Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)
http://dx.doi.org/10.1590/1983-21252022v35n407rc

AGRONOMIC PERFORMANCE OF WHEAT CULTIVARS IN RESPONSE TO INOCULATION (*Azospirillum brasilense*) AND NITROGEN APPLICATION[1]

RODRIGO LUIZ LUDWIG[2], THOMAS NEWTON MARTIN[3]*, DALVANE ROCKENBACH[4], SANDRO GIACOMINI[3], ROSANA TASCHETTO VEY[3]

ABSTRACT - The objective of this work was to verify the contribution of seed inoculation with the bacterium *Azospirillum brasilense* to promote growth on yield components in wheat crop. For this, experiments were carried out in different locations (Santa Maria and Cruz Alta, RS, Brazil), sowing times (June and July) and years of cultivation (2013 and 2014). A randomized block design with three replications was used. The treatments were composed by the combination of ten cultivars, three nitrogen managements and two types of inoculation (10 x 3 x 2). Inoculation was carried out with liquid inoculant in the seeds. The components of grain yield were evaluated. The average number of tillers per plant was influenced by seed inoculation (1.63 and 1.72) when uninoculated and inoculated, respectively. On the other hand, the hectoliter mass responded inversely, reducing to 75.7 kg hL^{-1}. Inoculation with *A. brasilense* increased the grain mass (1%) and had no positive influence on the variables number of ears and spikelets per ear, grain yield and hectoliter weight.

Keywords: Inoculant. Biological nitrogen fixation. Grain yield. *Triticum aestivum*.

DESEMPENHO AGRONÔMICO DE CULTIVARES DE TRIGO EM RESPOSTA À INOCULAÇÃO (*Azospirillum brasilense*) E APLICAÇÃO DE NITROGÊNIO

RESUMO - O objetivo deste trabalho foi verificar a contribuição da inoculação de sementes com a bactéria *Azospirillum brasilense* para a promoção de crescimento sobre os componentes de rendimento na cultura do trigo. Para isso, foram conduzidos experimentos em distintos locais (Santa Maria e Cruz Alta, RS, Brasil), épocas de semeadura (junho e julho) e anos de cultivo (2013 e 2014). Foi utilizado o delineamento de blocos casualisados com três repetições. Os tratamentos foram compostos pela combinação de dez cultivares, três manejos nitrogenados e dois tipos de inoculação (10 x 3 x 2). A inoculação foi realizada com inoculante líquido nas sementes. Foram avaliados os componentes da produtividade de grãos. O número médio de afilhos por planta foi influenciado pela inoculação das sementes (1,63 e 1,72) quando não inoculadas e inoculadas, respectivamente. Já a massa do hectolitro respondeu inversamente reduzindo para 75,7 kg hL^{-1}. A inoculação com *A. brasilense* aumenta a massa grãos (1%) e não tem influência positiva nas variáveis número de espigas e de espiguetas por espiga, produtividade de grãos e massa hectolítrica.

Palavras chave: Inoculante. Fixação biológica de nitrogênio. Produtividade de grãos. *Triticum aestivum.*

*Corresponding author
[1]Received for publication in 02/19/2021; accepted in 03/31/2022.
Paper extracted from the undergraduate thesis of the first author.
[2]Agronomy Departament, Instituto Federal de Educação Ciência e Tecnologia do Rio Grande do Sul, Ibirubá, RS, Brazil; rodrigo.ludwig@ibiruba.ifrs.edu.br - ORCID: 0000-0003-4057-3762.
[3]Crop Science Departament, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil; martin.ufsm@gmail.com - ORCID: 0000-0003-4549-3980, sjgiacomini@ufsm.br - ORCID: 0000-0001-8916-8958, rosanatasvey@gmail.com - ORCID: 0000-0003-1434-2813.
[4]Tarumã Trade and representations, São Borja, RS, Brazil; dalvane.rockenbach@gmail.com - ORCID: 0000-0003-3316-4768.

INTRODUCTION

The expansion of competitiveness in the world wheat market necessarily involves raising productivity levels, and seeking technologies that provide increased production with adjusted costs. Among the winter cereals produced in Brazil, the most prominent is wheat. For the 2020 harvest, 2,338.8 thousand hectares were destined for wheat cultivation, with a production of 6,354.8 thousand tons of the cereal. Crop productivity in Brazil is considered low, reaching an average of only 2,717 kg ha^{-1} (CONAB, 2020). This productivity is defined by some factors, such as cultivar, inputs used and the management adopted. Worldwide, wheat contributes approximately 20% of the calories and protein in the global diet (VELU et al., 2017).

