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Response of common bean to *Rhizobium* reinoculation in topdressing[1]

Resposta do feijoeiro à reinoculação de rizóbio em cobertura

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HIGHLIGHTS:

The number of nodules, nodule dry mass, and dry mass of each nodule of common bean increases with topdressing inoculation.
Reinoculation of rhizobia in the vegetative stage increases the dry mass of the common bean.
The common bean yield increases with the application of the inoculum in topdressing at the V4 stage.

ABSTRACT: The response capacity of the bean to fix atmospheric nitrogen is questionable, mainly due to its inability to supply all the nitrogen in the flowering and grain filling phases when the crop needs it most. Thus, a new application of inoculant can keep the population of rhizobia in the soil at adequate levels, meeting all the nitrogen demands of the plant. This study aimed to investigate the nodulation capacity and the production of beans submitted to doses and reinoculation of *Rhizobium* in topdressing under field conditions in two growth stages. For this, an experiment was conducted using a randomized block design with four replicates in a 4 × 2 + 2 factorial scheme. The treatments consisted of the application of four doses of liquid inoculant containing *Rhizobium tropici* (SEMIA 4088), in the concentration 2 × 109 CFU g^{-1}, in topdressing (0, 100, 200 and 400 mL ha^{-1}), in two development stages (V4 and R5) of plants, and two additional treatments (inoculation via seed at a dose of 100 g of the product per 50 kg of seeds and mineral nitrogen fertilization at a dose of 16 kg ha^{-1} applied at sowing and 60 kg ha^{-1} in topdressing, divided into two stages, with half being applied at the stage V3 and the other half in V4 stage).The inoculant application increased the nodulation rates of bean cultivar BRS Cometa and the dry biomass produced by plants, using doses of 232 and 221 mL ha^{-1}, respectively. The dose of 257mL ha^{-1} of the liquid inoculant applied in topdressing at the V4 stage, and the inoculation via seed provide greater common bean yield without supplementing mineral nitrogen.

Key words: *Phaseolus vulgaris* L., biological N fixation, application technology

RESUMO: A capacidade de resposta do feijoeiro em fixar o nitrogênio atmosférico é questionável, principalmente devido à sua incapacidade de fornecer todo o nitrogênio nas fases de floração e enchimento de grãos, quando a cultura mais necessita. Assim, uma nova aplicação de inoculante pode manter a população de rizóbios do solo em níveis adequados, atendendo toda a demanda de nitrogênio da planta. Deste modo, o este trabalho tem como objetivo investigar a capacidade de nodulação e a produção de feijão comum, submetido a doses de reinoculação de *Rhizobium* em cobertura, em dois estádios fenológicos da cultura. Para tanto, foi conduzido um experimento no delineamento de blocos casualizados, em esquema fatorial 4 × 2 + 2, com quatro repetições. Os tratamentos consistiram na aplicação de quatro doses de inoculante líquido *Rhizobium tropici* (SEMIA 4088), concentração de 2 × 109 CFU g^{-1}, adicionado em cobertura (0, 100, 200 e 400 mL ha^{-1}), em dois estádios de desenvolvimento da planta (V4 e R5) e dois tratamentos adicionais (inoculação via semente na dose de 100 g do produto por 50 kg de sementes e adubação mineral de nitrogênio na dose de 16 kg ha^{-1} aplicado na semeadura e 60 kg ha^{-1} em cobertura, fracionada em duas etapas, sendo metade aplicada no estádio V3 e a outra metade no estádio V4). A aplicação do inoculante aumentou a taxa de nodulação do feijoeiro cultivar BRS Cometa, bem como a biomassa seca produzida pelas plantas, com o emprego de doses respectivamente de 232 e 221 mL ha^{-1}. A dose de 257 mL ha^{-1} do inoculante líquido aplicado no estádio V4 e a inoculação via semente proporcionaram maior produtividade de feijão sem a necessidade de suplementação de nitrogênio mineral.

Palavras-chave: *Phaseolus vulgaris* L., fixação biológica de N, tecnologia de aplicação

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INTRODUCTION

The biological nitrogen fixation (BNF) is predominant in plants belonging to the Fabaceae family, like beans, providing a good amount of N necessary for the satisfactory development of the plants if a set of fully functional nodules is available during the plant cycle. For Barbosa et al. (2018), BNF under field conditions can supply up to 125 kg ha^{-1} of N for beans, depending on the availability of N in the soil, traits of the rhizobia, water availability, and genetic material cultivated.

A study conducted by Araújo et al. (2007) verified that only one inoculant application with SEMIA 4077/SEMIA 4080 strains in bean enabled to obtain the highest grain yield in bean, 1.783 kg ha^{-1}, compared to mineral fertilization with N and control (without inoculant or nitrogen fertilization), whose average yields were 1.314 and 950 kg ha^{-1}, respectively. Inoculation can also provide bean yields close to those obtained in crops that have adopted a high technological level in the activity, ranging from 2.500 to 3.500 kg ha^{-1} (Andraus et al., 2016).

Most of the nodules in Fabaceae roots are formed by applying the inoculant via seed before sowing since the host plant can self-regulate the nodulation, controlling the number of nodules (Tanabata & Ohyama, 2014). However, the initial formation of nodules is critical for common bean crops, beginning between 15 and 20 days after sowing (Araújo et al., 1996). In this sense, thepresence of nodules that makes available N$_2$ throughout the plant growth cycle may represent a key strategy to provide the nutrient N required by the common bean, contributing to yield gains (Moretti et al., 2018; Zhou et al., 2021), which can be achieved with the reinoculation in topdressing to the flowering stage. However, one of the limitations of rhizobia reinoculation in the bean is the lack of scientific studies on the issue.

