Research Article

Reinoculation of topdressing *Rhizobium tropici* combined or not with *Azospirillum brasilense* in common bean[1]

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ABSTRACT

The nitrogen (N) supplying capacity of common bean plants through biological fixation is still questioned by farmers. This study aimed to investigate the nodulation, growth and production of common bean cultivars under field conditions, when subjected to topdressing *Rhizobium tropici* reinoculation applied at different rates, combined or not with *Azospirillum brasilense*. A randomized blocks design was used, in a 2 × 4 × 2 + 2 factorial arrangement, with four replications. The treatments consisted of two common bean cultivars (BRS-Estilo and BRS-Esteio) reinoculated with four *R. tropici* rates (0-, 1-, 2- and 4-fold the reference rate), combined or not with *A. brasilense* in co-inoculation. The additional treatments consisted of nitrogen fertilizer applications (20 and 50 kg ha^{-1} at planting and as topdressing, respectively) for both cultivars. The variables evaluated at the R6 stage were nodulation (number of active nodules and nodule dry weight) and morphological plant characteristics (root length and dry weight, plant height, shoot dry weight and N content), while, at harvest, the number of pods per plant, number of grains per pod, 100-grain average weight and grain yield were evaluated. The topdressing reinoculation of *R. tropici* combined with *A. brasilense* at the V4 stage affected the nodulation process and agronomic characteristics of the common bean plants. When performed in addition to seed inoculation, it may totally replace the supply of mineral N in the crop, allowing the achievement of high yield levels.

KEYWORDS: *Phaseolus vulgaris*, mineral nutrition, biological nitrogen fixation.

RESUMO

Reinoculação de *Rhizobium tropici* em cobertura combinado ou não com *Azospirillum brasilense* em feijoeiro

A capacidade de fornecimento de nitrogênio (N) para o feijoeiro por meio de fixação biológica ainda é questionada por agricultores. Objetivou-se investigar a nodulação, crescimento e produção de cultivares de feijão comum em condições de campo, submetidas à reinoculação de *Rhizobium tropici* em cobertura aplicado em diferentes doses, em mistura ou não com *Azospirillum brasilense*. Empregou-se o delineamento de blocos casualizados, em esquema fatorial 2 x 4 x 2 + 2, com quatro repetições, sendo duas cultivares (BRS-Estilo e BRS-Esteio) reinoculadas com quatro doses de *R. trocipi* em cobertura (0; 1; 2; e 4 vezes a dose referência), em mistura ou não com *Azospirillum* em co-inoculação. Como tratamentos adicionais, utilizou-se adubação nitrogenada (20 e 50 kg ha^{-1} na base e em cobertura, respectivamente) para ambas as cultivares. No estádio R6, foram avaliadas as características de nodulação (número de nódulos ativos e massa seca de nódulos) e morfológicas das plantas (comprimento e peso seco de raiz, altura de planta, peso seco da parte aérea e teor de N), e, na colheita, o número de vagens por planta, número de grãos por vagem, peso médio de 100 grãos e rendimento de grãos. A reinoculação em cobertura de *R. tropici* com *A. brasilense* realizada no estádio V4 influenciou no processo de nodulação e características agronômicas do feijoeiro. Quando realizada em complementação à inoculação nas sementes, pode substituir totalmente o fornecimento de N mineral na cultura, permitindo a obtenção de elevados patamares de rendimento.

PALAVRAS-CHAVE: *Phaseolus vulgaris*, nutrição mineral, fixação biológica de nitrogênio.

INTRODUCTION

Nitrogen (N) is a nutrient with determinant effect on grain yield of common bean crops, which is usually supplied using mineral fertilizers that have high costs - its extraction by the crop is estimated in 140 kg ha^{-1} (Soratto et al. 2017). It is essential for plant growth; however, N-deficient soils are common (El-Beltagi et al. 2022), what limits the development and yield of most crops.

Common bean plants present a high nutritional demand and have a short cycle; thus, the immediate

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availability of N at stages with high demand is needed to avoid negative effects on grain yield (Arf et al. 2011, Nascente et al. 2012). The main sources used for supplying N to plants are: N fertilizers, organic matter and biological N fixation through inoculation with *Rhizobium* species, which are associated with roots of Fabaceae species, such as common bean (Pelegrin et al. 2009).

Rhizobium species are soil bacteria that transform N_2 in ammonia, which is absorbed by plants through the action of the nitrogenase enzyme (Silva et al. 2020). Inoculants for common bean are produced in Brazil with the *R. tropici* species, which is adapted to tropical soils, using the strains Semia 4077, Semia 4088 and Semia 4080 (Brasil 2011). This species can fix approximately 20-30 % of the needed N to the plant, representing up to 40 kg ha^{-1} of N (Soares et al. 2016), or even fully substitute the N fertilizer application (Sousa et al. 2021, Teixeira et al. 2022, Sousa et al. 2022).

In addition to the *Rhizobium* species, another bacterium that can improve crop yield is *Azospirillum brasilense* (Fukami et al. 2018). The number of researches involving this bacterium has increased in the last years in Brazil, with a proven success for soybean (Hungria et al. 2013, Hungria et al. 2015, Galindo et al. 2018), maize (Hungria et al. 2010, Galindo et al. 2016) and common bean (Peres et al. 2016, Steiner et al. 2019, Barbosa et al. 2020) crops.

The biological N fixation supplies a large part of the N demanded by common bean plants; however, it is characterized by a fast senescence of nodules, with a consequent decrease in total biological N fixation soon after flowering and beginning of the grain filling stage (Coelho et al. 2021), and the response capacity of plants depends on the cycle of the cultivar used (Andraus et al. 2016).

In this context, reinoculation, late reinoculation or additional inoculation are practices not very common in researches on biological N fixation in common bean crops. It consists of inoculation at sowing, applied directly to the seed (seed inoculation) together with inoculant application, after the implementation of the crop (topdressing inoculation). The product is directly applied to the soil, in order to promote the formation of new nodules and increase the N availability and contents during the crop cycle. Information on the use of topdressing reinoculation in common bean have been recently obtained and showed the potential of this technique for increasing grain yield (Sousa et al. 2021, Teixeira et al. 2022); however, more thoroughly studies on this subject are needed.

Thus, this study aimed to investigate the nodulation capacity and agronomic characteristics of common bean cultivars subjected to reinoculation with *Rhizobium tropici*, applied as topdressing at different rates, combined or not with *Azospirillum brasilense*.

MATERIAL AND METHODS

The experiment was carried out at the 2020 crop season, at the Universidade Estadual de Goiás, in Ipameri, Goiás state, Brazil (17º43'27''S, 48º08'55''W and mean altitude of 800 m). The soil of the area is classified as a Typic Hapludox (USDA 2014), or Latossolo Vermelho-Amarelo Distroférrico (Santos et al. 2018) of sandy loam texture. The climate of the region is Aw, according to the Köppen classification, with monthly temperatures higher than 20 ºC and annual rainfall of 1,100-1,800 mm (Cardoso et al. 2014). The maximum temperature recorded during the experiment was 34.9 ºC, while the minimum was 13.2 ºC. The total rainfall during the experiment was 397.7 mm (Figure 1).

A randomized blocks design was used, in a 2 × 4 × 2 + 2 factorial arrangement, with four replications. The treatments consisted of two common bean cultivars (BRS-Estilo and BRS-Esteio) reinoculated with four *Rhizobium tropici* rates (0-, 1-, 2- and 4-fold the reference rate, which was 150 L ha^{-1}), using a commercial inoculant, combined or not with *Azospirillum brasilense* in co-inoculation. The additional treatments consisted of nitrogen (N) fertilizer applications (20 and 50 kg ha^{-1} at planting and as topdressing, respectively), for both the tested cultivars.

