



# Effects of Foliar Silicon Application, Seed Inoculation and Splitting of N Fertilization on Yield, Physiological Quality, and Economic Viability of the Common Bean

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## Abstract

**Purpose** A field experiment was carried out to evaluate the effect of foliar Si application associated with inoculation of seeds and nitrogen management on the physiological quality and grain yield of common beans.

**Methods** The experiment was laid out in randomized blocks with a  $5 \times 2 \times 2$  factorial design (four replicates on common bean cultivar Pérola). The first factor was foliar Si application at the concentrations: 0, 1.0, 2.0, 3.0, and 4.0 g L<sup>-1</sup> of silicon. The second factor was the effect of the presence or absence of seed inoculation, while the third factor was the splitting of nitrogen topdressing (120 kg N ha<sup>-1</sup>). Parameters evaluated were relative chlorophyll index (RCI), foliar N and Si contents, gas exchanges, grain yield, and economic viability.

**Results** Split fertilization of 60 + 60 kg N ha<sup>-1</sup> at 20 and 40 days after emergence (DAE) showed better results of grain yield, approximately 41 % higher than splitting of 80 + 40 kg N ha<sup>-1</sup> at 20 and 40 DAE, regardless of fertilization containing Si. Foliar Si application increased grain yield in the order of 10 % (uninoculated seeds) and 25 % (inoculated seeds), regardless of splitting of nitrogen fertilization, using the highest Si concentration (4.0 g L<sup>-1</sup>). Regardless of seed inoculation, the splitting of 60 + 60 kg N ha<sup>-1</sup>, associated with the application of 3.0 g Si L<sup>-1</sup>, resulted in the maximum grain yield, approximately 20 % higher than the control.

**Conclusion** Application of 2.0 g Si L<sup>-1</sup> promoted differential profits of up to US\$ 236.72 regardless of seed inoculation and splitting of N fertilization.

**Keywords** *Phaseolus vulgaris* · Stabilized alkaline silicate · Biological nitrogen fixation · Topdressing · Seed treatment

## 1 Introduction

Common bean (*Phaseolus vulgaris* L.) is the most cultivated species among the *Phaseolus* genus and, currently, Brazil is the third largest bean producer in the world, reaching a production of 2.53 million tons in an area of 1.66 million hectares in the 2019/20 crop season [1]. However, the average national

grain yield is still considered low, approximately 1,520 kg ha<sup>-1</sup>, which highlights need for improvements in production chain through alternative with good return of investment to growers.

The common bean is considered a nutritionally demanding plant due to its superficial root system and short cycle [2]. As the N dynamics in the soil-plant system is highly complex, adequate management of nitrogen fertilization in legumes is essential for maximum grain yield [3]. Management techniques that maximize the judicious use of N fertilizers are extremely important because of the high cost coupled which low use efficiency [3].

In legumes, N fertilizer has been used to increase nutrient availability for the crop, aiming to reduce economic and environmental impacts associated with its use in productive systems [4]. In this context, seed inoculation with efficient strains of bacteria of the *Rhizobium* genus, associated with the use of

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N fertilizer, is a viable alternative to increase yield, especially for the common bean [5].

However, it is known that there is strong interaction between nitrogen and silicon (Si) for yield response in crops [6, 7], although still without consensus for the common bean. Several studies demonstrated synergistic effect between N and Si, with a proven yield gain, especially when applied together [8, 9]. Higher Si content in the plant improves its efficiency regarding N use, increasing protein content and grain nutritional quality [8, 9].

Previous studies have demonstrated the high capacity of Si to reduce effects of abiotic stress, with water and phytosanitary stress among them, which reflect in yield gains [10, 11]. This behavior is explained by its deposition in the outer epidermal wall, forming a double layer with the cuticle, increasing its resistance against deleterious effects of these stresses [12]. Si application improves the physiological quality of plants, increasing efficiency of transpiration rates and stomatal conductance and reflecting in better water use.

Si can be supplied to plants either through soil or foliar application [13, 14]. However, soil application demands large quantities due to its low solubility, which is common in the fertilizers available on the market. Foliar application, on the other hand, is an alternative, as its available sources are highly soluble and can be applied at low concentrations, reducing costs and being more economically feasible [14–16].

The appropriate supplying of nitrogen to the common bean, either through the use of nitrogen fertilizers or the use of N-fixing bacteria, associated with the foliar application of beneficial elements such as silicon, can result in greater productivity and quality of grains [17]. However, the use of these management practices can still influence the economic viability of grain production. Thus, it is necessary to look beyond the physiological and productive responses of the crop, and seek to understand how these practices affect cost economics of the common bean yield. According to Freire et al. [18], information regarding the economic viability of allows to maximize the use efficiency of fertilizers, making this study as an important tool in the transfer and its adoption by growers [19].

Therefore, in this study aimed to evaluate the effect of foliar silicon application and nitrogen supply management on the physiological quality, grain yield, and economic viability of common bean grown in an irrigated system was studied.

## 2 Materials and Methods

### 2.1 Characterization of the Experimental area

The experiment was conducted in the experimental field of the School of Agronomy at the Federal University of Goiás - UFG (16° 35" S and 49°21" W, 730 m altitude, average annual rainfall of 1600 mm), Goiás State, Brazil, 2017 crop season,

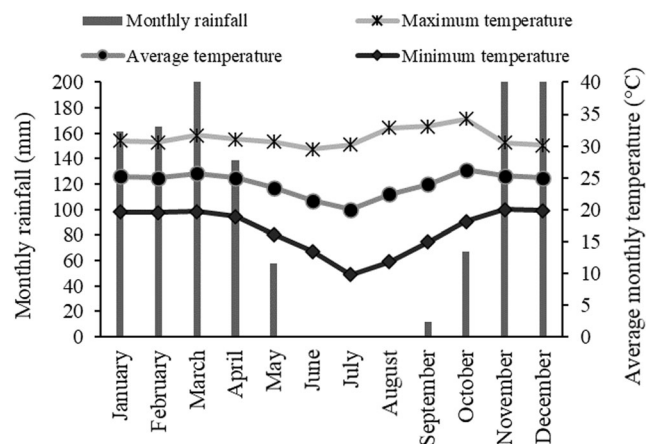
with the common bean crop, cultivar Pérola, in an area with central pivot irrigation system. The climate is Aw (megathermal) or tropical savannah, with dry winters and rainy summers. During conduction of the study with the course of the study, climatic conditions were monitored through a meteorological station located at the School of Agronomy at UFG, with the results shown below in Fig. 1.

The soil was classified as a RED LATOSOL, acric, clay sandy texture, according to the Brazilian Soil Classification System (SiBCS) [20]. In order to determine soil chemical and granulometric attributes, soil sampling was previously performed according to methods described by Teixeira et al. [21] at depths of 0–0.20 and 0.20–0.40 m (Table 1). Recommendation for soil correction and mineral fertilization was performed according to Souza and Lobato [22], with 20 kg N ha<sup>-1</sup>, 110 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, and 70 kg K<sub>2</sub>O ha<sup>-1</sup> applied to the planting furrow in the forms of urea, simple superphosphate, and potassium chloride, respectively.

### 2.2 Experimental Design

Planting was performed in June 2017, period included in the 3rd crop season or winter crop, with cultivar Pérola. The crop density was 300 thousand plants per hectare. Seed treatment (ST) was performed with application of 200 g of the insecticide Thiamethoxam for 100 kg<sup>-1</sup> of seeds. During the experiment, manual weeding was performed to control unwanted plants grown spontaneously.

The experiment was arranged in randomized blocks with a 5 × 2 × 2 factorial design, with four replicates, totaling 80 plots. Experimental units consisted of five rows spaced 0.45 m apart and 0.15 m per plant, 5 m long. The first factor was three foliar applications containing silicon (Si) in the concentrations of 0 (control), 1.0, 2.0, 3.0, and 4.0 g L<sup>-1</sup> applied in the form of potassium silicate and alkaline copper (Si = 107 g L<sup>-1</sup>; K<sub>2</sub>O = 28.4 g L<sup>-1</sup>; Cu = 14.9 g L<sup>-1</sup>; pH = 11.5).