Among the main challenges of the 21st century is the increase in crop productivity with reduced environmental impacts caused by agriculture. Among the limiting factors for wheat cultivation, nitrogen fertilization stands out (NUNES et al., 2015; TEIXEIRA-FILHO et al., 2010). Nitrogen (N) is one of the elements most needed by wheat crop and which most limits its productivity. Research indicates that to achieve 80% of the potential productivity in Europe, a nitrogen fertilization of 87 kg ha^{-1} with nitrogen fertilizer is necessary (SCHILS et al., 2018). It has an influence on obtaining high yield potential, such as number of tillers, number of grains per ear, grain mass and protein content (MANTAI et al., 2016). However, the economic, environmental and social impacts of using mineral nitrogen inappropriately should be highlighted. Regarding the economic impacts, it is highlighted that N in the soil is subject to losses, on average, 50% by the processes of ammonia volatilization and nitrate leaching (TEIXEIRA-FILHO et al., 2010). With this there will be an increase in the cost of producing the wheat crop. Integrated management practices are essential for farmers to increase grain production and nitrogen use efficiency and reduce environmental burden (LI et al., 2022). The higher or lower rate of loss can be circumvented by the form of application, management and source of the nutrient to be used (QUEIROZ et al., 2011). As a result, contamination of subsurface waters and water sources can occur.

An alternative to reduce dependence on nitrogen fertilizers, increase productivity and reduce costs, is the use of growth-promoting bacteria, which fix atmospheric nitrogen (N_2) in non-legume plants (HUNGRIA et al., 2010). Among them, the bacterium *Azospirillum brasilense* can be highlighted, which has been an alternative to increasing the grain production of many cereals (NUNES et al., 2015). In Brazil, *A. brasilense* has compatibility of use with mineral N (SANTOS et al., 2021; MUNARETO et al., 2019).

Inoculation has a low cost, so small increases in productivity justify its use. For Sala et al. (2008), increases above 15.43 kg ha^{-1} of grains would justify the practice of inoculation. Piccinin et al. (2013) highlighted that diazotrophic bacteria are effective in fixing atmospheric nitrogen and can provide savings of 50% in nitrogen fertilization.

Azospirillum brasilense can generate several stimuli for plant growth, especially the biological fixation of N (FUKAMI et al., 2016), plant hormone production, phosphate solubilization, greater root development (KAZI et al., 2016), increase in chlorophyll and stomatal conductance of contents (HUNGRIA, 2011), in addition to changes in the photosynthetic activity of plants (GORDILLO-DELGADO; MARÍN; CALDERÓN, 2016). Despite the benefits of inoculation being observed, there are still few studies that explain the factors involved in the fixation process, especially plant and *Azospirillum* interactions. This relationship is complex and interdependent, involving not only the plant and the bacteria, but other biotic and abiotic factors in the rhizosphere region. Some of these are root exudate composition, bacterial strain properties, soil conditions and activities of other microorganisms in the soil, among others (DUTTA; PODILE, 2010). It should be taken into account that there may be interaction of the bacteria with each genotype of the crop, type of soil, and chemical treatment of seeds. Thus, the hypothesis of this research is related to the increase in productivity and quality of wheat grains for the cultivars used and the possibility of using mineral nitrogen as an inoculant based on *Azospirillum brasilense*. Thus, the objective of this work was to verify the contribution of seed inoculation with the bacterium *Azospirillum brasilense* for the promotion of growth on the yield components of wheat.

MATERIALS AND METHODS

This study was carried out in the field, in 2013 and 2014, at the Federal University of Santa Maria (UFSM), Santa Maria, RS, Brazil (29º43'02.93"S and 53º44'00.10"W, at 119 meters of altitude) and at Cooperativa Central Gaúcha Ltda, Cruz Alta, RS, Brazil (28º26'14.06"S and 53º40'26.90"W, at 426 meters of altitude). The areas have a “Cfa”climate type, humid subtropical, with an average annual temperature of 19.3 and 18.9°C and precipitation of 1688 and 1736 mm in Santa Maria and Cruz Alta, respectively (HELDWEIN; BURIOL; STRECK, 2009). The soil of the Santa Maria area is classified as Argisol Dystrophic Red Argisol, and that of Cruz Alta, as a typical Dystrophic Red Latosol (EMBRAPA, 2018). Soil chemical characteristics of Cruz Alta and Santa Maria, respectively: clay content (%): 36 and 27, pH (H_2O):

5.7 and 5.0, SMP index: 6 and 5.5, phosphorus content (mg dm^{-3}): 40 and 18.9, K contents (mg dm^{-3}): 338 and 87.97, organic matter (%): 4.2 and 2.8, exchangeable Al (cmolc dm^{-3}): 0 and 0.7, Ca exchangeable ($cmol_c$ dm^{-3}): 6.6 and 8.3, Mg exchangeable (cmolc dm^{-3}): 2.1 and 3, H+Al ($cmol_c$ dm^{-3}): 4.4 and 7.7, CTC ($cmol_c$ dm^{-3}): 14 and 19.3, % CTC saturation Bases: 68.3 and 59.9 and Al: 0 and 5.7.