The seeds were firstly inoculated with *R. tropici* strains recommended for common bean plants (SEMIA 4088 and SEMIA 4077), using a liquid commercial inoculant (Biomax Premium; 2 x 10^9 CFU mL^{-1}) at the rate of 150 mL for each 50 kg of seeds (reference rate), following the manufacturer's recommendations. A reinoculation with *R. tropici* was carried out at the R4 stage, with the product directly sprayed to the soil, close to the plant roots, according to the rates of each treatment. *A. brasilense* was added to the spray solution for co-inoculation,

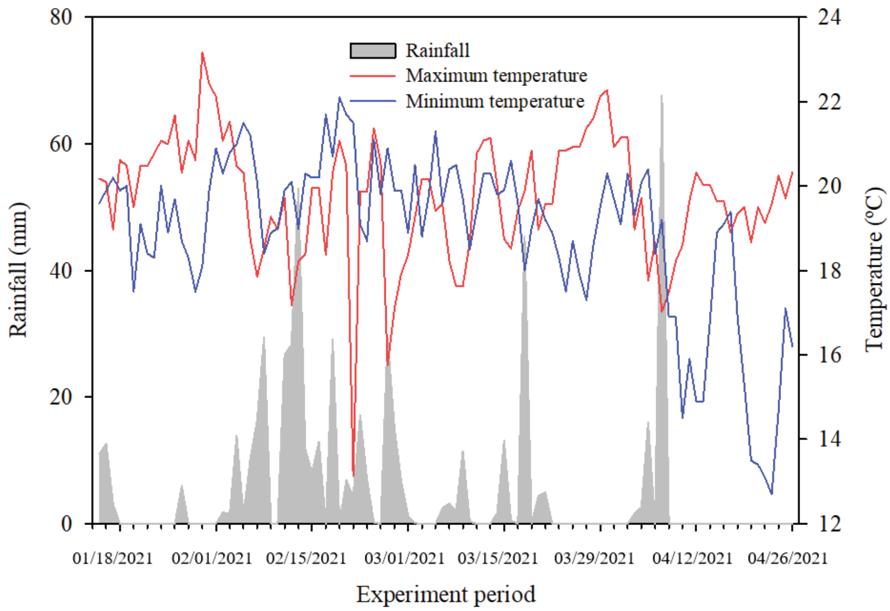

Figure 1. Rainfall, maximum and minimum temperatures in Ipameri (Goiás state, Brazil), between January 18 and April 26, 2021. Source: Brasil (2022).

using a commercial product (Biomax® Azum; 3 x 10^9 CFU mL^{-1}) at the rate of 1.0 L ha^{-1}, which is the recommended rate for common bean, according to the manufacturer. The solution was applied using a 20-L backpack sprayer with fan nozzles and application volume of 200 L ha^{-1}. Mineral N fertilizer was applied at sowing together with the other soil fertilizers and as topdressing.

The experimental area had a predominance of *Brachiaria decumbens* plants, and a burn down was carried out using the herbicides 2,4-D + glyphosate (2.5 L ha^{-1} of each product). After 7 days, conventional soil preparation was carried out, with one plowing and two light harrowings. Soil acidity correction was not necessary, according to the result of the soil analysis. Soil fertilizers were then applied using 400 kg ha^{-1} of the NPK formulation 00-20-20, except for the additional treatments, for which the 05-25-20 formulation was used at the same rate.

The experimental unit consisted of six 5-m rows spaced 0.50 m apart. The two central rows were used for the evaluation of nodulation and plant growth, and the other two central rows were used for the evaluation of agronomic characteristics at harvest. The two lateral rows were considered borders. The sowing density was 15 plants m^{-1}. Topdressing was carried out using mineral N (urea) at the beginning of the V4 stage, distributed continuously over the rows, close to the plants. Weed control was carried out using post-emergence herbicide application (Fusiflex®; 30 L ha^{-1}) at the beginning of the V3 stage, and then complemented with manual weeding. The Engeo® S (30 mL 100 L^{-1}) insecticide was used for the control of *Diabrotica speciosa* and *Bemisia tabaci*. The Approve® (100 g c.p. 100 L^{-1} of water) fungicide was applied for the preventive control of fungal diseases: white mold (*Sclerotinia sclerotiorum*), anthracnose (*Colletotrichum lindemuthianum*) and powdery mildew (*Erysiphe polygoni*). Three plants of each row of the evaluation area were collected at the R6 stage, with the aid of a spade, for the evaluations of nodulation (number of active nodules and nodule dry weight) and morphological characteristics of plants (root length, root dry weight, plant height, shoot dry weight and N contents).

The plants were placed in jute bags and immediately taken to the laboratory after the collection. They were then cut at the stem base region and washed. Active nodules (pink) with more than 1 mm were removed and counted, and the nodules were then taken to a forced air circulation oven for 48 hours, at 65 ºC, to determine the nodule dry weight. The main plant root length was measured with a ruler and the root dry weight was quantified after drying in an oven at 72 ºC, until constant weight. The shoot dry weight was determined after drying in an oven at 72 ºC. The N contents were determined according to Silva (2009).

Ten plants were harvested in the evaluation area of each plot at the end of the crop cycle to determine the following production components: number of pods per plant, number of grains per pod, 100-grain weight and grain yield. The mean 100-grain weight (g) and grain yield (kg ha^{-1}) were evaluated after correcting the grain moisture to 13 %. The final stand was evaluated by counting the number of plants in the plots at the end of the experiment.

The data were subjected to tests of homogeneity of variance and normality of residues (Levene and Shapiro-Wilk, respectively), and then subjected to analysis of variance, with the means compared to each other by the Tukey test ($p < 0.05$) and to the additional treatments by the Dunnett's test at 5 % of probability. The R software was used (R Core Team 2016) for the data analyses.

RESULTS AND DISCUSSION

The number of nodules per plant fitted to the quadratic polynomial and linear regression models as a function of the triple factorial. The number of nodules per plant was high in both cultivars treated with *Azospirillum brasilense* co-inoculation (Figure 2A). This result, combined with the applied reinoculation rates, improved the number of nodules per plant to appoint the maximum effect of the reinoculation rate, showing results of 24 nodules per plant for the BRS-Esteio cultivar with the rate of

