

## Reduction in nitrogen fertilization in *Triticum* sp. inoculated and co-inoculated

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**ABSTRACT:** Using techniques of inoculation and co-inoculation of plant growth-promoting rhizobacteria (PGPR) could be an alternative with great potential for the wheat agricultural market, aiming towards a higher fertilizer efficiency and a subsequent reduction in environmental impacts. The objective of this study was to evaluate the efficiency of rhizobia symbiont inoculants isolated from legumes, individually and combined with *Azospirillum brasilense*, in promoting the growth of the wheat crop. The experiments were performed with the cultivars BRS Parrudo and TBIO Sossego, both inoculated and co-inoculated with the rhizobia UFRGS Vp16 and UFRGS Lc348, and *A. brasilense*, and under nitrogen dosage levels recommended for wheat plants. Inoculation of rhizobia, isolated or combined with *A. brasilense*, promotes increases in the production of wheat grains with half of the nitrogen dose recommended in the two evaluated cultivars, standing as an economically viable and ecologically sustainable alternative for the agricultural market.

**Key words:** *Azospirillum* sp.; growth promotion; rhizobacteria; wheat; yield

## Redução na adubação nitrogenada em *Triticum* sp. inoculado e coinoculado

**RESUMO:** A utilização das técnicas de inoculação e coinoculação de rizobactérias promotoras do crescimento de plantas (RPCPs) pode ser uma alternativa com grande potencial para o mercado agrícola do trigo, visando uma maior eficiência no uso de fertilizantes e consequentemente redução do impacto ambiental. O objetivo deste trabalho foi avaliar a eficiência da inoculação de rizóbios simbiotes em leguminosas, isoladamente e em conjunto com *Azospirillum brasilense*, na promoção de crescimento da cultura do trigo. Os experimentos foram realizados com as cultivares BRS Parrudo e a TBIO Sossego, inoculadas e coinoculadas com os rizóbios UFRGS Vp16 e UFRGS Lc348, e *A. brasilense*, e sob níveis de doses de nitrogênio recomendadas para plantas de trigo. A inoculação de rizóbios, isoladamente ou em coinoculação com *A. brasilense* promove aumentos de produção de grãos de trigo com metade da dose de nitrogênio recomendada nas duas cultivares avaliadas, sendo uma alternativa economicamente viável e ecologicamente sustentável para o mercado agrícola.

**Palavras-chave:** *Azospirillum* sp.; promoção de crescimento; rizobactérias; trigo; produtividade

## Introduction

Wheat (*Triticum aestivum* L.) is among the vegetable species that are most prominent in the world economy, considered a staple food due to the diversified production of its grains derivatives (Scheuer et al., 2011). In Brazil, the production of the harvest from 2018 was of 5.5 million tons, with the South region responsible for 90% of it (Conab, 2018).

Like most grain-producing plant species, a good yield comes from the association of several factors, such as using cultivars adapted to local conditions, good soil fertility and a rigorous pest and disease management (Mumbach et al., 2017). Proper management of the nitrogen fertilization is one of the essential factors for obtaining good yields in the crop, mainly because this nutrient is absorbed in large quantities by the plants (Zagonel et al., 2002).

Nitrogen is the nutrient that most interferes in wheat production (Rodrigues et al., 2014), since it takes part in composing the organic compounds, acting in tillering and the biomass and seed production, directly influencing the yield and quality of the grains (Vogel & Fey, 2016). Using industrial fertilizer is the main available source of nitrogen for wheat plants, with high doses of this nutrient applied in order to obtain high yield. However, plants use only 50% of the applied, with the rest lost due to leaching, ammonia volatilization, denitrification, microbial immobilization and erosion. This overuse can result in damage to the environment such as the soil acidification and waters eutrophication, as well as raising the production costs (Pinto et al., 2017).

In the perspective of a more economically viable and environmentally sustainable agricultural production, the interest in using plant growth-promoting rhizobacteria (PGPRs) has increased. These PGPRs are capable of colonizing both the surface of the roots and internal tissues of the plants, promoting their growth through the production of hormones, phosphate solubilization, greater root development, increased chlorophyll content, in addition to changing the photosynthetic activity (Hungria, 2011; Gordillo-Delgado et al., 2016; Kazi et al., 2016).

