

## Full Length Research Paper

# Interaction of biological nitrogen fixation and fertilization: Effects on growth and yield of common bean in the dry season

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The inoculation with *Rhizobium* together with nitrogen (N) fertilization during sowing can maximize common bean yield cultivated in the rainy season, but this interaction was not studied in the dry season cultivation. Therefore, the objective of this study was to evaluate the effects of biological nitrogen fixation (BNF) and or N fertilization on growth and yield of common bean cultivated in the dry season. Two experiments were conducted in a randomized block design with four replications. The first experiment, in 2013, had three treatments: F-25 (only fertilized with 20 kg of N ha<sup>-1</sup> at sowing and with 40 kg of N ha<sup>-1</sup> at 25 days after emergence - DAE), I-25 (only inoculated with *Rhizobium tropici* at sowing and fertilized with 40 kg N ha<sup>-1</sup> at 25 DAE) and IF-25 (inoculated with *R. tropici* and fertilized with 20 kg N ha<sup>-1</sup> at sowing and with 40 kg N ha<sup>-1</sup> at 25 DAE). The second experiment, in 2014, had the same three treatments and an additional treatment I (inoculated with *R. tropici* with no N fertilization). Three plants were collected randomly weekly, for growth analysis, which showed the highest biomass and leaf area accumulation and, consequently, highest grain yield of common bean in the treatment IF-25. The results indicated that in the dry season, the inoculation with *Rhizobium tropici* might replace the N fertilization (20 kg ha<sup>-1</sup>) at sowing without yield loss for common bean cultivation in a low-cost agriculture. Nevertheless, the N fertilization (20 kg ha<sup>-1</sup>) together with inoculation with *Rhizobium tropici* at sowing did not inhibit root nodulation, increasing growth and yield of common bean for a high-cost agriculture. However, more studies are required with other cultivars and sites, to recommend these agronomic practices in the cultivation of common bean in the dry season.

**Key words:** Inoculation, fertilization, *Rhizobium*, *Phaseolus vulgaris*, growth.

## INTRODUCTION

Brazil is the largest world's producer and consumer of common bean (*Phaseolus vulgaris* L.), an important

source of protein for the increasing world population (Vieira et al., 2006), but its average yield is one of the

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**Table 1.** Soil chemical and physical characteristics.

pH	P (mg dm <sup>-3</sup> )	OM (g dm <sup>-3</sup> )	K <sup>+</sup>	Na <sup>+</sup>	Ca <sup>+2</sup>	Mg <sup>+2</sup>	H+Al	Al <sup>+3</sup>	V (%)
			cmol <sub>c</sub> dm <sup>-3</sup>						
5.8	86	10.7	115	0.03	1.5	1.4	0.6	0.0	84

lowest in the world (Hungria and Kaschuk, 2014). However, the common bean yield has significantly increased from 500 kg ha<sup>-1</sup> in the late 1970's to 1050 kg ha<sup>-1</sup> in 2015. This increase in yield occurred mainly due to a larger participation of big farmers, who use high-cost technologies for obtaining high yields, especially in the rainy season (first crop), but also in the dry season (second crop) and winter season (third crop), both in a less extent (Conab, 2015).

However, low technology of small farmers is one of the factors still reducing common bean crop yield in Brazil (Grange et al., 2007), due to their low input agriculture and environmental stresses, especially of water and N deficiency in the dry season (Pimentel, 2006), forcing them to concentrate their production in the rainy season (first crop) (Cardoso et al., 2012; Hungria and Kaschuk, 2014). On the other hand, if the efficiency of plant N use is improved, the yield of common bean cultivated in the dry season can be increased for a low-cost small farming, if there is no other environmental stress (Pimentel, 2006).

The common bean is a C<sub>3</sub> plant and thus, it is more sensitive to N deficiency than C<sub>4</sub> plants (Pimentel, 2006). The C<sub>3</sub> plants use more than 50% of the leaf N content for synthesizing the enzyme ribulose-1.5-bisphosphate carboxylase/oxygenase (RuBisCO), which is responsible for photosynthetic assimilation of CO<sub>2</sub> to maintain plant growth and consequently, grain yield (Long et al., 2006). Nowadays, the efficient use of N is essential for yield increases, decreasing production costs and lowering risks of environmental pollution, due to the losses of N in the soil, especially in the tropics, where they are generally poor in organic matter (Cardoso et al., 2012; Hungria and Kaschuk, 2014). Mineral N fertilizers are produced from non-renewable reserves using large quantities of fossil fuel energy and thus, have a high cost for food production. Therefore, research to improve the efficiency of N use by crops is a new paradigm for modern agriculture (Pimentel, 2006; Remigi et al., 2016).

Nevertheless, common bean crop can benefit from BNF, through symbiotic relations with rhizobia air-nitrogen fixers (Cardoso et al., 2012). However, the BNF does not meet the common bean N requirements for a high yield, thus needing an addition of mineral N fertilizer (Cardoso et al., 2012; Hungria and Kaschuk, 2014). Hungria et al. (2000) have shown that common bean crops in suitable environmental conditions in the rainy season can reach yields higher than 4 t ha<sup>-1</sup> only with the use of inoculation with rhizobia. Thus, technologies for increasing crop yield at lower costs are necessary, such

as the use of cultivars with greater BNF potential or inoculation with more competitive and better-adapted rhizobia strains (Hungria and Kaschuk, 2014). The BNF process is important for the Brazilian agriculture sustainability, generating organic N at low cost and with low environmental impact when compared with mineral N fertilizers use (Grange et al., 2007). Therefore, the objective of this study was to assess the effects of biological nitrogen fixation and or N fertilization on growth and yield of common bean cultivated in the dry season.