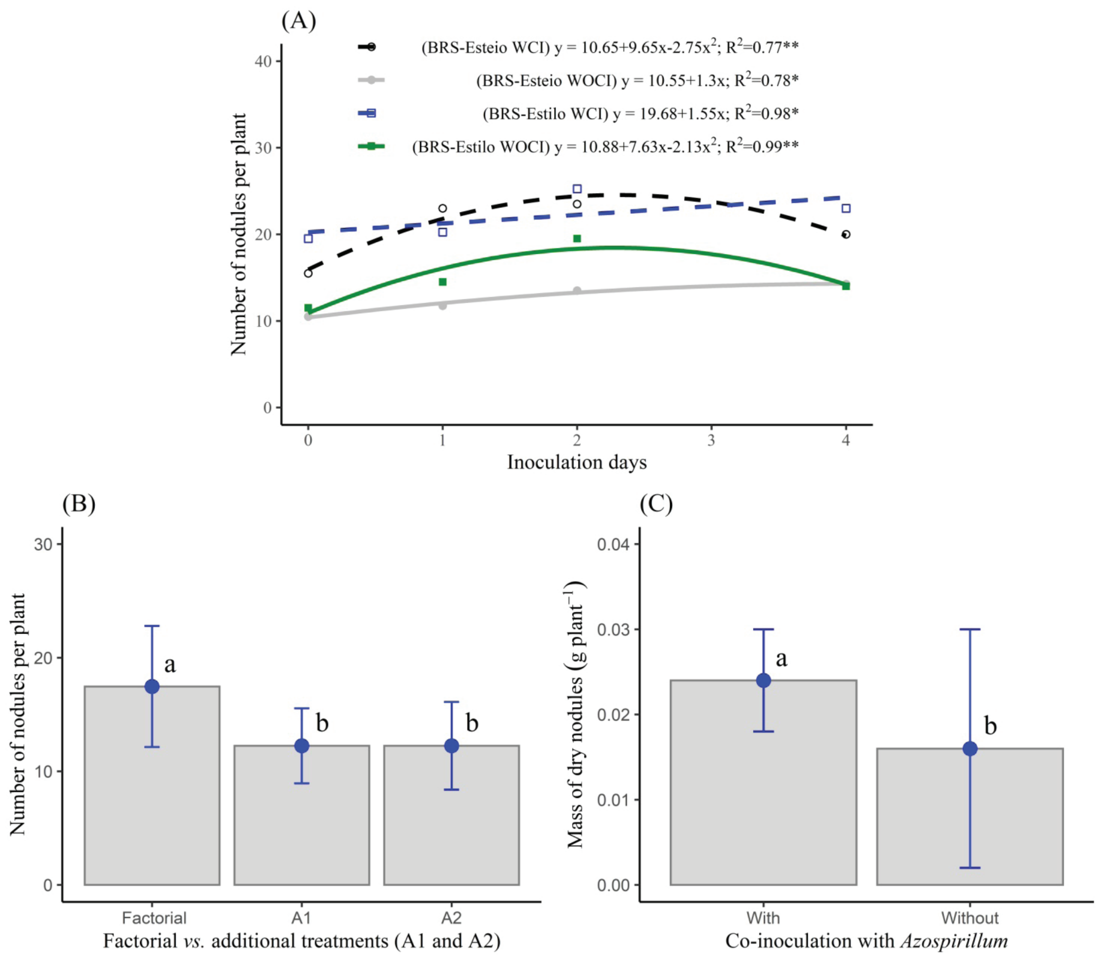

Figure 2. Number of nodules per plant as a function of triple factorial treatments (cultivars *vs.* reinoculation rates *vs.* co-inoculation) (A) and factorial *vs.* additional treatments (B); and nodule dry weight per plant as a function of co-inoculation with *Azospirillum brasilense* (C). A1: additional treatment 1 - BRS-Estilo with mineral N; A2: additional treatment 2 - BRS-Esteio with mineral N. Bars with the same letter are not different from each other by the Tukey test at 5 % of probability. WCI: with co-inoculation with *Azospirillun*; WOCI: without co-inoculation with *Azospirillum*.

1.7-fold the reference rate (262.5 L ha^{-1}), when using co-inoculation. The results found with co-inoculation for the BRS-Estilo cultivar fitted the linear model, as a function of reinoculation rates, with an increase of 1.5 in the number of nodules per plant for each unit increased in the reinoculation rate (Figure 2A).

Peres et al. (2016) and Steiner et al. (2019) also found increases in nodulation of common bean plants in response to co-inoculation with *R. tropici* + *A. brasilense*, when compared to those only inoculated with *R. tropici*. The presence of *A. brasilense* in the plant and its release of phytohormones, such as auxins and cytokinins, favor the root growth (Hungria et al. 2015, Rondina et al. 2020), contributing to increases in plant nodulation.

The triple factorial treatment presented a number of nodules per plant 43 % higher than the additional treatments (Figure 2B). The co-inoculation with *A. brasilense* increased the nodule dry weight, when compared to the treatment with no co-inoculation, representing an increase of up to 50 % (Figure 2C), regardless of the common bean cultivar used.

The plant height increased 29.8 and 24.8 cm, respectively for the BRS-Esteio and BRS-Estilo cultivars, when treated with the rate of 4-fold the reference rate (150 L ha^{-1}), if compared to the treatment without reinoculation (Figure 3A). Regarding the co-inoculation with *A. brasilense*, the plant height was 31 cm smaller in the treatment without co-inoculation.

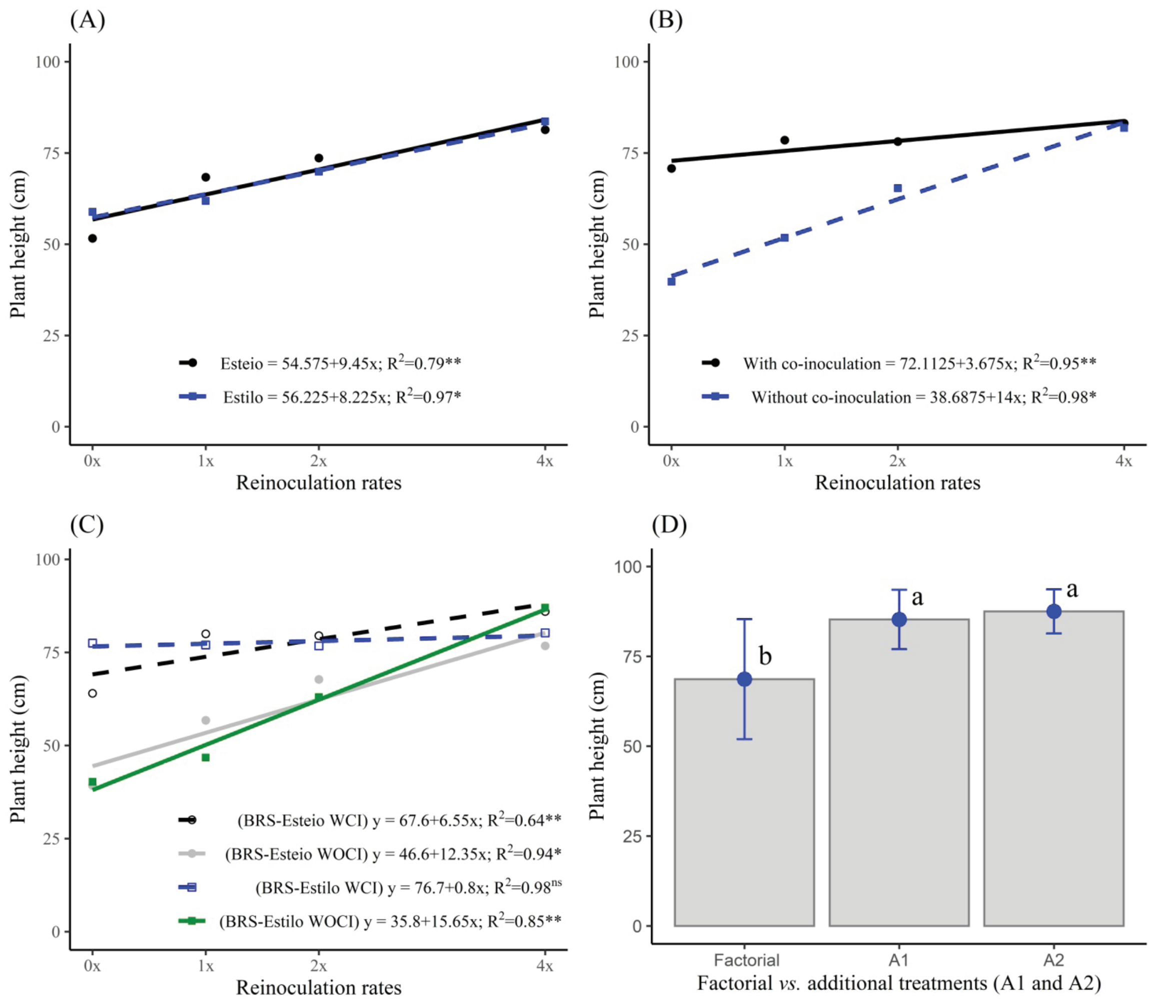

Figure 3. Plant height as a function of cultivars and reinoculation rates (A), co-inoculation with *Azospirillum brasilense* and reinoculation rates (B), triple factorial (cultivars *vs.* reinoculation rates *vs.* co-inoculation) (C) and factorial *vs.* additional treatments (D). A1: additional treatment 1 - BRS-Estilo with mineral N; A2: additional treatment 2 - BRS-Esteio with mineral N. Bars with the same letter are not different from each other by the Tukey test at 5 % of probability. WCI: with co-inoculation with *Azospirillun*; WOCI: without co-inoculation with *Azospirillum*.

The plant height found with 4-fold the reference rate (150 L ha^{-1}) was 1.3 cm higher than that found in the treatment without co-inoculation (Figure3B). Regarding the results found in the triple factorial treatments, both cultivars presented increases in plant height as a function of reinoculation rates, regardless of the co-inoculation. It was more pronounced for the BRS-Esteio cultivar with co-inoculation, which resulted in a maximum plant height of 86 cm with 4-fold the reference rate (150 L ha^{-1}) (Figure 3C). In addition, the results for plant height were 26 % higher in the additional treatments (BRS-Estilo and BRS-Esteio cultivars with application of mineral nitrogen fertilizer), when compared to the triple factorial treatments (Figure 3D).