Thus, this study aims to evaluate the nodulation capacity and the production of bean submitted to doses reinoculation of *Rhizobium* in topdressing, performed at two stages of plant growth.

MATERIAL AND METHODS

An experiment was conducted in the rainy season of the 2019/2020 harvest in the experimental area of the State University of Goiás, Ipameri Campus, in Ipameri, GO, Brazil at 17º 43' 27" S; 48º 08' 55" W, and 820 m of altitude. The regional climate is Aw-type, with precipitation and anaverage annual temperature of 1750 mm and 25 ºC, respectively (Cardoso et al., 2014).

Sowing was carried out manually, on November 16, 2019, in an area where no beans had been cultivated and without the use of any inoculants. With a predominance of *Brachiaria decumbens* destined for animal grazing, with a low quantitative potential of native nodulating bean bacteria.

The weather information regarding precipitation and maximum and minimum air temperatures during the experimental period is shown in Figure 1. They were obtained from the database of the National Institute of Meteorology (Inmet), conventional meteorological station (83522) in Ipameri, Goiás state, Brazil.

Soil samples, classified as Oxisol, were collected in the 0-20 cm layer, and physical-chemical analyses were performed. The results are shown in Table 1.

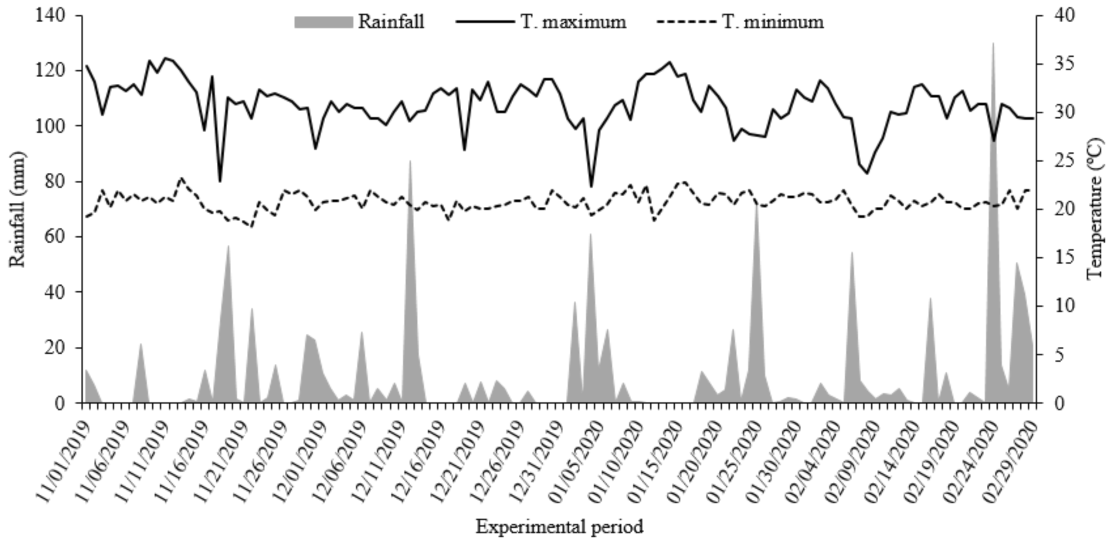


Figure 1. Rainfall (mm) and maximum and minimum air temperature (ºC) during the experimental period

Table 1. Physical-chemical attributes of the soil in the experimental area

Ca	Mg	Al	H + Al	K	P	Na	Zn	B	Cu	Fe	Mn
(cmol$_c$ dm^{-3})				(mg dm^{-3})							
5.0	1.2	0.0	3.4	188.2	11.2	10.4	9.3	0.19	1.1	27.9	33.8

V	MO	C	pH	CTC	Clay	Silt	Sand
(%)	(g dm^{-3})		(CaCl$_2$)	(cmol$_c$ dm^{-3})	(g kg^{-1})		
66.39	40	23.20	5.2	10.13	340.0	90.0	570.0

Ca - Calcium; Mg - Magnesium; Al - Aluminum; H - Hydrogen; K - Potassium; P (Melich) - Phosphorus; Na -Sodium; Zn - Zinc; B - Boron; Cu - Copper; Fe - Iron; Mn - Manganese; V - Base saturation; MO - Organicmatter; C - Carbon; pH - Hydrogenpotential; CEC - Cation exchange capacity

A randomized block design arranged in a 4 × 2 + 2 factorial scheme was used with four replicates. The treatments consisted of the inoculant liquid application in topdressing, besides the seed inoculation, with the doses 0, 100, 200 and 400 mL ha^{-1}. The liquid inoculant was applied in two growth stages (V4 and R5), plus two additional treatments (seed inoculation and fertilization with mineral nitrogen in sowing and topdressing).

Biomax Premium® was used, with strains of *Rhizobium tropici* (SEMIA 4088) recommended for common bean. In the inoculation via seed, the peat inoculant (2 × 10^9 UFC g^{-1}) was applied following the product recommendations, at the dose of 100 g of the product per 50 kg of seeds. The choice of inoculant liquid doses to be tested was based on the dosage of 150 mL ha^{-1} recommended by the manufacturer for common bean, adding quantities below and above the recommended.

The experimental unit consisted of six rows of 5.0 m length, spaced 0.50 m apart, using the four central rows as a useful area; two rows were removed to evaluate nodulation and plant growth, and the other two rows were used to assess agronomic traits at harvest. The two side rows were considered borders.