**Fig. 1** Rainfall and average monthly temperature from 2017, at pivot I of the School of Agronomy, of the Federal University of Goiás, Goiânia, Brazil

**Table 1** Chemical attributes of the soil, at a depth of 0 to 0.20 m, and 0.20 to 0.40 m before the installation of the experiment

Depth (m)	Clay %	Sand %	Silte %	Cu mg dm <sup>-3</sup>	Fe mg dm <sup>-3</sup>	Mn mg dm <sup>-3</sup>	Zn mg dm <sup>-3</sup>	OM %	pH (CaCl <sub>2</sub> )
0.00–0.20	57.0	39.0	4.0	1.8	26.0	28.0	2.9	1.1	5.6
0.20–0.40	57.0	37.0	6.0	2.1	30.0	18.0	2.6	0.7	5.5
Depth (m)	P mg dm <sup>-3</sup>	K mg dm <sup>-3</sup>	Ca cmolc dm <sup>-3</sup>	Mg cmolc dm <sup>-3</sup>	H+Al cmolc dm <sup>-3</sup>	Al cmolc dm <sup>-3</sup>	CEC cmolc dm <sup>-3</sup>	M %	V %
0.00–0.20	4.7	120.0	1.0	1.0	2.3	0.0	4.6	0.0	50.1
0.20–0.40	2.1	77.0	0.5	0.9	2.3	0.0	3.9	0.0	41.0

In each treatment containing Si, potassium (K) and copper (Cu) concentrations, in the form of potassium chloride and copper oxide, were balanced to eliminate the effect of these nutrients on the solution.

Because it is a non-accumulating silicon species, concentrations were applied via the leaf, as the culture has low root absorption of the element, due to the lack of specific transporters [23]. The applied product is an experimental compound, and there are still no recommendations for doses and method of application for spraying common bean. The applied concentrations were determined by observing the use of the product in other non-accumulating silicon plants such as tomato [24].

Foliar fertilizations containing Si were performed at 40, 55, and 70 days after emergence (DAE). The second factor was the presence or absence of seed inoculation before planting, using peat inoculant containing the strains SEMIA 4077 and SEMIA 4080 of *Rhizobium tropici* and the strain SEMIA 4088 of *R. freirei*, in the proportion 1:1:1 of the three strains. In order to improve adhesion of the peat inoculant to the seeds, a 10 % sugar solution was used in the proportion of 300 mL for 50 kg of seeds. The third factor was the splitting of nitrogen topdressing with 80 + 40 or 60 + 60 kg N ha<sup>-1</sup>, at 20 and 40 DAE, in the form of urea.

Fertilizers were applied based on the initial soil analysis report as recommended by Sousa and Lobato [22]. Thus, the following were applied in the planting furrow: 20 kg ha<sup>-1</sup> N, 110 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, and 70 kg ha<sup>-1</sup> K<sub>2</sub>O, in the form of urea, simple superphosphate, and potassium chloride, respectively. Values were calculated based on soil analysis collected from 0.0 to 20.0 cm and 20.0 to 40.0 cm in depth, following the methodology proposed by Teixeira et al. [21] (Table 1).

### 2.3 Physiological Parameters

Evaluations of nitrogen and silicon contents in leaves, relative chlorophyll index (RCI), and gas exchanges were performed at 80 DAE, i.e., 10 days after the last spray containing silicon. RCI was evaluated with the aid of a chlorophyll meter, model Falker®, ClorofiLOG CFL1030, in five plants chosen at

random within each plot, in the first completely expanded leaf from the apex of the main stem.

Similarly, gas exchanges (net photosynthetic rate (A), stomatal conductance (G), transpiration (E), and maximum quantum yield of FSII (ΦFSII) were evaluated using an Infrared Gas Analyzer (IRGA) with fluorometer, model iFL - Integrated Fluorometer and Gas Exchange System, with 6.25 cm<sup>2</sup> leaf chamber, between 9:00 am and 2:00 pm.

Leaf sampling was performed by collecting 20 diagnostic leaves + 3 (first matured leaf from the tip of the main stem) in each experimental unit, according to procedures proposed by Souza et al. [25]. Silicon contents were evaluated as proposed by Kraska and Breitenbeck [26].

### 2.4 Yield Evaluation

For yield evaluation experimental plots were manually harvested at 96 days after emergence. The two central rows were considered, discarding 0.5 m from each extremity, totaling 0.9 m<sup>2</sup> harvested per plot. Plots harvested were threshed manually and impurities were separated from seeds with the aid of sieves. Harvested seeds were weighed and used to calculate the yield in kg ha<sup>-1</sup>.

### 2.5 Cost Analysis

Economic analysis was performed using the partial budget technique, according to Noronha [27]. The method calculates the effects of additional costs and revenues in relation to a baseline, providing differential profits as an economic indicator, using the following equation:

$$Dp = Dr - Dc \quad (1)$$

Where:

Dp (US\$ ha<sup>-1</sup>) = Differential revenue, calculated from the variation of the yield obtained in each treatment in relation to the control, considered as baseline, multiplied by the historical record of the average price of common bean (Dr = differential yield x product price). The historical record of bean prices was

obtained from prices observed in Brazil in the last 11 years (2009–2019), which were deflated to the real values in 2020 and converted into dollars, at the rate of US\$ = R\$ 5.08 (12/12/2020). Prices were obtained from the Municipal Agricultural Survey (PAM/IBGE, 2020).

Dc (US\$ ha<sup>-1</sup>) = Differential cost was calculated directly from the price of the concentration of the product used in each treatment, as these were already differential in relation to the control. Analyses were performed in relation to the input price, resulting in the differential cost of the input and the cost of the product added to the operational cost of application, which subsequently resulted in the differential cost of operation. The operational cost of application was obtained from Róman et al. [28], who evaluated the operational efficiency of application for different spray volumes. This study allowed to calculate the updated value (US\$ – 2020) of US\$ 46.55 for three applications at spray volume of 100 L ha<sup>-1</sup>. Thus, it was possible to calculate from Eq. 1 the differential profit (Dp) for each treatment in relation to the control, which was subdivided into Dpi = Differential profit of input and Dpo = Differential profit of operation.

In addition, differential revenue was calculated between treatments with and without inoculation (considering the baseline) and between splitting of nitrogen fertilizations of 80 + 40 kg N ha<sup>-1</sup> and 60 + 60 kg N ha<sup>-1</sup> (considering the baseline), 20 and 40 days after seedling emergence (DAE), respectively.

## 2.6 Statistical Analysis

Data were subjected to analysis of variance by F test. When statistically significant differences were observed between treatments, means of qualitative parameters were compared by Tukey test at 5 % probability. For quantitative parameters statistically significant, regression analysis was performed.

## 3 Results

### 3.1 Foliar Contents of Nitrogen (N) and Silicon (Si) and Relative Chlorophyll Index (RCI)

Foliar nitrogen contents in common beans were not influenced by the management adopted ( $p > 0.05$ ), regardless of treatment, showing average content of 49.8 g kg<sup>-1</sup> (Table 2). When evaluating the effectiveness of treatments on the Si content in plants, observed the effect of interaction between doses of Si, seed inoculation, and splitting of nitrogen fertilization. Treatments with uninoculated seeds and silicon applications exhibited maximum silicon accumulation at 3.0 g Si L<sup>-1</sup> (Fig. 2a). As for plants that were inoculated with strains SEMIA 4077 and SEMIA 4080 of *Rhizobium tropici* and the

strain SEMIA 4088 of *R. freirei*, obtained the maximum Si content of 5.3 g kg<sup>-1</sup> with the dose of 4.0 g Si L<sup>-1</sup> (Fig. 2a).