The root length was significantly affected by the cultivars. BRS-Estilo presented a higher root length (20.4 cm) than BRS-Esteio (14.8 cm) (Figure 4A). The reinoculation rates had a strong effect on root length, with a linear increase up to the rate of 4-fold the reference rate (600 L ha^{-1}), presenting a maximum increase of 5.2 cm in root length, when compared to the treatment without reinoculation (Figure 4B). The co-inoculation with *A. brasilense* resulted in increases for root length, in comparison to those without co-inoculation, with a significant difference of 7.6 cm between treatments (Figure 4C); and the additional treatments resulted in a mean root length (24.9 cm) greater than that of the triple factorial (17.6 cm) (Figure 4D).

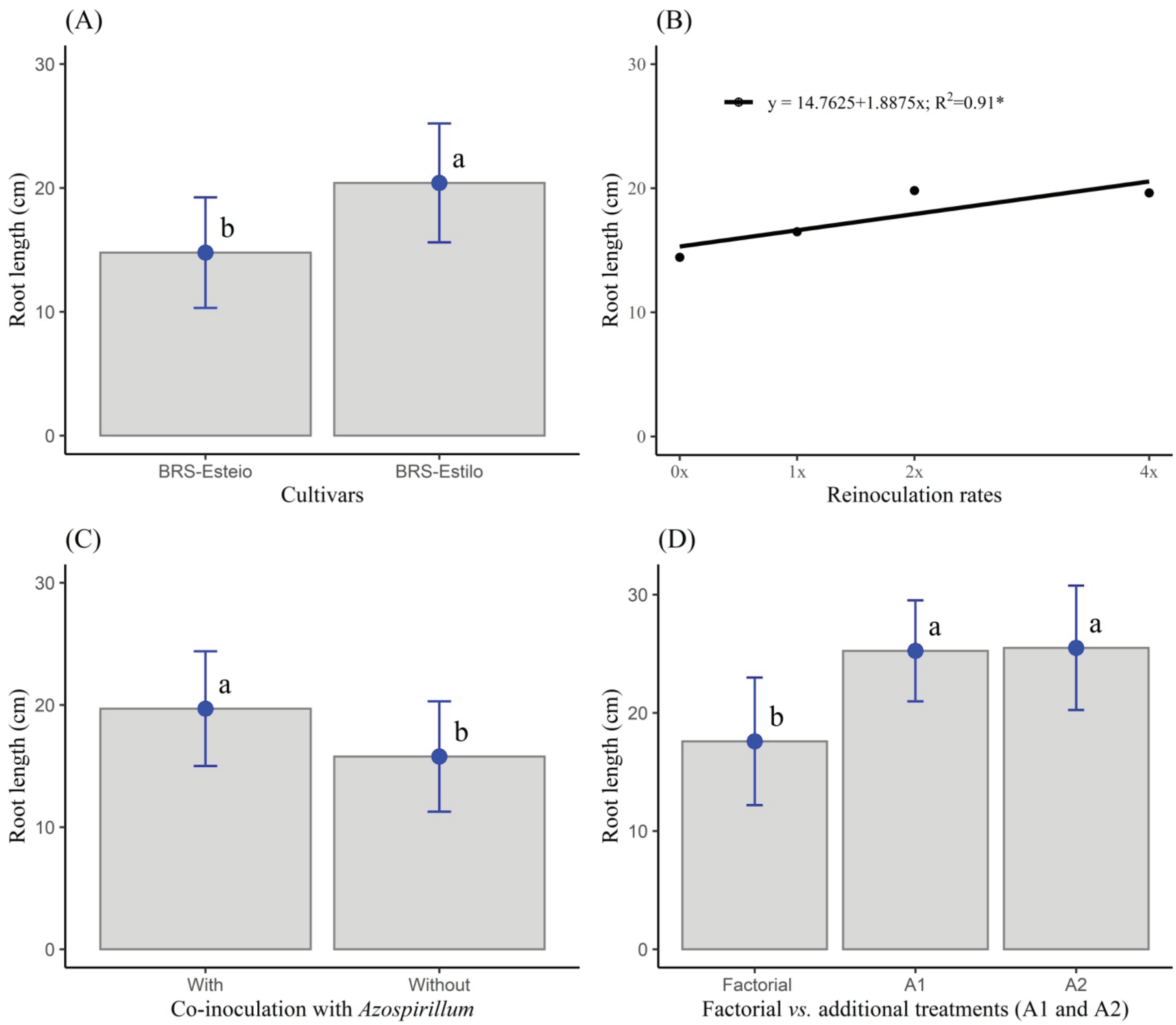

Figure 4. Root length as a function of cultivars (A), reinoculation rates (B), co-inoculation with *Azospirillum brasilense* (C) and factorial *vs.* additional treatments (D). A1: additional treatment 1 - BRS-Estilo with mineral N; A2: additional treatment 2 - BRS-Esteio with mineral N. Bars with the same letter are not different from each other by the Tukey test at 5 % of probability.

The shoot and root dry weight were affected by the evaluated factors (Figure 5). The rate of 4-fold the reference rate (600 L ha^{-1}) increased the shoot and root dry weights of plants in 65 and 115 %, respectively, when compared to those without co-inoculation (Figures 5A and 5D). The co-inoculation resulted in

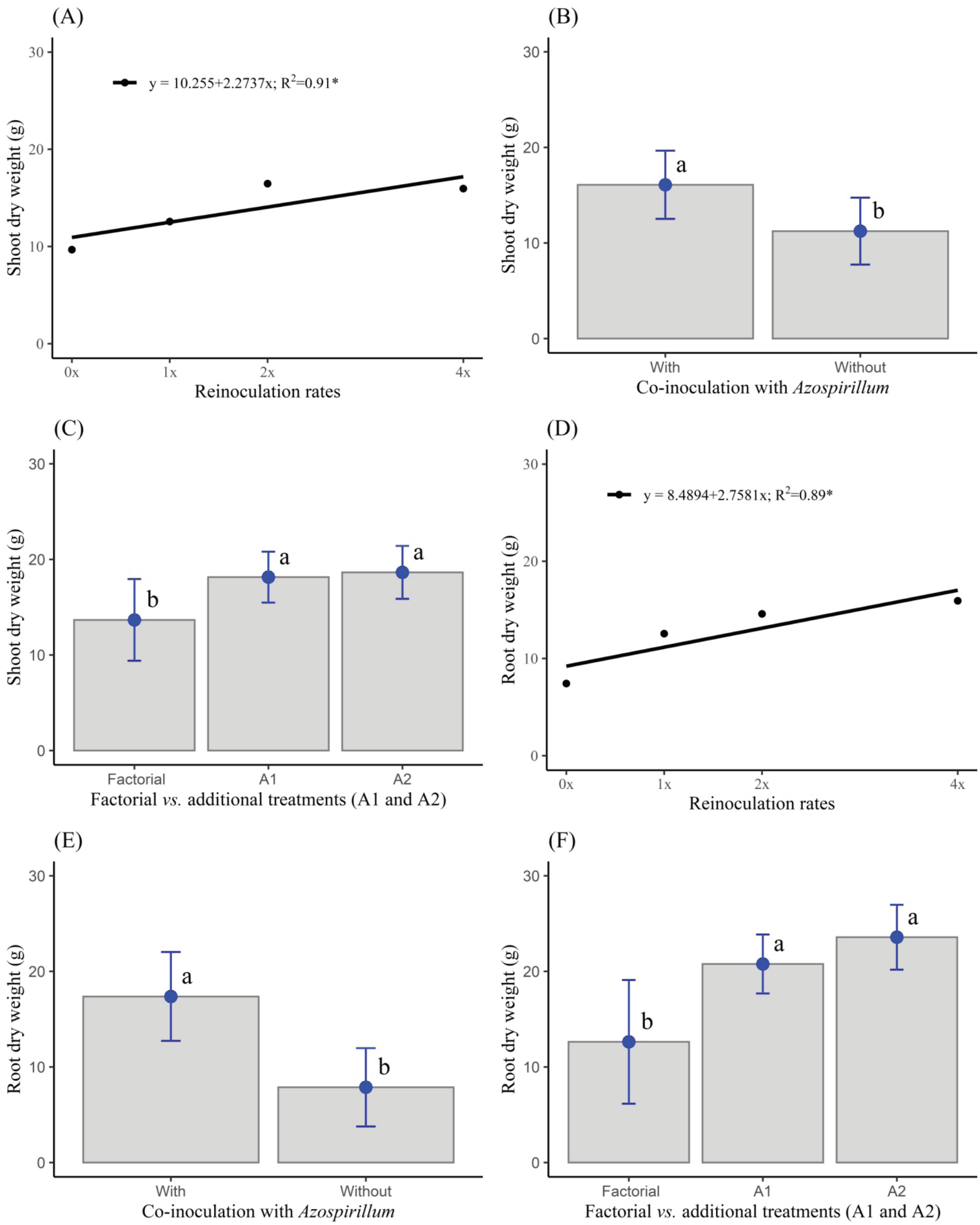

Figure 5. Shoot (A) and root dry weight (D) as a function of reinoculation rates, co-inoculation with *Azospirillum brasilense* (B and E) and factorial *vs.* additional treatments (C and F). A1: additional treatment 1 - BRS-Estilo with mineral N; A2: additional treatment 2 - BRS-Esteio with mineral N. Bars with the same letter are not different from each other by the Tukey test at 5 % of probability.