Initially, *Brachiaria decumbens* plants were desiccated using the herbicide Glyphosate (2.0 L ha^{-1}). Subsequently, the soil preparation was carried out with a plow and two harrowing operations. According to the soil analysis, the fertilization in the sowing furrow was carried out using 400 kg ha^{-1} of the NPK formulation 04:25:15 in all treatments. The use of fertilizer formulated at sowing, containing 16 kg N ha^{-1} was made to promote the greater initial start-up of the plants, since the cultivated soil presents a low amount of the population of bacteria native to bean, as suggested by Cassini & Franco (2006), and employed in other studies involving bean inoculation (Peres et al., 2016).

Initially, the inoculant was applied via seed with a 10% sugar solution to ensure greater product adhesion. After drying the seeds in the shade, sowing was carried out; no chemical treatment with insecticide/fungicide was applied to prevent the death of the inoculated bacteria. For treatments that received reinoculation with rhizobia, a 20 L backpack sprayer was used, using a fan-type nozzle and a spray volume of 200 L ha^{-1}, sprayed on the lower part of the plant at the height of 20 cm, aiming to reach the soil, always by the end of the afternoon (5:00 p.m.), with the soil showing adequate moisture, since planting occurred in the spring-summer season, with regular rainfall distribution.

Sowing was carried out with 15 seeds BRS Cometa cultivar per meter, and later thinning was carried out, adjusting for 12 plants per meter at the V2 phenological stage. At V3 and V4 stages, nitrogen fertilizer was applied in topdressing at 60 kg ha^{-1}, with urea as the source in a fractional dose, only in the mineral nitrogen fertilizer treatment.

Ants (*Atta* sp.) were controlled with the distribution of granulated Mirex - S (Sulfluramine) baits in the initial phase of the experiment. The insecticide Decis® 25 EC (30 mL 100 L^{-1}) was used to control leafhopper (*Empoasca kraemeri*), corn rootworm (*Diabrotica speciosa*), podborer (*Etiella zinckenella*), and aphid (*Aphis craccivora*). The insecticide Galil® SC (300 mL ha^{-1}) was used to control whitefly (*Bemisia tabaci*). For preventive control of fungal diseases caused by white mold (*Sclerotinia sclerotiorum*), anthracnose (*Colletotrichum lindemuthianum*), and powdery mildew (*Erysiphe polygoni*), the fungicide Cercobin® 700 WP (70 g p.c. 100 L^{-1} of water) was applied. The applications of these products were made only when necessary, obeying the doses and time of withdrawal of the products so as not to affect the activity of the N-fixing bacteria.

By the end of the R6 stage, corresponding to approximately 15 days after applications of the reinoculation treatments and coinciding with a high demand for photoassimilates due to the internal accumulation of reserves by the seeds, 20 plants (10 plants per row) were sampled in each plot, corresponding to the second and fifth row of the plot, to determine the characteristics of modulation (number of nodules, nodule dry mass, and dry mass of each nodule) and the total dry biomass, root dry mass, stem dry mass, leaf dry mass, pod dry mass, dry mass of each pod, stem diameter, plant height, and root length. The plants were sampled with a straight shovel aid, removing a soil block to keep the root system intact. Initially, the root and shoot were separated, and then the root system and shoot were washed, and evaluations were carried out.

After harvesting, carried out at the R9 stage, ten plants were taken randomly in each useful area of the plot (3rd and 4th rows) to evaluate the primary components (number of pods, number of grains per pod, and 100-grain weight). Subsequently, all plants were harvested to assess grain yield. The grain weight was corrected to 13% moisture.

The data were previously subjected to normality and homoscedasticity tests of variances. After these procedures, the collected data were subjected to analysis of variance. The Rhizobia doses were submitted to regression analysis. Using the F test ($p \leq 0.05$), the significance of the contrast was obtained, comparing the mean of the factorial treatments with additional treatments (factorial versus additional inoculated seed and factorial versus additional nitrogen fertilization). The statistical analysis software Sisvar® 5.6 was used to analyze the data (Ferreira, 2011).

RESULTS AND DISCUSSION

The analysis of variance of the data (Table 2) revealed a significant difference between the phenological stages of common bean for the variables total dry biomass (BIO) and grain yield (YIELD). As for the inoculant doses factor, applied in topdressing besides the seed inoculation, a significant difference was observed for the number of nodules (NN), nodule dry mass (NDM), BIO, leaf dry mass (LDM), dry mass of each pod (DMEP), number of grains per pod (NGPP) and YIELD. The interaction between the factors studied (Stages × Doses) was significant for root dry mass (RDM), stem dry mass (SDM), pod dry mass (PDM), plant height (PH), and the 100-grain weight (W100). When comparing the additional treatments with the phenological stages and inoculant doses, the F test found a significant effect only for the variables RDM, LDM and PDM.

Table 2. Summary of analysis of variance of the evaluated characteristics, according to the stages of inoculant application (S) and its referred doses (D), and two additional treatments (Ad.)