Results obtained through research with PGPRs inoculation in wheat cultivation have been promising (Mumbach et al., 2017; Pereira et al., 2017), demonstrating that this practice can supply, at least in part, the nitrogen need in plants, enabling reduced nitrogen doses. However, the agronomic feasibility of this practice in the soils from Rio Grande do Sul still needs to be better evaluated, requiring more scientific information about the interaction of PGPRs (mainly rhizobia, symbionts in legumes) with the used cultivars in the state crops.

Thereby, the objective of the present study was to evaluate the potential of inoculation and co-inoculation of two strains of rhizobia and commercial inoculant containing strains of *Azospirillum brasilense* bacteria, in the development and yield of grain from the wheat cultivars TBIO Sossego and BRS Parrudo.

## Materials and Methods

The study was conducted in the field during the agricultural harvest of 2016 in the Agricultural Experiment Station of the Federal University of Rio Grande do Sul (EEA/UFRGS), in the municipality of Eldorado do Sul-RS (30°05'S; 51°38'W). The EEA/UFRGS is located in the physiographic region of the Central Depression of Rio Grande do Sul (RS), at an average altitude of 46 meters above the sea level. Regarding wheat production, the region classification is as a Homogeneous Region for the Adaptation of Cultivars 2. The climate is subtropical with a hot humid summer, from the Cfa-type, according to the Koppen classification.

The experimental area soil, belonging to the São Jerônimo mapping unit, is characterized as a typical Dystrophic Red Argisol (Ultisol). Its chemical characteristics (0 – 0.20 m) prior to setting up the experiments were the following: Clay: 27%; pH (H<sub>2</sub>O): 5.6; P: 20 mg dm<sup>-3</sup>; K: 165 mg dm<sup>-3</sup>; Ca: 4.3 cmol<sub>c</sub> dm<sup>-3</sup>; Mg: 1.9 cmol<sub>c</sub> dm<sup>-3</sup>; OM: 21 g kg<sup>-1</sup> e CEC: 6.62 cmol<sub>c</sub> dm<sup>-3</sup>. Precipitation in the year of the experiment was of 1690 mm, with the average monthly temperature ranging from 14 to 30 °C, between the coldest and the warmest months.

Sowing was performed in June 2016, with each plot consisting of 10 planting rows three meters long, spaced 0.18 m apart each other. The used cultivars were BRS Parrudo and TBIO Sossego, both of medium cycle (130-135 days), medium-size and recommended for cultivation in the studied region, with the experiment conducted under dry conditions.

For each cultivar, an experiment was set up in a randomized block design, with twelve treatments and four replicates, totaling 48 experimental units. Sowing fertilization was composed by 150 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and 150 kg ha<sup>-1</sup> of K<sub>2</sub>O, and the top dressing fertilization, which differentiate the treatments, was of 50 kg ha<sup>-1</sup> (50% dose) and 100 kg ha<sup>-1</sup> (100% dose) of nitrogen (recommended and described by CQFS RS/SC, 2004), split into two applications, at the stages V2 (20 kg ha<sup>-1</sup> and 40 kg ha<sup>-1</sup>) and V6 (30 kg ha<sup>-1</sup> and 60 kg ha<sup>-1</sup>). Crop treatments were performed according to technical indications for the wheat crop (Reunion of the Brazilian Wheat and Triticale Research Commission, 2017), requiring two fungicides applications to control the head blight and leaf rust diseases.

The used rhizobacteria were the rhizobia UFRGS Vp16 (*Burkholderia* sp.), isolated from white clover (*Trifolium repens*) by Alves et al. (2012), and UFRGS Lc348 (*Mesorhizobium* sp.), isolated from plants of common bird's-foot trefoil (*Lotus corniculatus*) by Frizzo (2007), both belonging to the Collection of Rhizobia Strains from UFRGS, and the *A. brasilense* (commercial product containing the strains AbV5 and AbV6).

