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ORIGINAL PAPER

Innovative Soluble Silicon Leaf Source Increase Gas Exchange, Grain Yield and Economic Viability in Common Bean

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Abstract

Purpose New soluble sources of silicon (Si) have arrived in the agribusiness. Some of them are being considered efficient by their used composition, improving absorption of this beneficial element, photosynthetic parameters, and yield. However, the economic feasibility of using this source must be considered. Thus, the present study aimed to evaluate the effect of the foliar application of increasing concentrations of Si on the physiological aspects grain yield in common bean and the economic viability of silicon application.

Methods Randomized block design with seven Si concentrations: 0.00, 0.33, 0.66, 1.00, 1.33, 1.66 and 2.00 g L^{-1}. Solutions were applied via foliar spraying covering the whole treatment area. Solutions were provided in triplicates and three different days after sowing (40, 55, and 70 DAS). Si accumulation, gas exchange (stomatal conductance, transpiration, photosynthesis, and internal CO_2 concentration), photochemical efficiency, productivity, and economic viability were evaluated.

Results and conclusions Foliar applications of Si as sodium and potassium silicate stabilized with sorbitol were found to be efficient in providing Si for bean cultivation. The beneficial effect of Si application in the photosynthetic and yield parameters was observed in this study. However, when Si concentration is higher than 1.16 g L^{-1} it is considered not economically viable.

Keywords Beneficial element · Economic viability of silicon application · *Phaseolus vulgaris* · Photosynthesis · Plant nutrition

1 Introduction

The increase in the world population has created an overload on global agricultural resources and, consequently, the concern of producers and researchers from around the world about how to increase crop production without expanding new areas [1].Production must increase to 60–110 % by 2050 to supply the constant demand for food, considering 2005 as a base [2]. Bean (*Phaseolus vulgaris* L.) crop production has been highlighted due to its nutritional potential and its easy access, and thus a perfect source to the human diet [3].

Despite its easy adaptation to various regions, bean production is stagnated because of the lacking proper phytosanitary and phytotechnical management, especially about water stress and fertilization balance. The use of silicon (Si) is a strategy to improve plant development, and it was already mentioned in cotton [4, 5], peanut [6], and sunflower [7, 8]. Still, Si can minimize abiotic tress, acting in several defense mechanisms of the plant [9], as observed in cotton [4], field pea [10], and tomatoes [11].

The supply of Si to the plants can be done via root or leaf; however, bean is considered non-Si-accumulating due to its low efficiency to absorb Si by roots [12, 13]. For these kinds of plants, foliar application, with a soluble source of Si, can be systematically viable amending several variables that are important to the plant development [4, 5, 7].

There are some evidences that foliar application of soluble sources of Si can increase yield, as observed in beans [14] and other crops [14, 15]. The benefits of this foliar application can be explained by its positive effect on photosynthetic metabolism [14, 15]. Also, some studies under controlled situation showed the benefits of Si on the production of photosynthetic pigments [4, 5], the photosynthetically active area [8], and the photosynthetic efficiency [4]. It is also known that Si protects thylakoid cells from environmental stress, and may restore nitrogen (N) reductase activity, responsible for the reduction

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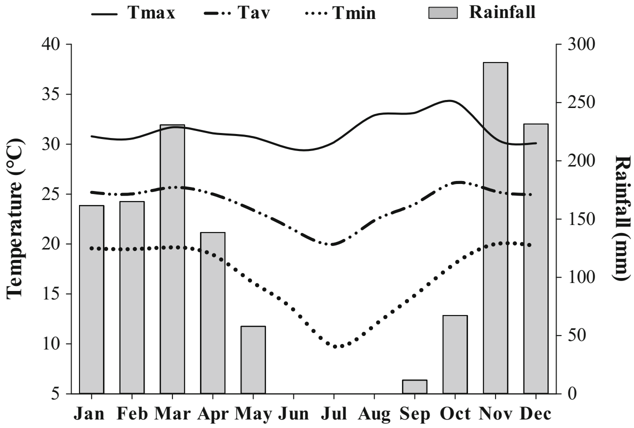


Fig. 1 Air temperature maximum (Tmax), minimum (Tmin) and average (Tav), and rainfall per month, from January/2017 to December/2017, in the School of Agronomy, Federal University of Goiás, Goiânia, Brazil

of NO_3^- and NO_2^-, observed in cucumber plants (*Cucumis sativus*), affecting the N ratio in plants [16].