## MATERIALS AND METHODS

### Site and climate description

Two experiments were conducted in the field, at the Experimental Station of the Department of Crop Science, Federal Rural University of Rio de Janeiro (Universidade Federal Rural do Rio de Janeiro, UFRRJ), Seropédica, RJ, Brazil (22°44' S, 43°42' W and 40 m of altitude), in May (dry season) of 2013 and 2014. The climate in the region is an Aw, according to the Köppen classification, with hot and rainy summers and dry winters.

The soil of the experimental area was classified as a Kanhapudalf soil with sandy loam texture. The chemical and physical characteristics of the soil layer 0-20 cm was presented in Table 1. During the experiments, total precipitation were of 153.5 (2013) and 139.2 mm (2014), evapotranspiration of 217.9 (2013) and 235.1 mm (2014), average maximum temperature of 28 (2013) and 28.3°C (2014) and minimum of 18.1 (2013) and 18.6°C (2014).

### Crop management

The common bean cultivar Carioca, used in the experiments, has a type-III indeterminate growth habit with an intermediate cycle (85 days) and seeds are beige with brown strips. The seeds were sowed manually, using 20 seeds per meter in rows spaced 0.5 m apart, which was thinned to a density of 12 plants per linear meter at 7 DAE. All the treatments of both experiments were fertilized with 30 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (superphosphate) and 45 kg K<sub>2</sub>O ha<sup>-1</sup> (potassium chloride) (Vieira et al., 2006). The inoculant for common bean SEMIA 4080, selected in low pH soils under high air temperatures, produced by the Embrapa Agrobiology with viable *Rhizobium tropici* cells was used to inoculate the seeds of the treatments with inoculation. During the experiments, the area was maintained free from weeds by manual control and irrigation was provided when necessary.

### Plant analysis

Three random plants were weekly collected in each plot for growth analysis, from 14 DAE to the end of the crop cycle. The leaf area was determined using a portable leaf area meter (LI-3000C, LI-COR Biosciences, USA) and the leaves, stems and roots collected were

dried at 65°C for 72 h. According to the methodology described by Hunt (1978), the TDW data was transformed to biomass per land area and the leaf area data were transformed to LAI. The CGR (crop growth rate) and NAR (net assimilation rate) were determined from the TDW and LAI data (Pereira and Machado, 1987).

In addition, samples of three plants per plot were collected at pollination stage (50% of plants in the plots with flower buds), when plants attain maximal growth and BNF (Vieira et al., 2006), which occurred at 33 DAE in 2013 and 31 DAE in 2014. In these plants, their BNF potential was assessed using the variables NN and NDW per plant, as stated by Hungria et al. (2003), and plant growth by the SDW (shoot dry weight) and RDW (root dry weight) per plant. The nodules were removed from the roots counted immediately to determine the NN and then dried at 65°C for 72 h to determine the NDW. The shoot and root were also dried at 65°C for 72 h to determine the SDW and RDW.

Furthermore, the central leaflet of the youngest fully expanded leaf of three plants were collected in each plot at the four development stages, as described by Vieira et al. (2006): vegetative (20 DAE), before N fertilization at 25 DAE; pollination (P); flowering (F) and grain filling (GF) for the first and second year of cultivation. The leaflets were immediately wrapped in foil and immersed in liquid N. These samples were used to quantify LSPC (leaf soluble protein content), according to the Bradford (1976) method.

Finally, the plants of the two central rows of each plot were collected at physiological maturity, excluding 0.5 m from each border, to determine the NP (number of pods per plant), NGP (number of grains per plant), DW100G (dry weight of 100 grains) and GY (grain yield).

### Experimental design and statistical analysis

The two experiments were conducted in a randomized block design, with three treatments in 2013 and four treatments in 2014, with four replications for both years. Each plot consisted of five rows of 5 m spaced 0.5 m apart, with a total area of 10 m<sup>2</sup> per plot. The treatments of the first experiment were: F-25 (only fertilized with 20 kg of N ha<sup>-1</sup> at sowing and with 40 kg of N ha<sup>-1</sup> at 25 days after emergence - DAE), I-25 (only inoculated with *Rhizobium tropici* at sowing and fertilized with 40 kg N ha<sup>-1</sup> at 25 DAE) and IF-25 (inoculated with *R. tropici* and fertilized with 20 kg N ha<sup>-1</sup> at sowing and with 40 kg N ha<sup>-1</sup> at 25 DAE). The second experiment was conducted in 2014 with four treatments, F-25, I-25, IF-25 and an additional treatment I (inoculated with *R. tropici* with no N fertilization). Data were subjected to analysis of variance (ANOVA), and the means were compared and segregated by the Tukey's test at 5% of significance level ( $P < 0.05$ ).

## RESULTS

### Total plant dry weight (TDW) and leaf area accumulation

The TDW of the treatments with nitrogen fertilization at sowing (IF-25 and F-25) were significantly higher than those without sowing nitrogen fertilization (I-25 and I), in some samplings from the vegetative stage to maturity (Figure 1). In 2013, differences in TDW among treatments were observed from 28 DAE (Figure 1A), and especially the TDW in IF-25 treatment was significantly higher than in F-25 and I-25 from 35 DAE until the last sampling day (77 DAE). The maximal TDW occurred at 70 DAE for both years (Figure 1). In 2014, differences in TDW were observed from 49 DAE (Figure 1B), and TDW