When evaluating the effect of interaction between splitting of nitrogen fertilization and foliar silicon fertilization, observed similar behavior, with quadratic adjustments. The splitting of 60 + 60 kg N ha<sup>-1</sup> at 20 and 40 DAE, respectively, obtained the maximum Si content (6.3 g kg<sup>-1</sup>) with the application of 3.0 g Si L<sup>-1</sup> (Fig. 2b). When we applied splitting of 80 + 40 kg N ha<sup>-1</sup> at 20 and 40 DAE, respectively, obtained maximum Si content (6.1 g kg<sup>-1</sup>) with the use of 4.0 g Si L<sup>-1</sup> (Fig. 2b).

When evaluated relative chlorophyll index (RCI), we observed effects of interaction between splitting of nitrogen fertilization, seed inoculation, and foliar silicon application (Table 2). Thus, in Fig. 3a we observe that seed inoculation obtained the best relative chlorophyll index, being 5.8 and 2.6 % higher than treatments without inoculation in splitting of 60 + 60 and 80 + 40 kg N ha<sup>-1</sup> at 20 and 40 DAE, respectively.

When evaluating the effect of interaction between the splitting of nitrogen fertilization and foliar silicon application, we observed linear behavior for the splitting of 60 + 60 kg N ha<sup>-1</sup>, obtaining 56.9 µg cm<sup>-2</sup> with the use of 4.0 g Si L<sup>-1</sup>, regardless of seed inoculation (Fig. 3b). However, when we applied the splitting of 80 + 40 kg N ha<sup>-1</sup>, there was a quadratic adjustment, reducing RCI with the increase of Si supply, reaching a minimum of 53.6 µg cm<sup>-2</sup> with foliar application of 2.0 g Si L<sup>-1</sup> (Fig. 3b).

### 3.2 Gas Exchanges and Photochemical Efficiency of Photosystem II (Fv/Fm)

When analyzed gas exchanges, treatments affected the physiological quality of common beans, as observed in Table 3, Figs. 4, 5, and 6. Regarding the transpiration rate of plants, was observed a significant effect of interaction among all variables (splitting of nitrogen fertilization, seed inoculation, and foliar silicon application). Seed inoculation showed a different behavior as a function of splitting nitrogen fertilization, as observed in Fig. 5a. For uninoculated plants, the transpiration rate with the splitting of 60 + 60 kg N ha<sup>-1</sup> at 20 and 40 DAE was 57 % higher than for splitting of 80 + 40 kg N ha<sup>-1</sup>, while for inoculated plants, the transpiration rate was 65 % higher for the splitting of 60 + 60 kg N ha<sup>-1</sup> in relation to the splitting of 80 + 40 kg N ha<sup>-1</sup> at 20 and 40 DAE, respectively.

When evaluated the effect of interaction between seed inoculation and foliar silicon application, in both cases the significant effect was quadratic. Uninoculated plants obtained the highest transpiration rate (7.3 mol m<sup>-2</sup> s<sup>-1</sup>) with the application of 1.0 g Si L<sup>-1</sup>, while inoculated plants obtained the highest transpiration rate (9.6 mol m<sup>-2</sup> s<sup>-1</sup>) with the application of 3.0 g Si L<sup>-1</sup>, regardless of splitting of nitrogen fertilization (Fig. 4b).

**Table 2** Transpiration (E), Stomatal conductance (Gs), Photosynthesis (A) and relative chlorophyll index (RCI) in common bean (*Phaseolus vulgaris* L.) as a function of seed inoculation, nitrogen fertilization split and application of Si via leaf

Treatments Inoculation (I)	E mol m <sup>-2</sup> s <sup>-1</sup>	Gs mol m <sup>-2</sup> s <sup>-1</sup>	A μmol m <sup>-2</sup> s <sup>-1</sup>	RCI μg cm <sup>-2</sup>
Without	6.27b	0.22	9.75a	54.24b
With	7.53a	0.22	8.47b	56.61a
<i>F</i> <sub>test</sub>	20.37**	0.18 <sup>ns</sup>	33.21**	50.44**
Subdivision of N (S)				
60+60	6.44b	0.25a	9.39a	55.45a
80+40	7.36a	0.19b	8.83b	55.40a
<i>F</i> <sub>test</sub>	10.88**	35.33**	6.12*	0.02 <sup>ns</sup>
Doses of Si (D)				
0	5.01c	0.13	7.00d	55.47b
1	7.14b	0.17	8.83c	55.79ab
2	8.47a	0.27	11.53a	55.23b
3	6.92b	0.27	10.24b	53.54c
4	6.98b	0.25	7.96 cd	57.10a
<i>F</i> <sub>test</sub>	15.82**	35.38**	52.11**	11.68**
Interaction				
IxP	273.69**	44.25**	359.87**	8.05**
IxD	28.80**	3.75**	1.76 <sup>ns</sup>	2.91 <sup>ns</sup>
DxP	20.69**	13.97**	3.79**	13.02**
IxDxP	3.77**	4.72**	11.71**	2.01 <sup>ns</sup>
<i>F</i> <sub>test</sub>	22.34**	4.62**	4.36**	5.01*
Mean	6.61	0.20	8.54	55.58
C.V. (%)	17.84	19.11	10.9	2.69

The averages followed by the same letter on the line do not differ by Tukey's test at 5 % probability. C.V.: Coefficient of variation. \*\*, \* and <sup>ns</sup> significant at 1 and 5 % and non-significant at 5 % probability by the F test, respectively

Significant quadratic adjustments also occurred in relation to the interaction between the splitting of nitrogen fertilization and foliar silicon application. In the splitting of 60 + 60 kg N ha<sup>-1</sup> at 20 and 40 DAE, the highest transpiration rate (8.2 mol m<sup>-2</sup> s<sup>-1</sup>) occurred with an application of 3.0 g Si L<sup>-1</sup>, while in the splitting of 80 + 40 kg N ha<sup>-1</sup>, the highest transpiration rate (8.2 mol m<sup>-2</sup> s<sup>-1</sup>) occurred with use of 2.0 g Si L<sup>-1</sup>, regardless of seed inoculation (Fig. 4c).

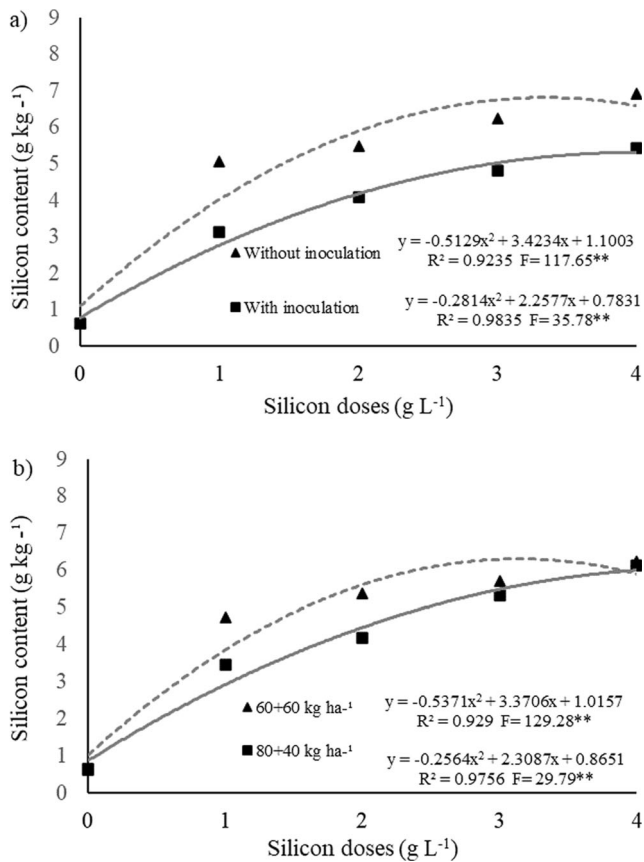
Similar to the observed for transpiration, the stomatal conductance of the common bean was also significantly affected by the interaction between all variables (splitting of nitrogen fertilization, seed inoculation, and foliar silicon application), as observed in Table 3; Fig. 5a. For uninoculated plants, stomatal conductance was not influenced by a splitting of nitrogen fertilization, showing an average value of 0.2 mol m<sup>-2</sup> s<sup>-1</sup>, while for inoculated plants, stomatal conductance was 75 % higher in the splitting of 60 + 60 kg N ha<sup>-1</sup> in relation to the splitting of 80 + 40 kg N ha<sup>-1</sup> at 20 and 40 DAE, respectively, regardless of foliar silicon application (Fig. 5a).