The 12 tested treatments were the following: no inoculation + fertilization with 50% N; no inoculation + fertilization with 100% N; inoculation with UFRGS Vp16 + fertilization with 50% N; inoculation with UFRGS Vp16 + fertilization with 100% N; inoculation with UFRGS Lc348 + fertilization with 50% N; inoculation with UFRGS Lc348 + fertilization with 100% N; inoculation with *A. brasilense* + fertilization with 50% N; inoculation with *A. brasilense* + fertilization with 100% N; co-

inoculation with UFRGS Vp16 + *A. brasilense* + fertilization with 50% N; co-inoculation with UFRGS Vp16 + *A. brasilense* + fertilization with 100% N; co-inoculation with UFRGS Lc348 + *A. brasilense* + fertilization with 50% N; co-inoculation with UFRGS Lc348 + *A. brasilense* + fertilization with 100% N.

For producing bacterial inoculum, the rhizobia (UFRGS Vp16 and UFRGS Lc348) were inoculated, in an isolate manner, in 1000 ml Erlenmeyer-type flasks containing the Liquid Yeast Mannitol (YM) culture medium at pH 6.8 (Vincent, 1970). The Erlenmeyer flasks were placed in an incubator with 120 rpm orbital shaking for six days at 28 °C. The bacteria inoculation was by spraying when the plants were in stages V2-V3. For the inoculated treatments, the used was a 200 ml ha<sup>-1</sup> dose of bacterial inoculum (containing about 10<sup>9</sup> CFU ml<sup>-1</sup>) and 100 ml ha<sup>-1</sup> of the commercial product (in the concentration guaranteed by the manufacturer of 2.0 x 10<sup>8</sup>, containing the strains Abv5 and Abv6 of *A. brasilense*), diluted in 10 liters of water for spraying on the plants.

The eight central rows, each one with two meters in length (2.88 m<sup>2</sup>), were considered as the useful area of the plot, and were evaluated for: (i) shoot dry matter in the flowering stage; (ii) number of ears per square meter; (iii) hectoliter weight (iv) weight of 1000 grains and (v) grain yield.

The yield component and grain yield data were subjected to analysis of variance by the Scott-Knott test at a significance level of 10%, aided by the statistical program SISVAR (Ferreira, 2000).

## Results and Discussion

The number of ears per square meter was significant ( $p < 0.10$ ) for the TBIO Sossego cultivar, ranging from 458.33 (treatment N/2 + Vp16) to 598.61 (treatment N + *Azospirillum*). For the BRS Parrudo cultivar, the number of ears per square meter and the weight of 1000 grains showed differences between treatments ( $p < 0.10$ ). The number of ears per square meter ranged from 359.72 (treatment N + Vp16) to 454.17 (treatment N + Lc348). For the weight of 1000 grains component, the inoculated or co-inoculated treatments had a weight equivalent to the control treatment with the 100% nitrogen dose, emphasizing the treatments that received half the nitrogen dose and the co-inoculation technique was applied: N/2 + *Azospirillum* + Vp16 (36.99 g) and N/2 + *Azospirillum* + Lc348 (36.98 g) (Table 1).

Regardless of the cultivar, a 50% reduction in the nitrogen dose reduced the number of ears per square meter in most treatments that received the inoculation or co-inoculation of rhizobia and *Azospirillum*. A similar result was reported in a study that worked with ways of applying nitrogen and *Azospirillum* (Mumbach et al., 2017). On the other hand, it was also reported that, differently from the results of this study, the reduced nitrogen dose, when associated with inoculation, did not result in a reduced number of ears in wheat (Rosário, 2013).

A noteworthy important characteristic is that the number of ears per square meter is variable between cultivars and this component is indirectly related to grain yield, since these

ears can abort during the development cycle. In other words, a greater number of ears per square meter may not mean an increased grain yield (Valério et al., 2008), as it can be seen in this study.

Regarding the weight of 1000 grains of the BRS Parrudo cultivar, Table 1 shows that the inoculated or co-inoculated treatments associated with 50% of the nitrogen dose (50 kg ha<sup>-1</sup>) had grain weight equivalent to the control treatment with 100% of nitrogen fertilization (with the exception of treatment N/2 + *Azospirillum*). These results suggest that inoculated PGPRs allowed the plants to allocate photoassimilates for later grain filling. Pereira et al. (2017), evaluating doses of nitrogen and *Azospirillum*, have already verified the benefits of PGPR inoculation on the weight of wheat grains.

