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Azospirillum brasilense in barley grown in the Brazilian Cerrado is capable of providing higher grain yield with less use of nitrogen

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Abstract

Bacteria such as Azospirillum brasilense are capable of increasing the productivity of several grasses, with a better use of

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nitrogen. Barley production for brewing purposes can be benefited by inoculation with the A. brasilense. The study aimed to test two varieties of barley for the Cerrado in terms of productivity, accumulation of biomass, and nitrogen by the inoculation with A. brasilense and doses of nitrogen. The experiment was carried out in field conditions, in Mato Grosso do Sul, Brazil. It was conduced in randomized blocks in a 2x2x4 factorial scheme corresponding to two cultivars of barley, Itanema and Manduri, with and without A. brasilense strain Ab-V5/Ab-V6 inoculation and four doses of N (0, 40, 80, and 120 Kg ha^{-1}). Aerial part dry mass, the nitrogen content of stem + leaf, spikes, and grains, the number of grains per spike, 100-grain weight, and grain yield were evaluated. The results obtained indicated that the barley cultivar Manduri accumulates less nitrogen in the plant tissues than the cultivar Itanema. However, it presents a higher grain yield. The seed inoculation with A. brasilense provides a higher nitrogen content in the plant tissues, increading barley yield when compared to non-inoculated plants and indicates a better use of the nitrogen supplied.

Key words: Diazotrophic bacteria, growth-promoting bacteria, nitrogen fixation, *Hordeum vulgare*, malt, hormone.

INTRODUCTION

Barley (*Hordeum vulgare* L.) worldwide production is predominantly for animal feed, followed by a demand from the beverage industries. In contrast, in Brazil, all commercially grown barley is destined for the manufacture of malt, with a higher price paid to producers due to the high demand of the beer industry. Although 95% of the malt produced is consumed by Brazilian breweries, 85% of the malt used in Brazil is imported, which reflects the insufficient national production for this purpose (De Mori and Minella 2012).

Among the managements that impact the crop yield, nitrogen fertilization is one of the most important, given its positive correlation with the development of the leaf area and the production of photoassimilates that will be used to fill the grains (Cai et al. 2012).

However, the supply of N must be judicious, as the plants are hardly able to take advantage of all the N supplied through fertilization, leading to economic and environmental losses (Good et al. 2007).

As an alternative, studies have shown advantages in the use of growth-promoting bacteria in cereals, due to their influence on nitrogen acquisition and metabolism, with gains in yield and nutritional quality of the final product (Ferreira et al. 2013; Piccinin et al. 2013). Inoculation of barley plants with *Azospirillum brasilense* can assist in the better N use, by the biological fixation capacity of the bacteria, by stimulating root development that can lead to a greater recovery of applied N, and by the greater activity of the enzyme nitrate reductase, essential in the N assimilation (Bashan and de-Bashan 2010; Martins et al. 2017; Pereira-Defilippi et al. 2017).

The study aimed to test two varieties of barley for the cerrado in terms of productivity, accumulation of biomass, and nitrogen by the inoculation with *A. brasilense* and doses of nitrogen.

MATERIAL AND METHODS

The experiment was carried out in 2014, in the experimental area of the Universidade Federal de Mato Grosso do Sul (the Federal University of Mato Grosso do Sul), in Chapadão do Sul, MS, Brazil, with an approximate location at 18º48'459" S, 52º36'003" W, and altitude of 820 meters. The region's climate, according to the Köppen classification, is Aw type, humid tropical, with an average temperature of 29 °C and annual precipitation of 1850 mm, with a dry season in winter (Santos et al. 2018). The temperature and precipitation data are shown in figure 1.

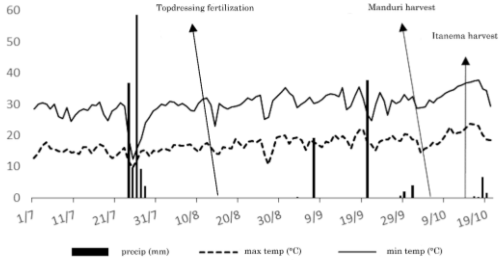

Figure 1. Data of maximum and minimum temperatures and rainfall during experiment period. Chapadão do Sul, Brazil, 2014.