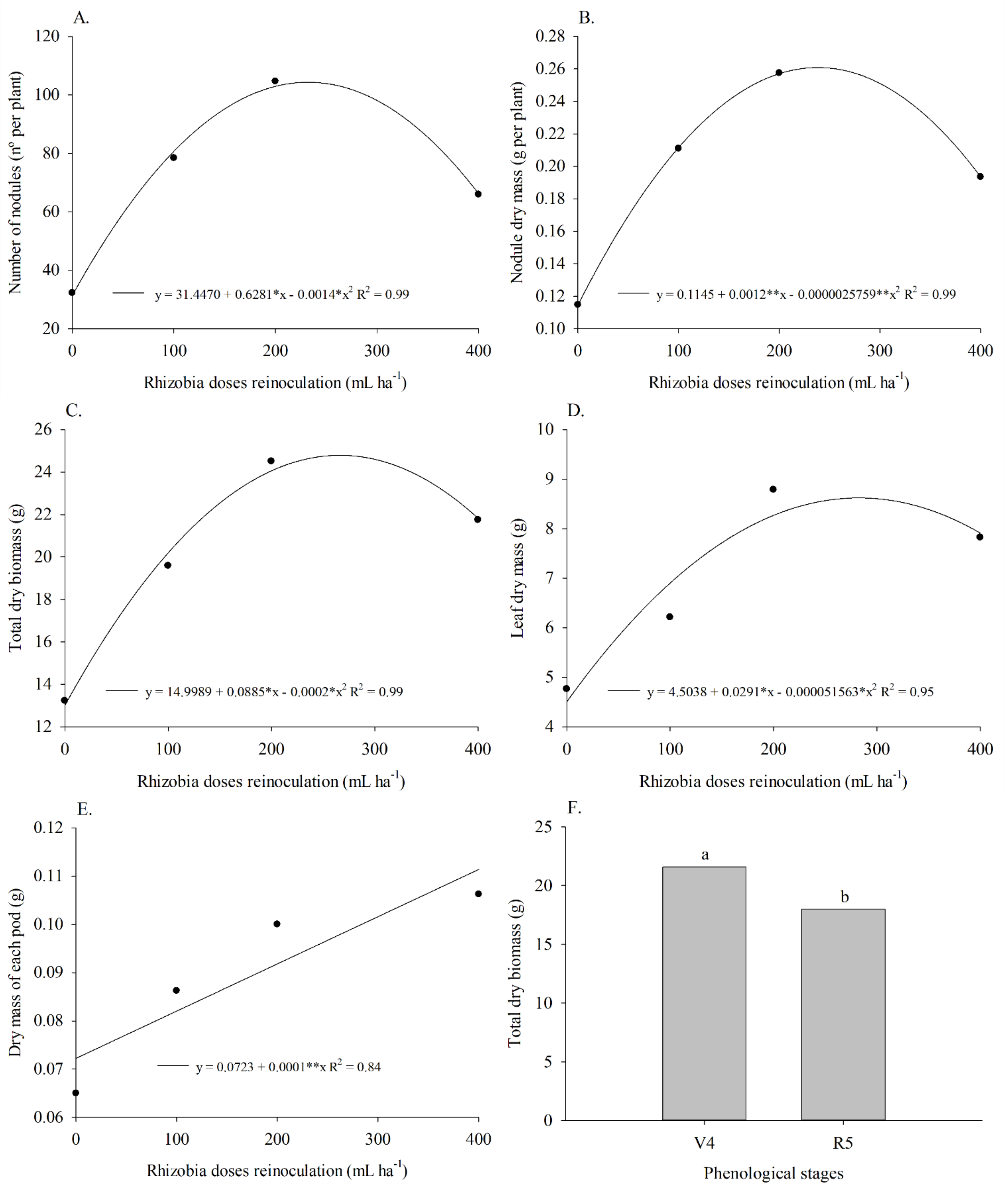
SV	DF	Mean squares			
		NN	**NDM**	**DMEN**	**BIO**
Blocks	3	783.485**	0.001^{ns}	0.000*	4.588^{ns}
Stages (S)	1	469.711^{ns}	0.000^{ns}	0.000^{ns}	102.961**
Doses (D)	3	7225.280**	0.028**	0.000^{ns}	184.752**
S x D	3	110.431^{ns}	0.001^{ns}	0.000^{ns}	3.387^{ns}
Factorial vs Ad. inoculated seed	1	178.101^{ns}	0.001^{ns}	0.002^{ns}	0.812^{ns}
Factorial vs Ad. mineral N	1	104.241^{ns}	0.052^{ns}	0.001^{ns}	9.346^{ns}
Residue	27	118.355	0.001	0.000	3.717
CV (%)	-	15.8	16.8	22.9	9.9
		RDM	**SDM**	**LDM**	**PDM**
Blocks	3	0.397^{ns}	2.180^{ns}	2.356^{ns}	0.117*
Stages (S)	1	0.245^{ns}	56.445**	3.315^{ns}	0.381**
Doses (D)	3	3.263**	63.437**	25.226**	0.289**
S x D	3	0.684*	4.184*	1.106^{ns}	0.481**
Factorial vs Ad. inoculated seed	1	1.744**	0.148^{ns}	7.464*	0.237**
Factorial vs Ad. mineral N	1	0.143^{ns}	0.158^{ns}	3.342^{ns}	0.047^{ns}
Residue	27	0.171	1.176	1.399	0.035
CV (%)	-	21.7	9.9	17.8	15.2
		DMEP	**SD**	**PH**	**RL**
Blocks	3	0.001^{ns}	0.008^{ns}	80.492*	5.640^{ns}
Stages (S)	1	0.001^{ns}	0.002^{ns}	10.811^{ns}	2.880^{ns}
Doses (D)	3	0.003**	0.002^{ns}	17.495^{ns}	2.0167^{ns}
S x D	3	0.001^{ns}	0.009^{ns}	72.631*	5.290^{ns}
Factorial vs Ad. inoculated seed	1	0.002^{ns}	0.146^{ns}	10.456^{ns}	0.345^{ns}
Factorial vs Ad. mineral N	1	0.017^{ns}	0.001^{ns}	13.314^{ns}	0.749^{ns}
Residue	27	0.000	0.005	19.642	3.613
CV (%)	-	22.3	13.6	6.2	10.7
		NP	**NGPP**	**W100**	**YIELD**
Blocks	3	8.979^{ns}	4.229^{ns}	58.415**	330610.412**
Stages (S)	1	23.120^{ns}	2.420^{ns}	0.500^{ns}	1648610.247**
Doses (D)	3	7.383^{ns}	10.962*	6.537^{ns}	1017124.754**
S x D	3	11.643^{ns}	1.693^{ns}	18.733**	119110.901^{ns}
Factorial vs Ad. inoculated seed	1	1.645^{ns}	0.152^{ns}	7.167^{ns}	109643.405^{ns}
Factorial vs Ad. mineral N	1	1.235^{ns}	4.463^{ns}	3.239^{ns}	94289.719^{ns}
Residue	27	5.705	2.608	3.025	61746.816
CV (%)	-	17.3	22.4	8.0	14.7

