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Azospirillum brasilense and nitrogen fertilization affecting wheat productivity

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In order to optimize the use of nitrogen (N), the aim of this work was to evaluate the efficiency of foliar application of *Azospirillum brasilense* Ab-V5 strain, as regards the productivity of wheat plantations combined with different N doses. The experiments were carried out in four municipalities in Minas Gerais (Brazil) under randomized block design with four replications. Forty percent of the N dose was applied at planting time and 60% as topdressing at tillering stage of the crop. *Azospirillum brasilense* was applied as foliar spray at a dose of 500 ml/ha. The treatments consisted of: (1) Control-without N or Ab-V5; (2) 50% of the N recommended; (3) 100% of the N; (4) Application of Ab-V5 strain; (5) 50% of N and Ab-V5 strain; (6) 100% of N and Ab-V5 strain; (7) 100% of N and seed inoculation with a commercial product (Master Fix). For all the locations, productivity increased with application of 100% of N recommended and foliar spray containing *A. brasilense* compared to the treatment that had only 100% of N recommended.

Key words: Foliar spray, nitrogen fertilization, diazotrophic bacteria, *Triticum aestivum* L.

INTRODUCTION

Wheat is the second cereal most produced in the world. In Brazil, it is grown in the South, Southeast and Midwest regions. In the 2015 to 2016 crop season, the cultivated area with wheat in Brazil was 2103 million hectares; with an average yield of 2770 kg ha^{-1} (Conab, 2016).

Nitrogen fertilization is essential, in order to ensure the production and quality of grains. This is the most limiting nutrient for wheat productivity (Rodrigues et al., 2014). Adequate N supplying determines the number of tillers, which may favor the nodes formation and cause the stem elongation. The increase in the number of tillers and greater elongation of the stem allow higher uptake of solar radiation and, therefore, greater productivity (Fornasieri Filho, 2008). In addition, the number of ears per area and the number of spikelets per ears increase the adequate availability and application of this nutrient

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Table 1. Chemical and physical characteristics of the soil at 0 to 20 cm depth.

Chemical and physical attributes		Madre de Deus de Minas	Uberaba	Lambari	Patos de Minas
pH in water		6.00	5.89	5.90	5.50
[9]P-rem	mg/ dm^3	14.17	41.08	12.93	6.05
[11]Organic matter	dag/kg	3.84	1.58	3.99	3.28
[1]P	mg/dm^3	42.85	72.15	10.64	50.31
[1]K		114.00	76.00	74.00	69.00
[2]Ca	$cmol/dm^3$	2.90	1.74	2.50	1.11
[2]Mg		0.80	0.61	0.90	0.37
[2]Al		0.00	0.04	0.10	0.15
[7]H+Al		3.24	1.74	3.62	4.62
[3]CTC (t)		3.99	2.58	3.69	1.81
[4]CTC (T)		7.23	4.28	7.21	6.28
[6]m	%	0.00	1.55	2.71	8.30
Base saturation		55.22	59.42	49.79	26.40
B	mg/dm^3	0.19	0.16	0.24	0.22
[1]Cu		2.55	1.20	1.31	11.30
[1]Fe		29.38	25.10	32.77	41.70
[1]Mn		29.38	8.50	12.57	82.20
[1]Zn		10.71	8.50	5.51	4.00
[10]S		20.59	2.63	21.20	25.04
[5]Clay	dag/kg	41.00	204.00	54	280.00
[5]Silt		42.00	26.00	13	226.00
[5]Sandy		17.00	770.00	33	494.00
[12]COT		-	0.92	-	1.90

[1]P, K, Fe, Zn, Mn, Cu - Mehlich I; [2]Ca, Mg, Al - KCl 1 mol/l; [3]CTC (t), effective cation exchange capacity; [4]CTC (T), total exchange capacity at pH 7; [5]Texture, pipette method; [6]m, aluminum saturation; [7]H + Al, SMP method; [8]B, hot water; [9]P-rem, remaining phosphorus, concentration of P of the equilibrium solution after stirring the air-dry soil during 1 h with $CaCl_2$ solution at 10 mmol/L, containing 60 mg/L of P (1:10); [10]S, monocalcium phosphate in acetic acid; [11]Oxidation, $Na_2Cr_2O_7$ 4N + H_2SO_4 10N; [12]COT, total organic carbon - oxidation with $Na_2Cr_2O_7$ 4N + H_2SO_4 10 mol/L.