The water supply for the crop was carried out through a sprinkler irrigation system, with a three-day watering interval. Irrigation management followed the recommendations of Guerra and Silva (1999).

The soil is classified as a Latossolo Vermelho distrófico with a clayey texture and a latosolic b horizon (Sanches et al. 2015), Annual crops were grown for many years in the area, and the last species was peas. Before barley sowing, soil samples from the 0-20 cm layer were obtained for chemical analysis. The results of chemical analysis were pH($CaCl_2$) - 4.9; OM - 33.5 mg dm^{-3}; P(resin) - 9 mg dm^{-3}; Ca^{2+} - 2.9 $cmol_c$ dm^{-3}; Mg^{2+} - 0.9 $cmol_c$ dm^{-3}; K^+ - 0.07 $cmol_c$ dm^{-3}; H+Al - 2.9 $cmol_c$ dm^{-3}; CEC - 6.77 $cmol_c$ dm^{-3}, and 53.7% base saturation.

Randomized blocks design with three replications, in a 2x2x4 factorial scheme, was used. Two barley cultivars (Itanema and Manduri), presence and absence of inoculation with *A. brasilense*, and four nitrogen doses (0, 40, 80, and 120 kg ha^{-1}) were evaluated. The plots consisted of five rows four meters length, spaced 0.17 m apart (3.4 m^2).

The soil preparation was carried out with a harrow operation. The fertilization was carried manually in the sowing furrow. The amount of fertilizer applied was calculated according to the results of the soil chemical analysis and the recommendations for the crop in the Cerrado (Sousa and Lobato 2004). Thus, 60 kg ha^{-1} of P_2O_5 and 50 kg ha^{-1} of K_2O were applied using single superphosphate and potassium chloride as sources.

The seeds were treated with Carboxin + Thiram in the dose of 50 mL 100 kg^{-1} of seeds, and Pyraclostrobin + Methyl Thiophanate + Fipronil in the dose of 100 mL 100 kg^{-1} of seeds. After drying, the inoculation treatments were applied, with the application of *A. brasilense* at a dose of 3 mL kg^{-1} of seeds (Masterfix® Gramíneas, strains Ab-V5 and Ab-V6, with $2x10^8$ viable cells mL^{-1}).

Sowing was carried out manually on July 2nd, 2014, and was used 250 seeds m^{-2}. The topdressing fertilization was performed 41 days after emergence (DAE), with urea as a nitrogen source, according to the doses indicated for each treatment. Also, 40 kg ha^{-1} of K_2O was applied to all plots using potassium chloride as a source. Phytosanitary management was carried out only for pest control, at

20 DAE for the control of caterpillars (2 g ha^{-1} of Deltamethrin), and at the grain filling stage for control of stink bugs (100 g ha^{-1} of Imidacloprid).

At the flowering stage, ten plants were collected in each plot, then submitted to drying in a forced air circulation oven at 65 °C for 72 hours. After drying, the dry matter of the shoot was weighed, followed by the separation of stems + leaves and spikes, which were ground in a Wiley-type mill. The milled samples were then used to determine the N content of shoots and spikes, according to the Kjeldahl method, adapted by Galvani and Gaertner (2006).

For the harvest, two central rows were used, and ten plants were randomly separated, in which the number of grains per spike was counted. The harvested material was subjected to manual threshing, and the grain nitrogen content, 100-grain weight, and grain yield corrected to 13% moisture (wet basis) were determined.

The collected data were subjected to analysis of variance and the F test. The means from cultivars and inoculation were compared by the Tukey test at 5% probability. The averages from N doses were submitted to regression analysis.

RESULTADOS

There was an interaction between the factors cultivar and inoculation, cultivar and nitrogen, and inoculation and nitrogen. Only the variables number of grains per spike and 100-grain weight were not influenced by the interaction between cultivar and inoculation (Table I).

Table I. Values of F-test for the interaction between the factors cultivar, inoculation and nitrogen doses, for aerial part dry mass (ADM), nitrogen content in stem and leaves (NSL), nitrogen content in spikes (NCS), nitrogen content in grains (NCG), number of grains per spike (NGS), 100-grain weight (W100) and grain yield (GY) of barley plants.