Wheat has a high capacity in compensating for the lack or excess of one yield component by modifying the other ones (Holen et al., 2001). Such modification, depending on the cultivar, environment, management and the interaction between these factors, may be sufficient in maximizing the productive potential (Valério et al., 2008). When analyzing the yield components results of the present study, it is possible to observe this compensatory effect of the cultivars, because when the treatments had a lower number of ear per square meter, there was an increase in the weight of the grains.

Regarding grain yield, there was a difference ( $p < 0.10$ ) between treatments in the two evaluated cultivars (Table 2). For the TBIO Sossego cultivar, wheat production ranged from 3.2 t ha<sup>-1</sup> (control treatment with 50% nitrogen dose) to 4.3 t ha<sup>-1</sup> (treatment inoculated with UFRGS Lc348 + 100% nitrogen). In the BRS Parrudo cultivar, production varied between 2.6 t ha<sup>-1</sup> in the control treatment with 50% of the nitrogen dose, and 3.6 t ha<sup>-1</sup> in the co-inoculated treatment with rhizobia UFRGS Lc348 + *Azospirillum* + 100% of the nitrogen dose.

Except for the N/2 + *Azospirillum* treatment for the TBIO Sossego cultivar, and the N/2 + *Azospirillum* and N/2 + *Azospirillum* + Lc348 treatments for the BRS Parrudo cultivar, using half the dose of nitrogen (50 kg ha<sup>-1</sup>) associated with the inoculation or co-inoculation of PGPRs showed similar yield to the plots that only received 100% nitrogen. Positive results with the reduced nitrogen dosage and the maintenance of yield were also found in studies by Rosário (2013), who did not obtain significant difference when evaluating wheat plants associated with the *A. brasilense* bacterium and reduced nitrogen fertilization.

Using rhizobia UFRGS Vp16 and UFRGS Lc348, and *Azospirillum* did not increase the grain yield when compared to the control treatment with the 100% nitrogen dose, but the yield was maintained in inoculated plants, allowing a reduction of 50% of the nitrogen fertilizer. Similar results were found in other studies with grain-producing plants, when the authors worked with different levels of nitrogen fertilization and inoculation with *Azospirillum* (Rosário, 2013; Pereira et al., 2017; Mumbach et al., 2017).

However, reports of inconsistent results on the PGPRs efficiency in both promoting growth and increasing plant yield are common in the literature (Oliveira et al., 2015). Studies

**Table 1.** Shoot dry matter, yield components (number of ears per square meter and weight of 1000 grains) and hectolitic weight of wheat plants inoculated and co-inoculated with plant growth-promoting rhizobacteria.

Treatment	Shoot dry matter (kg ha <sup>-1</sup> )	Ear/m <sup>2</sup>	Weight of 1000 grains (g)	HW (kg hl <sup>-1</sup> )
TBIO Sossego				
Control N/2 (50 kg)	4262.59 a	575.00 a	34.33 a	76.33 a
Control N (100 kg)	5333.33 a	558.33 a	35.58 a	76.18 a
N/2 + Vp16	4891.85 a	458.33 b	35.10 a	77.00 a
N + Vp16	4987.78 a	575.00 a	35.91 a	76.24 a
N/2 + Lc348	5142.22 a	506.94 b	35.56 a	76.35 a
N + Lc348	5574.81 a	598.61 a	36.34 a	76.54 a
N/2 + <i>Azosp.</i>	4739.63 a	515.28 b	34.28 a	76.99 a
N + <i>Azosp.</i>	5392.22 a	597.22 a	36.43 a	76.62 a
N/2 + <i>Azosp.</i> + Vp16	4888.52 a	469.44 b	35.24 a	77.12 a
N + <i>Azosp.</i> + Vp16	4784.44 a	513.89 b	36.25 a	76.48 a
N/2 + <i>Azosp.</i> + Lc348	4811.85 a	491.67 b	36.95 a	76.94 a
N + <i>Azosp.</i> + Lc348	5261.48 a	580.56 a	36.80 a	76.53 a
CV (%)	9.45	7.98	8.23	4.78
BRS Parrudo				
Control N/2 (50 kg)	4658.52 a	397.22 a	34.67 b	77.85 a
Control N (100 kg)	5613.33 a	425.56 a	35.85 a	76.69 a
N/2 + Vp16	4494.07 a	384.72 b	36.86 a	77.60 a
N + Vp16	5651.48 a	359.72 b	36.78 a	76.97 a
N/2 + Lc348	4794.07 a	427.78 a	35.94 a	77.87 a
N + Lc348	4760.00 a	454.17 a	36.09 a	76.57 a
N/2 + <i>Azosp.</i>	4968.15 a	390.28 b	34.78 b	77.16 a
N + <i>Azosp.</i>	5250.74 a	448.61 a	36.67 a	77.51 a
N/2 + <i>Azosp.</i> + Vp16	5118.52 a	361.50 b	36.99 a	77.37 a
N + <i>Azosp.</i> + Vp16	5414.44 a	426.39 a	36.42 a	76.84 a
N/2 + <i>Azosp.</i> + Lc348	4710.37 a	387.50 b	36.98 a	77.28 a
N + <i>Azosp.</i> + Lc348	5298.89 a	429.17 a	36.85 a	76.00 a
CV (%)	7.73	6.21	9.89	5.17

