ISSN 1807-1929
Revista Brasileira de Engenharia Agrícola e Ambiental
Brazilian Journal of Agricultural and Environmental Engineering
v.25, n.11, p.758-763, 2021
agriambi
Campina Grande, PB – http://www.agriambi.com.br – http://www.scielo.br/rbeaa

DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v25n11p758-763

Development of wheat plants coinoculated with rhizobium strains[1]

Desenvolvimento de plantas de trigo coinoculadas com estirpes de rizóbio

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HIGHLIGHTS:
Noduliferous bacteria can increase wheat yield.
Coinoculation of Azospirillum brasilense and Rhizobium tropici improved total grain mass in wheat plants.
Strains MT 08, MT15 and MT 16 isolated from legume nodules show potential for use in wheat plants.

ABSTRACT: This study aimed to evaluate the efficiency of diaztrophic bacteria coinoculation in wheat cultivars grown in Cerrado Oxisol. A randomized block design was used, with a 13 x 3 factorial scheme and four replicates. The treatments consisted of inoculation with *Azospirillum brasilense* (strains AbV5 and AbV6 strains combined) and coinoculation with *Bradyrhizobium japonicum (*strains SEMIA 5079 and SEMIA 5080 combined, and strain BR3267), as well as *Rhizobium tropici* (strains MT08 and MT15) and *R. leguminosarum* (strain MT16) combined or in isolation, tested in wheat cultivars BRS 394, BRS 264 and BRS 254. The variables analyzed were grain nitrogen concentration and accumulation and crude protein content, 100-grain weight and total grain mass. The treatment containing the commercial cowpea inoculant showed a higher total grain mass (5.8766 g). Interaction was observed for grain nitrogen concentration, particularly for *A. brasilense* + MT 15 (*R. tropici*) and MT 15 in wheat cultivar BRS 264. Coinoculation with diazotrophic bacteria isolated from leguminous plants shows potential for use in wheat cultivars.

Key words: *Triticum aestivum* L., associative bacteria, biofertilization

RESUMO: Objetivou-se com esse estudo avaliar a eficiência da coinoculação de bactérias diazotróficas no desenvolvimento de cultivares de trigo em Oxisol do Cerrado. O experimento foi conduzido em delineamento de blocos ao acaso, esquema fatorial 13 x 3 com quatro repetições. Os tratamentos foram compostos pela inoculação de *Azospirillum brasilense* (estirpes AbV5 e AbV6 combinadas) e coinoculação com *Bradyrhizobium japonicum* (estirpes SEMIA 5079 e SEMIA 5080 combinadas, e estipe BR3267), *Rhizobium Tropici* (estirpes MT08 e MT15), *R. leguminosarum* (estirpe MT16), de forma isolada ou combinada, testados em três cultivares de trigo: BRS 394, BRS 264 e BRS 254. As variáveis analisadas foram, concentração, acúmulo de nitrogênio e proteína bruta nos grãos, massa de 100 grãos e massa total dos grãos. O tratamento contendo a inoculação de inoculante comercial para feijão caupi apresentou a maior massa total de grãos (5,8766 g). Houve interação na concentração de nitrogênio nos grãos, com destaque da combinação de *A. brasilense* + MT 15 (*R. tropici*) e MT 15 isolados quando associados à cultivar BRS 264. A coinoculação com bactérias diazotróficas isoladas de plantas leguminosas tem potencial para uso em cultivares de trigo.

Palavras-chave: *Triticum aestivum* L., bactérias associativas, biofertilização

• Ref. 246208 – Received 03 Dec, 2020
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• Accepted 01 May, 2021 • Published 30 May, 2021
Edited by: Hans Raj Gheyi

Introduction

The Brazilian Midwest region accounts for 3% of national wheat production, with significant potential for expansion in the production of cereals (Pasinato et al., 2018). Studies aimed at determining optimal fertilizer doses and the efficiency of biological fertilization of wheat in zoning areas of the Brazilian Cerrado region suitable for new cultivars, showed good yields and qualitative performance for the crop (Bonfim-Silva et al., 2018; Galindo et al., 2018).