Interactions	Values of F-test						
	ADM	NSL	NCS	NCG	NGS	W100	GY
C*I	197.634*	249.657*	12.124*	5.200ns	2.765*	2.369ns	1202.169*
C*N	77.541*	203.807*	199.435*	62.568*	25.014*	10.361*	366.021*
I*N	119.821*	48.472*	67.775*	45.119*	28.690*	7.276*	807.287*
CV(%)	2.94	2.46	1.11	2.24	2.63	2.53	0.87

*Significant at 5% probability by the F test; nsnot significant by F test at 5% probability; CV (%) coefficient of variation. C = Cultivar; I = Inoculation e N = Nitrogen.

About the interaction between cultivar and inoculation, it was observed that the cultivar Manduri had a higher response to inoculation, with gains in the parameters evaluated, except for shoot dry matter. Inoculation also increased the grain yield of both cultivars (Table II). The grain yield of cultivar Manduri was 14.6% higher than the cultivar Itanema. The use of inoculation resulted in an increase of 24.9% in grain yield of cultivar Itanema and 3.0% in the cultivar Manduri.

Table II. Results of the interaction between the factors cultivar and inoculation of barley plants, for aerial part dry mass (ADM), nitrogen content in stem and leaves (NSL), nitrogen content in spikes (NCS), nitrogen content in grains (NCG), and grain yield (GY).

	ADM (g)		NSL (%)		NCS (%)	
	Itanema	Manduri	Itanema	Manduri	Itanema	Manduri
NI	70.33 bA	56.74 aB	1.70 aA	1.59 bB	2.39 aA	2.20 bB
I	77.42 aA	48.71 bB	1.60 bB	1.87 aA	2.39 aA	2.25 aB
	NCG		GY			
	Itanema	Manduri	Itanema	Manduri		
NI	2.24 aA	1.99 bB	1593.75 bB	2023.70 bA		
I	2.26 aA	2.08 aB	1990.30 aB	2083.85 aA		

Averages followed by the same letter, uppercase on the line, and lowercase on the column, do not differ by the Tukey test (p> 0.05). NI = non-inoculated; I - inoculated.

The cultivar Itanema had higher aerial part dry mass than cultivar Manduri, with the highest accumulation achieved with 49 kg ha^{-1} of nitrogen. For the Manduri cultivar, the highest aerial part dry mass was reached with 65 kg ha^{-1} of nitrogen (Figure 2a). The inoculated plants obtained the highest aerial part dry mass with 46 kg ha^{-1} of nitrogen and the non-inoculated plants with 61 kg ha^{-1} of nitrogen. However, in the absence of nitrogen fertilization, the inoculated plants had a higher accumulation of aerial part dry mass than non-inoculated plants (Figure 2b).

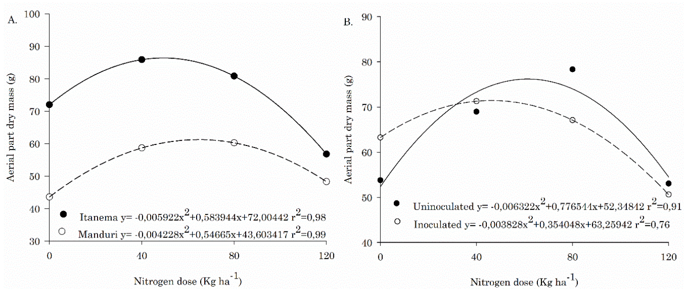

Figure 2. Aerial part dry mass of two barley cultivars (A) and inoculated or uninoculated with *A. brasilense* (B) under nitrogen doses.

The highest N content in stem and leaves was observed in the cultivar Manduri, with the highest nitrogen dose. The Itanema cultivar had an increasing accumulation of nitrogen up to the dose of 61 kg ha^{-1}. This result represented an increase in the N content of 51 and 24% concerning plants without nitrogen for the cultivars Itanema and Manduri, respectively (Figure 3a).

The inoculation with *A. brasilense* provided an increase in N content of stem and leaves of barley plants up to the N dose of 82 kg ha^{-1}. Above this dose, nutrient accumulation occurred only for non-inoculated plants, up to a dose of 101 kg ha^{-1}. The inoculated plants had an increase of 23% in the N accumulation concerning the non-inoculated plants, in the absence of nitrogen (Figure 3b).