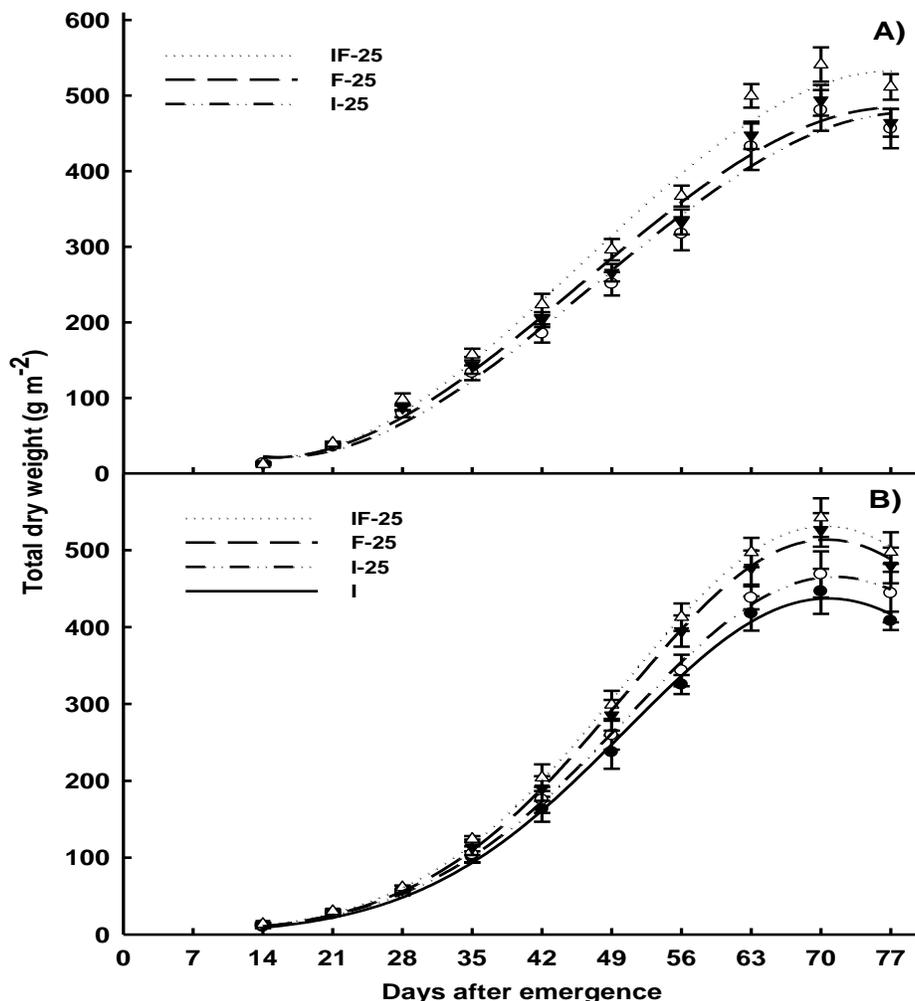
for IF-25 and F-25 treatments were significantly higher than the other two treatments until 77 DAE. In addition, significant differences of the LAI among the treatments were found in both years. The treatment IF-25 presented higher LAI in both years (Figure 2), with a peak at 63 DAE in 2013 and at 56 DAE in 2014, that is, the beginning of the grain filling stage. The treatment I-25 had a significant lower LAI than the others treatments in 2013, especially from 21 to 70 DAE (Figure 2A). In 2014, the LAI of the IF-25 and F-25 treatments were significant higher than the treatments without sowing N fertilization from 42 to 70 DAE (Figure 2B), with significantly higher LAI values than especially the I treatment, but also for I-25 treatment in several samplings.

The CGR had maximum values before the LAI peak, at 56 (2013) and 49 (2014) DAE, with significant differences between treatments in both years (Figure 3). As for TDW and LAI, the treatment IF-25 had significant higher CGR than the others treatments in the initial and final samplings of 2013 (Figure 3A). While in 2014, this treatment IF-25 showed significant differences only on samplings at 28 and 42 DAE (Figure 3B), with significantly higher CGR than the treatment I, but the treatments F-25 and I-25 did not differ from IF-25 and I (Figure 3B). The TDW peaked (Figure 1) at the end of the cycle (70 DAE), when the CGR reached negative values (Figure 3).

The NAR was high in the first sampling (14 DAE) in both years (Figure 4), and gradually decreased from this time, however, it was similar in all treatments and years evaluated, with values near zero or negative from 63 DAE (beginning of grain filling stage), together with the LAI decrease (Figure 2).

### Nodulation and plant dry weight at the pollination stage

The BNF potential was evaluated by the NN and NDW, and the plant dry weight accumulation at the pollination stage was evaluated by the SDW and RDW, which occurs at 33 (2013) and 31 (2014) DAE (Table 2). The NN and NDW of the treatments were different in both years, with significantly higher values in the treatment I-25 than in the others two treatments with N fertilization at sowing (F-25 and IF-25) in 2013. In 2014, the treatments with only inoculation without N fertilization at sowing (I and I-25) presented significantly higher NN values than the treatments F-25 but not for IF-25, as shown in Table 2. The NDW of the treatments were also significantly different (Table 2), with higher values in the treatment I-25 than F-25 and IF-25 in 2013 and in 2014, the treatment I was significantly higher as compared to the F-25, and the treatments I-25 and IF-25 did not differ from I and F-25. On the other hand, the treatment IF-25 had SDW significantly higher than the I-25 in 2013, without any significant differences in 2014 as for RDW in both years (Table 2).



**Figure 1.** Total dry weight (TDW) of common bean, under the treatments F-25 ( $\blacktriangledown$ ), I-25 ( $\circ$ ), and IF-25 ( $\Delta$ ) in the first year (A), and a fourth treatment I ( $\bullet$ ) included in the second year (B).

### During ontogeny (LSPC)

The LSPC was similar for all treatments at the vegetative stage, before the fertilization with  $40 \text{ kg N ha}^{-1}$  at 25 DAE, at the pollination stage and at the grain filling stage, in both years (Figure 5). However, the treatments were significantly different at the flowering stage, at 39 (2013) and 38 DAE (2014), in which the treatment IF-25 had significantly higher LSPC than the treatment I-25 in 2013 (Figure 5A) and I in 2014 (Figure 5B). At the grain filling stage, the LSPC was much lower than in the stages before indicating an increased N remobilization and leaf senescence (Figure 5).