It should be noted that high nitrogen levels are needed to obtain satisfactory wheat yields and grain quality in this region (Carneiro et al., 2017; Bonfim-Silva et al., 2018). Inoculants formulated from diazotrophic bacteria can be used as an alternative to nitrogen fertilization in crops such as wheat since, in addition to making nitrogen available, these bacteria stimulate plant growth, support root development and facilitate the absorption of water and nutrients from the soil (Stefen et al., 2016).

In grasses, biological nitrogen fixation occurs due to the association between these plants and several genera of microorganisms, such as *Azospirillum*, *Herbaspirillum*, *Gluconacetobacter*, *Burkholderia*. These bacteria produce and exude hormones such as cytokinin, gibberellin and indolacetic acid, promoting plant growth, ensuring better use of phosphorus, potassium and iron and providing the increased energy needed for nitrate reductase activity (Guimarães et al., 2015; Chen et al., 2017; Grageda-Cabrera et al., 2018).

Thus, the aim of this study was to evaluate the efficiency of diazotrophic bacteria coinoculation in wheat cultivars grown in Cerrado Oxisol.

Material and Methods

The experiment was conducted in a greenhouse at the Federal University of Rondonópolis (16º 28’ 42” S; 50º 34’ 37” W; 284 m) in Mato Grosso state (MT), Brazil. Climate in the region is Aw according to the Koppen’s classification, denoting a megathermal climate with no winter and frequent summer rain (Dubreuil et al., 2018).

A randomized block design was used, in a 13 x 3 factorial scheme (13 inoculation treatments x 3 wheat cultivars). The 13 inoculation treatmets were: T1 (commercial wheat inoculant consisting of *A. brasilense* strains AbV5 and AbV6); T2 (commercial soybean inoculant composed of *B. japonicum* strains SEMIA 5079 and SEMIA 5080); T3 (commercial cowpea inoculant containing the *B. japonicum* strain BR3267); T4 (*R. tropici* strain MT08, isolated from cowpea lure plants); T5 (*R. leguminosarum* strain MT16, isolated from cowpea lure plants); T6 (*R. tropici* strain MT15, isolated from cowpea lure plants); T7 (wheat inoculant + cowpea inoculant); T8 (wheat inoculant + MT08); T9 (wheat inoculant + MT16); T10 (wheat inoculant + MT15); T11 (wheat inoculant + soybean inoculant); T12 (control with mineral nitrogen); T13 (absolute control - no inoculation or nitrogen fertilizer), and three wheat cultivars (BRS 394, BRS 264 and BRS 254), with four replicates, totaling 156 experimental units containing five plants each.

Three wheat cultivars adapted to the Cerrado and developed by the Empresa Brasileira de Pesquisa Agropecuária (Brazilian Agricultural Research Company) (EMBRAPA) were used: BRS 394, with a high grain yield, BRS 264, a precocious cultivar whose grains produce type 1 flour (Fioreze et al., 2020), and BRS 254, a breeding cultivar with high yields (Freitas et al., 2018).

For inoculant preparation, strains MT08, MT16, MT15 and the commercial cowpea inoculant for beans were multiplied in YMA culture medium, in an orbital shaker for 48 hours, at 30 ºC (Lazzaretti & Melo, 2005). Inoculant viability met the required standards of 1 x 10^9 CFU mL^{-1} (Brazil, 2010). Commercial liquid inoculants for wheat and soybeans were stored according to the manufacturers’ recommendations and purity testing was performed by counting bacterial colonies in the Petri dishes. The commercial inoculant concentrations were consistent with the manufacturers’ standards (wheat inoculant = 2 x 10^8 CFU mL^{-1}; soybean inoculant = 7 x 10^9 CFU mL^{-1}).