SV - Source of variation; vs - Versus; NN - Number of nodules; NDM - Nodule dry mass; DMEN - Dry mass of each nodule; BIO - Total dry biomass; RDM - Root dry mass; SDM - Stem dry mass; LDM - Leaf dry leaf; PDM - Pod dry mass; DMEP - Dry mass of each pod; SD - Stem diameter; PH - Plant height; RL - Root length; NP - Number of pods; NGPP - Number of grains per pod; W100 - 100-grain weight, and YIELD - Grain yield; SV - Source of variation; DF - Degrees of freedom; CV - Coefficient of variation.
^{ns}, *, ** - Not significant and significant at $p \leq 0.05$ and $p \leq 0.01$ by the F test, respectively

The data referring to the regression analysis for the number of nodules per plant (NN) and nodule dry mass (NDM) fitted to a quadratic polynomial regression (Figure 2); the estimated doses of 224 and 232mL ha^{-1} of the inoculant resulted in the maximum point of NN (Figure 2A) and NDM (Figure 2B) with values of 101.89 units per plant and 0.25 g per plant, respectively. Thus, it is possible to infer that the inoculant applications in topdressing in the mentioned doses enabled the bean plant to enhance nodulation in terms of nodule quantity, as well as in their dry mass, results attributed to the fact that the plants overcome the limitations of self-regulation of nodulation in the initial phase, which allowed the development of new functional nodules with the realization of the reinoculation in coverage to support the increased demand for N in later stages of the growth cycle, mainly during the filling of the pods, thus corroborating the reports obtained by Moretti et al. (2018).

The total dry biomass (BIO) and leaf dry mass (LDM) also presented the quadratic polynomial as the best regression adjustment curve (Figure 2), being the estimated doses of 221 and 282 mL ha^{-1} as those of maximum effect, obtaining, respectively, of 24.79 g for BIO (Figure 2C) and 8.61g for LDM (Figure 2D). For the dry mass of each pod (DMEP), there was a linear increasewiththe doses of inoculant applied in topdressing, with an increment of 0.1 mg for each unit mL ha^{-1}of inoculant applied (Figure 2E).The results indicate that the dose of up to 252 mL ha^{-1}of the rhizobia inoculant in topdressing can further grow bean plants, according to the BIO and LDM characteristics. This result is possible due to the bean nodulatingpotential since this is a great contributing factor in the greater shoot dry mass accumulation (Brito et al., 2015).

As for the phenological stages factor of the bean, BIO presented a higher result in the V4 stage (21.56 g) when compared to the R5 stage (17.97 g) (Figure 2F). Because BIO was a product resulting from the sum of the root, leaf, and stem dry masses, corresponding to the crop vegetative period explains the greater accumulation of dry mass in these organs, generally the longest vegetative period of the plant (Santos & Gavilanes, 2006); it results in higher values of BIO in the V4 stage than in the V5 stage. This greater accumulation of biomass produced at the V4 stage is essential to guarantee the supply of photoassimilates for the plant's reproductive stages, including the flowering and grain filling stages, in which there is greater demand for the plant.