The experimental design used was randomized blocks with four replications per treatment, distributed in a trifactorial (10x3x2) with the factors cultivar, nitrogen dose and inoculation of *Azospirillum brasilense*. The experiment resulted in 60 combinations, composed of 10 wheat cultivars (TBIO Itaipu, FUNDACEP Bravo, TBIO Pioneiro 2010, TEC Frontale, BRS 331, OR/TBIO Quartzo, TEC 07-244, TEC 6219, TEC 10 and BRS 327), with and without inoculation of *A. brasilense*, and three doses of N in coverage (0, 50 and 100 kg N ha^{-1}). N doses were defined according to values used for low, medium and high productivity expectations (CQFSRS/SC, 2004).

The experiment was carried out at two distinct sowing times, with an interval of approximately one month between them. Sowing in Santa Maria was carried out on June 6th and July 3rd in 2013, and on June 11th and July 14th in 2014, for the first and second seasons, respectively. In Cruz Alta, it took place on June 1 and July 1, 2013, and June 12 and July 15, 2014 for the first and second seasons, respectively. Inoculation was carried out two hours before sowing with a liquid inoculant, composed of a culture of *A. brasilense* bacteria, using strains Ab-V5 and Ab-V6, at a concentration of 2,0 x 10^8 colony forming units (CFU) mL^{-1}, at a dose of 5 mL kg^{-1} of seed. To carry out the sowing, a fertilizer seeder was used, previously regulated to distribute 300 to 330 seeds m^2 and fertilizer of the commercial formula 00-20-20, (350 kg ha^{-1}) according to the need predicted by the soil analysis, for productivity above 3 t ha^{-1} (CQFSRS/SC, 2004).

Nitrogen topdressing, for treatments with 50 and 100 kg of N ha^{-1}, was divided into two plots. The first application when the crop was at the beginning of the tillering phase (approximately 35 days after sowing (DAS)), and the second, applied in the stalk elongation phase (approximately 50 DAS). The nitrogen fertilizer used was urea (45% of N), and the applications were carried out manually, on the surface. The control of weeds, insects and diseases was carried out whenever necessary to avoid competition and damage to the crop, following the technical indications for the wheat crop (EMBRAPA, 2017).

After the physiological maturation of the crop, and when the humidity was close to 17%, the harvest was carried out. In Santa Maria, the harvested useful area was 3.2 m^2, and then they were threshed on an electric threshing machine and cleaned in a seed classifier. In Cruz Alta, a useful area of 3 m^2 was harvested with a plot harvester. The number of plants, number of tillers (only average data presented) and number of ears in one linear meter (in each repetition) and transformed to square meter were evaluated. The number of spikelets per ear represented the average of ten spikelets randomly harvested in the plots (in each repetition), transformed to spikelets per ear. The grains were weighed and the grain yield calculated with humidity corrected to 13% on a dry basis. The mass of the hectoliter was determined on a hectoliter balance. A sample of the harvested grains was taken to define the mass of 1000 grains at 13% humidity.

The experimental data were submitted to test the assumptions of the mathematical model. The joint analysis of variance of the two years, sites and times was performed by the F test ($p \leq 0,05$), after which the variables that obtained significance were deployed for the study of interaction or main effects by the Scott-Knott test ($p \leq 0,05$). The software used for the analyses was Sisvar (FERREIRA, 2011).

RESULTS AND DISCUSSION

The quotients obtained between the highest and lowest mean square residue (QMRes) for the variables were as follows: number of ears per square meter (2.9), number of spikelets per ear (2.7), grain yield (2.7), hectoliter mass (8.8) and thousand grain mass (4.0). According to Pimentel-Gomes (2009), trials in different locations can be grouped together in a single analysis, as long as the quotient between the highest and lowest QMRes is less than 7, otherwise, subgroups of homogeneous sites can be considered, with QMRes that satisfy the quotient, in order to build as many joint analyses as there are subgroups created. The hectoliter mass variable exceeded the limit mentioned by Pimentel-Gomes (2009), who chose to analyze this variable separately (the Cruz Alta trial, year 2013, second growing season). The new quotient between the highest and lowest QMRes was 4.3, and can be analyzed together.

The population of emerged plants per square meter was 246.11 plants (average of two sites, two years and two sowing times). This value is below that indicated by the authors Silveira et al. (2010) which is 330 plants m^2. This is due to the fact that there was no optimal soil moisture condition at the time of sowing (2014). Also, there was intense rainfall in the months of June and July, right after sowing, making it difficult to establish the crop. Wheat is able to linearly compensate for the lack of plants by stimulating tillering. Since mineral nitrogen has linear responses to the number of fertile tillers (FERRARI et al., 2016).

The average number of tillers per plant was influenced by seed inoculation, being 1.63 and 1.72 when uninoculated and inoculated, respectively. This evaluation was carried out after the first topdressing nitrogen application, which positively influenced tiller emission. In this study, 1.21; 1.82 and 1.99 tillers per plant were observed for doses of 0, 50 and 100 kg of N ha^{-1}, respectively, separated into groups of different means by the Scott-Knott test at 5% error probability (shown core values).