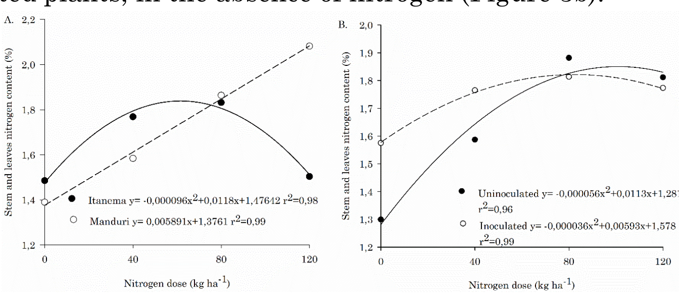

Figure 3. Steam and leaves nitrogen content of two barley cultivars (A) and inoculated or uninoculated with *A. brasilense* (B) under nitrogen doses.

The cultivar Itanema showed the highest N content in spikes, except for the highest nitrogen doses. The highest accumulation for this cultivar was at a dose of 64 kg ha^{-1}, 5% higher than in the absence of nitrogen. The cultivar Manduri responded to fertilization with N until the maximum tested dose, with a gain of 25% concerning the lack of nitrogen (Figure 4a). The N content in spikes followed the behavior of the N accumulated in the shoot and leaves of the barley plants.

The plants had similar responses in terms of nitrogen fertilization, regardless of inoculation, and reached the highest N content in spikes at the maximum dose of nitrogen fertilizer. However, when N was not supplied, the inoculation increased its content in the spikes by 8% when compared to non-inoculated plants (Figure 4b). This result shows that the inoculation can favor the obtaining of N by the plant, resulting not only in higher growth but also in better conditions for the development of the reproductive structure.

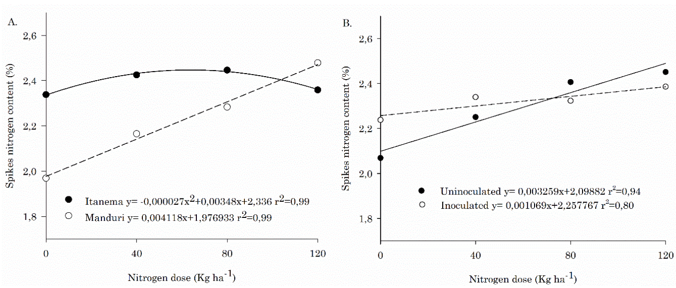

Figure 4. Spikes nitrogen content of two barley cultivars (A) and inoculated or uninoculated with *A. brasilense* (B) under nitrogen doses.

About N content in the grains, cultivar Itanema also had higher values than Manduri. The difference was 20% in the absence of nitrogen fertilization. However, the cultivar Manduri responded to the nitrogen fertilization up to the maximum tested dose, and Itanema up to the dose of 73 kg ha^{-1} (Figure 5a).

The inoculation of barley plants provided an increase in N content in the grains up to the dose of 89 kg ha^{-1} of nitrogen (Figure 5b). After that dose, only the non-inoculated plants continued to accumulate the nutrient up to the maximum dose, with 7% higher N content than that obtained by the inoculated plants. In the absence of fertilization, the inoculated plants accumulated 12% more N in the grains than the non-inoculated ones.

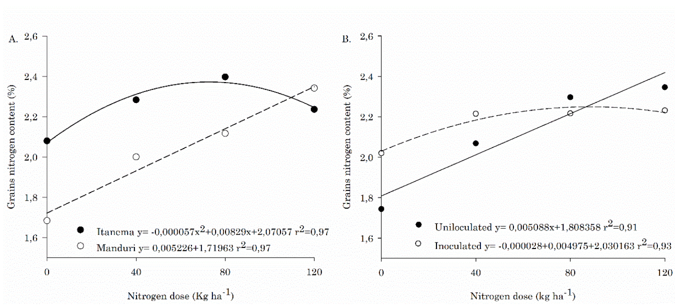

Figure 5. Grains nitrogen content of two barley cultivars (A) and inoculated or uninoculated with *A. brasilense* (B) under nitrogen doses.