When evaluated the effect of interaction between seed inoculation and foliar silicon application, in both cases significant effect was quadratic. Uninoculated plants obtained the

highest stomatal conductance (0.28 mol m<sup>-2</sup> s<sup>-1</sup>) with the application of 3.0 g Si L<sup>-1</sup>, while inoculated plants obtained the highest stomatal conductance (0.26 mol m<sup>-2</sup> s<sup>-1</sup>) with the application of 3.0 g Si L<sup>-1</sup>, regardless of splitting of nitrogen fertilization (Fig. 5b).

Regarding the effects of interaction between the splitting of nitrogen fertilization and foliar silicon the application, were also observed significant quadratic adjustments. For splitting of 60 + 60 kg N ha<sup>-1</sup> at 20 and 40 DAE, the highest stomatal conductance (0.34 mol m<sup>-2</sup> s<sup>-1</sup>) was observed with the application of 4.0 g Si L<sup>-1</sup>. On the other hand, for the splitting of 80 + 40 kg N ha<sup>-1</sup>, the highest transpiration rate (0.22 mol m<sup>-2</sup> s<sup>-1</sup>) was observed with the use of 2.0 g Si L<sup>-1</sup>, regardless of seed inoculation (Fig. 5c).

Regarding net photosynthetic rate, significant effects of interaction occurred between splitting of nitrogen fertilization, seed inoculation, and foliar silicon application, as observed in Table 3; Fig. 6a. For uninoculated plants, net photosynthetic rate was 47 % higher for splitting of 80 + 40 kg N ha<sup>-1</sup> at 20 and 40 DAE compared to splitting of 60 + 60 kg N ha<sup>-1</sup>, while for inoculated plants, net photosynthetic rate was 79 % higher for splitting of 60 + 60 kg N ha<sup>-1</sup> compared to splitting of



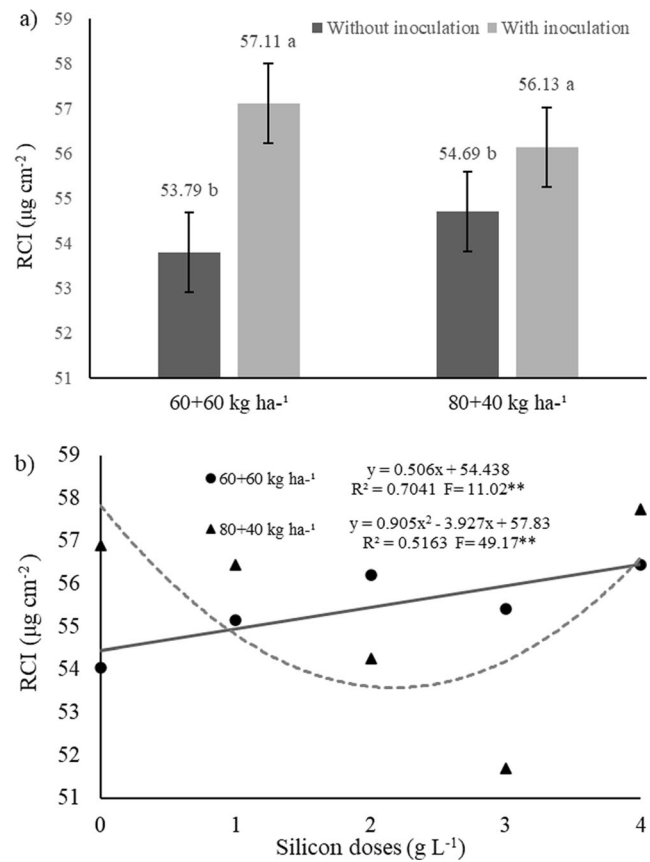
**Fig. 2** Effect of the interaction between seed inoculation and application of Si via leaf (a) and of the interaction between the split of nitrogen fertilization and application of Si via leaf (b), on the leaf silicon content of common bean (*Phaseolus vulgaris* L.). \*\* significant at 1 % probability by the F test

80 + 40 kg N ha<sup>-1</sup> at 20 and 40 DAE, respectively, regardless of foliar silicon application (Fig. 6a).

Regarding the effects of interaction between splitting of nitrogen fertilization and foliar silicon application, significant quadratic adjustments were observed. For splitting of 60 + 60 kg N ha<sup>-1</sup> at 20 and 40 DAE, the highest net photosynthetic rate (11.4  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) occurred with the application of 2.0 g Si L<sup>-1</sup>. While, for splitting of 80 + 40 kg N ha<sup>-1</sup>, the highest net photosynthetic rate (10.3  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) occurred with the application of 2.0 g Si L<sup>-1</sup>, regardless of seed inoculation (Fig. 6b).

For the photochemical efficiency of photosystem II (Fv/Fm), significant interaction occurred for all treatments, as observed in Table 3; Fig. 7a. For uninoculated plants, the highest Fv/Fm (0.75) was observed with the splitting of 60 + 60 kg N ha<sup>-1</sup> at 20 and 40 DAE. While for inoculated plants, the highest Fv/Fm (0.74) was observed with the splitting of 60 + 60 kg N ha<sup>-1</sup> at 20 and 40 DAE, respectively, regardless of foliar silicon application (Fig. 7a).

On the evaluated the effect of interaction between seed inoculation and foliar silicon application in uninoculated plants, a linearly decreasing behavior was observed, resulting



**Fig. 3** Effect of the interaction between seed inoculation and nitrogen fertilization split (a) and interaction between nitrogen fertilization split and Si application via leaf (b), on the relative chlorophyll index of common bean (*Phaseolus vulgaris* L.). \*\*significant at 1 % probability by the F test

in the lowest Fv/Fm (0.72) with the application of 4.0 g Si L<sup>-1</sup>. In the inoculated plants occurred a quadratic adjustment, with the highest Fv/Fm (0.75) been reached with the application of 2.0 g Si L<sup>-1</sup>, regardless of splitting of nitrogen fertilization (Fig. 7b).

In relation to the effects of interaction between splitting of nitrogen fertilization and foliar silicon application, significant quadratic adjustments were observed. For splitting of 60 + 60 kg N ha<sup>-1</sup> at 20 and 40 DAE, the highest Fv/Fm (0.76) occurred with the application of 2.0 g Si L<sup>-1</sup>. While, for splitting of 80 + 40 kg N ha<sup>-1</sup>, the highest Fv/Fm (0.75) occurred with the application of 2.0 g Si L<sup>-1</sup>, regardless of seed inoculation (Fig. 7c).

### 3.3 Grain Yield and Cost Analysis

All evaluated treatments (splitting of nitrogen fertilization, seed inoculation, and foliar silicon application) affected significantly the grain yield of common bean, as observed in Table 2; Fig. 8a. For uninoculated plants, the splitting of nitrogen fertilization did not affect the grain yield of common bean, showing mean grain yield of 3,866.3 kg ha<sup>-1</sup>. On the

**Table 3** Photochemical efficiency of photosystem II, nitrogen and foliar silicon content and productivity of common bean (*Phaseolus vulgaris* L.) as a function of seed inoculation, nitrogen fertilization split and Si application via leaf