The experimental units consisted of 4 dm^{-3} pots filled with dystrophic red Oxisol, classified in accordance with soil taxonomy (EMBRAPA, 2013; United States, 1999) and collected in the Cerrado area at a depth of 0 to 0.20 m. Chemical and granulometry analyses showed the following characteristics: pH($CaCl_2$) = 4.10; Al^{3+} = 1.0 $cmol_c$ dm^{-3}; H^+ + Al^{+3} = 4.90 $cmol_c$ dm^{-3}; CEC = 5.69 $cmol_c$ dm^{-3}; V(%) = 13.90; P = 0.40 mg dm^{-3}; K^+ = 34.0 mg dm^{-3}; Ca^{2+} = 0.40 $cmol_c$ dm^{-3}; Mg^{2+} = 0.30 $cmol_c$ dm^{-3}; sand = 545.00 g kg^{-1}; silt = 100.00 g kg^{-1}; clay = 355.00 g kg^{-1} and organic matter = 17.70 g kg^{-1}.

Based on the soil analysis results, base saturation was increased to 50% by incorporating dolomitic limestone (TNP = 80%), which reacted for 30 days to correct acidity.

Soil moisture content in the pots was maintained using the gravimetric method (Bonfim-Silva et al., 2011), preserving the same moisture content in the experimental units and replacing water lost through evapotranspiration. Invasive plants were removed manually and aphids and caterpillars were controlled by applying 0.5% Azaradctin.

Based on soil chemical analysis, after liming, fertilization was carried out with phosphorus (P_2O_5 - 300 mg dm^{-3}), potassium (K_2O - 100 mg dm^{-3}) and micronutrients (50 mg dm^{-3} with FTE BR12 as source: 9% Zn - 1.8% B - 0.8% Cu - 2% Mn - 3.5% Fe - 0.1% Mo), according to the recommendations of Freitas et al. (2018). For nitrogen control, 100 mg dm^{-3} of urea was used, divided into two applications: 10 days after germination and at the onset of tillering (Freitas et al., 2018).

Ten seeds were sown in each experimental unit and thinning was performed five days after emergence, leaving five plants per pot. After thinning, 5 mL of the bacterial broth was applied per pot, 1 mL to each plant, close to the root system (Guimarães et al., 2015). The plants reached physiological maturity at 75 days and were harvested to analyze the following variables: grain nitrogen concentration, accumulation and crude protein content, 100-grain weight and total grain mass.

Grain nitrogen concentration was determined using the Kjeldahl method. In order to quantify crude protein, the nitrogen concentration was multiplied by 5.7, in line with the methodology described by Malavolta et al. (1997). Nitrogen accumulation in the grains was determined by multiplying nitrogen concentration by the dry weight of the samples (Viana & Kiehl, 2010).

The variables 100-grain weight and total grain mass were determined according to the Brazilian Regulations for Seed Analysis (Regras para Análise de Sementes, Brazil, 2009), with a standardized moisture content of 13%.

The results were submitted to analysis of variance by the F-test and, when significant, grouped by the Scott-Knott test ($p \leq 0.05$) for treatments with inoculants alone or those exhibiting interaction. Sisvar software version 5.7 was used (Ferreira, 2019). Total grain mass and nitrogen accumulation in the grains were transformed by the square root of Y + 1.0.

Results and Discussion

Analysis of variance showed no significant effect ($p \leq 0.05$) for cultivars and their interaction with inoculants for the variables total grain mass, 100-grain weight and nitrogen accumulation in grains. Interaction was observed between treatments and wheat cultivars for the variables grain nitrogen concentration and crude protein content, as well as a significant effect between inoculant treatments for all the variables analyzed. The cultivars were significant for grain nitrogen concentration and crude protein content (Table 1).