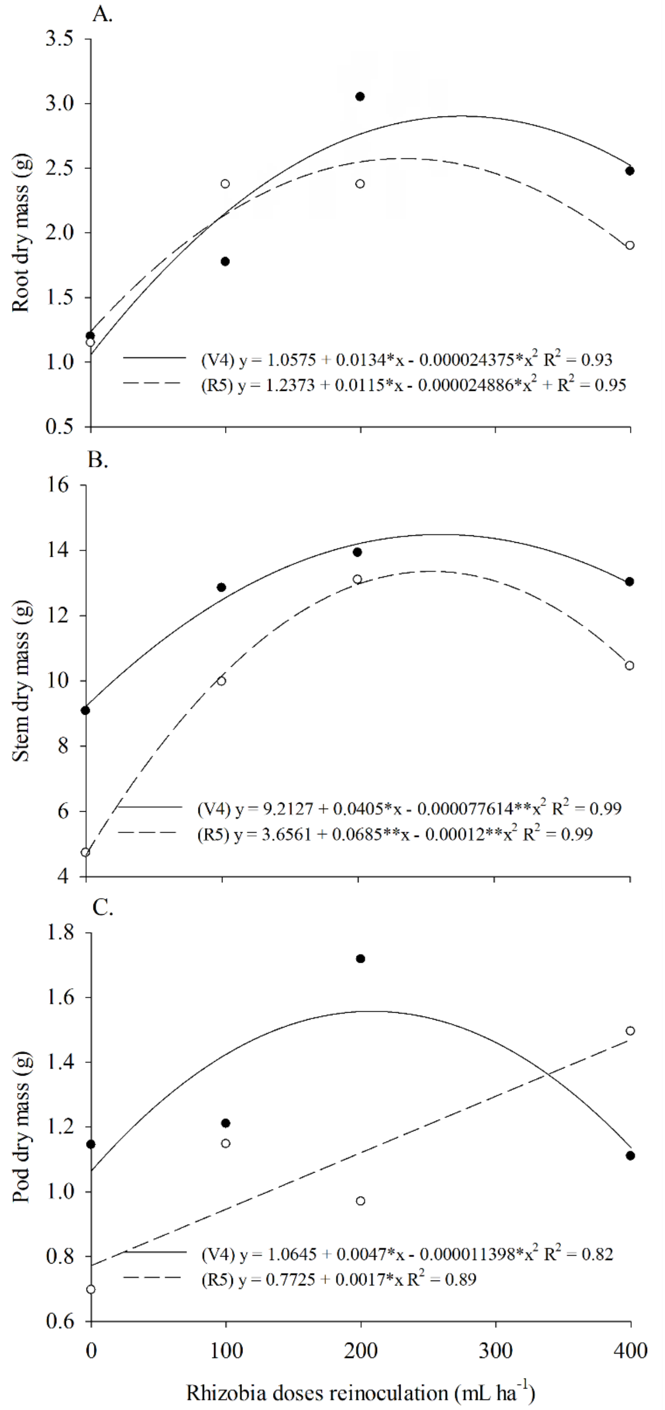


*and** - Significant at $p \leq 0.05$ and $p \leq 0.01$ by the F test, respectively; Columns followed by the same lowercase letter do not differ from each other by the F test ($p \leq 0.05$)

Figure 2. Number of nodules (A), nodule dry mass (B), total dry biomass (C), leaf dry mass (D), and dry mass of each pod (E) of the beans according to Rhizobia doses reinoculation in topdressing. Average values for total dry biomass (F) of common bean according to the phenological stages

The inoculant application in topdressing and the phenological stages of the bean showed interaction fitting better to quadratic polynomial regression for the variables root dry mass (RDM), stem dry mass (SDM), and pod dry mass (PDM) (Figure 3). There is no adjustment of the regression model to the plant height characteristic due to the great variability of the data obtained in measuring the growth of the main stem of the bean plant of Cometa cultivar, hampered by the closing of the space between rows at the time of the evaluation.

The maximum mean increment referring to the inoculant dose was found when applied at the V4 stage with an increase of 2.90 g in the RDM (Figure 3A), 14.49 g in the SDM (Figure 3B), and1.55 g in the PDM (Figure 3C), in the respective



**, *, ^{ns}- Significant at $p \leq 0.01$ and $p \leq 0.05$, and not significant by the F test, respectively

Figure 3. Root dry mass (A), stem dry mass (B), and pod dry mass (C)of the bean according to Rhizobia doses reinoculation in topdressing at twoplant phenological stages

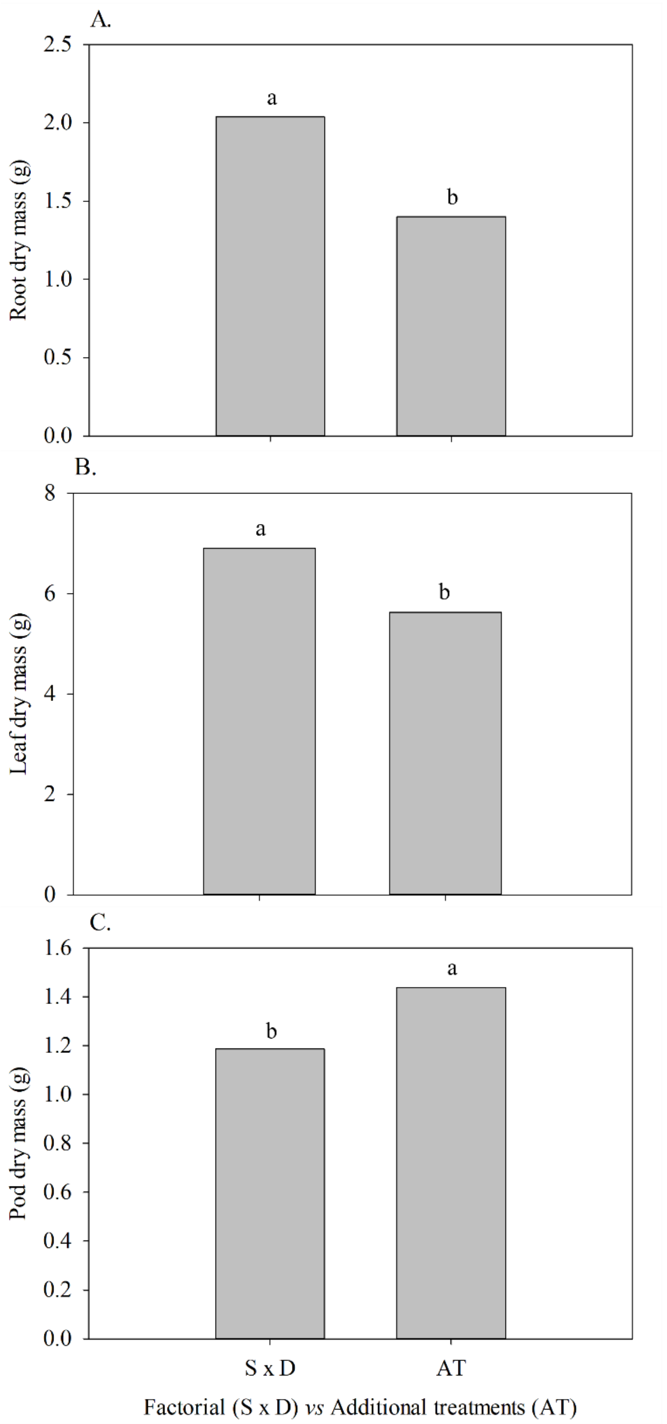
doses equivalent to 274 and 206mL ha^{-1} of the inoculant. It is noteworthy that there was a linear increase for PDM when the doses were applied in the R5 stage of the common bean. For each increased inoculant unit (mL ha^{-1}), a raise of 1.7 mg was observed for PDM (Figure 3C). This behavior is attributed to the fact that V4 is the longest growth stage of the plant when it reaches the highest dry matter accumulation rates in its different organs (Santos & Gavilanes, 2006).