### Yield components

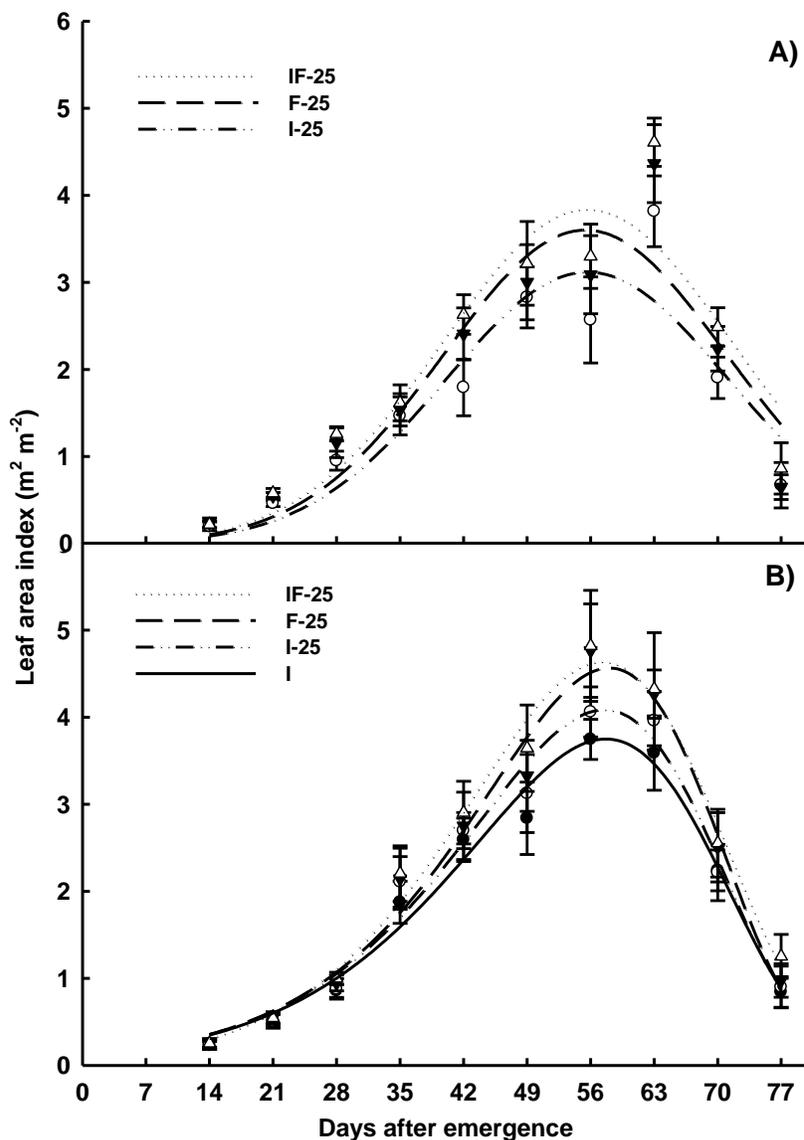
The yield components NP, NGP and DW100G of the treatments were similar in both years (Table 3), while GY of the treatments was significantly different. The treatment IF-25 had significantly higher GY than the

others in 2013 and higher than the I in 2014, however, the treatments I-25 and F-25 did not differ from IF-25 and I (Table 3).

## DISCUSSION

### Total plant dry weight and leaf area accumulation

The TDW of all treatments were significantly different in both years (Figure 1A, B). The treatments with N fertilization at sowing (F-25 and IF-25), showed significantly higher TDW from the vegetative stage to the end of the cycle, in both years. This result was probably due to an incomplete nodulation in the vegetative stage (Hungria et al., 2000), resulting in some samplings with significantly higher TDW from the vegetative stage to the end of the cycle, in both years. This result was probably due to an incomplete nodulation in the vegetative stage (Hungria et al., 2000), resulting in some samplings with lower TDW in the treatments that was only inoculation at