The number of ears per area is an important component of wheat yield, being dependent on tillering and tiller survival. The number of ears m^2 for the cultivars TBIO Itaipu, FUNDACEP Bravo, TBIO Pioneiro 2010 and TEC Frontale presented values close to or greater than 500 ears m^{-2} (Table 1), which is considered a reference value for obtaining high yields (EMBRAPA, 2017). The variables evaluated in this research refer to the income components. In which the relationship of variables (number of ears, spikelets, grains per ear and mass of a thousand grains) should approximate the grain yield. With the increase in nitrogen fertilization, there was an increase in the number of ears (Table 1).

Inoculation was not efficient in increasing the number of ears m^{-2}. Only in cultivar BRS 327, at doses of 50 and 100 kg ha^{-1} of nitrogen, was there a significant increase for this trait (Table 1). Although the inoculation showed an increase in the number of shoots per plant, there was no increase in the number of ears, indicating that some of the shoots emitted by the plants did not generate ears, that is, the inoculation was not enough to meet their demands and promote the growth and development of an ear. Tillling is an important variable as long as they are fertile tillers, that is, they produce ears. This occurs when the environment is able to support the "mother plant" and the offspring (VALÉRIO et al., 2013). Otherwise, the infertile offspring becomes a competitor.

Table 1. Number of ears per m^2 of different wheat cultivars, seed inoculation, nitrogen doses.

Cultivar	Number of ears m^{-2}		
	Nitrogen Doses (kg ha^{-1})		
	0	50	100
	Without inoculant		
TBIO Itaipu	381.7 a B α*	495.6 a A α**	499.8 a A α
FUNDACEP Bravo	400.5 a B α	496.1 a A α	498.5 a A α
TBIO Pioneiro 2010	375.0 a B α	480.7 a A α	508.5 a A α
TEC Frontale	395.8 a B α	502.4 a A α	511.7 a A α
BRS 331	327.3 b C α	372.8 c B α	417.7 b A α
OR/ BIOTRIGO Quartzo	330.6 b C α	396.0 c B α	433.3 b A α
TEC 07-244	367.1 a C α	438.0 b B α	490.0 a A α
TEC 6219	354.7 b B α	420.4 b A α	440.7 b A α
TEC 10	378.6 a B α	413.8 b B α	488.0 a A α
BRS 327	320.0 b B α	366.8 c A β	392.4 c A β
Mean	363.1	438.3	468.1
	With inoculant		
TBIO Itaipu	380.5 a C α	442.8 a B β	526.9 a A α
FUNDACEP Bravo	389.6 a B α	443.1 a A β	446.7 b A β
TBIO Pioneiro 2010	383.3 a C α	458.6 a B α	508.2 a A α
TEC Frontale	369.2 a C α	478.0 a B α	517.7 a A α
BRS 331	300.0 b B α	385.5 b A α	406.8 c A α
OR/ BIOTRIGO Quartzo	317.6 b B α	393.5 b A α	408.8 c A α
TEC 07-244	383.1 a B α	449.4 a A α	455.2 b A α
TEC 6219	362.5 a A α	388.6 b A α	382.9 c A β
TEC 10	360.0 a B α	413.8 b A α	431.3 b A β
BRS 327	337.3 b B α	407.5 b A α	443.2 b A α
Mean	358.3	426.1	452.8
CV (%)	5.24		

*Means not followed by the same letter differ at 5% probability of error by the Scott-Knott test; ** Lowercase letters presented in the column represent the effect of cultivars within each level of N dose and inoculation. Capital letters presented in the line represent the interaction of N doses in each cultivar. Greek letters presented in the column represent the inoculation interaction within each cultivar level and N dose.

Nitrogen fertilization proved to be efficient in stimulating the emission of ears, significantly increasing the number of ears m^2, except for the cultivar TEC 6219, which was indifferent to N applications when inoculated. Contrary to what was observed in the inoculation factor, the application of N, in addition to increasing the number of tillers, provided conditions for their development to generate ears.

Nitrogen fertilization significantly increased the number of spikelets per ear, demonstrating the limitation that nitrogen exerts on this yield component (Table 2). Prando et al. (2012) concluded that increasing the N doses applied in topdressing, regardless of the evaluated genotype and the form of urea used, positively influenced the wheat yield components.

Table 2. Number of spikelets per ear of different wheat cultivars and nitrogen rates.