Agronomic interest characteristics of common bean such as PDM may be a possible grain yield indicator, as they are strongly correlated (Didonet & Costa, 2004). Pods with higher dry mass accumulation in the grain filling period will promote heavier grains formation, decisive for production success (Araújo et al., 2012). Thus, it was found that the inoculant applications carried out in the V4 stage provided better results for RDM and SDM with a maximum efficiency point than the application made in the R5 stage; the PDM increased linearly with the inoculant doses. However, applying the dose of 187 mL ha^{-1} of the inoculant in the V4 stage, the result for PDM was 1.56 g, and to achieve the same result with application in the R5 stage, the required dosage would be 450 mL ha^{-1}. Therefore, it is possible to obtain the same result in the increase of PDM by applying the inoculant in topdressing at the V4 stage, with lower doses, and further potentiate the root and stem development of common beans, whose characteristics are essential to overcome abiotic stresses (Cury et al., 2011).

The interaction between stages and doses showed higher results for RDM (Figure 4A) and LDM (Figure 4B), with a difference in the order of 0.64 g for RDM and 1.27 g for LDM. The results were superior for PDM (Figure 4C) when using additional treatment inoculation via seed, with a statistical difference of 0.252 gper pod compared to the factorial. The inoculant in topdressing provided greater results in the variable of root and leaf dry mass than with additional treatment, which received inoculation with *Rhizobium tropici* via seed, regardless of the dose and application stage. Conversely, the pod dry mass stood out with the additional treatment inoculation with *Rhizobium tropici* via seedin terms of averages, compared to factorial treatments, that is, in topdressing.

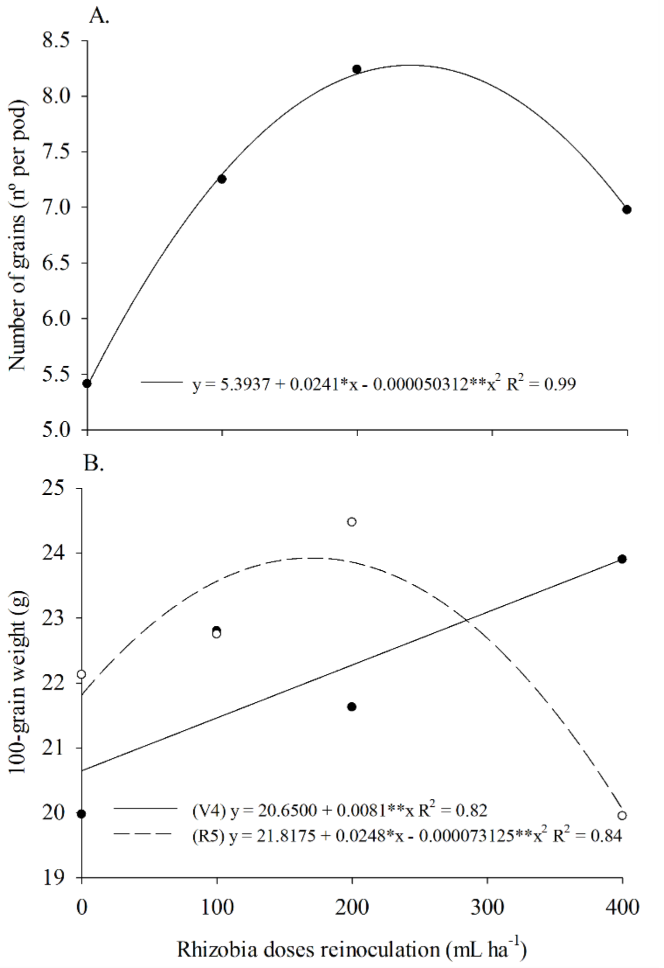
Dry matter accumulation in the plant vegetative organs results from the nodulating potential in beans; thus, being intrinsic characteristics to the plant and the rhizobia (Matoso & Kusdra, 2014). Another important characteristic to be highlighted in this process is that nodule formation occurs up to a certain period during the crop cycle. Early and semi-early cycle cultivars culminate in the formation of nodules at the R8 stage (Andraus et al., 2016). Therefore, the BRS Cometa cultivar used in this research has an early cycle when the inoculant is applied at the V4 stage. Its efficiency in forming the nodules is potentiated when applied at the R5 stage due to the greater action interval of rhizobia between the inoculant application and the final nodule formation period (R8), reflecting significantly on the accumulation of root and stem dry mass in beans.