The highest number of grains per spike was found for the cultivar Manduri, except in the absence of nitrogen fertilization. The highest number of grains per spike was achieved at the dose of 61 kg ha^{-1} of nitrogen with 26 grains for Manduri. For the cultivar Itanema, the highest number of grains per spike (25 grains) was found with 25 kg ha^{-1} of nitrogen (Figure 6a). The inoculated plants obtained the highest number of grains per spike, 26.2 grains, achieved with 62 kg ha^{-1} of nitrogen. The non-inoculated plants obtained a higher number of grains per spike than inoculated plants only in the treatment that did not receive nitrogen fertilization, and the highest number of grains per spike was 25 grains (Figure 6b).

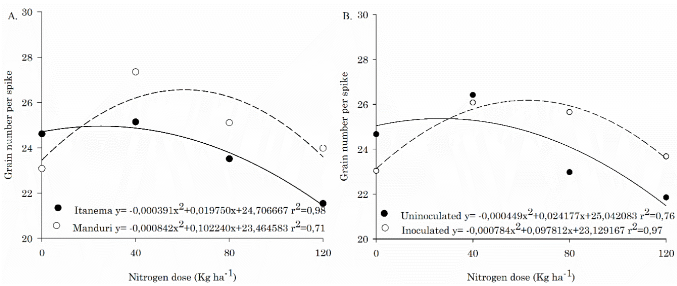

Figure 6. Grains number per spike of two barley cultivars (A) and inoculated or uninoculated with *A. brasilense* (B) under nitrogen doses.

There was a decrease in the 100-grain weight for the cultivar Manduri with nitrogen fertilization. The nitrogen dose of 81 kg ha^{-1} resulted in the 100-grain weight around 7% lower the best treatment, which did not receive N (Figure 7a). The nitrogen fertilization caused a reduction of the 100-grain weight of inoculated plants. The reduction was greater, the higher the nitrogen dose (Figure 7b).

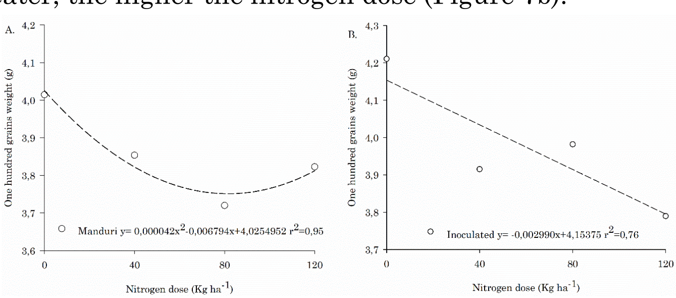

Figure 7. One hundred grains weight of Manduri cultivar barley (A) and inoculated with *A. brasilense* (B) under nitrogen doses.

The highest grain yield was found for cultivar Manduri in all nitrogen doses. The highest grain yield for cultivar Manduri was 2383 kg ha^{-1}, obtained with 62 kg ha^{-1} of nitrogen. For the cultivar Itanema, the highest grain yield was2076 kg ha^{-1} with 68 kg ha^{-1} of nitrogen (Figure 8a). About the inoculation, the highest grain yield (2319 kg ha^{-1}) was found in the inoculated plants, with 55 kg ha^{-1} of nitrogen. In the non-inoculated plants, the nitrogen dose of 85 kg ha^{-1} provided the highest grain yield, 2304 kg ha^{-1}. When N was not used, the inoculation provided a 45% higher yield than the non-inoculated plants (Figure 8b).

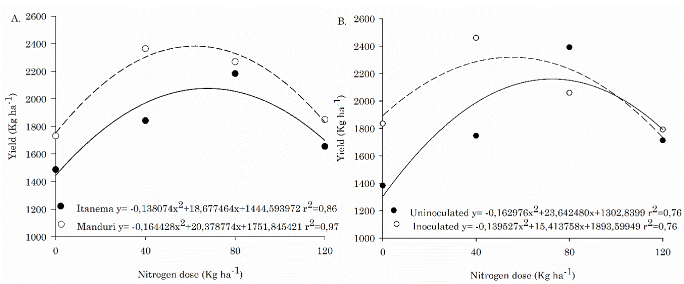

Figure 8. Grains yield of two barley cultivars (A) and inoculated or uninoculated with *A. brasilense* (B) under nitrogen doses.