Cultivar	Number of spikelets per ear		
	Nitrogen Doses (kg ha^{-1})		
	0	50	100
TBIO Itaipu	13.3 c B*	14.5 c A**	14.7 b A
FUNDACEP Bravo	12.8 d B	14.0 d A	14.1 c A
TBIO Pioneiro 2010	13.9 b B	14.8 b A	14.8 b A
TEC Frontale	13.8 b B	14.0 d B	14.4 b A
BRS 331	14.1 a B	15.3 a A	15.2 a A
OR/ BIOTRIGO Quartzo	14.2 a B	15.1 a A	15.3 a A
TEC 07-244	13.1 c C	14.2 d B	14.6 b A
TEC 6219	13.7 b B	14.3 c A	14.4 b A
TEC 10	13.1 c B	13.8 d A	14.0 c A
BRS 327	13.8 b B	14.4 c A	14.6 b A
Mean	13.6	14.4	14.6
CV (%)	2.16		

*Means not followed by the same letter differ at 5% probability of error by the Scott-Knott test; ** Lowercase letters presented in the column represent the effect of cultivars within each N dose level. Capital letters presented in the row represent the interaction of N doses in each cultivar.

The inoculation showed significant interaction with the cultivar factor, for the variable number of spikelets, however, only for the cultivar TEC Frontale were there differences, with a negative effect of inoculation being observed. The other cultivars did not respond to inoculation with *A. brasilense.*

The cultivars TBIO Itaipú, OR/BIOTRIGO Quartzo and TEC 6219 showed the highest grain yields at each level of applied nitrogen dose (Table 3). The application of N resulted in significant increases in grain yield, corroborating the results obtained by Nunes et al. (2015). From the yield averages in each N dose, one can calculate the productivity increase provided by each kg of N applied. The increment was of 14.35 and 5.79 kg of grains kg^{-1} of N applied for doses of 50 and 100 kg of N ha^{-1} respectively. These values were obtained from the ratio of the increase in productivity in relation to the control.

For the cultivars FUNDACEP Bravo and TEC 10, there were no significant differences between the levels of 50 and 100 kg of N ha^{-1}, indicating that these materials are not responsive to high doses of N, being a good alternative for medium or low technology crops, where the investment is lower.

On the other hand, there was a reduction in productivity with seed inoculation. This reduction may be associated with growing conditions (BRZEZINSKI et al., 2014). Bacteria are attracted to the rhizosphere by exudates released from plant roots and used as a source of energy promoting growth (HUNGRIA et al., 2010). However, the interaction depends on factors such as the composition of exudates (BIANCHET et al., 2013), biotic and abiotic factors in the rhizosphere region (DUTTA; PODILE, 2010) and the competition of *Azospirillum* with diazotrophic bacteria native to the soil (MOREIRA et al., 2010). In addition, regarding competition for space and food, among the various microorganisms present in the soil, inoculation does not completely replace nitrogen fertilizer, but promotes better absorption and use of available N. However, in Brazil (PICCININ et al., 2013; CLEMENTE et al., 2016; FUKAMI et al., 2016; GALINDO et al., 2017; GALINDO et al., 2019; MUNARETO et al., 2019), many studies have reported increased grain yield. In addition, some important producing countries highlight and encourage the use of *A. brasilense* as strategies to increase the profitability and sustainability of crops [Argentina (CASSÁN et al., 2015; CASSÁN; DIAZ-ZORITA, 2016; CASSÁN et al., 2020), Iran (ARZANESH et al., 2011), Russia (SHELUD'KO et al., 2010) and Australia (KAZI et al., 2016)].

Table 3. Grain yield (kg ha^{-1}) of different wheat cultivars, nitrogen doses and seed inoculation.

Cultivar	Grain yield (kg ha^{-1})		
	Nitrogen Doses (kg ha^{-1})		
	0	50	100
TBIO Itaipu	2433.8 a C*	3211.6 a B**	3616.5 a A
FUNDACEP Bravo	2230.3 b B	2749.0 c A	2789.6 d A
TBIO Pioneiro 2010	2208.7 b C	2926.8 b B	3182.3 c A
TEC Frontale	2240.2 b C	2889.3 b B	3188.7 c A
BRS 331	1897.7 c C	2630.0 c B	3089.2 c A
OR/ BIOTRIGO Quartzo	2339.8 a C	3192.9 a B	3666.0 a A
TEC 07-244	2322.7 a C	3026.1 b B	3337.7 b A
TEC 6219	2509.4 a C	3297.8 a B	3538.3 a A
TEC 10	2379.7 a B	2941.4 b A	3090.0 c A
BRS 327	2352.5 a C	3225.6 a B	3485.5 b A
Mean	2291.5	3009	3298.4
Inoculant	Without inoculant	With inoculant	Mean
	2906.4 A	2826.2 B	2866.3
CV (%)	5.28		

*Means not followed by the same letter differ at 5% probability of error by the Scott-Knott test; ** Lowercase letters presented in the column represent the effect of cultivars within each N dose level. Capital letters presented in the row represent the interaction of N doses in each cultivar.

The value of hectoliter mass (MH) is used as a measure of wheat commercialization, and indirectly expresses the quality of grains. It is known that the higher the MH, the greater the acceptance and market valuation of the product. The increase in nitrogen fertilization caused a reduction in the MH value in 50% of the evaluated cultivars (Table 4). This reduction may be associated with the increase in the number of tillers by the addition of N, as well as the later emission of tillers. In this case, late tillers have a small number of grains per ear, few fertile spikelets and few grains per spikelet, making it difficult to use wheat genotypes with high tillering potential (VALÉRIO et al., 2013). Thus, a greater contribution of tillers in grain production may have reduced the hectoliter mass of these samples.