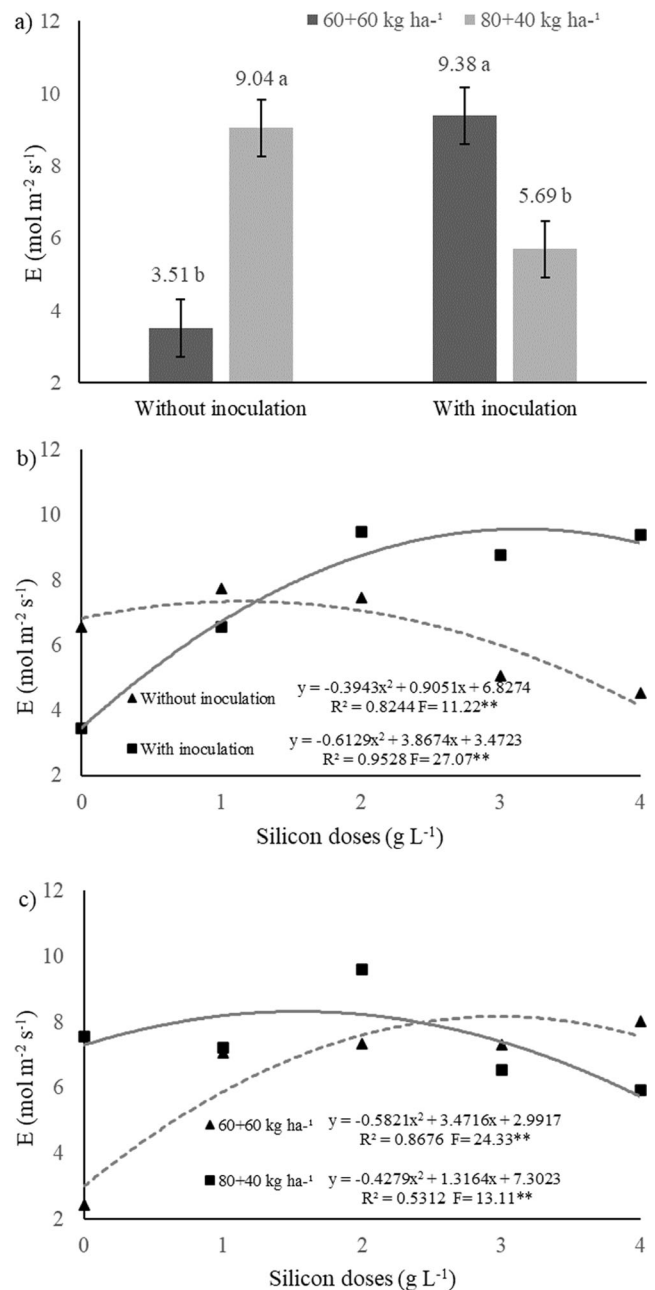
Treatments	Fv/Fm	N g kg <sup>-1</sup>	Si g kg <sup>-1</sup>	Yield kg ha <sup>-1</sup>
<b>Inoculation (I)</b>				
Without	0.75a	50.16a	4.87a	3866.03a
With	0.73b	49.13b	3.61b	3387.53b
<i>Ftest</i>	15.29**	7.17**	126.78**	28.80**
<b>Subdivision of N (S)</b>				
60+60	0.74a	49.35a	4.53a	3975.58a
80+40	0.74a	49.94a	3.94b	3277.97b
<i>Ftest</i>	1.70 <sup>ns</sup>	2.34 <sup>ns</sup>	27.78**	61.22**
<b>Doses of Si (D)</b>				
0	0.74a	48.77a	0.63e	3245.76
1	0.74a	50.28a	4.09d	3537.76
2	0.75a	49.38a	4.77c	3803.29
3	0.75a	50.09a	5.53b	3659.05
4	0.71b	49.70a	6.18a	3888.02
<i>Ftest</i>	18.87**	1.95 <sup>ns</sup>	300.41**	6.38**
<b>Interaction</b>				
IxP	24.84**	0.47 <sup>ns</sup>	0.64 <sup>ns</sup>	28.03**
IxD	14.64**	1.35 <sup>ns</sup>	8.45**	3.01*
DxP	3.93**	2.27 <sup>ns</sup>	6.05**	3.32*
IxDxP	8.44**	0.67 <sup>ns</sup>	8.93**	5.28**
<i>Ftest</i>	5.99**	2.89 <sup>ns</sup>	0.54 <sup>ns</sup>	2.40 <sup>ns</sup>
Mean	0.74	49.8	2.89	3640.71
C.V. (%)	1.96	3.44	8.99	10.28

The means followed by the same letter in the column do not differ by Tukey's test at 5 % probability. C.V: Coefficient of variation. \*\*, \* and <sup>ns</sup>, significant at 1 % and not significant at 5 % probability by the F test, respectively

other hand, for inoculated plants, grain yield was 42 % higher for splitting of 60 + 60 kg N ha<sup>-1</sup> as compared to splitting of 80 + 40 kg N ha<sup>-1</sup> at 20 and 40 DAE, regardless of Si application (Fig. 8a).

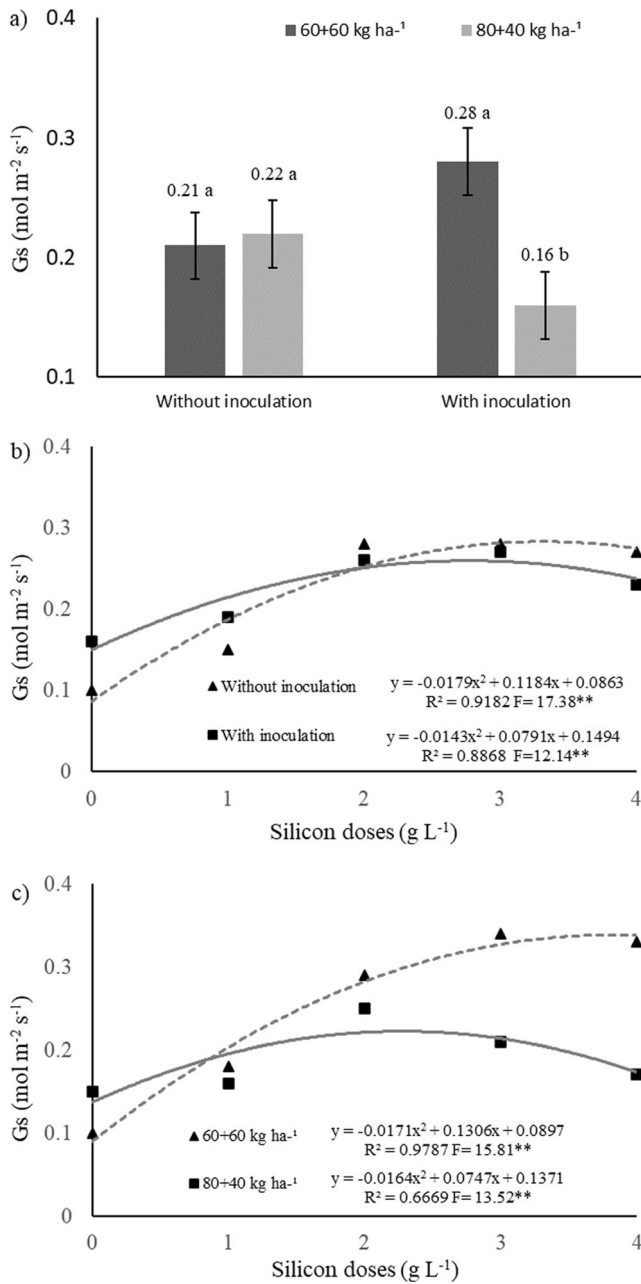
When evaluating the effect of interaction between seed inoculation and foliar silicon application, a linear behavior was observed for uninoculated plants, with the highest grain yield (3,803.47 kg ha<sup>-1</sup>) observed for application of 4.0 g Si L<sup>-1</sup>. For the inoculated plants, a quadratic behavior was observed, reaching the highest grain yield (3,275.14 kg ha<sup>-1</sup>) by the application of 4.0 g Si L<sup>-1</sup>, regardless of splitting of nitrogen fertilization (Fig. 8b).

