DIAZOTROPHIC BACTERIA, YIELD AND GRAIN QUALITY OF WHEAT INOCULATED WITH *Azospirillum brasilense* AND FERTILIZED WITH BASALT POWDER

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ABSTRACT - Brazilian wheat (*Triticum aestivum* L.) is currently produced mainly with industrialized chemical fertilizers. Biological nitrogen fixation (BNF) and fertilization with basalt powder (BP) are possible alternatives for a more sustainable production. The aim of this study was to evaluate plant growth, yield, grain industrial quality and the amount of endophytic diazotrophic bacteria present in wheat grains inoculated with *Azospirillum brasilense* and fertilized with basalt powder. Wheat was cultivated under field conditions in the 2018/19 season with the recommended chemical fertilization (NPK) and in residual doses of 40, 80 and 160 t ha^{-1} of basalt powder. The plots were subdivided, being half inoculated with *A. brasilense* and other half fertilized with topdressing urea. Shoot dry matter production (SDM), yield, protein content and endophytic bacteria in the grains were determined. Fertilization with basalt powder, regardless of dose, associated with inoculation with *A. brasilense* resulted in shoot dry matter production, grain yield and protein content equal to NPK. The amount of endophytic bacteria in wheat grains is higher when *A. brasilense* is inoculated. Alternative fertilization of basalt powder associated with *A. brasilense* results in yield and grain quality similar to NPK chemical fertilization.

Keywords: Biological nitrogen fixation, endophytic bacteria, rock dust, rocking, *Triticum aestivum*.

BACTÉRIAS DIAZOTRÓFICAS, PRODUTIVIDADE E QUALIDADE DOS GRÃOS DO TRIGO INOCULADO COM *Azospirillum brasilense* E FERTILIZADO COM PÓ DE BASALTO

RESUMO - O trigo (*Triticum aestivum L.*) brasileiro atualmente é produzido principalmente com fertilizantes químicos industrializados. A fixação biológica de nitrogênio (FBN) e a adubação com pó de basalto (PB) são possíveis alternativas para uma produção mais sustentável. O objetivo deste estudo foi avaliar o crescimento das plantas, a produtividade, a qualidade industrial dos grãos e a quantidade de bactérias diazotróficas endofíticas presentes nos grãos do trigo inoculado com *Azospirillum brasilense* e fertilizado com PB. O trigo foi cultivado em condições de campo na safra 2018/19 com a fertilização química (NPK) recomendada e nas doses residuais de 40, 80 e 160 t ha^{-1} de PB. As parcelas foram subdivididas, sendo metade inoculada com *A. brasilense* e metade adubada com ureia em cobertura. Foram determinadas a produção de matéria seca da parte aérea (MSPA), a produtividade, o teor de proteína e quantificadas as bactérias endofíticas nos grãos. A fertilização com PB, independente da dose, associada com a inoculação de *A. brasilense* resultou em produção de

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MSPA, produtividade e teor de proteína dos grãos igual ao NPK. A quantidade de bactérias endofíticas nos grãos de trigo é superior quando há inoculação do *A. brasilense.* A fertilização alternativa do PB associado com *A. brasilense* resulta em produtividade e qualidade de grãos semelhante a da fertilização química NPK.

Palavras-chave: Bactérias endofíticas, Fixação biológica de nitrogênio, Pó de rocha, Rochagem, *Triticum aestivum*.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the second most produced cereal in the world (Camponogara et al., 2015). The states of Rio Grande do Sul and Paraná produce 95% of Brazilian wheat, making it the main winter cereal (Chavarria et al., 2015). However, Brazilian wheat has low grain quality, which results in large volumes being imported annually to supply the bakery industry (Costa et al., 2008; Caierão et al., 2014).

Among the factors that have a direct influence on the productivity and quality of wheat grains, the adequate availability of nutrients for plants, especially nitrogen (N), stands out (Mendes et al., 2011). Wheat production is mainly carried out with chemical fertilizers, which come from non-renewable sources, have a high acquisition cost, can be leached or volatilized in the crop, which reduces its efficiency and contaminates natural resources (Gleissman, 2005; Van Straaten, 2006). As a result, alternatives have been sought to partially or fully replace the use of these fertilizers in crops.