Means followed by the same letter in the row do not differentiate from each other by the Scott-Knott test at the significance level of 10%. N/2 = 50 kg of N; N = 100 kg of N; *Azosp.* = *Azospirillum brasilense*.

**Table 2.** Yield of wheat grains in function of the inoculation and co-inoculation with rhizobia and *Azospirillum brasilense*.

Treatment	Grain yield (kg ha <sup>-1</sup> )	
	TBIO Sossego	BRS Parrudo
Control N/2 (50 kg)	3229.91 b	2695.78 b
Control N (100 kg)	4240.51 a	3524.51 a
N/2 + Vp16	3801.53 a	3047.81 a
N + Vp16	4116.07 a	3526.42 a
N/2 + Lc348	3934.42 a	3045.89 a
N + Lc348	4324.75 a	3535.99 a
N/2 + <i>Azosp.</i>	3488.13 b	2816.16 b
N + <i>Azosp.</i>	4317.09 a	3292.86 a
N/2 + <i>Azosp.</i> + Vp16	3965.05 a	3107.15 a
N + <i>Azosp.</i> + Vp16	4083.53 a	3518.76 a
N/2 + <i>Azosp.</i> + Lc348	4051.20 a	2800.84 b
N + <i>Azosp.</i> + Lc348	4240.51 a	3601.08 a
CV (%)	12.54	14.26

Means followed by the same letter in the row do not differentiate from each other by the Scott-Knott test at the significance level of 10%. N/2 = 50 kg of N; N = 100 kg of N; *Azosp.* = *Azospirillum brasilense*.

indicate that the inoculation success is directly related to the interaction between plant genotypes and bacterial isolates, as well as other related factors such as the environment (Pandolfo et al., 2015).

In the experimental conditions of this study, the TBIO Sossego cultivar was more productive than the BRS Parrudo

cultivar. Considering the mean yield (10 years) of wheat crops in the state of Rio Grande do Sul (2089.00 kg ha<sup>-1</sup>) (Conab, 2017), the TBIO Sossego cultivar yield was up to 52% higher than the mean, while the BRS Parrudo cultivar was up to 41% higher.

Due to the high existing costs in relation to nitrogen fertilizers, using alternative sources in the partial replacement of nitrogen fertilization can become an economically viable option (Hungria, 2011), able to promote the wheat crop yield maintenance. Thus, the results found in this study show that using PGPR inoculation or co-inoculation allows reducing half the amount of the used nitrogen fertilizer, with no losses in grain production, representing an important saving in Brazilian agriculture.

## Conclusions

Inoculation and co-inoculation of the rhizobia UFRGS-VP16 and UFRGS Lc348, as well as of the *A. brasilense*, only interferes in the yield component of number of ears per square meter when cultivating TBIO Sossego, while for BRS Parrudo, the inoculation and co-inoculation change the number of ears per square meter and weight of 1000 grains.

Inoculating rhizobia, isolated or in co-co-inoculation with *A. brasilense*, maintains the wheat grains production with only 50% of the nitrogen dose.

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