Interaction between splitting of nitrogen fertilization and foliar silicon application resulted in a quadratic behavior for splitting of 60 + 60 kg N ha<sup>-1</sup> at 20 and 40 DAE, for which the highest grain yield (4,217.2 kg ha<sup>-1</sup>) was achieved with application of 3.0 g Si L<sup>-1</sup>. For the splitting of 80 + 40 kg N ha<sup>-1</sup>



**Fig. 4** Effect of the interaction between seed inoculation and nitrogen fertilization split (a), interaction between seed inoculation and Si application via foliar (b) and interaction between nitrogen fertilization split and Si application via foliar (c), on the stomatal conductance (Gs) of common bean (*Phaseolus vulgaris* L.). \*\* significant at 1 % probability by the F test

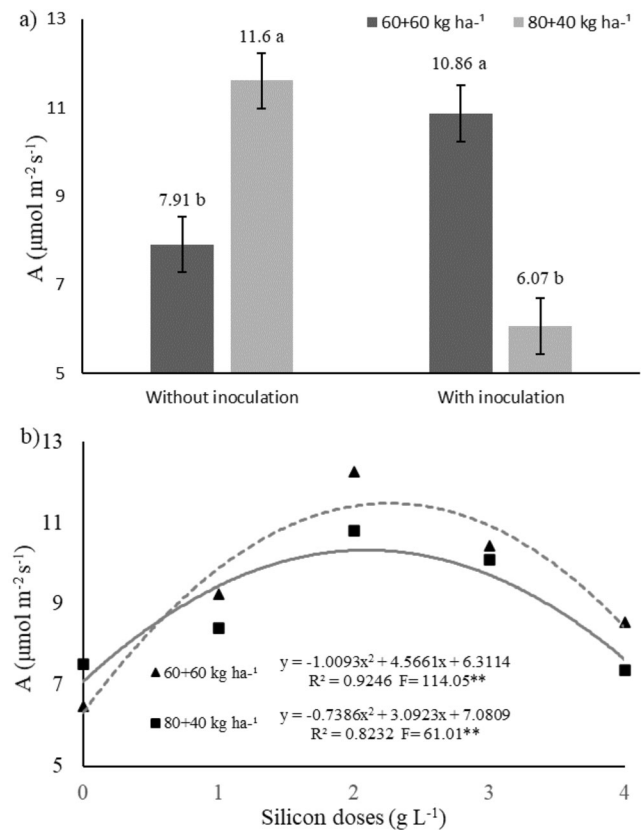
<sup>1</sup>, the highest grain yield (3,729.8 kg ha<sup>-1</sup>) was obtained with the application of 4.0 g Si L<sup>-1</sup>, regardless of seed inoculation (Fig. 8c). Figure 9 shows the results of differential profits of inputs and operation in relation to silicon application in common beans, regardless of splitting of nitrogen fertilization and seed inoculation. For all doses of Si, positive differential profit was observed, with the application of 2.0 g Si L<sup>-1</sup> showing



**Fig. 5** Effect of the interaction between seed inoculation and nitrogen fertilization split (a), interaction between seed inoculation and Si application via foliar (b) and interaction between nitrogen fertilization split and Si application via foliar (c) on Transpiration (E) of common bean (*Phaseolus vulgaris* L.). \*\* significant at 1 % probability by the F test

the best return (US\$ 236.7 ha<sup>-1</sup>), considering three sprays. Suppressing the cost of operation, there are increases of US\$ 46.5 ha<sup>-1</sup> in the differential profit, regardless of Si dose.

By the evaluation of seed treatment separately, a mean loss of revenue of US\$ 308.5 ha<sup>-1</sup> was observed in relation to its reference (treatments with uninoculated seeds). Similarly, the splitting of 80 + 40 kg N ha<sup>-1</sup> showed a



**Fig. 6** Effect of the interaction between seed inoculation and nitrogen fertilization split (a) and interaction between nitrogen fertilization split and Si application via leaf (b) on Photosynthesis (A) of common bean (*Phaseolus vulgaris* L.). \*\* significant at 1 % probability by the F test

mean loss of revenue of US\$ 449.8 ha<sup>-1</sup> in relation to its reference (60 + 60 kg N ha<sup>-1</sup>), both at 20 and 40 days after seedling emergence (DAE).

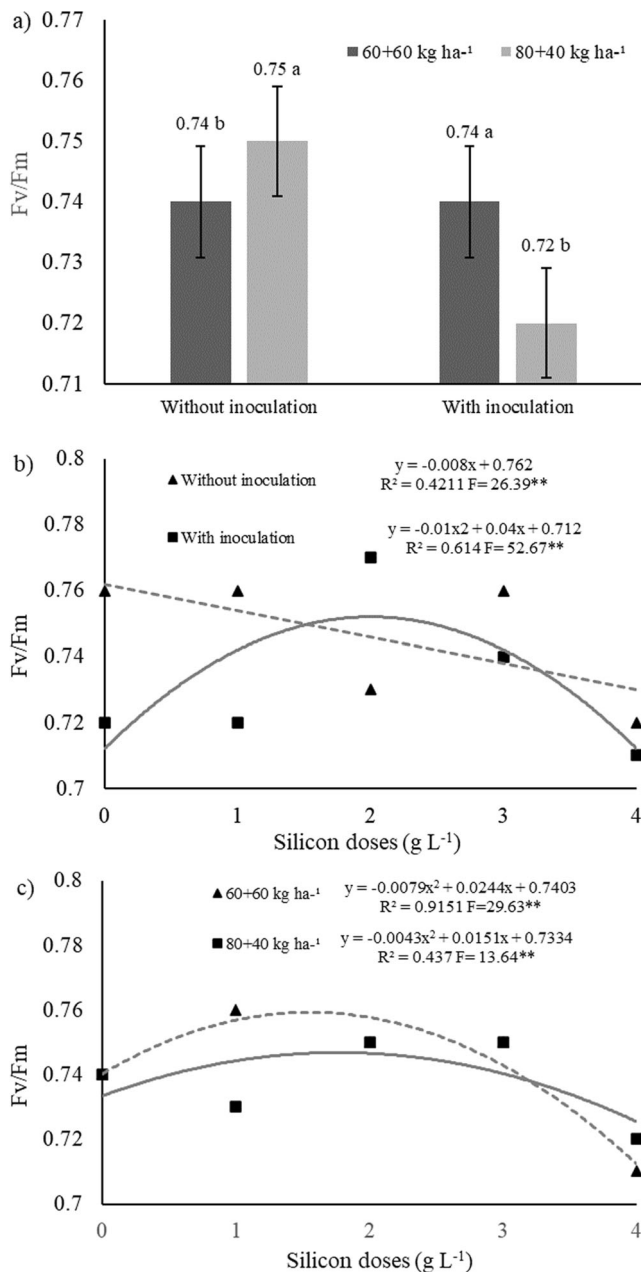
## 4 Discussion

### 4.1 Foliar Contents of Nitrogen (N) and Silicon (Si) and Relative Chlorophyll index (RCI)

Higher relative RCI were recorded for inoculated plants regardless of splitting of nitrogen fertilizer. This could be common bean plants are absorbed nitrogen is assimilated more efficiently Soratto et al. [29].