DISCUSSION

One of the known effects of *A. brasilense* is its ability to promote plant growth (Martins et al. 2017). This is because the bacteria produce substances that promote plant growth as auxins (Vega-Celedón et al. 2016), besides being able to perform biological nitrogen fixation (Bashan and de-Bashan 2010). These effects were observed in the accumulation of shoot dry matter in cultivar Itanema, and in the nitrogen content in stem and leaves of cultivar Manduri (Table 2).

It is observed, however, that for cultivars, the effects were contrary to the variables mentioned above. The Itanema cultivar had an increase in dry matter with a reduction in the shoot N content, and the cultivar Manduri had a higher N content with less accumulation of dry matter. This may be due to the dilution effect of the nutrient by its partitioning in plant tissues in the first case, and a higher N concentration in the second case. The variation in the responses of barley genotypes to inoculation has already been verified by Lade et al. (2018), and one of the reasons for this includes a difference in growth mechanisms regulated by hormones such as auxins, causing changes in the size, surface area and the number of initial roots of plants.

The Itanema cultivar had, as a characteristic, greater production of vegetable matter than Manduri, as this was obtained in any dose of nitrogen applied (Figure 2a). About the result of the accumulation of aerial part dry mass and inoculation, it was observed that nitrogen fertilization interfered with the growth-promoting effect of the bacteria in the shoot of plants, since in the absence of the application of the nutrient, the highest value was found with the inoculation (Figure 2b). This effect may have occurred due to greater investment in root biomass of the inoculated plants (Lade et al. 2018), as a result of the production of auxins, to the detriment of the shoot growth, which may have provided higher uptake of nitrogen applied (Figure 3b).

The nitrogen content in the stem and leaves of the cultivar Itanema reflected in the accumulation of aerial part dry mass of the plants. However, in the cultivar Manduri, there was no decrease in the shoot nitrogen content, even in the highest dose. However, the highest nitrogen doses led to a reduction in the dry matter accumulation of plants (Figures 2a and 2b). This may be the result of harmful effects caused by the high availability of N (Araújo et al. 2012) or by the use of carbohydrates to assimilate N into proteins (Cai et al. 2011), which results in lower photosynthetic efficiency.

The use of inoculation provided a higher nitrogen content in the stem and leaves of plants up to around 80 kg ha^{-1}. The highest accumulation of nitrogen in the shoot is due to the effect of the greater root development of the inoculated plants, with higher absorption of water and nutrients, and the biological N fixation carried out by the bacterium.

Besides the influence on plant growth, the amount of N applied in barley directly impacts the quality of the grain for the brewing industry, since high protein content impairs the preparation of the product (Wamser and Mundstock 2007).

The inoculation with *A. brasilense* is capable of altering the nitrogen metabolism in plants, through the fixation of atmospheric N, the presence of bacterial nitrate reductase and higher recovery of applied N, due to the stimulus to root growth. These effects directly impact the availability of the nutrient for the various tissues of the plant and increase the N content in the grains. This same behavior was verified in the study by Souza et al. (2014), with an increase in the protein content of wheat grains caused by the inoculation of *A. brasilense*.

While the cultivar Itanema produced more shoot dry matter, the cultivar Manduri reverted the production to the reproductive structures, which is evidenced by the greater number of grains per spike. The inoculation proved to be beneficial when combined with the nitrogen supply, which was also verified in work by Piccinin et al. (2013) in wheat crop. According to Alves et al. (2017), adequate availability of N favors the nutritional status of plants at the time of floral differentiation, which leads to an increase in the number of grains formed.

The increase in the doses of N supplied led to a greater number of grains per spike, but reduced the 100-grain weight, both in the cultivar factor and in the inoculation factor. This effect is due to the distribution of photoassimilates for seed formation. This same trend was seen in the production of barley in the study of Cai et al. (2012).