increases of 4.9 and 9.5 g, respectively for the shoot and root dry weights of plants, when compared to those without co-inoculation (Figures 5B and 5E). The additional treatments stood out with statistical differences of 4.7 and 9.5 g for the shoot and root dry weights, respectively (Figures 5C and 5F).

The N contents in plants of the BRS-Esteio cultivar were greater than those of the BRS-Estilo cultivar, with a difference of 3.6 g kg^{-1} between them (Figure 6A). The co-inoculation with *A. brasilense* had a significant interaction with the reinoculation rates, and the use of co-inoculation combined with the reinoculation rates, including the rate 0, resulted in statistically greater N contents, when compared to the rates of 0- and 4-fold the reference rate (600 L ha^{-1}) without co-inoculation. A significant difference was also found between the best combinations of treatments with 1- and 2-fold the reference rate (150 and 300 L ha^{-1}, respectively) without co-inoculation. The results showed that the use of the highest rate (4-fold the reference rate) combined with co-inoculation resulted in a higher N accumulation in the plants than the treatment without co-inoculation and the reinoculation rate of 0, with a significant difference of 26.9 g kg^{-1} (Figure 6B). The comparison between factorial *vs.* additional treatments showed greater N contents for the cultivars with mineral N (additional treatments), with a difference of 13.5 % (Figure 6C).

The rate of 2.3-fold the reference rate (345 L ha^{-1}) presented the highest mean increase in number of pods

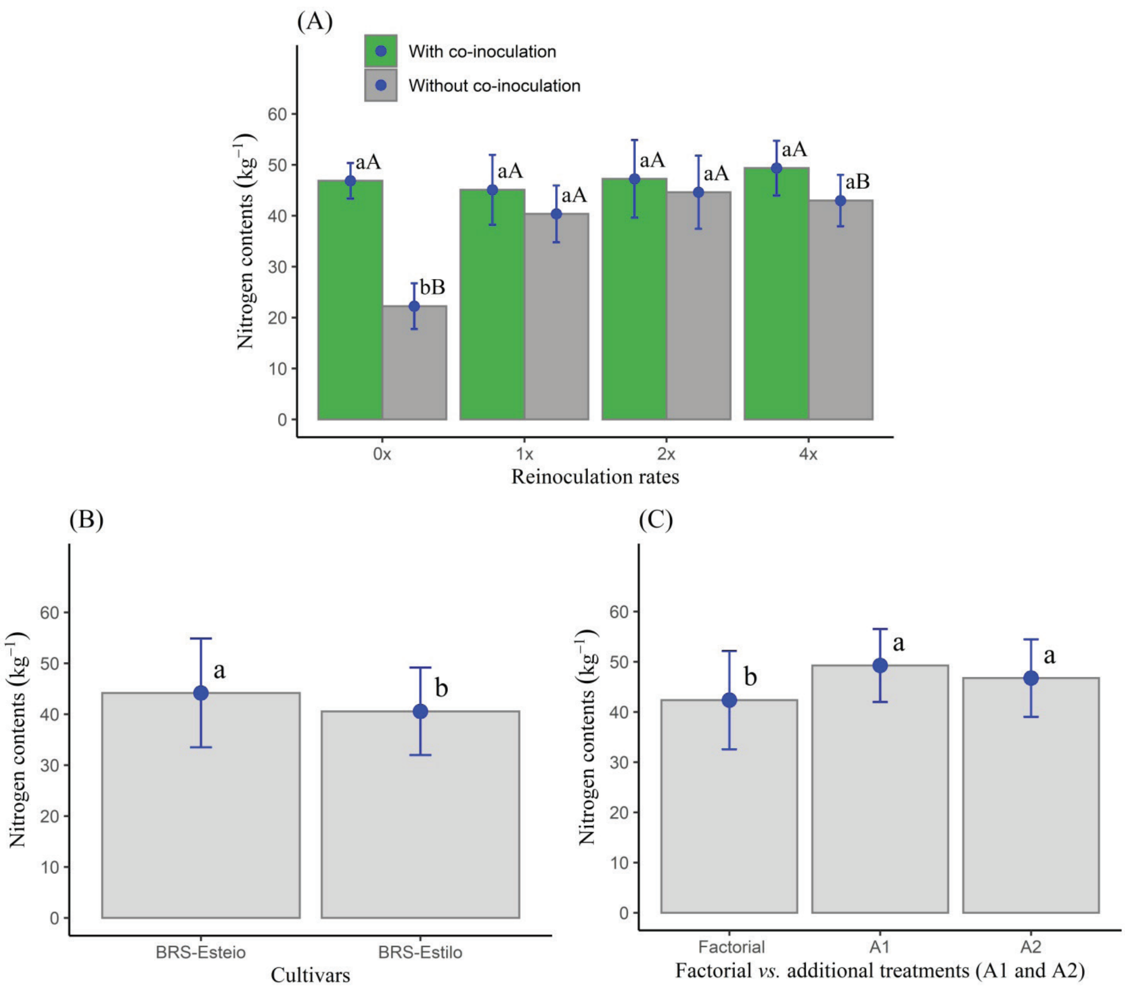

Figure 6. Nitrogen contents as a function of cultivars (A), reinoculation rates with or without co-inoculation with *Azospirillum brasilense* (B) and factorial *vs.* additional treatments (C). A1: additional treatment 1 - BRS-Estilo with mineral N; A2: additional treatment 2 - BRS-Esteio with mineral N. Fitting was not significant for linear or quadratic regression models. Bars with the same letter are not different from each other by the Tukey test at 5 % of probability. Lowercase letters compare reinoculation rates within each co-inoculation and uppercase letters compare co-inoculation within each reinoculation rate.

per plant, resulting in 19 pods plant^{-1} (Figure 7A). The co-inoculation with *A. brasilense* increased the number of pods per plant in 58 %, if compared to the treatment without co-inoculation (Figure 7B). Regarding the factorial *vs.* additional treatments, the number of pods per plant was 46 % higher in the treatments with N fertilizer (additional treatment), when compared to the triple factorial treatments (Figure 7C).

The rate of 150 L ha^{-1} (reference rate) resulted in the highest mean number of grains per pod, if compared to the reinoculation rate of 0, with a difference of 4 grains per pod. However, it did not statistically differ from the rates 2- and 4-fold the rate of reference (300 and 600 L ha^{-1}, respectively) (Figure 7D).

Regarding the100-grain weight, the interaction between the factors cultivar and reinoculation rates fitted to an increasing linear model as a function of reinoculation rates for the BRS-Estilo cultivar, with increases of 0.73 g for each unit of the reinoculation rate increased. The results found for the BRS-Esteio cultivar fitted to a quadratic regression model, with an estimated rate of 2.1-fold the reference rate (315 L ha^{-1}), resulting in the highest 100-grain weight (23.8 g) (Figure 8A). The factorial *vs.* additional treatments showed higher results for the cultivars fertilized with mineral N, when compared to the triple factorial, with a difference for 100-grain weight of 3.4 g (Figure 8B).