The hectoliter mass of the Cruz Alta experiment, year 2013, second growing season, was not analyzed together with the others. There were no significant interactions in this experiment. Inoculation negatively affected the crop, causing a reduction in MH, with mean values of 75.9 and 75.7 kg hL^{-1}, respectively, for the uninoculated and inoculated plots.

In relation to the weight of a thousand grains, the cultivars present significant variations among themselves, due to their genetic characteristics and responses to management (Table 5). This variable appears to have little influence on management, being strongly coordinated by genetic factors.

The cultivar BRS 327, which presented the highest values for this variable and TEC Frontale and FUNDACEP Bravo, with the lowest can be highlighted. When comparing the grain yield of these two cultivars, it is clear that the mass of 1000 grains was important in contributing to the crop yield, which showed high yields for the BRS 327 genotype and lower yields for the FUNDACEP Bravo and TEC Frontale cultivars.

Nitrogen fertilization resulted in a reduction in the mass of 1000 grains (Table 5). This reduction may be associated with the greater contribution of tillers in grain production, which were stimulated by the increase in nitrogen fertilization. Usually tiller ears have smaller grains and in smaller quantity (VALÉRIO et al., 2013) result in lower grain mass. This reduction, as with MH, may be associated with the inverse relationship between yield and quality. Rodrigues et al., (2014) obtained higher mass averages of 100 grains in seeds that were inoculated with *A. brasilense*, without the application of N. In conditions of low nitrogen fertilization, the plant changes the direction of partitioning of the photoassimilates, investing in the mass of the grains and, in adequate amounts, they are directed towards increasing the number and length of the reproductive structures (RODRIGUES et al., 2014). The high yields provided by the higher doses of N may have shown a lower accumulation of photoassimilates in the grains. However, Teixeira-Filho et al. (2010) and Piccinin et al. (2013) did not observe differences regarding N doses for this variable.

Table 4. Hectoliter mass (kg hL^{-1}) of different wheat cultivars and nitrogen rates.

Cultivar	Hectoliter mass (kg hL^{-1}) Nitrogen Doses (kg ha^{-1}) 0	50	100
TBIO Itaipu	74.2 d A*	73.9 d A**	74.2 c A
FUNDACEP Bravo	74.2 d A	73.0 e B	72.9 e B
TBIO Pioneiro 2010	75.9 b A	76.1 b A	75.2 b B
TEC Frontale	76.1 b A	76.1 b A	75.4 b B
BRS 331	74.7 c A	73.8 d B	73.6 d B
OR/ BIOTRIGO Quartzo	74.8 c A	74.5 c A	74.5 c A
TEC 07-244	74.7 c A	74.8 c A	74.7 b A
TEC 6219	76.6 a A	76.9 a A	76.2 a A
TEC 10	73.9 d A	73.7 d A	73.2 e A
BRS 327	76.7 a A	76.5 a A	75.8 a B
Mean	75.2	74.9	74.6
CV (%)	0.65		

*Means not followed by the same letter differ at 5% probability of error by the Scott-Knott test; ** Lowercase letters presented in the column represent the effect of cultivars within each N dose level. Capital letters presented in the row represent the interaction of N doses in each cultivar.

Table 5. Mass of 1000 grains (g) of different wheat cultivars, inoculation and nitrogen doses.

Mass of 1000 grains (g) — Nitrogen doses (kg ha^{-1}) 0	50	100	Mean
31.3 A*	31.1 B**	30.8 C	31.1

Inoculant Without	With	Mean
30.9 B	31.2 A	31.1

Cultivar	
TBIO Itaipu	31.7 d
FUNDACEP Bravo	26.9 h
TBIO Pioneiro 2010	29.5 g
TEC Frontale	26.5 h
BRS 331	31.1 e
OR/ BIOTRIGO Quartzo	33.5 b
TEC 07-244	30.3 f
TEC 6219	32.1 c
TEC 10	30.9 e
BRS 327	38.1 a
Mean	31.1
CV (%)	1.97

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

Piccinin et al. (2013) observed that the use of *Azospirillim brasilense* resulted in an increase in the number of grains and in wheat yield. Braccini et al. (2012) obtained similar results for corn, noting that seed inoculation with *Azospirillum brasilense* in the liquid formula promoted an increase in plant height and grain yield, compared to the control.

According to Corassa et al. (2013), the inoculation of wheat seeds with *Azospirillum brasilense*, when not associated with nitrogen fertilization, causes a decline in grain yield, and also, the replacement of nitrogen fertilization at the base by the use of *Azospirillum brasilense*, provided that its association with nitrogen fertilization in topdressing, promotes an increase in wheat grain yield under the conditions that were performed.