The effect of Si input in the system is already well-defined but it is still fundamental to consider how the application can be economically feasible. Some studies using Si-solutions in the non-accumulated plants are indicate positive results with quadratic adjustment, but equally indication that high concentrations may not be the best option to yield [8] or the plant development [5, 7, 11].

Therefore, the present study are claiming that (a) the foliar application of Si, in a soluble form, can affect the common bean in terms of quality, Si concentration in leaves, and yield; and (b) in spite of the phytotechnical responses, it has a limit Si input in the system which remain economically feasible. Thus, the present study aimed to evaluate the effect of the foliar application of Si concentrations on the physiology, nutrition, grain yield and economic viability of common bean.

2 Materials and Methods

2.1 Characteristics of the Experimental Area and Bean Cultivation

The experiment was carried out with the bean cultivar BRS Estilo, in June 2017 at the experimental area of the School of Agronomy of the Federal University of Goiás (EA-UFG), Goiânia, Brazil.

The climate of the region is Aw type (tropical savanna), with a well-defined rainfall regime, with dry and rainy seasons [17]. During the experimental period, the average, maximum and minimum air temperature and rainfall were recorded (Fig. 1).

The soil was classified as Rhodic Hapludox [18]. The chemical analysis of the 0-0.20 m soil layer was performed according to Teixeira et al. [19] (Table 1). Si in the soil was quantified by sulfuric extraction as proposed by Embrapa [20].

Fertilization recommendations for the crop were made based on the results from the soil analysis [21], 20 kg ha^{-1} of N, 110 kg of P_2O_5, and 70 kg ha^{-1} of K_2O, with urea ($CO(NH_2)_2$ − 45 % N), simple superphosphate (18 % - P_2O_5) and potassium chloride (58 % - K_2O) as sources, respectively. As topdressing fertilization, 80 and 40 kg ha^{-1} of N, were applied at 20 and 40 days after seedling sowing (DAS), respectively, in the form of urea.

For the cultivation of beans, the area was irrigated by sprinkling (center-pivot), and the uniformity of application of the water depth was checked using the Christiansen Uniformity Coefficient (CUC). The center pivot showed a CUC of 88 %, an index considered adequate by the Brazilian standard (NBR 14,244) [22], with a gross irrigation depth of 7.8 mm day^{-1} (in 21 h), totaling the application of the net irrigation depth of 267.52 mm, throughout the growing period. The water depth was calculated taking into consideration the crop coefficient (Kc) recommended by Gonzaga [23], and the reference evaporation data, calculated by the Penman-Monteith method, based on the climatological data collected at the UFG meteorological station.

Before sowing, seed treatment was carried out with the application of 200 g 100 kg^{-1} of seeds with Thiamethoxan insecticide. Sowing was carried out on June 16, 2017, in the winter harvest, with a final plant population around 150,000 plants per hectare. The BRS Estilo cultivar has indeterminate growth habit, erect plants, a 100-grain weight of around 26 g, and normal cycle between 85 and 90 days [24]. The monitoring of pests and diseases was carried out weekly, with no need to use chemicals during the conduct of the experiment.

Table 1 Chemical and physical attributes of the soil in the study area, before the implementation of the experiment

Layer	pH	OM	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al
m	($CaCl_2$)	g dm^{-3}	mg dm^{-3}	cmol$_c$ dm^{-3}				
0.0-0.2	5.60	11.00	4.70	0.31	1.00	1.00	0.00	2.30
	Cu	Fe	Mn	Zn	SiO_2	Clay	Silt	Sand
	mg dm^{-3}				%	g kg^{-1}		
0.0-0.2	1.80	26.00	28.00	2.90	10.25	570	40	390

OM = Organic Matter of soil

2.2 Experimental Design and Silicon Application

The experiment was designed in randomized blocks with seven Si concentration supplied by foliar application: 0.00 (T1 - control), 0.33 (T2), 0.66 (T3), 1.00 (T4), 1.33 (T5), 1.66 (T6) and 2.00 (T7) g L^{-1}, with three replicates. The Si source used was sodium and potassium silicate stabilized with sorbitol, and copper sulfate (SiAl − 107 g L^{-1} of Si; 14.9 g L^{-1} of Cu; 28.8 g L^{-1} of K; 60.5 g L^{-1} of Na_2O; 20 mL L^{-1} of sorbitol; pH 11.8; and soluble in water). K, Cu and Na contents were balanced between treatments with the addition, respectively, in the following amounts (g L^{-1}): 0.53, 1.12 and 0.27 for T1; 0.44, 146 0.93 and 0.23 for T2; 0.36, 0.75 and 0.18 for T3; 0.27, 0.56 and 0.14 for T4; 0.18, 0.37 and 0.09 for T5; 0.09, 0.18 and 0.05 for T6; and no applications were needed for T7.