About grain yield, the cultivar Itanema obtained the highest accumulation of aerial part dry mass, but with the lowes grain yield, even with the highest N content in the tissues and grains. This indicates a lower physiological efficiency in the use of N in these conditions (Cai et al. 2012) and a lower harvest index. Inoculation was shown to be positive for barley, increasing its grain yield, due to the higher use of N. This result shows that, similar to the use of *A. brasilense* in other grasses, the inoculation of the bacterium in barley is capable of altering the dynamics of obtaining and using N by the plant, whether due to greater efficiency in absorption (Ferreira et al. 2013) or by its biological fixation (Piccinin et al. 2013). It should be emphasized in this way, that the supply of *A. brasilense* via seed for the cultivation of barley is a good alternative to improve the efficiency of nitrogen fertilization and obtain higher grain yield.

CONCLUSIONS

The barley, cultivar Manduri, accumulates less nitrogen in plant tissues than the cultivar Itanema; however, it presents a higher grain yield.

The inoculation of barley seeds with *A. brasilense* provides a higher nitrogen content in the plant tissues and increases the crop grain yield when compared with non-inoculated plants.

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REFERÊNCIAS

1. Alves, Cleiton J., Orivaldo Arf, Ane F. Ramos, Fernando F. Galindo, Lais M. Nogueira, and Ricardo A. F. Rodrigues. "Irrigated wheat subjected to inoculation with *Azospirillum brasilense* and nitrogen doses as top-dressing." *Revista Brasileira de Engenharia Agrícola e Ambiental* 21, no. 8 (2017): 537–542. https://doi.org/10.1590/1807-1929/agriambi.v21n8p537-542.
2. Araújo, Josinaldo Lopes, Valdemar Faquin, Neiva Maria B. Vieira, Marcos Vanner C. de Oliveira, Antônio Alves Soares, Carlos Ribeiro Rodrigues, and Alessandro Carlos Mesquita.. "Crescimento e Produção do Arroz Sob Diferentes Proporções de Nitrato e de Amônio." *Revista Brasileira de Ciências do Solo* 36, no. 3 (2012): 921–930. https://doi.org/10.1590/S0100-06832012000300022.
3. Bashan, Yoav, and Luz E. de-Bashan. "How the plant growth-promoting bacterium *Azospirillum* promotes plant growth - A critical assessment." *Advances in Agronomy* 108 (2010): 77-136. https://doi.org/10.1016/S0065-2113(10)08002-8.
4. Cai, Jian, Dong Jiang, Fulai Liu, Tingbo Dai, and Weixing Cao. "Effects of split nitrogen fertilization on post-anthesis photoassimilates, nitrogen use efficiency and grain yield in malting barley." *Acta Agriculturae Scandinavica Section B* 61, no. 5 (2011): 410–420. https://doi.org/10.1080/09064710.2010.497158.
5. Cai, Jian, Dong Jiang, Bernd Wollenweber, Tingbo Dai and Weixing Cao. "Effects of nitrogen application rate on dry matter

redistribution, grain yield, nitrogen use efficiency and photosynthesis in malting barley." *Acta Agriculturae Scandinavica Section B* 62, no. 5 (2012): 410–419. https://doi.org/10.1080/09064710.2011.637508.

6. De Mori, Cláudia and Euclydes Minella. "Aspectos econômicos e conjunturais da cultura da cevada." *Embrapa Trigo Documentos Online* 139 (2012): 28 p. http://www.cnpt.embrapa.br/biblio/do/p_do139.htm.
7. Ferreira, A. S., R. R Pires, P. G. Rabelo, R. C. Oliveira, J. M. Q. Luz and C. H. Brito. "Implications of *Azospirillum brasilense* inoculation and nutrient addition on maize in soils of the Brazilian Cerrado under greenhouse and field conditions." *Applied Soil Ecology* 72 (2013): 103–108. https://doi.org/10.1016/j.apsoil.2013.05.020.
8. Galvani, F., and Eliney Gaertner. "Adequação da metodologia Kjeldahl para determinação de nitrogênio total e proteína bruta." *Embrapa Pantanal Circular Técnica* 63 (2006): 9 p. https://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/812198.
9. Good, Allen G, Susan J. Johnson, Mary De Pauw, Rebecka T. Carroll, Nic Savidov, John Vidmar, Zhongjin Lu, Gregory Taylor, and Virginia Stroeher. "Engineering nitrogen use efficiency with alanine aminotransferase." *Canadian Journal of Botany* 85, no. 3 (2007): 252–262. https://doi.org/10.1139/B07-019.
10. Guerra, Antônio Fernando, and Dijalma Barbosa da silva. "Coeficientes de cultura (Kc) para a cevada BRS 180, cultivada em áreas irrigadas do cerrado." *Embrapa Cerrados Guia Técnico do Produtor Rural* 48 (1999): 2 p. https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/546140/1/gtec48.pdf.
11. Lade, Sarah B., Carla Román, Ana I. Cueto-Ginzo, Laura Maneiro, Pilar Muñoz and Vicente Medina. "Root development in agronomically distinct six-rowed barley (*Hordeum vulgare*) cultivars inoculated with *Azospirillum brasilense* Sp7." *Plant Breeding* 137, no. 3 (2018): 338–345. https://doi.org/10.1111/pbr.12593.
12. Martins, Marcio Reis, Claudia Pozzi Jantalia, Verônica M. Reis, Ingbert Döwich, José Carlos Polidoro, Bruno José, and Rodrigues Alves. "Impact of plant growth-promoting bacteria on grain yield, protein content, and urea-^{15}N recovery by maize in a Cerrado Oxisol." *Plant and Soil* 422, no. 1-2 (2017): 239–250. https://doi.org/10.1007/s11104-017-3193-1.
13. Pereira-Defilippi, L, E. M. Pereira, F. M. Silva and G. V. Moro. "Expressed sequence tags related to nitrogen metabolism in maize