Table 1. Analysis of variance summary for total grain mass, 100-grain weight and grain nitrogen concentration, nitrogen accumulation and crude protein content as a function of cultivars (C), treatments with inoculants (I) and interaction (C x I)

Variable	Sources of variation/ Degrees of freedom				CV (%)
	B	C	I	C x I	
	3	2	12	24	
	p-value				
Total grain mass	0.56^{ns}	0.12^{ns}	0.000*	0.69^{ns}	19.70[1]
100-grain weight	0.28^{ns}	0.38^{ns}	0.000*	0.89^{ns}	24.18
Grain nitrogen concentration	0.40^{ns}	0.04*	0.000*	0.04*	8.91
Nitrogen accumulation in grains	0.37^{ns}	0.23^{ns}	0.000*	0.55^{ns}	26.45[1]
Grain crude protein	0.40^{ns}	0.04*	0.000*	0.04*	8.91

*, ns - Significant at $p \leq 0.05$, and not significant according to the F-test, respectively; CV - Coefficient of variation; [1] - Data transformed by the square root of Y + 1.0; B - Block; C - Cultivar; I - Inoculant

For the treatments containing diazotrophic bacteria, the results demonstrated no grain production for the absolute control. This reflects the low availability of essential nutrients in the soil, which compromised the development of wheat plants (Galindo et al., 2018).

There was a significant difference in total nitrogen accumulation in grains between the absolute control and other treatments. The nitrogen control obtained the highest average value (277.30 g kg^{-1}), the commercial wheat inoculant + MT15 (228.61 g kg^{-1}) (Table 2).

With respect to grain nitrogen accumulation, the rhizobium strains performed similarly to the commercial wheat inoculant, indicating that the autochthonous organisms influence plant growth (Grageda-Cabrera et al., 2018) and may be an economically viable alternative to nitrogen fertilizer in regions where wheat has been bred for local soil and climate conditions. The MT 15 and MT 08 *R. tropici* strains, MT16 *R. leguminosarum* strain and cowpea inoculant containing *B. japonicum* strain BR3267 contributed to improving nitrogen availability in treatments without *A. brasilense* coinoculation.

Grain quality attributes in wheat cultivars may also be related to inoculation with growth-promoting bacteria. Galindo et al. (2018) found that inoculation with *A. brasilense* promoted a higher nitrogen concentration in wheat grains.

Rhizospheric bacteria fix significant amounts of atmospheric nitrogen and produce high levels of indolacetic acid and other organic acids, which improve phosphate solubilization, increasing grain production and the accumulation of nutritious extracts in wheat crops (Kumar et al., 2017).

Bonfim-Silva et al. (2018) analyzed the correlation between nitrogen doses applied during wheat cultivation and the accumulation of nutrients in the grains and found that nitrogen concentration increased linearly in relation to the mineral dose. This relationship may justify the results obtained here for nitrogen accumulation in the grains, proving that the nutrient was assimilated by plants inoculated with diazotrophic bacteria.

With respect to 100-grain weight, *Rhizobium* and *Bradyrhizobium* bacteria showed statistically similar average values to those recorded for the commercial wheat inoculant. *A. brasilense* strains Abv5 and Abv6 of obtained the best result

Table 2. Average values for nitrogen accumulation in grains, 100-grain weight and total grain mass in wheat coinoculated with rhizobium strains

Treatments	Variables		
	Nitrogen accumulation in grains (g kg^{-1})	100-grain weight	Total grain mass
		(g)	
Commercial wheat inoculant	205.03 a	2.8043 a	5.4583 a
Commercial soybean inoculant	197.48 a	2.5672 a	5.1866 a
Commercial cowpea inoculant	223.69 a	2.7886 a	5.8766 a
MT08 *R. tropici* strain	203.89 a	2.4310 a	5.2950 a
MT16 *R. leguminosarum* strain	202.52 a	2.5451 a	5.3358 a
MT15 *R. tropici* strain	209.67 a	2.7183 a	5.2383 a
Commercial wheat inoculant + Commercial cowpea inoculant	208.74 a	2.4838 a	5.1550 a
Commercial wheat inoculant + MT08 Strain	218.68 a	2.6404 a	5.7358 a
Commercial wheat inoculant + MT16 Strain	213.61 a	2.5715 a	5.2150 a
Commercial wheat inoculant + MT15 Strain	228.61 a	2.7702 a	5.6516 a
Commercial wheat inoculant + Soybean inoculant	223.58 a	2.7198 a	5.8316 a
Nitrogen control	277.30 a	2.7180 a	6.7616 a
Absolute control	0.00 b	0.00 b	0.00 b