Rock powders are fertilizers rich in macro and micronutrients, inexpensive and less polluting compared to chemical fertilizers (Van Straaten, 2006; Silva et al., 2015; Writzl et al., 2019). Among these, basalt powder (BP) is the one with the greatest potential for use as a fertilizer in agricultural crops due to its diverse mineralogical composition and wide geographic distribution. As it does not contain N in its composition, in systems that seek sustainability this nutrient can be supplied to plants through biological nitrogen fixation (BNF) (Writzl et al., 2019; Van Straaten, 2006). Bacteria of the genus *Azospirillum* are easily found in association with forage cereals and grasses, inhabiting the soil, the rhizosphere and also roots, stems, leaves and grains of plants (Döbereiner et al., 1995; Didonet et al., 1996; Reis Júnior et al., 2004; Silva et al., 2004; Silva et al., 2007; Silva et al., 2011; Barros, 2022).

The inoculation of *Azospirillum brasiliense* provides several benefits to plants, the environment and farmers; only in Brazil with wheat and corn this practice could result in savings of $1.2 billion of nitrogen fertilizers per year (Hungria et al., 2010; Hungria et al., 2016). Therefore, the use of inoculation of this bacterium in agricultural cultures has increased in recent years, especially in those demanding N, in view of the various benefits that it provides to the plants. In wheat, the increase in grain production stands out (Sala et al., 2007; Hungria et al., 2010), increases in the initial development of plants, in the accumulation of N in plants, in the reallocation of N present in the biomass for grains (Didonet et al., 1996; Sala et al., 2005; Rapim et al., 2012) and improvement in grain quality, through an increase in hectoliter weight and an increase in N content (Rodrigues et al., 2000; Mendes et al., 2011). In grains, the external surface is the preferred place for colonization of bacteria of the genus *Azospirillum* (Silva et al., 2011). However, in wheat it is more relevant to identify/quantify the communities present in the inner part of the grain (endophytic), because in the manufacture of traditional commercial flour, the outer surface of the wheat grain is removed.

The studies on the combined effect of inoculation of *A. brasilense* with fertilization with BP are rare if not non-existent, especially when one seeks to quantify the community of these bacteria in grains and analyze their influence on the performance of the crop and on the quality of the grains. Therefore, the aim of this study was to evaluate plant growth, yield, grain industrial quality and the amount of endophytic diazotrophic bacteria present in wheat grains inoculated with *Azospirillum brasilense* and fertilized with basalt powder.

MATERIALS AND METHODS

The wheat cultivar TBIO Toruk® was cultivated in the field and without irrigation from May to October 2018, in Bom Progresso, RS, Brazil, (27°33’49.17” S, 53°51’31.87” W, level 480 m), under a typical Red Oxissol (Santos et al., 2018). The climate of the Cfa-type study region, with mean annual temperature close to 19°C and rainfall between 1800 to 2000 mm (Silva et al., 2014).

The basalt powder was applied in a single dose to the soil surface in September 2017, and black beans (*Phaseolus vulgaris* L.) were subsequently cultivated in the study area, until December 2017. Afterwards, the area remained fallow and wheat was sown in 10 May 2018, in five treatments: Three residuals doses of basalt powder (40, 80 and 160 t ha^{-1}), treatment with chemical fertilizer (NPK) recommended in accordance with the Manual de

Adubação e Calagem do RS e SC (CQFS, 2016) performed at the time of sowing and another treatment without adding fertilizer (control). The experimental plots measured 3x2 m and were arranged in a randomized block design with three replications.