The grain yield was significantly affected by the evaluated factors. Plants of the BRS-Estilo

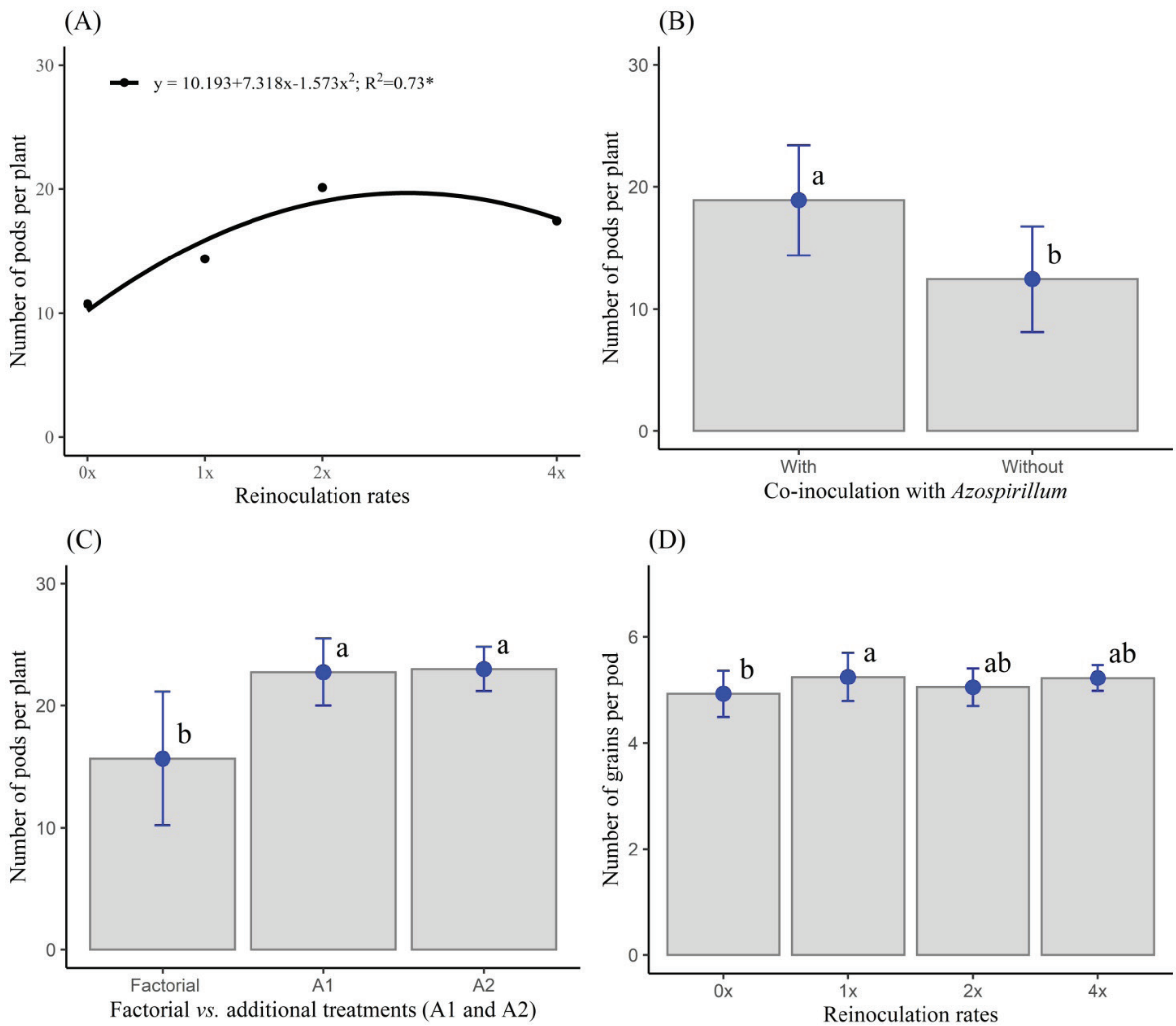

Figure 7. Number of pods per plant as a function of reinoculation rates (A), co-inoculation with *Azospirillum brasilense* (B), factorial *vs.* additional treatments (C) and number of grains per plant as a function of reinoculation rates (D). A1: additional treatment 1 - BRS-Estilo with mineral N; A2: additional treatment 2 - BRS-Esteio with mineral N. Bars with the same letter are not different from each other by the Tukey test at 5 % of probability.

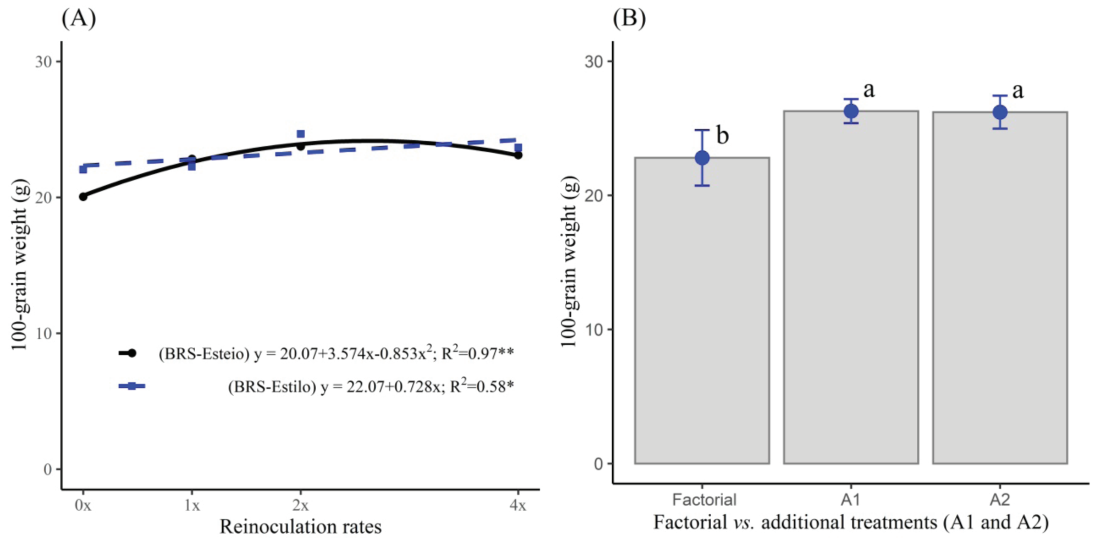

Figure 8. One-hundred grain weight as a function of cultivars and reinoculation rates (A) and factorial *vs.* additional treatments (B). A1: additional treatment 1 - BRS-Estilo with mineral N; A2: additional treatment 2 - BRS-Esteio with mineral N. Bars with the same letter are not different from each other by the Tukey test at 5 % of probability.

cultivar co-inoculated with *A. brasilense* presented a higher production potential (1,883 kg ha^{-1}) than those without co-inoculation (1,226 kg ha^{-1}). Similar results were found for the BRS-Esteio cultivar, to which the co-inoculation increased the grain yield in 111 %, if compared to that of plants without co-inoculation (Figure 9A). Regarding the co-inoculation effects, the maximum grain yield was found in the treatment with co-inoculation as a function of reinoculation rates, with the rate of 2.5-fold the reference rate (375 L ha^{-1}) resulting in a grain yield of 2,148 kg ha^{-1}.

The highest grain yield found in the treatments without co-inoculation was 1,317 kg ha^{-1}, with the reinoculation rate of 2.1-fold the reference rate (315 L ha^{-1}) (Figure 9B). The highest grain yield found in the treatment with *Rhizobium tropici* reinoculation at 2.5-fold the reference rate (150 L ha^{-1}) and co-inoculation with *A. brasilense* was lower than that found in the treatment with mineral N. However, this is a comparison between the mean of all the factorial treatments involving the application of *R. tropici* and the mean of the treatment with mineral N fertilizer. The mean grain yield found in the treatments with the highest *R. tropici* + A. *brasilense* reinoculation rates resulted in a yield higher than 2,000 kg ha^{-1} for the BRS-Esteio cultivar, being close to the means found in the treatment with mineral N.