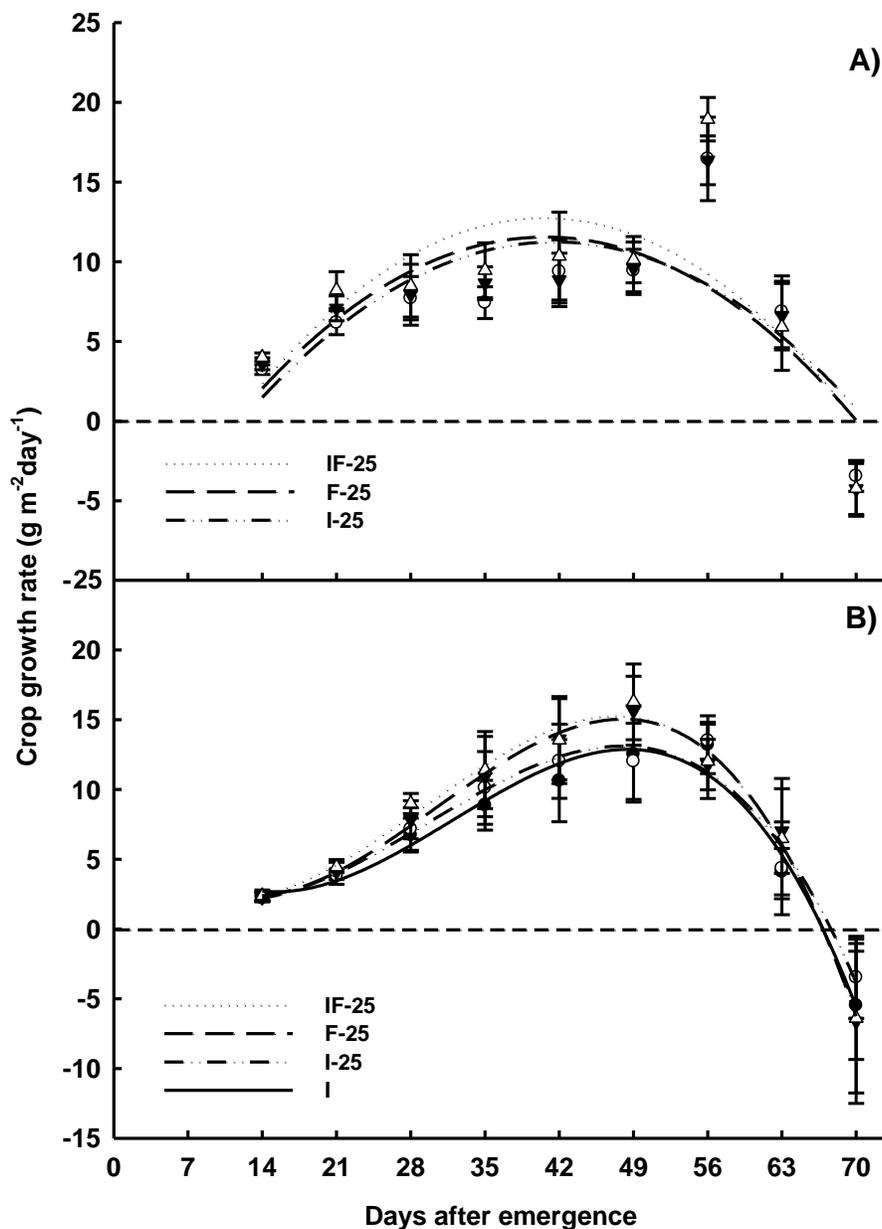


**Figure 2.** Leaf area index (LAI) of common bean, under the treatments F-25 ( $\blacktriangledown$ ), I-25 ( $\circ$ ), and IF-25 ( $\Delta$ ) in the first year (A), and a fourth treatment I ( $\bullet$ ) included in the second year (B).

sowing (I-25 and I). The treatment IF-25 presented significantly higher TDW than the others treatments in most samplings, with a peak at 70 DAE for all treatments during the grain filling stage in both years, in accordance with Gomes et al. (2000). Therefore, the start dose of 20 kg N ha<sup>-1</sup> at sowing in the dry season cultivation induced higher plant growth at initial developmental stages before the increase in BNF, as stated by Hungria et al. (2003).

In addition, the LAI of all treatments was also significantly different in both years (Figure 2A, B). As for TDW, the treatment IF-25 presented higher LAI in both years. The LAI peak occurred at 63 DAE in 2013 (Figure 2A) and at 56 DAE in 2014 (Figure 2B), during the beginning of the grain filling stage, indicating a decrease

in the LAI (leaf senescence) and photosynthesis from this stage (Pimentel et al., 1999), when photoassimilate requirements for grains is high to maintain embryo growth (Pimentel, 2006). The treatment I-25 had a significant lower LAI than the others treatments in 2013 (Figure 2A), especially from 21 to 70 DAE, while in 2014, there were more significant differences of LAI between the treatments from 42 to 70 DAE (Figure 2B). Therefore, the treatments IF-25 and F-25 had significantly higher LAI than especially the treatment I but they were also higher than the I-25 in several samplings of both years. Thus, the increase in TDW of the treatment IF-25 and F-25 promoted an increased leaf expansion and consequently LAI, which will increase total leaf photosynthesis to



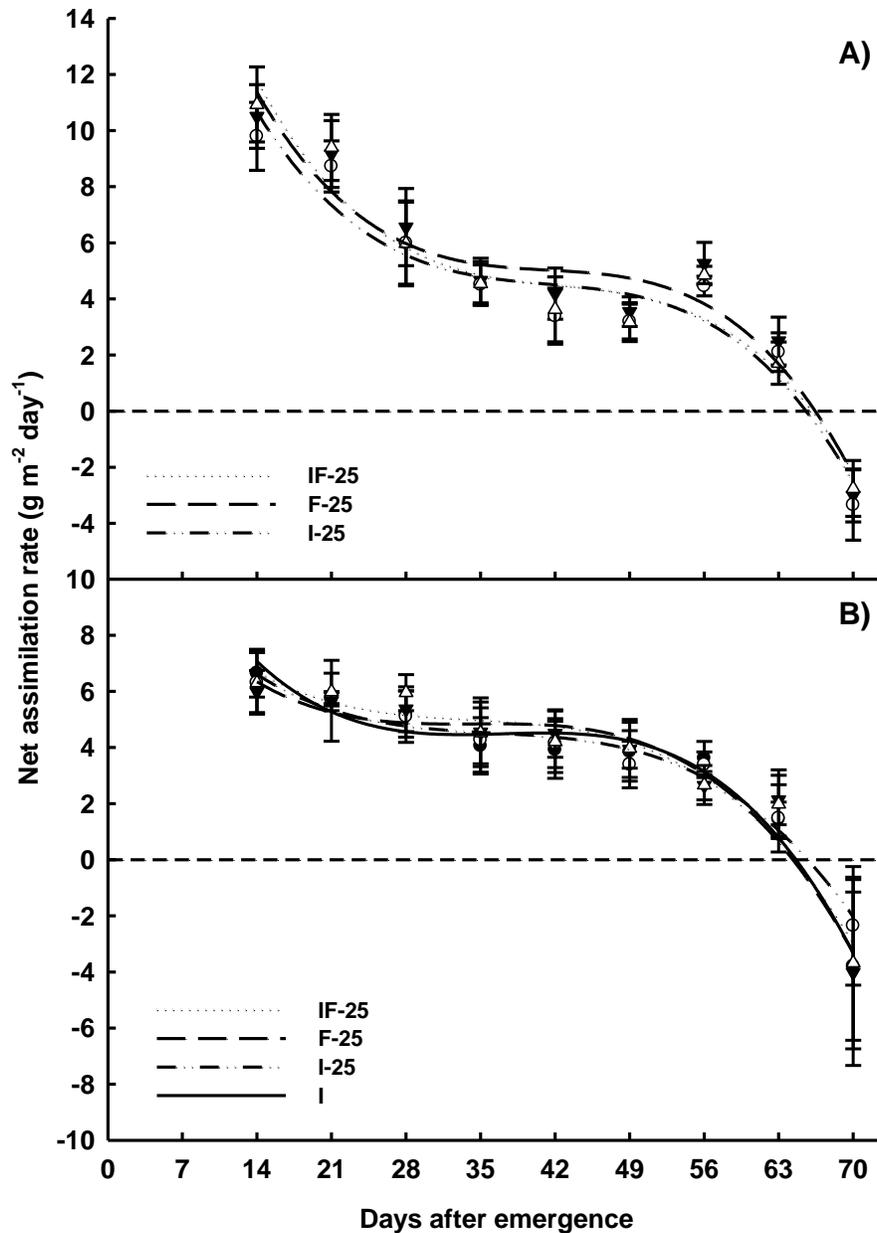
**Figure 3.** Crop growth rate (CGR) of common bean, under the treatments F-25 ( $\blacktriangledown$ ), I-25 ( $\circ$ ), and IF-25 ( $\Delta$ ) in the first year (A), and a fourth treatment I ( $\bullet$ ) included in the second year (B).