Means followed by the same letter do not differ according to the the Scott-Knott test ($p \leq 0.05$)

(2.80 g), followed by the *B. japonicum* strain BR3267 from the commercial cowpea inoculant (2.78 g). *R. tropici* strain MT08 (2.43 g) exhibited the lowest value for this variable. The respective increases for the commercial wheat inoculant, commercial cowpean inoculant and commercial wheat inoculant + MT15 coinoculations treatments in relation to nitrogen control were 3.07, 2.59 and 1.92% (Table 2).

The inoculant treatments studied here obtainined similar average values to those recorded for the nitrogen control. These results differ from those reported by Guimarães et al. (2015), who found that the average 100-grain weight recorded for the MTb3 strain (similar to *Burkholderia* spp.) was 7% higher than that of the nitrogen control and commercial wheat inoculant.

A similar result was obtained by Guimarães et al. (2015), who observed a 7% increase for the MTb3 strain (similar to *Burkholderia* spp.) when compared to the nitrogen control and commercial wheat inoculant.

These findings corroborate those reported by Ullah et al. (2017) for 100-grain weight, whereby the best results were obtained for the treatment involving coinoculation with different rhizobia strains (SRL5 + SRC8 = 3.43 g), followed by SRC8 (3.12 g), SRL5 (2.91 g) and the absolute control (2.45 g). The authors attributed this productive gain to P-solubilization, the production of organic acids, siderophores and indole-3-acetic acid (IAA), as well as a positive oxidase reaction and catalase activity in *Rhizobium* strains inoculated in wheat. These substances act on the plant directly or indirectly (Galindo et al., 2018), contributing to high yields.

For total grain mass, the nitrogen control (6.76 g), commercial cowpea inoculant (5.87 g) and commercial wheat inoculant + comecial soybean inoculant coinoculation treatment stood out among the average values obtained. The treatments with the lowest grain yield were coinoculation with the commercial wheat inoculant + commercial cowpea inoculant (5.15 g), and the commercial soybean inoculant (5.18 g) (Table 2).

The performance of diazotrophic bacteria in nitrogen fixation and the availability of growth hormones promoted dry matter accumulation, resulting in nutrient reserves in the leaves and stem during the growth phase, which were subsequently relocated, reflecting in higher grain yields and nutrient concentrations (Galindo et al., 2018).

In a study investigating the coinoculation of wheat plants in India, Kumar et al. (2017) reported that the combination of three rhizobacteria (*Enterobacter*, *M. arborescens* and *S. marcescens*) provided greater nitrogen, phosphorus, copper, zinc, magnesium and iron absoprtion, suggesting that different strains can be combined to produce inoculants effective at promoting growth and sustainable agricultural production. The authors observed that rhizobacteria inoculation alone or coinoculation with different strains increases wheat growth and yield when compared to the nitrogen and absolute controls, confirming the results obtained in the present study.

In regard to interaction between the cultivars and inoculants, the best-performing treatments for grain nitrogen concentration in the BRS 254 cultivar were commercial wheat inoculant + MT16 (43.75 g kg^{-1}), nitrogen control (41.65 g kg^{-1}) and commercial wheat inoculant + MT 08 (40.60 g kg^{-1}). Still in relation to BRS 254, the treatments with the lowest average values were the commercial cowpea inoculant (35.00 g kg^{-1}), commercial soybean inoculant (35.17 g kg^{-1}), commercial wheat inoculant + MT15 (37. 97 g kg^{-1}) and the MT16 strain alone (37.45 g kg^{-1}) (Table 3).