Wheat sowing was performed manually, with a spacing of 17 cm between rows and an approximate density of 186 plants per m^2. The fertilization in the NPK treatment line was 100 kg ha^{-1} ammonium sulfate, 58 kg ha^{-1} potassium chloride and 166 kg ha^{-1} triple superphosphate. After sowing, the plots were subdivided. In half of the area, the bacteria *A. brasilense* was used through the liquid inoculant (SimbioseMaíz®, Simbiose, Cruz Alta, Rio Grande do Sul, Brazil) with 5.0 x 10^8 CFU mL^{-1} of the mixture of the Ab-V5 and Ab-V6 strains, which was applied at a dosage of 100 mL ha^{-1} to wheat seeds, two hours before sowing. Another 200 mL ha^{-1} of the inoculant diluted in 10 liters of water were sprayed on the plants 30 days after emergence. The other half of the plot received, according to technical recommendations, 89 kg ha^{-1} N mineral in the form of urea, divided into two applications, the first at 30 days and the second at 45 days after emergence, in the tillering and tillering stages elongation of the culm, respectively. Weeds were controlled with manual weeding, as needed. Pest and disease control was carried out with chemical insecticides and fungicides, in accordance with technical recommendations.

The evaluation of shoot dry matter production of wheat plants was carried out at full flowering stage with two linear segments of 0.75 m from the interior of each subplot, which were dried in an oven at 65ºC until reaching constant mass. At the physiological maturation stage of the plants, both ears were harvested in two linear segments of 0.75 m in the two central lines of each subplot. The grains were separated from the ears and dried, weighed, and grain yield was determined, corrected for 13% moisture. The protein content of the grains was determined by near infrared spectroscopy (NIRS) in a Foss equipment (DS 2,500, Denmark), with approximately 300 grams of wheat, clean and without impurities, with 13% moisture, following the standard methodology developed and validated by the equipment manufacturer.

To determine the community of endophytic diazotrophic bacteria in wheat grains, 10 grams of grains from each sample were used. The grains were disinfected in a laminar flow chamber by immersion in 5% NaOCl for two minutes, according to Silva et al. (2011). The disinfected grains were finely ground in a sterilized grain until flour granulometry. After 1 g of flour was mixed in 9 ml of sterilized water for serial dilution 10^{-3}, 10^{-4}, 10^{-5} and inoculation of 0.1 ml of each dilution in five flasks with 5 ml of semi-solid NFb medium. The flasks were incubated at 27°C for 14 days, when the positive tubes with a characteristic film of diazotrophic bacteria growth in the microaerophilic condition were counted. To estimate the Most Probable Number (MPN) we used the McCrady table, described by Döbereiner et al. (1995).

Data of shoot dry matter production, grain yield, protein content and endophytic community of diazotrophic bacteria were subjected to analysis of variance, followed by Tukey's test at 5%. The effect of inoculated and non-inoculated conditions on the variables analyzed within each treatment was compared using the 5% T test. Analyzes were performed using the Sisvar statistical package (Ferreira, 2011).

RESULTS AND DISCUSSION

The inoculation of *A. brasilense* resulted in an increase in the amount of diazotrophic endophytic bacteria in wheat grains in both treatments (Table 1). These increases were 494, 136, 985, 117 and 1.416% for control, NPK and basalt powder doses 40, 80 and 160 t ha^{-1}, respectively, compared to non-inoculated treatments. When inoculated with *A. brasilense*, the unfertilized plants (control) and those fertilized with 160 t ha^{-1} of basalt powder stood out for presenting a community of endophytic diazotrophic bacteria present in wheat grains superior to all other inoculated treatments.

The amount of naturally occurring diazotrophic bacteria was numerically similar, showing no significant differences when comparing the treatments without inoculation with each other (Table 1), thus indicating a basal value of colonization of this habitat, that is, the soil of the experiment site. However, the results showed that the use of mineral N associated with the fertilizers tested reduced the amount of autochthonous diazotrophic bacteria by 51, 88, 38 and 58% in NPK and in basalt powder doses 40, 80 and 160 t ha^{-1} respectively, compared to unfertilized (control). Silva et al. (2007) and Silva et al. (2011) also found a reduction in these microorganism communities in the leaves and roots of irrigated rice plants with application of mineral N in topdressing. This occurs, according to Sylvia et al. (1998), Silva et al. (2007) due to the high availability of N in the soil at periods time, which contributes to this community being easily supplanted by others that become dominant, given that the community of diazotrophic bacteria is highly competitive with the others only under conditions of low availability of N in the soil.