produce more photoassimilates for growth.

The evaluation of CGR during plant development (Figure 3) showed that a maximal accumulation of biomass per area of soil occurred at 56 (2013) and 49 (2014) DAE, before the LAI peak, with significant differences between treatments in both years. To ensure higher TDW and LAI, the treatment IF-25 had significant higher CGR than the others treatments in the initial and final samplings of 2013 (Figure 3A), while in 2014 (Figure 3B), this treatment IF-25 showed significant differences only on samplings at 28 and 42 DAE, with significantly

higher CGR than the treatment I, but the treatments F-25 and I-25 did not differ from IF-25 and I in 2014. The TDW peaked at the end of the cycle (70 DAE), when the CGR reached negative values, and this is probably related to the well-known high leaf senescence rate of common bean from the flowering stage (Pimentel et al., 1999; Vieira et al., 2006), resulting in a leaf area decrease at the grain filling stage from 63 (2013) and 56 (2014) DAE, when the need of photoassimilates for the grain is high.

The NAR is a growth variable that represents the biomass produced per leaf area and time, that is, the



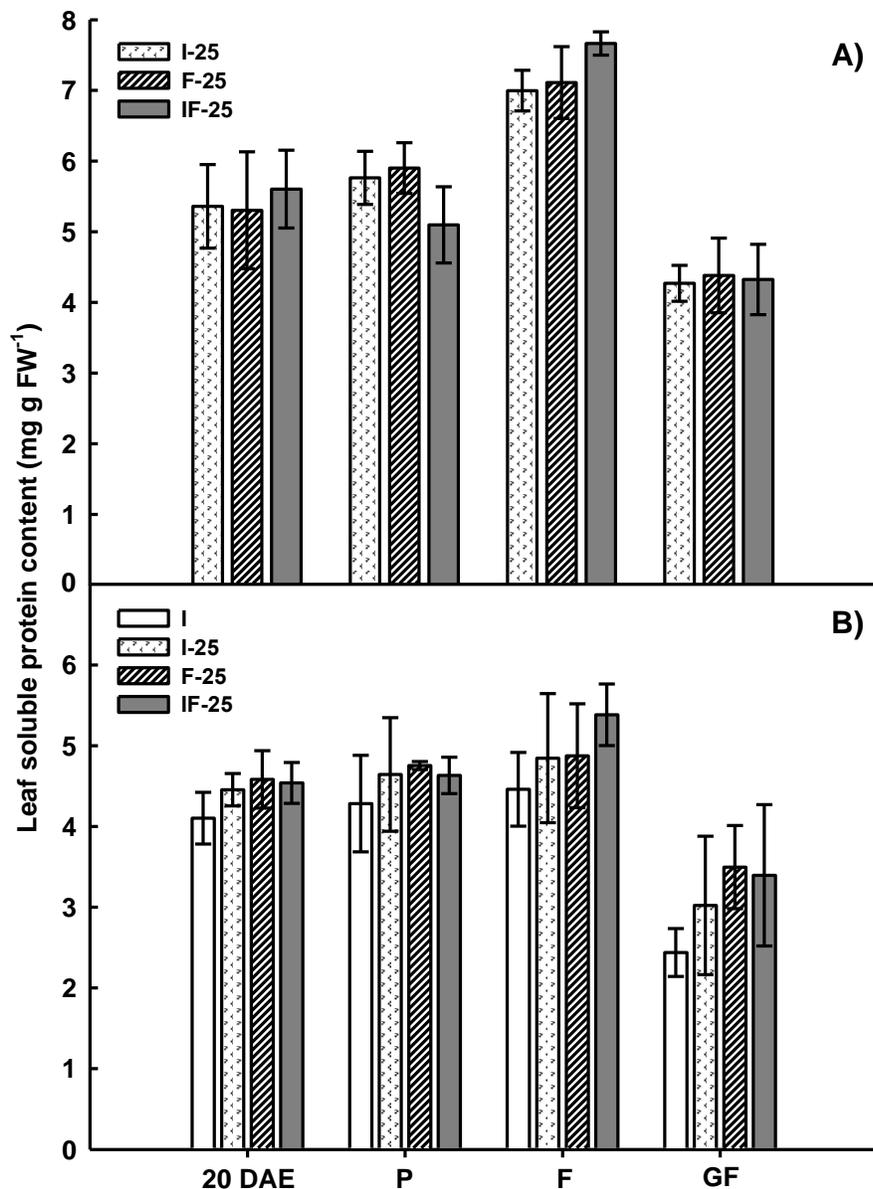
**Figure 4.** Net assimilation rate (NAR) of common bean, under the treatments F-25 ( $\blacktriangledown$ ), I-25 ( $\circ$ ), and IF-25 ( $\Delta$ ) in the first year (A), and a fourth treatment I ( $\bullet$ ) included in the second year (B).