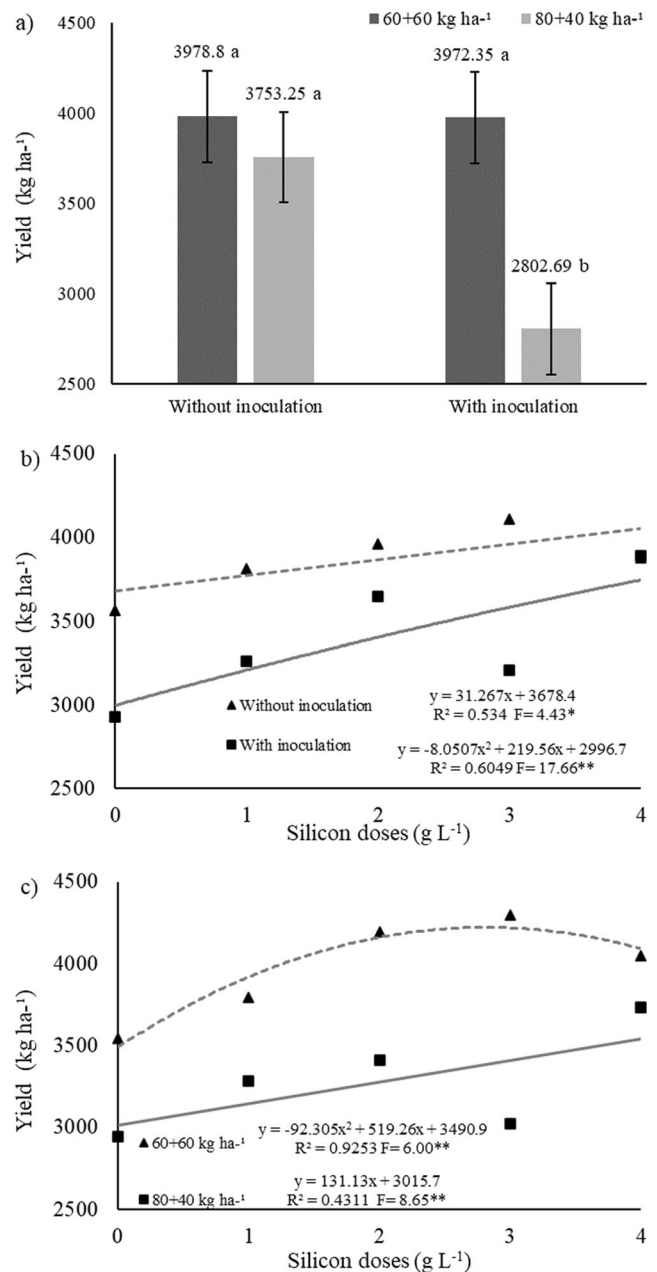
De Araújo et al. [30], who studied cowpea cultivation, attributed the highest RCI values to N fertilizer application, followed by seed inoculation. However, Soratto et al. [31] reported that increased supply of N fertilizer can increase chlorophyll contents in common beans, cultivar Pérola, up to a saturation point, with no gains in pigment production beyond plant needs.





**Fig. 7** Effect of the interaction between seed inoculation and nitrogen fertilization split (a), interaction between seed inoculation and Si application via foliar (b) and interaction between nitrogen fertilization split and Si application via foliar (c), on the photochemical efficiency of the photosystem of common bean (*Phaseolus vulgaris* L.). \*\* significant at 1 % probability by the F test

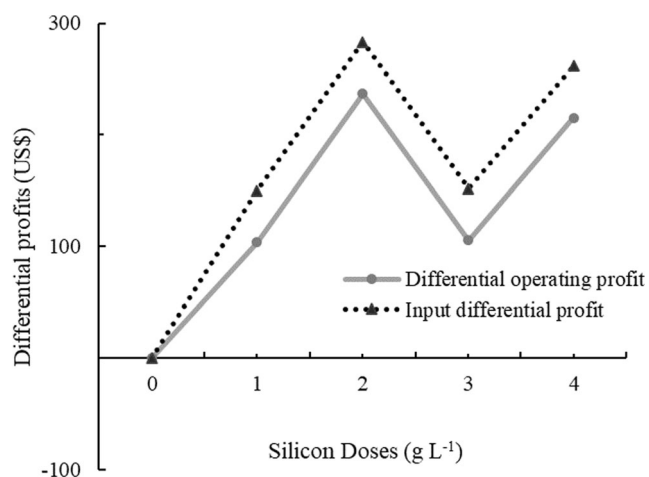
The behavior for the relative chlorophyll index was different when submitted to the splitting of nitrogen fertilization. There is an increasing trend in relative chlorophyll contents for splitting of 60 + 60 kg N ha<sup>-1</sup> at 20 and 40 DAE, respectively. Studies reported that Si assists in the retention of photosynthetic pigments and carotenoids, especially when plants are subjected to stress conditions (nutritional, phytosanitary or water), relieving stress in



**Fig. 8** Effect of the interaction between seed inoculation and nitrogen fertilization split (a), interaction between seed inoculation and Si application via foliar (b) and interaction between nitrogen fertilization split and Si application via foliar (c), on the yield of common bean (*Phaseolus vulgaris* L.). \* and \*\* significant at 5 and 1 % probability by the F test respectively

chloroplasts [32]. The results of our study corroborate this statement, as although increases in foliar nitrogen content have not been observed, in splitting of 60 + 60 kg N ha<sup>-1</sup> at 20 and 40 DAE, higher foliar silicon content, RCI, and grain yield were found.

Moreover, relative chlorophyll index, considers the relationship between *chlorophyll a* and *chlorophyll b*, which can reveal the stress the plant is undergoing.



**Fig. 9** Economic differential analysis of common bean (*Phaseolus vulgaris* L.) in function of Si application via foliar

Studies reveal the existence of direct relationship between increasing silicon and *chlorophyll a* contents in the plant [33] and, consequently, *chlorophyll a* overtime. The results of our study raise the hypothesis that, in splitting of 60 + 60 kg N ha<sup>-1</sup> at 20 and 40 DAE, the common bean has better use of the N fertilizer, especially when associated with increased Si supply.

Highlight that Si contents obtained in common beans leaves are within the range considered adequate (6.3 g kg<sup>-1</sup>) by Korndörfer et al. [34]. However, plants subjected to seed inoculation and splitting of 80 + 40 kg N ha<sup>-1</sup> at 20 and 40 DAE required higher doses of Si to reach foliar contents adequately.

#### 4.2 Gas Exchanges and Photochemical Efficiency of Photosystem II (Fv/Fm)

Besides facilitating cooling of leaves and roots with solute transport, transpiration promotes the movement of water and dissolved nutrients to root surfaces through a mass flow. In this context, in the present study, uninoculated plants required more supply of N fertilizer to increase transpiration rates, while inoculated plants were more efficient in splitting 60 + 60 kg N ha<sup>-1</sup> at 20 and 40 DAE (Fig. 5a). This behavior is explained by the contribution of the fixing bacteria from in the initial N supply for common bean.

Was observed this same behavior for the other physiological variables, such as stomatal conductance and net photosynthetic rate. The experiment was conducted in an irrigated system by central pivot sprinkling, without water restrictions for the cultivation of common beans, maximizing the expression of stomatal conductance. Moreover, diazotrophic bacteria can increase water use by plants, especially by increasing the root system [35]. Thus, the crop absorbs more water and maintains the transpiration rate high for a longer time period, favoring nutrient absorption, especially those absorbed through mass

flow, such as nitrogen, ensuring adequate mineral nutrition of plants [36].

As reported, for net photosynthetic rate, according to Flores et al. [37] and Juge et al. [38], the use of nitrogen-fixing bacteria in soybean plants increases the production of carbon-dependent compounds, such as ureids and flavonoids, which can be attributed to increased assimilation of carbon fixed by photosynthesis. The nitrogen fertilization of 80 + 40 kg N ha<sup>-1</sup> at 20 and 40 DAE, suggests a reduction of biological N fixation efficiency, by the increased supply of N fertilizer in the first 20 days after plant emergence, like experiments of Hungria et al. [4]. However, when applied in smaller quantities, at sowing of the common bean crop, it increases nodule growth and biological N fixation efficiency, and very low N contents in the soil may even limit symbiotic activity.

When supplying silicon to plants, was observed significant increases in transpiration rates and stomatal conductance of common bean. Was believe that this behavior is also associated with Si being accumulated in the epidermal cells of shoots, improving the contact angle of the leaf opening, making them more erect, and improving light capture [39], reflecting on photosynthetic capacity. Si is deposited as solid amorphous silica, directly affecting water relations in plants, which can explain quadratic behavior with reduction of transpiration rates after a certain dose of Si. De Moraes et al. [24], who studied the tomato crop subjected to foliar silicon fertilization and water restriction, observed increases in transpiration rates with increasing Si supply, especially when plants were not under water stress, corroborating our data, as the common bean was grown in an irrigated system, i.e., without water restriction.