Table 1 - Number of endophytic diazotrophic bacteria in wheat grains (*Triticum aestivum* L.) inoculated or not with *A. brasiliense,* without fertilization, NPK chemical fertilizer or with three residual doses of basalt powder (BP) in a Red Oxisol.

Treatments	Without inoculation	With inoculation
	UFC g^{-1} of grain	
Without fertilization	2,46 x 10^4 B b*	1,46 x 10^8 A a
NPK	1,20 x 10^4 B b	2,83 x 10^4 B a
40 t ha^{-1} BP	2,90 x 10^3 B b	3,16 x 10^4 B a
80 t ha^{-1} BP	1,53 x 10^4 B b	3,33 x 10^4 B a
160 t ha^{-1} BP	1,03 x 10^4 B b	1,57 x 10^8 A a

*Equal uppercase letters in columns and equal lowercase letters in rows do not present statistical difference by the Tukey test and T 5%, respectively.

The inoculation with *A. brasilense* in wheat plants increased the production of shoot dry matter when fertilized with basalt powder and without fertilization (Figure 1). The increment was 19.9; 18.7; 14.9 and 18.5% in treatments with 40, 80 and 160 t ha^{-1} of basalt powder and plants without fertilization, respectively. Inoculation did not influence the production of shoot dry matter of plants fertilized with NPK and those fertilized with basalt powder did not differ statistically from plants fertilized with NPK (Figure 1). However, when not inoculated, plants fertilized with the lowest doses of basalt powder (40 and 80 t ha^{-1}) showed reductions of 16.2 and 14.4%, respectively, compared to the NPK treatment. On the other hand, plants fertilized with basalt powder not inoculated at the highest dose and plants inoculated at both doses produced shoot dry matter similar to plants fertilized with NPK. The production of shoot dry matter did not show statistical difference when comparing the different doses of basalt powder tested against each other, regardless of inoculation. However, an increase in the production of shoot dry matter was observed according to the increase in the tested basalt powder dose, an increase of 24.4% when compared to the doses of 40 and 160 t ha^{-1} of basalt powder, whereas when inoculated the increase was 19.3%.

The better development of wheat plants (dry part of the aerial part) inoculated with *A. brasilense* is according to Mehnaz (2015), a consequence of the production of phytohormones gibberellin, cytokinin, abscisic acid, auxin and the better use of N, caused by the bacteria. Auxin production by *A. brasilense*, mainly indole-3-acetic acid (IAA), together with BNF capacity are mechanisms that contribute to increased plant growth (Licea-Herrera et al., 2020; Cassán et al., 2020). Furthermore, according to Silva et al. (2017), the availability of nutrients from basalt powder may have contributed to improving the nutrition and development of wheat plants.

As verified for shoot dry matter production (Figure 1), plants fertilized with basalt powder and those without fertilization (control) significantly increased grain yield with *A. brasilense* inoculation (Figure 2). The inoculation of *A. brasilense* provided an increase in grain yield of 27.4% in the control (1002 kg ha^{-1}) and in plants fertilized with basalt powder of 15, 18 and 15% for doses 40, 80 and 160 t ha^{-1}, respectively. Furthermore, inoculation did not increase the grain yield of plants fertilized with NPK, on the contrary, there was a reduction of 3.7%, compared to non-inoculated.

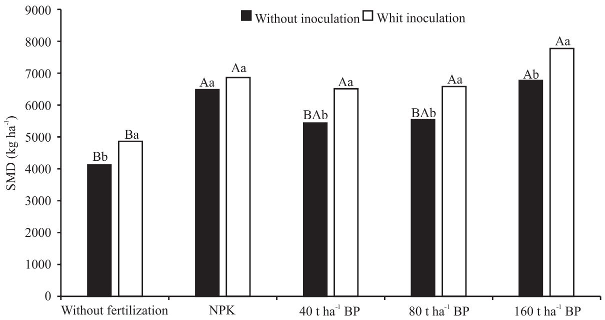

Figure 1 - Production of shoot dry matter (SDM) of wheat inoculated or not with *A. brasiliense,* without fertilization, NPK chemical fertilizer or with three residual doses of basalt powder (BP) in a Red Oxissol. Equal uppercase letters do not show significant difference between treatments (Tukey 5%) anda equal lowercase letters do not show significant difference between conditions with and without inoculation within each treatment (T Test 5%).