In regard to the BRS 264 cultivar, except for the absolute control, the remaining inoculant treatments and nitrogen control obtained the following average values, considered statistically identical: commercial wheat inoculant + MT 16 (42.87 g kg^{-1}), commercial wheat inoculant + MT 15 strain (42.70 g kg^{-1}), commercial wheat inoculant + commercial cowpea inoculant (42.00 g kg^{-1}) MT 15 strain inoculation (41.82 g kg^{-1}) (Table 3).

The best results for cultivar BRS 394 were recorded for the commercial wheat inoculant + commercial cowpea inoculant (42.17 g kg^{-1}), nitrogen control (41.82 g kg^{-1}), commercial wheat inoculant + MT 15 (41.65 g kg^{-1}) and MT 16 alone (41.12 g kg^{-1}). The commercial wheat inoculant + MT 16 treatment (36.40 g kg^{-1}) obtained the lowest average value for this interaction (Table 3).

The superior performance of inoculant treatments for the BRS 264 cultivar is attributed to genetic factors inherited from progenies that ensure a better response to inoculation with diazotrophic bacteria, such as higher grain yield and quality, giving this cultivar an advantage over BRS 254 (Freitas et al., 2018; Fioreze et al., 2020). Fioreze et al. (2020) also reported

Table 3. Average grain nitrogen concentration (g kg^{-1}) in wheat cultivars coinoculated with rhizobium strains

Treatment	Cultivar		
	BRS 254	BRS 264	BRS 394
Commercial wheat inoculant	35.00 aB	38.85 aA	39.20 aA
Commercial soybean inoculant	35.17 bB	40.25 aA	40.42 aA
Commercial cowpea inoculant	35.00 aB	40.25 aA	38.85 aA
MT08 *R. tropici* strain	39.02 aA	40.25 aA	38.50 aA
MT16 *R. leguminosarum* strain	37.45 aB	36.92 aA	41.12 aA
MT15 *R. tropici* strain	39.20 aA	41.82 aA	40.42 aA
Commercial wheat inoculant + Commercial cowpea inoculant	38.50 aA	42.00 aA	42.17 aA
Commercial wheat inoculant + MT08 Strain	40.60 aA	38.67 aA	37.45 aA
Commercial wheat inoculant + MT16 Strain	43.75 aA	42.87 aA	36.40 bA
Commercial wheat inoculant + MT15 Strain	37.97 aA	42.70 aA	41.65 aA
Commercial wheat inoculant + Commercial soybean inoculant	39.37 aA	37.97 aA	38.74 aA
Nitrogen control	41.65 aA	40.25 aA	41.82 aA
Absolute control	0.00 aC	0.00 aB	0.00 aB

Means followed by the same lowercase letter in the rows and uppercase letter in the columns do not differ according to the Scott-Knott test ($p \leq 0.05$)

Table 4. Average grain crude protein concentration (g kg^{-1}) in wheat cultivars coinoculated with rhizobium strains

Treatment	Cultivar		
	BRS 254	BRS 264	BRS 394
Commercial wheat inoculant	199.50 aB	221.44 aA	223.44 aA
Commercial soybean inoculant	200.49 bB	229.42 aA	230.42 aA
Commercial cowpea inoculant	199.50 aB	229.42 aA	221.44 aA
MT08 *R. tropici* strain	222.44 aA	229.42 aA	219.45 aA
MT16 *R. leguminosarum* strain	213.46 aA	210.47 aA	234.41 aA
MT15 *R. tropici* strain	223.44 aA	238.40 aA	230.42 aA
Commercial wheat inoculant + Commercial cowpea inoculant	219.45 aA	239.40 aA	240.39 aA
Commercial wheat inoculant + MT08 Strain	231.42 aA	220.44 aA	213.45 aA
Commercial wheat inoculant + MT16 Strain	249.37 aA	244.38 aA	207.48 bA
Commercial wheat inoculant + MT15 Strain	216.45 aA	243.39 aA	237.40 aA
Commercial wheat inoculant + Soy commercial inoculant	224.43 aA	216.45 aA	220.84 aA
Nitrogen control	237.40 aA	229.42 aA	238.40 aA
Absolute control	0.00 aC	0.00 aB	0.00 aB

Means followed by the same lowercase letter in the rows and uppercase letter in the columns do not differ according to the Scott-Knott test ($p \leq 0.05$)

similar average grain yields for cultivars BRS 394 and BRS 264 in Inceptisols in Southern Brazil, which produced high quality grains and can be used to improve wheat flour.