(Megda et al., 2009).

Bacteria that belong to the *Azospirillum* genus are known to associate symbiotically with grass forming specialized structures in the roots in which there is conversion of N_2 to NH_3 (Radwan et al., 2004). For the establishment of a symbiotic relationship, germinating seeds and roots exude molecules that chemically attract the N fixing bacteria and stimulate its development in the rhizosphere and the gene expression of the bacteria related to the biological N fixation (Hungria et al., 2001).

The use of *Azospirillum* spp. in the development of grasses has been researched in recent years, not only regarding the yields but also regarding physiological effects. Recent reports states its positive effects on the growth and on the N accumulation in wheat plants (Sala et al., 2005), maintaining the fertile tillers, higher extraction and N accumulation in grains (Jezewski et al., 2010), more efficient translocation of N to the grains, heavier and full grains, better distribution of N in the grains biomass (Didonet et al., 2000) and root development. However, some authors did not find any difference between treatments with or without inoculation (Campos et al., 1999). Studies on the application of *Azospirillum brasilense* via foliar spray in wheat plants are inconclusive and scarce. Farther, N fertilization is one of the most expensive management practices for the growers and the application of *A. brasilense* may increase the profits as well the use efficiency of this nutrient.

The objective of this study was to evaluate in field conditions the efficiency of foliar application of *A. brasilense,* Ab-V5 strain, as regard to the productivity of wheat cultivated with different N doses.

MATERIALS AND METHODS

The experiments were carried out in four experimental fields located in the following municipalities of Minas Gerais state: Madre de Deus de Minas, Uberaba, Lambari and Patos de Minas. The plots were 6.0 m long and 4.0 m wide and presented 10 rows spaced at 0.17 m with 80 seeds/linear meter at sowing; the four central rows were considered the useful plot (12 m^2). The experiment was designed as randomized block with 4 replications. The chemical and physical characteristics of the soil are presented in Table 1.

The dose of N indicated for the crop under irrigation was 60 kg

Table 2. Variance analysis for the productivity of wheat (bags/ha) evaluated in Madre de Deus de Minas, Uberaba, Lambari and Patos de Minas municipalities.

FV	FD	Mean square
Location	3	3443.34**
Treatment	6	920.43**
Location * Treatment	18	341.96**
Replication	3	426.71
Residue	18	114.32
CV (%)		16.86
Average		63.40

**Significant at 1% probability by the F test.

per hectare. It was applied as 40% of the N dose at planting time and 60% as topdressing at tillering stage. The *A. brasilense* strain Ab-V5 was used at a concentration of $1×10^8$ colony forming units per ml. The Ab-V5 bacterium has been widely studied by "*Embrapa Soja*" and Federal University of Paraná; nowadays it is in the list of inoculant bacteria approved by Ministry of Agriculture, Livestock and Food Supply (MAPA) for corn and wheat. Ab-V5 was applied via foliar spray at the dose of 500 ml/ha at the beginning of tillering stage with a flow of 200 l/ha.

Nitrogen fertilization varied according to the treatments as it follows: (1) Absolute control, without N or Ab-V5 strain; (2) 50% of the N dose recommended for the crop; (3) 100% of the N dose recommended for the crop; (4) Ab-V5 without N; (5) 50% of the N recommended for the crop with Ab-V5; (6) 100% of the N recommended for the crop with Ab-V5; (7) 100% of the N recommended for the crop and seeds inoculation with Master Fix (100 ml/20 kg of seeds) which is a commercial product containing *A. brasilense*.

Inoculation with the commercial product "Master Fix" composed by *A. brasilense* (Ab-V5 and Ab-V6 strains) was directly performed in the seed previous to sowing at a dose of 100 ml ha^{-1} of the inoculant liquid, containing around $2.0 × 10^8$ colony forming units/ ml.

The crop management practices were uniform in all the experiments, except for N fertilization and in the experiment of Madre de Deus de Minas city where the wheat was grown in rainfed system. Sowing was made using a seeder for grooves opening, as well as marking the sowing lines and the N fertilizer was manually distributed. The experiments were performed in four different locations, as described as follow:

(a) Madre de Deus de Minas municipality in a field of "Fazenda Liberdade" located at "21°27'04" S and "44°19'14" W at the altitude of approximately 1026 m. Coodetec 108 wheat cultivar was cultivated. Sowing was performed on February 29th 2012 and the harvest was done on June 08th 2012.