Regarding seed inoculation, uninoculated plants performed better after foliar application of 2.0 g Si L<sup>-1</sup>, regardless of splitting of nitrogen fertilization. When evaluating the effect of splitting of nitrogen fertilization and Si supply, better stomatal conductance was observed for splitting of 60 + 60 kg N ha<sup>-1</sup> at 20 and 40 DAE, reflecting in better net photosynthetic rates. Khan et al. [40] attributed increases in photosynthetic rate to the increase in CO<sub>2</sub> uptake and stomatal opening. Si accumulation in regions with increased leaf transpiration allows the maintenance of open stomata and increased influx of CO<sub>2</sub> and water, which may result in increased CO<sub>2</sub> content per water molecule transpired in dicotyledonous plants [41].

However, high Si concentrations can reduce the metabolic rates of plants, as observed in our study for Si supply in concentrations above 3.0 g L<sup>-1</sup>. This behavior is attributed to the polymerization of Si, reducing the flexibility of stomatal walls, inducing stomata to remain closed [42]. After depositing Si on the leaf, silica-cuticle and silica-cellulose double layers are formed [43], promoting stomatal closure and water loss through transpiration, reducing photosynthesis and internal CO<sub>2</sub> concentration. This behavior was also observed by do

Couto et al. [15] for rice, where the application of doses above  $1.68 \text{ g Si L}^{-1}$  reduced the internal  $\text{CO}_2$  concentration of plants. Several studies in the literature suggested that foliar silicon application in concentrations higher than  $3.0 \text{ g L}^{-1}$  negatively affect the metabolic rates of plants, as in rice [15], tomato [24], and sunflower [16], suggesting this concentration as a limit for foliar application.

When was evaluated maximum quantum efficiency of photosystem II, results proved the behavior aforementioned, as regardless of treatment, Si application in concentrations above  $3.0 \text{ g L}^{-1}$  decreased Fv/Fm considerably. The ratio between variable and maximum fluorescence (Fv/Fm) expresses the maximum quantum efficiency of electron transport through FSII, when all reaction centers of the FSII are open [44]. It is used to detect disturbances in the photosynthetic system caused by environmental and biotic stresses, indicating inhibition of the photochemical activity of the plant. Our results show the best use of light energy to perform photosynthetic activities in common beans with Si application at the maximum concentration of  $3.0 \text{ g L}^{-1}$ . In addition, decrease in Fv/Fm may be related to decreases in transpiration, stomatal conductance, and net photosynthesis, indicating reduction in plant metabolism due to increased deposition of Si in leaves.

### 4.3 Grain Yield and Cost analysis

Splitting of nitrogen fertilization of  $60 + 60 \text{ kg N ha}^{-1}$  at 20 and 40 DAE<sup>1</sup> in inoculated plants, provided the best grain yields, regardless of foliar silicon application. As aforementioned, increased supply of N fertilizers in inoculated plants may have reduced the efficiency of rhizobacterial inoculation and, consequently, the grain yield of common bean.

Even with reduction of metabolic rates of plants with Si application in concentrations above  $3.0 \text{ g L}^{-1}$ , the maximum grain yield was achieved with the application of  $4.0 \text{ g Si L}^{-1}$ . Several factors regulate crop yield, being summarized in environment, soil, and plant factors. In this sense, even with losses of physiological quality of plants with applications higher than  $3.0 \text{ g Si L}^{-1}$ , the common bean benefited from Si application, converting it into grain yield. As suggested by Epstein and Bloom [45], increase in crop yield may be related to the adequate mineral nutrition of plants associated with the beneficial effect of Si regarding its induction of resistance to pests and diseases, besides improving tolerance to possible abiotic stresses.

Crusciol et al. [46], studied foliar silicon application in the form of stabilized silicic acid, observed increases of 14, 15 and 9.6 % in the grain yield of soybeans, common beans, and peanuts, using the dose of  $2 \text{ L ha}^{-1}$  of a commercial product containing 0.8 % soluble Si. However, de Melo Peixoto et al. [16] who studied the sunflower crop, observed increases of up to 39 % in biomass production with application of up to  $2.52 \text{ g}$

$\text{Si L}^{-1}$  in the form of potassium and sodium silicate. All the above studies highlighted the increase in biomass/grain production following application of silicon. The optimal dose of silicon varied in studies, as the sources were different. In our study, the best grain yield in common bean has been recorded at  $3.0 \text{ g Si L}^{-1}$ .

When performing the economic analysis in relation to Si application for the common bean crop, it was observed that differential profit was positive for all doses, reaching US\$  $236.7 \text{ ha}^{-1}$  regardless of splitting of nitrogen fertilization and seed inoculation. If was consider crop production systems with high technology, foliar fertilizers are often used in order to increase crop yields. De Farias Guedes et al. [47] also observed the possibility of applying potassium and sodium silicate as Si source together with Zn, in the form of chelates (Zn-EDTA 14 %), with stabilizers sorbitol, fulvic acid, and salicylic acid in the sorghum crop. Therefore, the Si source used in the present study can be associated with other foliar fertilizers, which can reduce the operational costs of application and make Si application economically viable for the common bean.

However, in the conditions of the present study, seed inoculation proved to be economically inefficient, which could compromise the positive results of differential profits from Si application in the order of up to US\$  $308.5 \text{ ha}^{-1}$ , while splitting of nitrogen fertilization of  $80 + 40 \text{ kg N ha}^{-1}$  implies an average loss of revenue of US\$  $449.8 \text{ ha}^{-1}$  in relation to its reference ( $60 + 60 \text{ kg N ha}^{-1}$ ), both applied at 20 and 40 days after seedling emergence (DAE).

## 5 Conclusions

The physiological quality of common bean, when there is no seed inoculation, is better with splitting of nitrogen fertilization of  $80 + 40 \text{ kg N ha}^{-1}$  at 20 and 40 DAE, while for inoculated plants, splitting of  $60 + 60 \text{ kg N ha}^{-1}$  at 20 and 40 DAE show better metabolic rates, regardless of silicon application.

In general, application of up to  $3.0 \text{ g Si L}^{-1}$  promotes the best physiological rates for the common bean, regardless of seed inoculation and splitting of nitrogen fertilization.

Splitting of nitrogen fertilization with  $60 + 60 \text{ kg N ha}^{-1}$  at 20 and 40 days after emergence of common beans promotes the best grain yields ( $3,975.6 \text{ kg ha}^{-1}$ ), regardless of Si supply and seed inoculation.

Foliar silicon application improves grain yield in approximately 10 % (uninoculated seeds) and 25 % (inoculated seeds), regardless of splitting of nitrogen fertilization, with the use of the highest concentration applied ( $4.0 \text{ g Si L}^{-1}$ ).

Regardless of seed inoculation, for splitting of  $60 + 60 \text{ kg N ha}^{-1}$ , the higher grain yield occurs with application of  $3.0 \text{ g Si L}^{-1}$ , approximately 20 % higher than the control, while for splitting of  $80 + 40 \text{ kg N ha}^{-1}$ , grain yield is maximum with

the use of 4.0 g Si L<sup>-1</sup>, approximately 17.4 % higher than the control, without adding Si.

Application of 2.0 g Si L<sup>-1</sup>, associated with splitting of 60 + 60 kg N ha<sup>-1</sup> at 20 and 40 days after seedling emergency and without seed inoculation result in the best differential profit, reaching US\$ 283.3 per hectare, depending on spraying costs.

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**Authors' Contributions** AMB, RAF, EPBF, AFA, FRSL led the data analysis and led the writing with input of all co-authors. RAF, EPBF, KOA, MM and RMP designed the experiment and provided overall project leadership. AMB, AFA and FRSL grew the plants, applied the treatments and collected data. AMB, AFA, FRSL and JPSJ was responsible for the lab analysis. KOA conducted the economic analysis of data. AMB, RAF, EPBF, AFA, FRSL, JPS, KOA, MM and RMP carried out the final review of the article.

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**Data Availability** Data is available upon request to the corresponding author.

## Declarations

**Conflicts of Interest/Competing Interests** There is no conflict of interest.

**Ethics Approval** All experiments were conducted ethically and no issues regarding ethical issues arouse during the experiments or the manuscript confection.

**Consent to Participate** All authors freely agreed and gave their consent to participate on the experiment.

**Consent for Publication** All authors freely agreed and gave their consent for the publication of this paper.

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