Si concentrations were chosen based on the visual polymerization test. A previous test was performed with 50 mL of solution at a concentration of 2.00 g L^{-1} of Si (highest concentration studied). The solution was prepared with the SiAl source, and the pH was adjusted to 7 ± 0.2 using HCl (1 M) or NaOH (1 M). No change in the color of the spray solution was observed, there was no polymerization process that could prevent leaf absorption by the plants. Each experimental unit was composed of five rows of 5 m length spaced at 0.45 m, totaling 11.25 m². As usual, just the central rows were considered useful with a total of 5.4 m², so it was excluded 0.5 m at each zone.

Silicon application in the plants were carried out in three dates, at 40, 55, and 70 days after sowing (DAS). The silicate spray volume was prepared and immediately applied to the leaves. The applications were carried out with the aid of a backpack sprayer, pressurized with CO_2, and a 20-liter tank, with a standardized spray volume of 120 L ha^{-1}. The volume of each application was calculated to cover the entire leaf area of the plant. During the application, the temperature max and the relative air humidity was < 25 °C and > 60 %, respectively.

2.3 Physiological and Nutritional Assessment

As physiological parameters, five days after each silicon foliar application (45, 60 and 75 DAS), the relative chlorophyll content (RCC), gas exchange (stomatal conductance, internal CO_2 concentration, net photosynthesis rate, and maximum quantum yield of FSII (Fv Fm^{-1})), were measured on the first leaf completely expanded from the apex of the main stem.

The evaluations of relative chlorophyll content were performed using a chlorophyll meter (Falker® - ClorofiLOG CFL 1030 model), in five plants chosen at random within each experimental unit, in the first completely expanded leaf from the apex of the main stem [25]. Similarly, gas exchanges (stomatal conductance, internal CO_2 concentration, net photosynthesis rate, and maximum quantum yield of FSII (Fv Fm^{-1})) were evaluated using an Infrared Gas Analyzer (IRGA) with fluorometer, model iFL - Integrated Fluorometer and Gas Exchange System, with 6.25 cm^2 leaf chamber, between 9:00 am and 2:00 pm [26].

The nutritional status of beans was evaluated through three-leaf analyses at 45, 60, and 75 DAS, in which 20 diagnostic leaves were collected (first leaf completely expanded the plants) [27].

Silicon was determined according to Korndörfer et al. [28] after wet digestion of plant material with H_2O_2 and NaOH in a water bath and autoclave. Si estimation was made in a spectrophotometer after a colorimetric reaction with ammonium molybdate, hydrochloric acid, and oxalic acid. The nitrogen content was determined through wet digestion with sulfuric acid followed by distillation in the presence of NaOH, and titration with sulfuric acid (0.02 N), as described by Silva [29].

2.4 Grain Yield and Economic Evaluation

In each plot, one meter of the three central rows was manually harvested when the crop reached physiological maturity (90 DAS).

For the economic analysis procedure, the partial budgeting technique was used Noronha [30]. This technique is recommended to analyze decisions that involve partial modifications in the organization of productive activity. From the differential cost and revenue budgets, the differential profit is calculated according to the reference enterprise (control treatment). The best alternative will be the one that offers higher net benefits or higher profit margins. Differential revenues and costs for the application of different treatments concerning the control were determined for each treatment.

Based on the average bean yield for each treatment, the increase in grain yield provided by the different treatments relative to the control was calculated. The production value (Pv) in each treatment was obtained by multiplying the additional grain yield by the price received by the bean producers in Brazil, according to the Eq. 1:

$$Pv = Gy * P \tag{1}$$

Where:
Gy = Grain yield gain.
P = Price received by the bean producers in Brazil.