(b) Uberaba municipality in a field of IFTM (Federal Institute of Triângulo Mineiro) located at "19°43'6.94" S and "47°57'28.29" W at the altitude of approximately 810 m. Coodetec 108 wheat cultivar was cultivated. Sowing was performed on May 24th 2012 and the harvest on September 02nd 2012.

(c) Lambari municipality in a field of EPAMIG (Agricultural Research Company of Minas Gerais) located at "21°56´40.85" S and "45°18´40.91" W at the altitude of approximately 883 m. Coodetec 207 cultivar was cultivated. Sowing was performed on April 03rd 2013 and harvest on September 15th 2013.

(d) Patos de Minas municipality in a field of EPAMIG (Agricultural Research Company of Minas Gerais) located at "18°31´01.90" S and "46°26´19.08" W at the altitude of approximately 926 m. Coodetec 207 cultivar was cultivated. Sowing was performed on May 17th 2013 and the harvest on September 17th 2013.

In all experiments, the ears were harvested from the useful plots, corresponding to 12 m^2 (four central rows). To determine the grain yield, the ears were threshed and weighed in threshing electric machine. The grains had moisture corrected to 13% on wet basis.

The experimental data were submitted to variance analysis and the effects of treatments and interactions were evaluated by F test, whereas the treatment means were compared by the Scott-Knott test (Ferreira et al., 2014).

RESULTS AND DISCUSSION

The individual variance analyses showed significant results for productivity. In this experiment, for most of the treatments, wheat cultivars showed higher yield than the national average which is 47.65 bags/ha (Conab, 2016). The analysis showed significant results for different locations, treatments and their interaction (Table 2).

Three groups of treatments were performed in Uberaba and Patos de Minas municipalities and two groups in Lambari and Madre de Deus de Minas. For all of them, the use of 100% N + *Azospirillum* via foliar spray yielded the best results. For the treatments with no application of *A. brasilense* (0, 50 and 100% of N) the productivity was significantly lower compared to the treatments with Azospirillum, however they belonged to the same group (Table 3). This lack of response may be related to the residual effect of N fertilizers applied to cultivate previous crops in all of these areas. In these regions the adoption of crop rotation system with leguminous and horticulture crops which leaves high amounts of N in the soil is common. It ensures the positive effect of Azospirillum on the N use efficiency.

As this bacterium was applied via foliar spray, probably this increasing productivity might be related to changes in phytohormone metabolism and N metabolism, even in rainfed systems such as Lambari municipality. However, Azospirillum`s action on the plant`s metabolism is found to be controversial.

Recent reports states its increasing ability to fix N from atmosphere (Huergo et al., 2008); increasing effects on activity of nitrate reductase when they grow endophytically (Cassán et al., 2008), production of hormones such as auxins, cytokinins (Tien et al., 1979), gibberellins (Bottini et al., 1989); ethylene (Strzelczyk et al., 1994) and a variety of other molecules in the cell (Perrig et al., 2007); phosphate solubilization (Rodriguez et al., 2004) and biological control of pathogens (Correa et al., 2008). So they are able to promote the development of roots and shoots, increase water and mineral absorption and optimize the tolerance to abiotic stresses such as salinity or drought (Roscoe and Miranda, 2013).

It is widely known that nitrate reduction occurs in the cytosol and involves the action of the nitrate reductase producing nitrite; it enters the plastids of roots or chloroplasts of leaves and it is reduced to ammonia by the action of the enzyme nitrite reductase, which is

Table 3. Average of the grain productivity of wheat evaluated in Madre de Deus de Minas, Uberaba, Lambari and Patos de Minas and conjoint analysis of the locations.

Treatments	Madre de Deus de Minas	Uberaba	Lambari	Patos de Minas	Conjoint analysis
0% N	64.06[b]	50.26[c]	54.68[b]	54.38[b]	55.85[b]
50% N	58.37[b]	56.83[b]	56.29[b]	59.22[b]	57.68[b]
100% N	68.12[b]	59.17[b]	58.54[b]	59.48[b]	61.33[b]
0% N + Ab-V5	92.62[a]	46.37[c]	59.75[b]	34.77[c]	58.53[b]
50% N + Ab-V5	88.50[a]	47.08[c]	66.86[a]	51.20[b]	63.41[b]
100% N + Ab-V5	93.50[a]	66.62[a]	75.27[a]	72.37[a]	76.95[a]
100% N + Master Fix	90.31[a]	56.38[b]	64.56[a]	68.89[a]	70.04[a]

[1]Means followed by the same letter in the columns do not differ at 5% probability, except in Lambari that was 10% probability, by Scott Knott test.

attached via glutamate synthase/glutamine synthase (GS/GOGAT) in amino acids such as glutamine and glutamate which, in its turn serve as substrate for transamination reactions that are essential for the production of amino acids and proteins (Donato et al., 2004).