biomass accumulation from the photosynthesis (Pereira and Machado, 1987). The NAR was high in the first sampling (14 DAE) in both years (Figure 4A and B), and gradually decreased after this date; however, it was similar in all treatments and years evaluated, with values near zero or negative from 63 DAE (beginning of grain filling stage), along with the LAI decreasing (Figure 4). The annulment of NAR in the grain filling stage confirm the high rate of abortion of last formed reproductive organs in common bean (Vieira et al., 2006) due to a decrease in photosynthesis in this final stage (Pimentel et al., 1999). The treatment IF-25, which showed higher va-

lues of TDW and LAI did not show significant differences of NAR as compared to the other treatments (Figure 4A and B).

#### **Nodulation and plant dry weight at the pollination stage**

At the pollination stage of common bean, at 33 (2013) and 31 (2014) DAE, when the BNF is considered maximal (Vieira et al., 2006), the BNF potential was estimated by the NN and NDW (Table 1), which are considered



**Figure 5.** Leaf soluble protein content (LSPC) of common bean, under the treatments F-25 (▼), I-25 (○) and IF-25 (Δ) in the first year (A), and a fourth treatment I (●) included in the second year (B).

proportional to the nitrogenase activity, as stated by Hungria et al. (2003). At this time, the plant dry weight accumulation was also evaluated by the SDW and RDW (Table 2). The NN and NDW of the treatments were significantly different in both years, with significantly higher values in the treatment I-25 than in the others two treatments with N fertilization at sowing (F-25 and IF-25) in 2013. In 2014, the treatments only inoculated at sowing (I and I-25) presented significantly higher NN values than the treatment F-25, but not for IF-25. The NDW of the treatments were different, with significantly higher values in I as compared to the F-25, however, the

treatments I-25 and IF-25 did not differ from F-25 and I (Table 2). Therefore, the N fertilization at sowing reduced BNF potential in 2013 for F-25 and IF-25, but in 2014, the N fertilization associated with inoculation of the treatment IF-25 produced the same BNF potential as compared to the only inoculated at sowing treatments I and I-25.

At this pollination stage, the treatment IF-25 had SDW significantly higher than the I-25 in 2013; however, in 2014, the treatment F-25 did not differ from IF-25 and I-25 (Table 2). The SDW of all the treatments in 2014 were similar, indicating that the application of 20 kg N ha<sup>-1</sup> at sowing reduced, but did not inhibit the BNF potential and

**Table 2.** Number of nodules (NN), NDW (nodules dry weight), shoot dry weight (SDW) and root dry weight (RDW) per plant of common bean at the pollination stage, under the treatments F-25, I-25, and IF-25 in the first year, and a fourth treatment I included in the second year.

Treatment	NN	NDW (mg)	SDW (g)	RDW (g)
<b>2013</b>				
I-25	57.75 <sup>a</sup>	170.19 <sup>a</sup>	7.37 <sup>b</sup>	0.640 <sup>a</sup>
F-25	38.00 <sup>b</sup>	129.03 <sup>b</sup>	7.67 <sup>ab</sup>	0.537 <sup>a</sup>
IF-25	40.50 <sup>b</sup>	130.24 <sup>b</sup>	8.850 <sup>a</sup>	0.594 <sup>a</sup>
Pr>Fc	0.0071	0.0109	0.0348	0.2467
CV%	12.98	10.52	7.87	14.05
<b>2014</b>				
I	70.25 <sup>a</sup>	182.75 <sup>a</sup>	6.74 <sup>a</sup>	0.70 <sup>a</sup>
I-25	65.00 <sup>a</sup>	165.50 <sup>ab</sup>	7.45 <sup>a</sup>	0.63 <sup>a</sup>
F-25	41.75 <sup>b</sup>	111.78 <sup>b</sup>	7.86 <sup>a</sup>	0.58 <sup>a</sup>
IF-25	54.64 <sup>ab</sup>	158.52 <sup>ab</sup>	8.22 <sup>a</sup>	0.61 <sup>a</sup>
Pr>Fc	0.0107	0.0151	0.1089	0.1317
CV%	17.21	15.91	10.23	10.04

Mean values followed by the same letter in the column do not differ by the Tukey's test ( $p < 0.05$ ). Pr>Fc = F probabilities.

**Table 3.** NP (number of pods per plant), NGP (number of grains per plant), DW100G (dry weight of 100 grains) and GY (grain yield) of common bean, under the treatments F-25, I-25, and IF-25 in the first year, and a fourth treatment I included in the second year.