The average real price of the last ten years (from 2010 to 2020) was used as a reference, whose value was US $ 34.70 for 60 kg of grains [31], considering an exchange rate of US $ 1 = R$ 5.83. The differential profit was obtained from the difference between differential revenue and the differential cost of applying Si concentrations, relative to the control, according to the following Eq. 2:

$$Pd = Rd - Cd \tag{2}$$

Where:
Pd: differential profit;
Rd: differential revenue (Rdti - RdIt0);
Cd: differential cost Cd (Cdti - Cdt0);
ti: treatment i and;
t0: control.

The cost of the silicon was obtained at current market value, converted into dollars once the historical increase in fertilizer prices in Brazil was verified [32].

2.5 Statistical Analyses

The data were submitted to analysis of variance (F-test) and, when significant, they were submitted to the study of polynomial regression. Linear and quadratic mathematical models were tested, applying the models that obtained the best data adjustments. The magnitude of the significant regression coefficients at a 5 % of probability by the t-test was adopted as a model choice criterion. When significant, the maximum and minimum points were obtained by deriving the equations. Statistical analyses were performed with the aid of the AgroEstat® software [33].

3 Results

Foliar applications of Si were efficient in providing Si for the bean culture, which increase linearly the Si leaf content due to the increase in the applied Si concentration in all evaluated periods (Fig. 2). The higher Si concentration used in the treatments reached the Si content in leaves was 4.08, 5.56, and

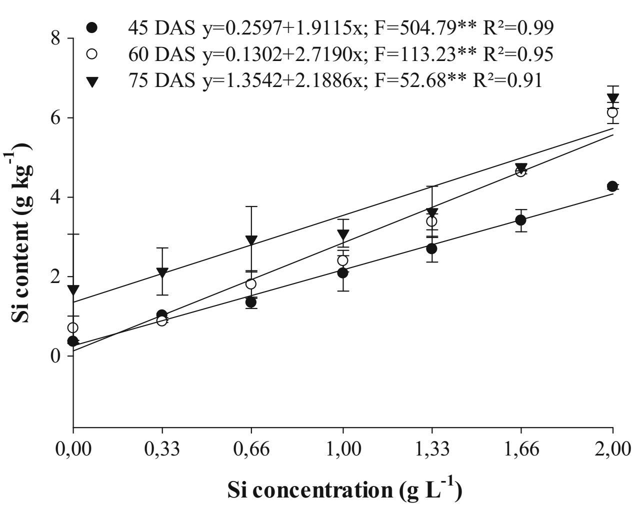


Fig. 2 Silicon leaf content of the bean plants in function with Si-concentrations applied via leaf at 45, 60, and 75 DAS, respectively. ** - significant by the F-test at 1 % probability

5.72 g Si kg^{-1} in the leaves at 45, 60, and 75 DAS, respectively (Fig. 2).

Increases of Si application via foliar in common beans was no-significant to N uptake which preserves an average of 52.61 g kg^{-1} of N. The same response was observed to RCC with values in the zone of 52.91, 51.07 and 51.36 µg cm^{-2} at 45, 60, and 75 DAS, respectively (Table 2).

Si application demonstrated positive effects in photosynthetic parameters. In 45 DAS soon after Si applications, the stomatal conductance (Fig. 3a) and the transpiration index (Fig. 3b) increased linearly reaching 0.34 mol m^{-2} s^{-1} and 10.19 mol m^{-2} s^{-1}, respectively, with 2.00 g L^{-1} of Si. Liquid photosynthesis rate (Fig. 3c) presented a quadratic curve as the higher value that was 11.18 µmol m^{-2} s^{-1} with the application of 1.10 g L^{-1} of Si. In circumstances different from those presented, Si did not affect internal concentration index of CO_2 (Fig. 3d) and quantum efficiency of photosystem II (Fig. 3e), which reached figures of 318.61 µmol mol^{-1} and 0.74, respectively.

Si concentration increased linearly to the stomatal conductance (Fig. 3a) and transpiration index (Fig. 3b) when the experiment was evaluated at 60 DAS. Both variables had performed with the application of 2.00 g L^{-1} of Si, with 0.53 mol m^{-2} s^{-1} and 11.60 mol m^{-2} s^{-1}, respectively. However, it was noticed quadratic adjustments to liquid photosynthesis rate (Fig. 3c), internal CO_2 concentration (Fig. 3d), and quantum efficiency of photosystem II (Fig. 3e) with 14.57 µmol m^{-2} s^{-1}, 323.33 µmol mol^{-1} and 0.75 with the application of 1.35, 0.79 and 1.38 g L^{-1} of Si, respectively.