The results can be interpreted as demonstrating excellent symbiotic affinity between the cultivars and the strains tested, reinforcing the hypothesis that in order for microorganism inoculation of wheat plants to be viable, specific chemical signals are needed, whereby the better the genetic affinity between the plant and microorganisms, the more successful the exchange between them (Cardoso et al., 2017).

In regard to interaction between treatments and cultivars, wheat inoculant + MT16 performed better for grain crude protein content when combined with the BRS 254 cultivar (249.3 g kg^{-1}), followed by the commercial wheat inoculant + strain MT16 with BRS 264 (244.38 g kg^{-1}) and commercial wheat inoculant + strain MT15, also with cultivar BRS 264 (243.39 g kg^{-1}). The lowest grain crude protein results were obtained for the commercial wheat and cowpea inoculants combined with BRS 254, with 199.50 g kg^{-1} of crude protein (Table 4).

Inoculation of BRS 394 and BRS 254 with the MT15 *R. tropici* strain, under controlled cultivation conditions, showed potential for field testing under rainfed conditions, as described by Pasinato et al. (2018), who studied the use of drought-resistant wheat cultivars during the grain filling phase. These cultivars are considered more reliable and robust, with superior grain quality for Cerrado regions in Mato Grosso state, Brazil, at altitudes below 600 m (Bonfim-Silva et al., 2018).

According to Feldmann et al. (2018), inoculation response is also related to the genotype of the cultivars used and low levels of soil organic matter. This finding was corroborated in the present study, whereby inoculation and coinoculation were found to be efficient in improving the grain quality of cultivars even in soil with a low organic matter content (OM = 1.77%).

Grain protein concentration and nitrogen concentration are essential in determining the quality of wheat flour (Bonfim-Silva et al., 2018) and fundamental variables in gluten formation. Stefen et al. (2016) found wet gluten concentration between 18 and 38% when grain crude protein concentration was greater than 11%, with positive correlation between the general strength of gluten, protein concentration and nitrogen concentration (Petroczi et al., 2018). The overall average of grain crude protein concentration in the present study was 207.87 g kg^{-1} (20.78%), varying according to the cultivar and treatment used.

Ferreira et al. (1987) attributed increased nitrogen in plant tissues to nitrate reductase performed by associative bacteria in the wheat rhizospheric region. The higher the grain nitrogen concentration, the greater the crude protein content in grains, resulting in better grain quality. This is essential for producers, since superior quality grains command a higher selling price.

According to Zang et al. (2019), plant growth-promoting bacteria such as *A. brasilense* can act in association with rhizobia in leguminous species as well as grasses such as wheat. This relationship promoted an increase in grain yield, which is vital for the wheat market. In addition, the present study found that bacterial strains isolated from soils in the region are suitable for wheat inoculation and produce good results in cultivars adapted to the Cerrado (Caneiro, 2017).

This may be influenced by adequate nitrogen supplementation by nodular bacteria during the crop cycle (Chen et al., 2017). The results of the present study are promising in terms of using nodular bacteria in wheat, since the treatments analyzed resulted in satisfactory production when compared to nitrogen fertilization and commercial wheat inoculant (*A. brasilense*).

Conclusions

1. Coinoculation with *Bradyrhizobium* and *Rhizobium* strains, alone or combined with *A. brasilense*, increased the grain quality of wheat cultivars, particularly BRS 264.
2. Coinoculation with diazotrophic bacteria isolated from legumes showed positive effects, especially for 100-grain weight and grain dry mass.

Acknowledgements

To the Coordination for the Improvement of Higher Education Personnel (CAPES) for the financial support.

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