Treatment	NP	NGP	DW100G (g)	GY (kg ha <sup>-1</sup> )
<b>2013</b>				
I-25	13.75 <sup>a</sup>	5.75 <sup>a</sup>	25.55 <sup>a</sup>	2346.00 <sup>b</sup>
F-25	13.25 <sup>a</sup>	5.50 <sup>a</sup>	25.76 <sup>a</sup>	2248.69 <sup>b</sup>
IF-25	15.00 <sup>a</sup>	5.50 <sup>a</sup>	25.69 <sup>a</sup>	2509.42 <sup>a</sup>
Pr>Fc	0.7327	0.9190	0.7609	0.2437
CV%	22.51	17.66	1.55	15.64
<b>2014</b>				
I	15.00 <sup>a</sup>	5.25 <sup>a</sup>	25.68 <sup>a</sup>	2207.52 <sup>b</sup>
I-25	18.50 <sup>a</sup>	6.50 <sup>a</sup>	26.44 <sup>a</sup>	2529.85 <sup>ab</sup>
F-25	17.25 <sup>a</sup>	6.00 <sup>a</sup>	26.17 <sup>a</sup>	2474.77 <sup>ab</sup>
IF-25	20.00 <sup>a</sup>	5.50 <sup>a</sup>	26.00 <sup>a</sup>	2642.58 <sup>a</sup>
Pr>Fc	0.3178	0.7733	0.1059	0.0137
CV%	20.35	19.25	1.47	17.97

Mean values followed by the same letter in the column do not differ by the Tukey's test ( $p < 0.05$ ). Pr>Fc = F probabilities.

plant growth. Hungria et al. (2003) also found the same results in the rainy season cultivation of common bean, applying 15 kg N ha<sup>-1</sup> at sowing. On the other hand, the RDW of all the treatments were similar in both years (Table 2), in agreement with Pimentel. (2006) considering root growth of annual crops.

### LSPC during ontogeny

The LSPC is proportional to the leaf RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase) content and thus, it regulates the net photosynthetic rate and crop growth and yield (Long et al., 2006). The LSPC was similar

in all treatments at the vegetative stage, before the fertilization with 40 kg N ha<sup>-1</sup> at 25 DAE and in the pollination stage, in both years (Figure 5). Therefore, the inoculation without the N fertilization (20 kg ha<sup>-1</sup>) at sowing, in the treatments I-25 in 2013 and I-25 and I in 2014, provided enough N by BNF for the initial LSPC synthesis in the same level as with sowing N fertilization (20 kg ha<sup>-1</sup>) in the treatments F-25 and IF-25, for both years (Figure 5). Nevertheless, the treatments with N fertilization (20 kg ha<sup>-1</sup>) at sowing, F-25 and especially IF-25, had the highest TDW (Figure 1), LAI (Figure 2) and high CGR (Figure 3) than the only inoculated at sowing treatments I-25 and I. Consequently, the total LSPC in a plant with increased LAI, as in the treatments F-25 and IF-25, will be higher than in the treatments only inoculated at sowing treatments with lower LAI.

However, in the flowering stage, at 39 (2013) and 38 DAE (2014), the treatment IF-25 with high TDW had also a significantly higher LSPC than the treatment I-25 in 2013 (Figure 5A) and I in 2014 (Figure 5B). The higher LSPC probably ensured an increased RuBisCO activity and photoassimilates production per plant (Pimentel et al., 1999; Long et al., 2006) increasing TDW (Figure 1), LAI (Figure 2) and CGR (Figure 3). At the grain filling stage, there were no significant differences for LSPC in both years (Figure 5). The LSPC was lower at this stage than for the stages before confirming the increased leaf senescence and reduced photosynthesis (Pimentel et al., 1999) in this important stage causing an accentuated abortion of reproductive organs well-known in common bean reducing its potential yield (Vieira et al., 2006).

### Yield components

The yield components NP, NGP and DW100G of the treatments were similar in both years (Table 3), while the GY of the treatments was significantly different. The treatment IF-25 had significantly higher GY than the others in 2013 but only higher than I in 2014, when the treatments I-25 and F-25 did not differ from IF-25 and I (Table 3). Thus, the treatment IF-25, which had significantly higher TDW, LAI, CGR and LSPC for both years, produced a significantly higher GY than the others treatments in 2013 and a high GY in 2014.

The results of these experiments indicate that in the dry season, the use of inoculation in the treatments I and I-25 resulted in a similar yield as compared to the treatment with N fertilization at sowing (20 kg N ha<sup>-1</sup>) and at 25 DAE (40 kg N ha<sup>-1</sup>), but at lower cost. Therefore, these treatments can be recommended to small farmers with limited technological resources, as proposed by Pimentel (2006), and it can increase common bean yield and production by these small farmers especially in the dry season (second crop). On the other hand, the mineral N fertilization (20 kg ha<sup>-1</sup>) combined with inoculation and 40 kg N ha<sup>-1</sup> at 25 DAE (treatment IF-25) resulted in the highest yield, and thus, this treatment can be recommended

for farmers with higher technological resources to have high yield and annual production. However, further studies evaluating other cultivars and others locations are needed to support these recommendations.

### Conclusions

In the dry season, the inoculation with *R. tropici* may replace the N fertilization (20 kg ha<sup>-1</sup>) at sowing without yield loss for a low-cost agriculture for small farmers. However, the N fertilization (20 kg ha<sup>-1</sup>) combined with inoculation with *R. tropici* at sowing and 40 kg N ha<sup>-1</sup> at 25 DAE did not inhibit the root nodulation of common bean and increased its growth and grain yield for a high-cost agriculture for big farmers. In addition, the LSPC at the flowering stage was higher in the treatment with higher growth and yield, and thus, this physiological parameter can be used for selection of more productive common bean genotypes. However, more studies are required with other cultivars and sites, before recommending these agronomic practices of inoculation with or without sowing N fertilization to improve common bean yield in the dry season.

### CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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### Abbreviations

**NN**, Number of nodules; **NDW**, nodules dry weight; **SDW**, shoot dry weight; **RDW**, root dry weight; **NP**, number of pods per plant; **NGP**, number of grains per pod; **DW100G**, dry weight of 100 grains; **GY**, grain yield; **TDW**, total plant dry weight; **LAI**, leaf area index; **CGR**, crop growth rate; **NAR**, net assimilation rate; **LSPC**, leaf soluble protein content; **DAE**, days after emergence; **BNF**, biological nitrogen fixation.

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