inoculated with *Azospirillum brasilense*." *Genetics and Molecular Research* 16, no. 2 (2017): 1–14. https://doi.org/10.4238/gmr16029682.

14. Piccinin, Gleberson G., Alessandro L. Braccini, Lilian G. M. Dan, Carlos A. Scapim, Thiago T. Ricci and abriel L. Bazo. "Efficiency of seed inoculation with *Azospirillum brasilense* on agronomic characteristics and yield of wheat." *Industrial Crops and Products* 43, no 1 (2013): 393–397. https://doi.org/10.1016/j.indcrop.2012.07.052.
15. Sanches, Fernando Mendes, Fernando França Cunha, Osvaldir Feliciano dos Santos, Epitácio José Souza, Aguinaldo José F. Leal and Gustavo de Faria Theodoro. "Desempenho agronômico de cultivares de cevada cervejeira sob diferentes lâminas de irrigação." *Semina: Ciencias Agrarias* 36, no. 1 (2015): 89–102. https://doi.org/10.5433/1679-0359.2015v36n1p89.
16. Santos, H. G., P. K. T. Jacomine, L. H. C. Anjos, V. A. Oliveira, J. F. Lumbreras, M. R. Coelho, J.A. Almeida, J. C. Araujo Filho, J. B. Oliveira and T. J. F. Cunha. 2018. *Sistema Brasileiro de Classificação de Solos*. 5ª ed. Brasília: Embrapa.
17. Sousa, Djalma M. G., and E. Lobato, eds. 2004. *Cerrado correção do solo e adubação*. 2ª ed. Brasília: Embrapa Informação Tecnológica.
18. Souza, Thiago Montagner, André M. Prando, Cássia R. Takabayashi, Joice S. dos Santos, Angélica T. Ishikawa, Ana Lúcia de S. M. Felício, Eiko N. Itano, Osamu Kawamura, Claudemir Zucareli, and Elisa Y. Hirooka. "Composição química e desoxinivalenol em trigo da região Centro-Sul do Paraná: adubação nitrogenada em cobertura associada com *Azospirillum brasilense*." *Semina: Ciencias Agrarias* 35, no. 1 (2014): 327–342. https://doi.org/10.5433/1679-0359.2014v35n1p327.
19. Vega-Celedón, Paulina, Hayron C. Martínez, Myriam González, and Michael Seeger. Review: Biosynthesis of indole-3-acetic acid and plant growth promoting by bacteria. *Cultivos Tropicales* 37 (2016): 33–39. http://dx.doi.org/10.13140/RG.2.1.5158.3609.
20. Wamser, Anderson Fernando, and Cláudio Mário Mundstock. "Teor de proteínas nos grãos em resposta à aplicação de nitrogênio em diferentes estádios de desenvolvimento da cevada." *Ciência Rural* 37, no. 6 (2007): 1571–1576. http://dx.doi.org/10.1590/S0103-84782007000600011.