Fertilization with basalt powder both doses studied and regardless of inoculation provided grain yield statistically similar to chemical fertilization with NPK without inoculation (Figure 2). In the absence of inoculation, at the lowest levels of basalt powder studied (40, 80 t ha^{-1}) the grain yield was 6.1 and 10.9% lower than the treatment with NPK, respectively; on the other hand, at the highest dose of basalt powder, 160 t ha^{-1}, the productivity was 5.9% higher compared to NPK. With inoculation, both basalt powder doses showed higher grain yield than the inoculated NPK treatment, although only the 160 t ha^{-1} dose differed statistically, due to a 26.5% increase in grain yield. The control, regardless of inoculation, presented grain yield statistically lower than both other treatments.

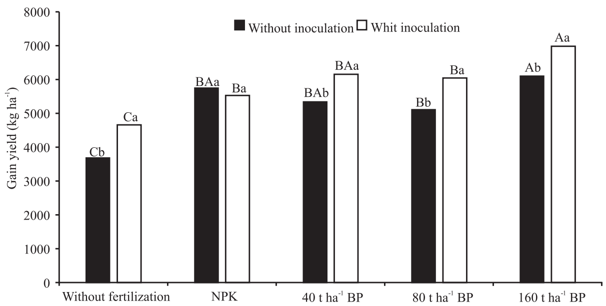

Figure 2 - Grain yield of wheat inoculated or not with *A. brasiliense*, without fertilization, NPK chemical fertilizer or with three residual doses of basalt powder (BP) in Red Oxissol. Equal uppercase do not show significant difference between treatments (Tukey 5%) and equal lowercase letters do not show significant difference between conditions with and without inoculation within each treatment (T Test 5%).

The inoculation of *A. brasilense* in plants fertilized with basalt powder regardless of the dose tested provided the production of grains with a protein content of 13%, equal to that verified in the NPK chemical fertilization and higher than the control and both basalt powder doses associated with mineral N which presented 12% of protein in the grain (Figure 3). Inoculation of *A. brasilense* in plants without fertilization and with NPK did not provide an increase, on the contrary, with NPK there was a reduction with inoculation, whereas in plants without fertilization there was no increase or decrease in the protein content in the grains.

The increase in grain yield and protein content using basalt powder and inoculation of *A. brasilense* occurred due to the greater number of these bacteria community present in wheat grains. Yet, this probably also occurred in other plant organs, such as the roots and rhizosphere (not evaluated). Silva et al. (2019) report that bacteria contribute positively to length, number, hair density and lateral roots. Several authors report that the larger the community of these organisms, the more efficient is the availability of nutrients for plants, especially those existing in basalt powder (Mehnaz, 2015; Silva et al., 2017; Silva et al., 2019). This information can be associated with a greater community of endophytic diazotrophic bacteria observed in wheat grains that received inoculation at the highest dose of basalt powder (160 t ha^{-1}), consequently, also with greater production of shoot dry matter and grain yield. The greater availability of nutrients in the soil caused by the dose of 160 t ha^{-1} basalt powder favored diazotrophic bacteria. According to Hungria et al. (2010) there is greater efficiency of inoculated bacteria compared to autochthonous soil bacteria, and this proves that doses of 40 and 80 t ha^{-1} of inoculated basalt powder produced more shoot dry matter and grains than non-inoculated doses, although they had a population of *A. brasilense* lower than a dose of 160 t ha^{-1}.