The statistical breakdown of the triple factorial (cultivars *vs.* reinoculation rates *vs.* co-inoculation) showed a better fit to the quadratic model, except for the BRS-Estilo cultivar with co-inoculation, which better fitted to a linear model as a function of reinoculation rates, denoting increases in grain yield of 214 kg ha^{-1} for each unit of the increased reinoculation rate (L ha^{-1}). The co-inoculated BRS-Esteio cultivar combined with the rate of maximum reinoculation effect (2.3-fold the reference rate) resulted in a grain yield of 2,197 kg ha^{-1}. The rate of maximum reinoculation effect without co-inoculation was also 2.3-fold the reference rate (345 L ha^{-1}), with a maximum grain yield of 1,014 kg ha^{-1}.

The BRS-Estilo cultivar without co-inoculation treated with 2-fold the reference rate (300 L ha^{-1}) resulted in a grain yield of 1,625 kg ha^{-1}, and the use of co-inoculation resulted in a maximum grain yield of 1,990 kg ha^{-1} (Figure 9C). The grain yield of the additional treatments (mineral N fertilizer application) was 67 % higher (2,344 kg ha^{-1}) than that of the triple factorial (1,403 kg ha^{-1}). Thus, the control treatments without reinoculation resulted in grain yields of only 548 kg ha^{-1} (BRS-Estilo) and 426 kg ha^{-1} (BRS-Esteio) (Figure 9C). A similar result (487 kg ha^{-1}) was observed for the grain yield of plants without co-inoculation, regardless of the cultivar (Figure 9B).

This is a pioneer study regarding combination of seed inoculation + reinoculation combined with co-inoculation. The results were compared and showed to be consistent with those found by Sousa

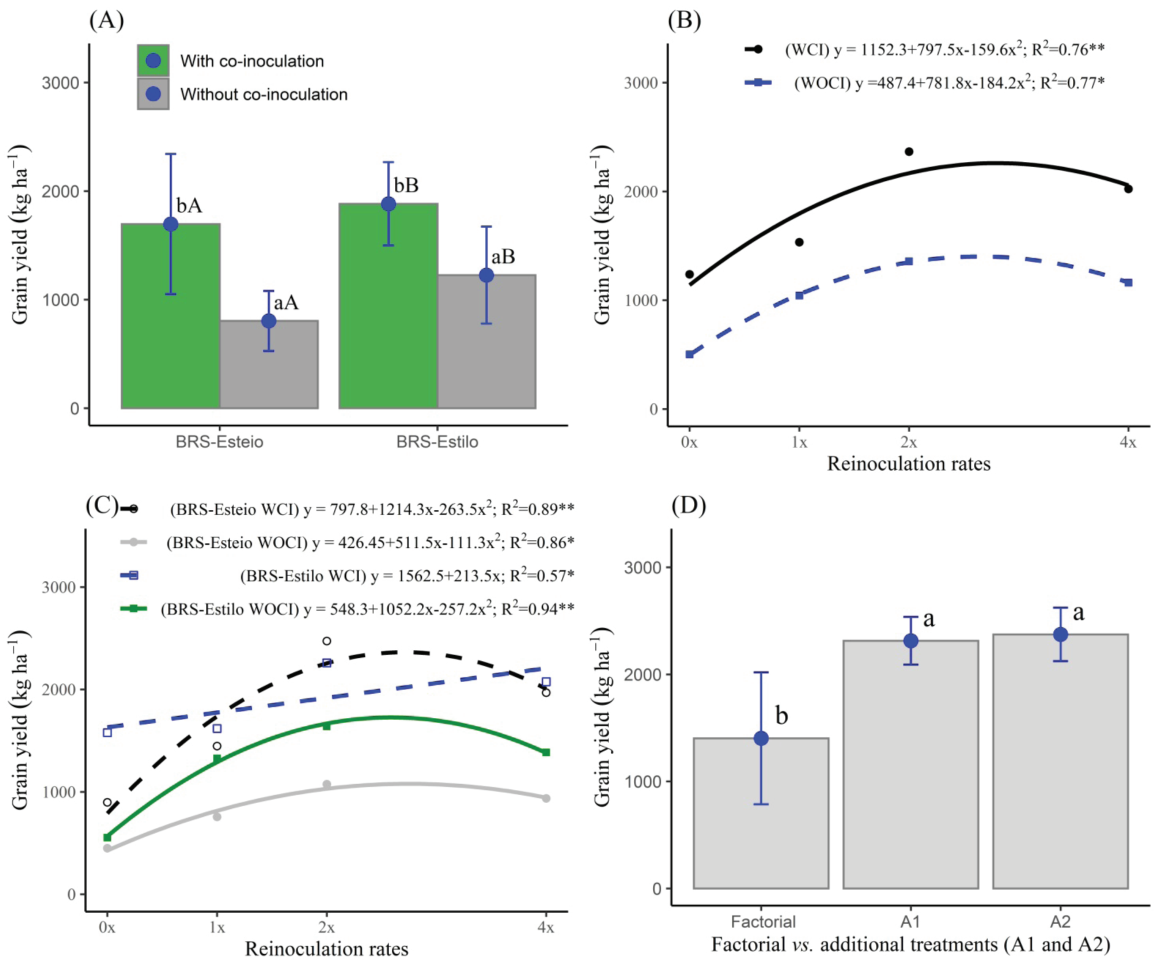

Figure 9. Grain yield as a function of cultivars with or without co-inoculation with *Azospirillum brasilense* (A), co-inoculation and reinoculation rates (B), triple factorial (cultivars *vs.* reinoculation rates *vs.* co-inoculation) (C) and factorial *vs.* additional treatments (D). A1: additional treatment 1 - BRS-Estilo with mineral N; A2: additional treatment 2 - BRS-Esteio with mineral N. Bars with the same letter are not different from each other by the Tukey test at 5 % of probability. Lowercase letters compare cultivars within each co-inoculation and uppercase letters compare co-inoculation within each cultivar. WCI: with co-inoculation with *Azospirillun*; WOCI: without co-inoculation with *Azospirillum*.

et al. (2021), who evaluated the response of two common bean cultivars to topdressing reinoculation and found that seed inoculation combined with additional topdressing inoculation (reinoculation) resulted in an increase of 2,827 kg ha^{-1} in grain yield for the BRS-Valente cultivar, when compared to N fertilizer applications with 20 and 40 kg ha^{-1} of mineral N at planting and as topdressing, respectively. Despite the fact that they did not use co-inoculation with *A. brasilense*, it is possible to note a positive trend for the use of reinoculation with *Rhizobium tropici* as topdressing, what is shown in the present study for the co-inoculation with *A. brasilense*; an unusual practice that has shown promising results (Sousa et al. 2021, Teixeira et al. 2022).

CONCLUSIONS

1. Reinoculation involving the combination of *Rhizobium tropici* with *Azospirillum brasilense* affects nodulation and morphological characteristics of common bean plants;
2. Reinoculation with *R. tropici* at the rate of 2.5-fold the reference rate (375 L ha^{-1}), combined with co-inoculation with *A. brasilense* at the rate of 100 L ha^{-1}, at the V4 stage, results in higher grain yields for the BRS-Esteio common bean cultivar, which is a grain yield similar to those found with the application of mineral N fertilizer;
3. The reinoculation with *R. tropici* combined with *A. brasilense* for complementation of seed

inoculation can totally substitute the supplying of mineral N to common bean crops.

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REFERENCES

ANDRAUS, M. P.; CARDOSO, A. A.; FERREIRA, E. P. B. Differences in nodulation and grain yield on common bean cultivars with different growth cycles. *Communications in Soil Science and Plant Analysis*, v. 47, n. 9, p. 1148-1161, 2016.

ARF, M. V.; BUZETTI, S.; ARF, O.; KAPPES, C.; FERREIRA, J. P.; GITTI, D. C.; YAMAMOTO, C. J. T. Fontes e épocas de aplicação de nitrogênio em feijoeiro de inverno sob sistema plantio direto. *Pesquisa Agropecuária Tropical*, v. 41, n. 3, p. 430-438, 2011.

BARBOSA, C. K. R.; REIS, J. N.; BRIGANTE, G. P.; FRANCO JUNIOR, K. S. Adubação nitrogenada, inoculação e coinoculação na cultura do feijoeiro-comum. *Caderno de Ciências Agrárias*, v. 12, n. 1, p. 1-6, 2020.

BRASIL. Instituto Nacional de Meteorologia. *Banco de dados meteorológicos*. 2022. Available at: https://portal.inmet.gov.br/. Access on: Jan. 25, 2022.