The number of grains per pod (NGPP) according to the inoculant doses in topdressing presented a better quadratic polynomial fit of the curve, showing a maximum point in the dose of 239 mL ha^{-1} and NGPP of 8.27 units per pod in that dose (Figure 5A). The same regression adjustment occurred for an average 100-grain weight (W100) when the inoculant was applied at the R5 stage. It resulted in 23.92g for W100 at the maximum point of the 169 mL ha^{-1} dose of the inoculant in topdressing (Figure 5B). When the inoculant is applied at the V4 stage, there is a linear increase for the W100, increasing 8.1 mg for each incremented inoculant unit (mL ha^{-1}).



vs - Versus; Bars followed by the same lowercase letter do not differ from each other by the F test ($p \leq 0.05$)

Figure 4. Root dry mass (A), leaf dry mass (B), and pod dry mass (C) of reinoculated beans with rhizobia in topdressing in the factorial and additional treatments inoculated seed



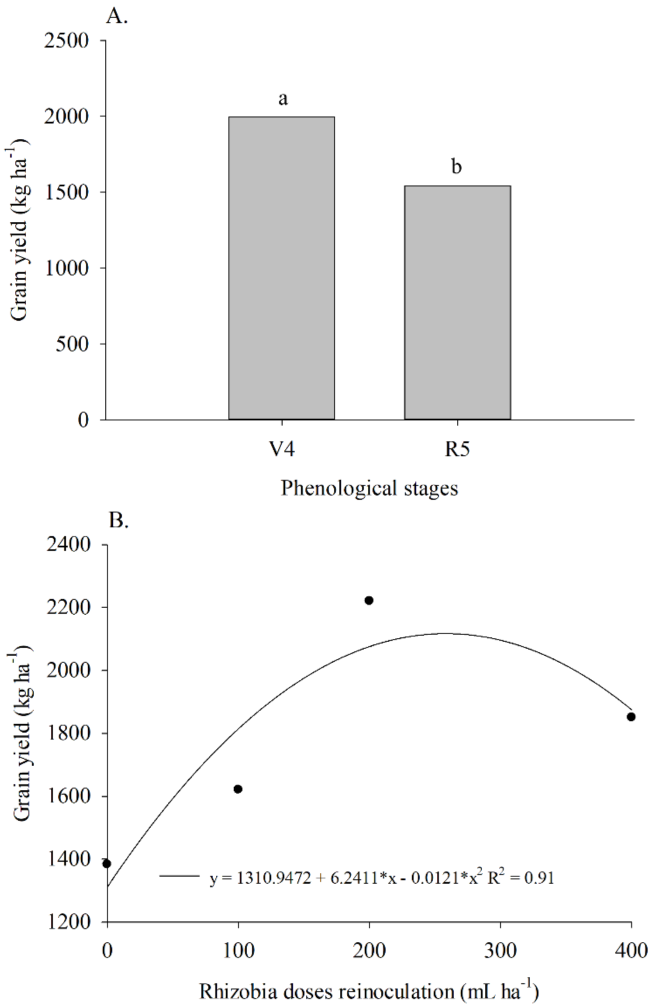
*and** - Significant at $p \leq 0.05$ and $p \leq 0.01$ by the F test, respectively

Figure 5. Number of grains per pod (A) according to Rhizobia doses reinoculation in topdressing in common bean and 100-grain weight (B) according to *Rhizobium* a doses reinoculation in topdressing applied in two phenological stages of common bean

Despite the agronomic characteristics of beans being genetically controlled (Silva et al., 2009) and suffering little influence from fertilization (Moreira et al., 2013), in the current study, NGPP was much higher than that described by Lemos et al. (2004), which is 4.8 grains per pod. This result can be inferred as a reflection of the nodulation potentiated by inoculant applications in topdressing since the N fixation and its availability to the plant are of great importance in forming pods and grain filling (Moretti et al., 2018). In this way, the optimization of BNF with applications via rhizobia in topdressing can supply N of the bean demand and increase the agronomic components.

The common bean yield was influenced by the inoculant application time in topdressing. The V4 stage application provided a higher yield (2000 kg ha^{-1}) than the application in the R5 stage (1500 kg ha^{-1}) (Figure 6A). This result can be attributed to the fact that the inoculant's earlier application provided a longer time of effectiveness of the population of bacteria inoculated in the plant root system. Regarding the effect of reinoculation doses, it can be ascertained that grain yield showed quadratic polynomial regression as the best fit (Figure 6B), which in the inoculant dose of 257 mL ha^{-1}, was obtained the maximum yield estimated at 2.116 kg ha^{-1}. The difference between the maximum effective and zero doses (without inoculant application intopdressing) was 734 kg ha^{-1}.

Finally, it is noteworthy that this study was conducted in spring-summer, known as the "rainy season", with a predominance of adequatewater availability and high temperatures, thus favoring the survival of bacterial populations in the soil, whether native or inoculated. On the other hand, in the other two bean cultivation crops, autumn-



Bars followed by the same lowercase letter do not differ from each other by the F test $(p \leq 0.05)$; *and** - Significant at $p \leq 0.05$ and $p \leq 0.01$ by the F test, respectively

Figure 6. Common bean grain yield according to the plant phenological stages (A) and Rhizobia doses reinoculation in topdressing (B)

winter and winter, called “dry” and properly “winter”, there is water restriction requiring the use of complementary (dry) or complete (winter) irrigation to the development of the crop, in addition to the occurrence of milder temperatures, especially in winter, which can affect the efficiency of populations of nodulating bacteria. Furthermore, factors such as chemical-physical soil conditions, growing environment, genetic materials also influence the ability of N-fixing bacteria to common bean plants. Thus, studies like this must be conducted in the “dry” and “winter” cropping seasons, aiming to generate knowledge for each one of them, ensuring the respective scientific validation of the reinoculation technique in the bean.

CONCLUSIONS

1. The application of the inoculant in topdressing, at the dose of 232 and 221 ml ha^{-1}, increasesthe nodulationand total dry biomass of beans BRS Cometa, respectively.

2. The dose of 257 mL ha^{-1} of the inoculant liquid applied at the V4 stage and the inoculation via seed provide greater common bean yield without supplementing mineral N.

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