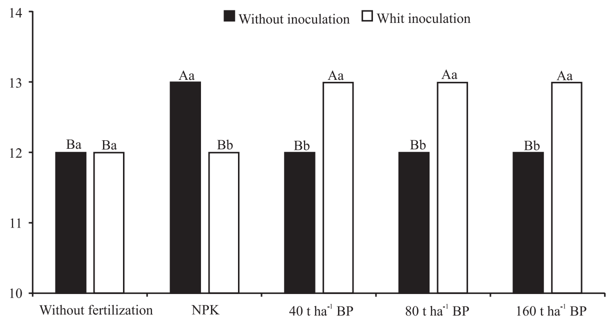

Figure 3 - Protein content of wheat grains inoculated or not with *A. brasiliense*, without fertilization, NPK chemical fertilizer or with three residual doses of basalt powder (BP) in Red Oxissol. Equal uppercase do not show significant difference between treatments (Tukey 5%) and equal lowercase letters do not show significant difference between conditions with and without inoculation within each treatment (T Test 5%).

As already well elucidated by Silva et al. (2004) and Silva et al. (2007), the inoculation of *A. brasilense* to the chemical fertilizer NPK did not increase the community of diazotrophic bacteria in wheat grains due to the inhibitory effect that N has on these soil organisms. Consequently, inoculation also reduced grain yield and protein content, although it was significant when compared to uninoculated NPK. According to Silva et al. (2007) due to the fact that *A. brasilense* uses the nutrients in NPK, especially N for its growth and survival, it decreases the availability in the soil solution, consequently, in the absorption by plants, which directly reflects on the productivity and on the protein of the grains.

The *A. brasilense* promotes better translocation of accumulated N in biomass to grains, greater absorption and utilization of available N by BNF; considering that N is the main forming component, its availability to plants exerts a direct influence on wheat grain protein (Didonet et al., 2000; Pinnow et al., 2013; Mehnaz, 2015). Thus, this effect was observed in our study, showing that basalt powder inoculated with *A. brasilense* both doses increased the amount of diazotrophic bacteria in the grains, concomitantly increasing the protein content of the grains, compared to those without inoculation. However, the inoculated control did not showed an increase in grain protein content, thus indicating that isolated inoculation was not responsible for the increase in grain protein in the study, but that this was due to the combination of *A. brasilense* and basalt powder. Thus, these results confirm the need to use fertilizers in the soil in order to improve the availability of nutrients to plants, even when inoculated with *A. brasilense.*

The fertilization of wheat with basalt powder, especially associated with *A. brasilense* proved efficient alternative to chemical fertilizer, as it provides of shoot dry matter production, grain yield and grain protein content equivalent to NPK, as well as studies by Writzl et al. (2019) and Melo et al. (2012). The association of *A. brasilense* with basalt powder proved to be an efficient alternative for more sustainable wheat production, as it allows production without the use of mineral N, both in sowing and in coverage, which reduces production costs, in addition to environmental contamination, since the greater part of the applied mineral N is lost by denitrification or leaching (Dobbelaere et al., 2002; Mendes et al., 2011). Finally, insufficient production and especially the low quality of grains are the main obstacles to Brazilian wheat production. Thus, management techniques, especially sustainable ones, such as the association of *A. brasilense* with basalt powder, which increase productivity and protein content of wheat grains, are necessary, as the grain protein exerts a direct influence on the quality of the flour, which determines its purpose of use (Gutkoski et al., 2003; Marchetti et al., 2012).

CONCLUSIONS

The incidence of endophytic diazotrophic bacteria in wheat grains is higher when the culture is inoculated with *A. brasilense*.

The doses of basalt powder, regardless of *A. brasilense* association, provide of shoot dry matter production equal to NPK fertilization.

The basalt powder, regardless of the dose, provides grain production with protein content equal to those obtained in NPK fertilization, only when inoculated *with A. brasilense*.

Doses of 40 and 80 t ha^{-1} of basalt powder, associated with *A. brasilense*, are the best alternatives in the search for substitutes to chemical NPK fertilization and elimination of nitrogen fertilizers for wheat grain yield, shoot dry matter production and grain protein content.

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