In general, when the effects on inoculation are verified, it was noticed that there are many associated causes, from the production of phytohormones (not evaluated in this research), to root growth, and consequently, increased absorption of water and nutrients. The saving of financial resources given by the replacement of mineral N is fundamental for economic and environmental sustainability. Thus, the search for understanding the relationships between bacteria of the genus *Azospirillum* and the edaphoclimatic environment are important to enable the functionalities of bioinputs. Thus, it is complex to dissociate the causes and the effects provided. It is understood with this research that the uses of bacteria such as *Azospirillum brasilense* should be referred to as growth promoters. The results with the inoculation of *A. brasilense* do not always follow the same trend, varying a lot, mainly among the studied cultivars, thus needing to expand the research, in view of the greater sustainable applicability of the theme.

CONCLUSIONS

Inoculation with *A. brasilense* increases wheat grain mass and does not influence the variables number of ears and spikelets per ear, grain yield and hectoliter mass of wheat grains.

ACKNOWLEDGMENTS

To the National Council for Scientific and Technological Development (CNPq – Processe 312480/2020-2) and the Coordination for the Improvement of Higher Education Personnel (CAPES) and the Rio Grande do Sul Research Support Foundation (FAPERGS) for granting the scholarships to the authors. To scholarship students and volunteers for their assistance in data collection.

REFERENCES

ARZANESH, M. H. et al. Wheat (*Triticum aestivum* L.) growth enhancement by *Azospirillum* sp. under drought stress. **World Journal of Microbiology and Biotechnology**, 27: 197-205, 2011.

BIANCHET, P. et al. Formulações simples e mista de inoculantes com bactérias diazotróficas, sob diferentes doses de nitrogênio na cultura do arroz irrigado. **Semina: Ciências Agrárias**, 34: 2655–2666, 2013.

BRACCINI, A L. et al. Seed inoculation with *Azospirillum brasilense*, associated with theuse of bioregulators in maize. **Revista Caatinga**, 25: 58-64, 2012.

BRZEZINSKI, C. R. et al. Nitrogênio e inoculação com *Azospirillum* na qualidade fisiológica e sanitária de sementes de trigo. **Revista de Ciências Agrárias**, 57: 257-265, 2014.

CASSÁN, F.; DIAZ-ZORITA, M. *Azospirillum* sp. in current agriculture: From the laboratory to the field. **Soil Biology and Biochemistry**, 103: 117-130, 2016.

CASSÁN, F. et al. Everything you must know about *Azospirillum* and its impact on agriculture and beyond. **Biology and Fertility of Soils**, 56: 461-479, 2020.

CASSÁN, F. et al. **Handbook for *Azospirillum*: Technical issues and protocols**. Switzerland: Springer International Publishing; 2015.

CLEMENTE, J. M. et al. *Azospirillum brasilense* and nitrogen fertilization affecting wheat productivity. **African Journal of Agricultural Research**, 11: 2179-2184, 2016.

CQFSRS/SC - Comissão de Química e Fertilidade do Solo. **Manual de adubação e de calagem para os estados do Rio Grande do Sul e Santa Catarina**. 10 ed. Porto Alegre, RS: Sociedade Brasileira de Ciência do Solo/Núcleo Regional Sul, 2004. 400 p.

CONAB - Companhia Nacional de Abastecimento. **Levantamentos de safra**. Disponível em: <https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos>. Acesso em: 20 nov. 2020.

CORASSA, G. M. et al. Inoculação com *Azospirillum brasilense* associada à adubação nitrogenada em trigo na região norte do Rio Grande do Sul. **Enciclopédia Biosfera**, 9: 1298-1308, 2013.

DUTTA, S.; PODILE, A. R. Plant growth promoting rhizobacteria (PGPR): the bugs to debug the root zone. **Critical Reviews in Microbiology**, 36: 232–244, 2010.

EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária. **Informações Técnicas para trigo e triticale**. Brasília, DF: Embrapa, 2017. 240 p. Disponível em: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/155787/1/Informacoes-Tecnicas-para-Trigo-e-Triticale-Safra-2017-OL.pdf. Acesso em: 20 nov. 2020.

EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária. **Sistema Brasileiro de Classificação de Solos**. Brasília, DF: Embrapa Solos, 2018. 355 p.

FERRARI, M. et al. Manejos e fontes de nitrogênio nos componentes de afilhamento no trigo. **Revista Brasileira de Ciências Agrárias**, 11: 178-185, 2016.

FERREIRA, D. F. Sisvar: a computer statistical analysis system. **Ciência e Agrotecnologia**, 35: 1039–1042, 2011.

FUKAMI, J. et al. Acessing inoculation methods of maize and wheat with *Azospirillum brasilense*. **AMB Express**, 6: 1-13, 2016.