Nitrate reductase is one of the most sensitive enzymes to any stress in the plants, because it is highly dependent on NADPH derived from photosynthesis. Therefore, factors that enhances the photosynthetic efficiency, probably improves the N use efficiency. Nitrate reductase has been widely studied, because it controls protein synthesis in plants that absorbs nitrate as the main source of N (Marschner, 2011).

Possibly *A. brasilense* applied via foliar spray had a great effect on nitrate reductase and it increased the use efficiency of N applied via fertilization. Besides this, *A. brasilense* can also work fixing N from the atmosphere, which may help the plants save energy with the N reduction. Adequate nitrate reductase activity is primordial to guarantee high productivity; once N is one of the most limiting nutrients to form organic molecules (Taiz and Zeiger, 2013). This effect was observed in this experiment.

According to Sala et al. (2007) some wheat cultivars may present increases around 27 to 45% on grain production with *A. brasilense* inoculation. Nozaki et al. (2013) observed a significant increase on wheat productivity applying 290 kg/ha with 1.5 ml of *Azzospirillum* spp. Martins et al. (2012) observed that *A. brasilense* inoculation as foliar spray was more efficient in different corn hybrids and showed an excellent choice for use on grass, because it coincided with the herbicide application phase. However, Mendes et al. (2011) did not observed any difference between the treatments with reduction of the N fertilization and inoculation of *A. brasilense* for the number of tillers, number of ears and weight of 1000 grains.

According to Kapulnik et al. (1983), wheat plants inoculated with *A. brasilense* increased the contents of N, P and K. The contents of nitrate in the vacuole are directly related to nitrate reductase activity (Li and Gresshoff, 1990). Panwar (1991) observed that seeds of wheat inoculated with *A. brasilense* increased intensively the activity of this enzyme. Didonet et al. (2000) inoculated 245 strains with 10 isolates of *A. brasilense* in wheat plants with different doses of N and concluded that they provide better use of N accumulated in the biomass, translocating N more efficiently. Swędrzyńska (2000) concluded that *A. brasilense* can be a factor to increase vigor and yield of wheat. In water stress conditions the author observed an increase of 27% in wheat productivity.

Initially, some authors expected that the benefits with the use of *A. brasilense* were basically derived from biological N fixation (Dobbelaere et al., 2004). But it seems that the positive effects provided by these microorganisms are mainly derived from the morphological and physiological changes in the roots of inoculated plants, causing an increase in the uptake of water and nutrients (Okon and Vanderleyden, 1997).

Probably this is the reason that the productivity had been so high, even in rainfed systems such as in Lambari municipality. Previous studies show an increasing concentration of the following phytohormones when Ab-V5 strain was inoculated in the plants: Kinetin which induces root growth; salicylic acid which may have an acclimatization effect providing increased tolerance to many different kinds of abiotic stresses; jasmonic acid that may induce gene expression regarding stress defense; indolbutyric acid which is a root promoter; indoleacetic acid which is growth promoter and gibberellic acid that stimulates plant growth.

Quadros et al. (2014) concluded that the use of Azospirillum stimulated the growth of plants in the vegetative period, which increased the uniformity of plant stand, greater resistance to stress and greater concentration of chlorophyll in leaves. The Ab-V5 strain induces the production of these phytohormones in a balanced way and possibly is capable to be absorbed by leaves, demonstrating the effectiveness of foliar application. Therefore, in the present study the use of *A.*

brasilense may have a combined effect on wheat productivity, by phytohormones and N metabolism.

Conclusion

The foliar application of *A. brasilense*, Ab-V5 strain, promotes an increasing productivity combined with 100% of the N dose. *A. brasilense* is a complementary technology focused on increasing wheat productivity especially under water stress conditions.

Conflict of Interests

The authors have not declared any conflict of interests.

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