments with the use of the bacteria *A. brasilense* associated with growth stimulator, differing statistically from the control treatment. Studies indicated the possibility of an increase on wheat productivity in situations of hydric and nutritional stress with the use bacteria from genus *Azospirillum*. This is due to the increase of roots superficial area. Silva et al. (2004) demonstrated that the roots of wheat and barley plants increased their superficial area in the presence of *A. brasilense*, in a way that secondary roots presented lower number but wider contact surface, while control plants presented higher number of disordered and thinner secondary roots.

With the results obtained in this work one observed an increase of grains productivity in the association of the bacteria with stimulators. This is due to the higher nitrogen absorption by the plants generated from the seed treated with the bacteria, as to the higher production of phytohormones that stimulate the plants root growth, obtaining a better exploitation of the nutrients and water and increasing the plant photosynthetic rate. However, the wheat grains productivity does not increase with the inoculation of *Azospirillum brasilense*, in accord with Lucio et al. (2014), who assigned the result to genetic material, which was required high level of nitrogen, and obtained better result with the use of 240 kg of N/ha, reaching productivity of 8 t/ha. Even with years of research, the results found still very divergent, demonstrating that there is a necessity of conducting more experiments in field and studies about the subject (SALA et al., 2007).

The use of stimulators via seed (experiment 2) did not present the expected effect, or, the obtained results did not differ from control (with exception of plants height, that presented better results only with the inoculation of the bacteria), just as in the work of Silva et al. (2004), where in the use of stimulators on seeds the plants that were inoculated with the bacteria obtained better development. In another work, Rampim et al. (2012) observed that the use of phytohormones-based biostimulators in association with *Azospirillum brasilense* or isolated in wheat seeds showed an increase in the initial development of wheat seedlings.

Table 2. Comparation of experiments means for the variables number of plants per m² (NP), number of cobs per m² (NE), plants height (AP), number of spikelets per plant (Nesp.plant^{-1}), grains per cob (GE), one thousand grains mass (MMG, g), hectoliter mass (kg. 100L^{-1}), and grains productivity (PG, kg ha^{-1}). Santa Maria, Rio Grande do Sul State, Brazil, 2020.

EXPERIMENT 1

TREAT.	NP	NE	AP	Nesp	GE	MMG	MH	PG
I	145.00 b	389.29 b	81.61 ns	18.61 ab	39.41 ns	30.48 b	59.81 ns	2,094.83 b
II	139.29 b	465.71 a	82.86	18.93 a	41.57	31.33 ab	60.98	2,321.95 ab
III	186.43 a	361.43 b	82.5	18.07 ab	38.7	32.29 a	61.11	2,543.39 a
IV	194.29 a	383.57 b	82.2	17.87 b	40.08	31.76 ab	61.35	2,276.88 ab
V	207.14 a	401.43 a	81.71	18.17 ab	38.07	31.90 ab	61.31	2,260.84 ab
VI	183.57 a	362.86 b	81.74	18.76 ab	39.36	32.09 a	60.06	2,116.16 b

EXPERIMENT 2

TREAT.	NP	NE	AP	Nesp	GE	MMG	MH	PG
I	145.00 ab	389.28 ns	81.61 b	18.61 ns	39.41 ns	30.47 ns	59.81 ns	2,094.83 ns
II	175.00 a	397.14	84.12 a	18.72	40.65	31.90	59.76	2,202.65
III	167.85 ab	441.42	82.34 ab	18.47	38.49	31.90	60.66	2,305.30
IV	139.28 b	465.71	82.85 ab	18.92	41.57	31.33	60.98	2,321.95
V	145.00 ab	402.14	81.14 b	18.72	40.24	31.14	61.35	2,132.86

*Means that are not connected by the same letter in the column did not differ at 5% probability by Duncan test.
*ns: non-significant.

CONCLUSION

The inoculation wheat with *Azospirillum brasilense*, via seed or foliar pulverization, increases grains productivity, and one thousand grains mass of wheat culture.

The association of stimulators and biofertilizers can be a tool in the construction of wheat productivity, providing an energy input for the moments of stress during the culture cycle.

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