GALINDO, F. S. et al. Nitrogen fertilization efficiency and wheat grain yield affected by nitrogen doses and sources associated with *Azospirillum brasilense*. **Acta Agriculturae Scandinavica, Section B Soil & Plant Science**, 69: 606-17, 2019.

GALINDO, F. S. et al. Wheat yield in the Cerrado as affected by nitrogen fertilization and inoculation with *Azospirillum brasilense*. **Pesquisa Agropecuária Brasileira**, 52: 794-805, 2017.

GORDILLO-DELGADO, F.; MARÍN, E.; CALDERÓN, A. Effect of *Azospirillum brasilense* and *Burkholderia unamae* bacteria on maize photosynthetic activity evaluated using the photoacoustic technique. **International Journal of Thermophysics**, 37: 1-11, 2016.

HELDWEIN, A. B.; BURIOL, G. A.; STRECK, N. A. O clima de Santa Maria. **Ciência & Ambiente**, 38: 43–58, 2009.

HUNGRIA, M. et al. F. O. Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. **Plant and Soil**, 331: 413–425, 2010.

HUNGRIA, M. **Inoculação com *Azospirillum brasilense*: inovação em rendimento a baixo custo**. Londrina, PR: Embrapa Soja, 2011. 36 p. (Documentos, 325).

KAZI, N. et al. The response of wheat genotypes to inoculation with *Azospirillum brasilense* in the field. **Field Crops Research**, 196: 368-78, 2016.

LI, C. et al., Optimizing nitrogen fertilizer inputs and plant populations for greener wheat production with high yields and high efficiency in dryland areas. **Field Crops Research**, 276: 1-12, 2022.

MANTAI, R. D. et al. The dynamics of relation oat panicle with grain yield by nitrogen. **American Journal of Plant Sciences**, 7: 17-27, 2016.

MOREIRA, F. M. S. et al. Bactérias diazotróficas associativas: Diversidade, ecologia e potencial de aplicações. **Comunicata Scientiae**, 1: 74-99, 2010.

MUNARETO, J. D et al. Nitrogen management alternatives using *Azospirillum brasilense* in wheat. **Pesquisa Agropecuária Brasileira**, 54: e00276, 2019.

NUNES, P. H. M. P. et al. Produtividade do trigo irrigado submetido à aplicação de nitrogênio e à inoculação com *Azospirillum brasilense*. **Revista Brasileira de Ciência do Solo**, 39: 174–182, 2015.

PICCININ, G. G. et al. Rendimento e desempenho agronômico da cultura do trigo em manejo com *Azospirillum brasilense*. **Agrarian**, 6: 393–401, 2013.

PIMENTEL-GOMES, F. **Curso de estatística experimental**. 15. ed. Piracicaba, SP: FEALQ, 2009. 451 p.

PRANDO, A. M. et al. Formas de ureia e doses de nitrogênio em cobertura na qualidade fisiológica de sementes de trigo. **Revista Brasileira de Sementes**, 34: 272-279, 2012.

QUEIROZ, A. M. et al. Avaliação de diferentes fontes e doses de nitrogênio na adubação da cultura do milho (*Zea mays* L.). **Revista Brasileira de Milho e Sorgo**, 10: 257-266, 2011.

RODRIGUES, L. F. O. S. et al. Características agronômicas do trigo em função de *Azospirillum brasilense*, ácidos húmicos e nitrogênio em casa de vegetação. **Revista Brasileira de Engenharia Agrícola e Ambiental**, 18: 31–37, 2014.

SALA, V. M. R. et al. Novas bactérias diazotróficas endofíticas na cultura do trigo em interação com a adubação nitrogenada, no campo. **Revista Brasileira de Ciência do Solo**, 32: 1099-1106, 2008.

SANTOS, M. S. et al. Outstanding impact of *Azospirillum brasilense* strains Ab-V5 and Ab-V6 on the Brazilian agriculture: Lessons that farmers are receptive to adopt new microbial inoculants. **Revista Brasileira de Ciência do Solo**, 45: e0200128, 2021.

SCHILS, R. et al. Cereal yield gaps across Europe. **European Journal of Agronomy**, 101:109-120, 2018.

SHELUD'KO A.V. et al. Wheat root colonization by *Azospirillum brasilense* strains with different motility. **Microbiology**, 79: 688-695, 2010.

SILVEIRA, G. et al. Efeito da densidade de semeadura e potencial de afilhamento sobre a adaptabilidade e estabilidade em trigo. **Bragantia**, 69: 63-70, 2010.

TEIXEIRA-FILHO, M. C. M. et al. Doses, fontes e épocas de aplicação de nitrogênio em trigo irrigado em plantio direto. **Pesquisa Agropecuária Brasileira**, 45: 797–804, 2010.

VALÉRIO, I. P. et al. Seeding density in wheat: the more, the merrier? **Scientia Agricola**, 70: 176-184, 2013.

VELU, G. et al., Genetic impact of Rht dwarfing genes on grain micronutrients concentration in wheat. **Field Crops Research**, 214: 373 - 377, 2017.