BRASIL. Instrução normativa SDA nº 13, de 24 de março de 2011. Aprova as normas sobre especificações, garantias, registro, embalagem e rotulagem dos inoculantes destinados à agricultura, bem como as relações dos micro-organismos autorizados e recomendados para produção de inoculantes no Brasil, na forma dos Anexos I, II e III, desta instrução normativa. *Diário Oficial da União*, Brasília, DF, 25 mar. 2011. Seção 1, p. 3.

CARDOSO, M. R. D.; MARCUZZO, F. F. N.; BARROS, J. R. Classificação climática de Köppen-Geiger para o estado de Goiás e o Distrito Federal. *Acta Geográfica*, v. 8, n. 16, p. 40-55, 2014.

COELHO, L. G. F.; BONFIM, C. A.; MENDES, I. C.; VALE, H. M. M.; REIS JÚNIOR, F. B. *Inoculação do feijoeiro no Brasil*: alternativas para aumentar a produtividade utilizando microrganismos promotores do crescimento vegetal. Planaltina, DF: Embrapa Cerrados, 2021.

EL-BELTAGI, H. S.; HASHEM, F. A.; MAZE, M.; SHALABY, T. A.; SHEHATA, W. F.; TAHA, N. M. Control of gas emissions (N_2O and CO_2) associated with applied different rates of nitrogen and their influences on growth, productivity, and physio-biochemical attributes of green bean plants grown under different irrigation methods. *Agronomy*, v. 12, n. 2, e249, 2022.

FUKAMI, J.; OLLERO, F. J.; LA OSA, C.; VALDERRAMA-FERNÁNDEZ, R.; NOGUEIRA, M. A.; MEGIA, M.; HUNGRIA, M. Antioxidant activity and induction of mechanisms of resistance to stresses related to the inoculation with *Azospirillum brasilense*. *Archives of Microbiology*, v. 200, n. 8, p. 1191-1203, 2018.

GALINDO, F. S.; TEIXEIRA FILHO, M. C. M.; BUZETTI, S.; LUDKIEWICZ, M. G. Z.; POLIANA, A. L. R.; TRITAPEPE, C. A. Technical and economic viability of coinoculation with *Azospirillum brasilense* in soybean cultivars in the Cerrado. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v. 22, n. 1, p. 51-56, 2018.

GALINDO, F. S.; TEIXEIRA FILHO, M. C. M.; BUZETTI, S.; SANTINI, J. M. K.; ALVES, C. J.; NOGUEIRA, L. M.; LUDKIEWICZ, M. G. Z.; ANDREOTTI, M.; BELLOTTE, J. L. M. Corn yield and foliar diagnosis affected by nitrogen fertilization and inoculation with *Azospirillum brasilense*. *Revista Brasileira de Ciência do Solo*, v. 40, e0150364, 2016.

HUNGRIA, M.; CAMPO. R. J.; SOUZA, E. M.; PEDROSA, F. O. Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. *Plant and Soil*, v. 331, n. 1, p. 413-425, 2010.

HUNGRIA, M.; NOGUEIRA, M. A.; ARAUJO, R. S. Alternative methods of soybean inoculation to overcome adverse conditions at sowing. *African Journal of Agriculture Research*, v. 10, n. 23, p. 2329-2338, 2015.

HUNGRIA, M.; NOGUEIRA, M. A.; ARAUJO, R. S. Co-inoculation of soybeans and common beans with rhizobia and azospirilla: strategies to improve sustainability. *Biology and Fertility of Soils*, v. 49, n. 7, p. 791-801, 2013.

NASCENTE, A. S.; KLUTHCOUSKI, J.; CRUSCIOL, C. A. C.; COBUCCI, T.; OLIVEIRA, P. Adubação de cultivares de feijoeiro comum em várzeas tropicais. *Pesquisa Agropecuária Tropical*, v. 42, n. 4, p. 407-415, 2012.

PELEGRIN, R.; MERCANTE, F. M.; OTSUBO, I. M. N.; OTSUBO, A. A. Resposta da cultura do feijoeiro à adubação nitrogenada e à inoculação com rizóbio. *Revista Brasileira de Ciências do Solo*, v. 33, n. 1, p. 219-226, 2009.

PERES, A. R.; RODRIGUES, R. A. F.; ARF, O.; PORTUGAL, J. R.; CORSINI, D. C. D. C. Co-inoculation of *Rhizobium tropici* and *Azospirillum brasilense* in common beans grown under two irrigation depths. *Revista Ceres*, v. 63, n. 2, p. 198-207, 2016.

R CORE TEAM. *R:* a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2016.

RONDINA, A. B. L.; SANZOVO, A. W. S.; GUIMARÃES, G. S.; WENDLING, J. R.; NOGUEIRA, M. A.; HUNGRIA, M. Changes in root morphological traits in soybean co-inoculated with *Bradyrhizobium* spp. and *Azospirillum brasilense* or treated with *A. brasilense* exudates. *Biology and Fertility of Soils*, v. 56, n. 4, p. 537-49, 2020.

SANTOS, H. G.; JACOMINE, P. K. T.; ANJOS, L. H. C.; OLIVEIRA, V. A.; LUMBRERAS, J. F.; COELHO, M. R.; ALMEIDA, J. A.; ARAUJO FILHO, J. C.; OLIVEIRA, J. B.; CUNHA, T. J. F. *Sistema brasileiro de classificação de solos.* 3. ed. Brasília, DF: Embrapa, 2018.

SILVA, E. A.; BARBOSA, E. R.; COSTA, C. M. da; SILVA, G. G. da; TEODORO, H. L. C.; CUNHA, L. T. Ação de fungicidas na fixação biológica do nitrogênio em feijoeiro. *Revista Agroveterinária do Sul de Minas*, v. 2, n. 1, p. 21-32, 2020.

SILVA, F. C. D. S. *Manual de análises químicas de solos, plantas e fertilizantes.* Rio de Janeiro: Embrapa Solos, 2009.

SOARES, B. L.; FERREIRA, P. A. A.; RUFINI, M.; MARTINS, F. A. D.; OLIVEIRA, D. P.; REIS, R. P.; ANDRADE, M. J. B.; MOREIRA, F. M. S. Agronomic and economic efficiency of common-bean inoculation with hizobia and mineral nitrogen fertilization. *Revista Brasileira de Ciência do Solo*, v. 40, e0150235, 2016.

SORATTO, R. P.; CATUCHI, T. A.; SOUZA, E. F. C.; GARCIA, J. L. N. Densidade de plantas e fertilização com nitrogênio na nutrição e produtividade do feijoeiro. *Revista Caatinga*, v. 30, n. 2, p. 670-678, 2017.

SOUSA, W. S.; SORATTO, R. P.; PEIXOTO, D. S.; CAMPOS, T. S.; SILVA, M. B.; SOUZA, A. G. V.; TEIXEIRA, I. R.; GITARI, H. I. Effects of Rhizobium inoculum compared with mineral nitrogen fertilizer on nodulation and seed yield of common bean: a meta-analysis. *Agronomy for Sustainable Development*, v. 42, e52, 2022.

SOUSA, W. S.; TEIXEIRA, I. R.; CAMPOS, T. S.; SILVA, G. C.; SILVA, M. B.; MOREIRA, S. G. Supplementary reinoculation in topdressing of *Rhizobium tropici* in common bean crop: effects on nodulation, morphology, and grain yield. *Journal of Plant Nutrition*, v. 45, e20, 2021.

STEINER, F.; FERREIRA, H. C. P.; ZUFFO, A. M. Can co-inoculation of *Rhizobium tropici* and *Azospirillum brasilense* increase common bean nodulation and grain yield? *Semina: Ciências Agrárias*, v. 40, n. 1, p. 81-98, 2019.

TEIXEIRA, I. R.; LOPES, P. R.; SOUSA, W. S.; SILVA, G. C. Response of common bean to rhizobium reinoculation in topdressing. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v. 26, n. 4, p. 274-282, 2022.

UNITED STATES DEPARTMENT OF AGRICULTURE (USDA). Soil Survey Staff. *Keys to soil taxonomy.* 12. ed. Washington, DC: USDA, 2014.