The last day of evaluation was at 75 DAS. The stomatal conductance (Fig. 3a) and transpiration index (Fig. 3b) behaves with quadratic adjustment in function of increasing Si-concentrations. The first variable reached the lower value of 0.13 mol m^{-2} s^{-1} and 3.53 mol m^{-2} s^{-1} with the application of 1.08 and 0.88 g L^{-1} of Si, respectively. Contrarily, liquid photosynthesis rate (Fig. 3c) responded linearly and reached 6.47 µmol m^{-2} s^{-1} with the application of 2.00 g L^{-1} of Si. Those foliar application did not affect internal CO_2 concentration (Fig. 3d) neither quantum efficiency of photosystem II (Fig. 3e), that presented figures around 320.86 µmol mol^{-1} and 0.75, respectively.

The increase in the productive parameters proved the beneficial effect of Si application. When evaluating the production parameters, it is observed that the Si application increased the 100-grain weight (Fig. 4a) and the grain yield (Fig. 4b) up to the concentration of 1.05 and 1.21 g L^{-1} of Si, respectively. In these concentrations, the 100-grain weight was 29.00 g, and the grain yield was 3,005.34 kg ha^{-1}, corresponding to an increase of 18 and 33 % relative to the control treatment, respectively.

It was possible to observe that all doses were economically efficient, providing positive differential profit compared to the

Table 2 Nitrogen (N) content and relative chlorophyll content (RCC) of bean plants cultivated in function of Si applied via leaf

Si concentration (g L^{-1})	N content (g kg^{-1})	RCC^{1} µg cm^{-2}	RCC^{2}	RCC^{3}
0	51.63±0.63	51.97±1.27	56.03±0.03	51.00±0.72
0.33	51.80±2.92	53.77±1.22	55.87±2.60	51.20±0.20
0.66	52.10±2.42	54.90±0.95	57.30±1.49	52.47±0.43
1.00	52.23±0.88	53.43±0.32	57.33±0.82	52.30±1.33
1.33	53.47±0.44	52.67±0.55	57.87±2.54	51.00±0.35
1.66	53.60±0.50	52.13±0.59	57.63±1.99	50.77±1.06
2.00	53.33±0.33	51.50±0.98	57.43±0.84	50.80±1.01
F-test	0.26^{ns}	2.84^{ns}	0.20^{ns}	0.79^{ns}
C.V. (%)	5.41	2.31	5.39	2.71

C.V.: coefficient of variation; RCC^{1}, RCC^{2} and RCC^{3} – evaluations performed at 45, 60, and 75 days after sowing (DAS), respectively; ^{ns} – non-significant by the F-test at 5 % probability

control treatment, without adding Si. However, the dose with the greatest differential return was 1.00 g L^{-1}. Si foliar application has economic viability until 1.17 g L^{-1} dose, and after that, it is not considered economically satisfying. The evidence demonstrates that differential revenue was lesser than the differential costs with the addition of Si to the system (Fig. 5).

4 Discussion

The use of soluble sources of Si, such SiAl, for foliar applications in common bean (Fig. 2) was found efficient in increasing the leaf Si content. Plants absorb Si in the form $SiOH_4$ [34]; SiAl source is efficient in keeping Si stable in the form of monomer species by increasing the absorption of the beneficial element in different crops like cotton [4, 5], sunflower [7], soybean [35, 36], and orchids [37]. Polymerization dependent of pH [38] solution and it is one of the limiting factors for choosing the adequate Si source. Sorbitol is used to balance spraying solution [39, 40] with positive improving in leaves absorption [4, 41]. This is the explanation claimed in this present study about high Si content in the composition of leaves (Fig. 2). Sorbitol decreases the deliquescence point of the drop solution on the leaf surface, and then, decreasing the water evaporation rate [39, 42] favoring the absorption elements by leaves [43].

Some studies evaluated turbidity index of four different Si sources (sodium and potassium silicate stabilized with sorbitol; monosilicic acid stabilized with PEG400; nanosilica Bindizil® and potassium silicate without stabilizer) in time (among 30 and 390 min after the preparation of the solution) [5]. This same study concluded the lesser index to sodium and potassium silicate stabilized with sorbitol being the most recommendable Si source among the other ones.

Leaf absorption of Si did not affect the absorption of N (Fig. 3). Despite the report of antagonistic relationships between N and Si in some species, such as Poaceae [44], in legume plants, this effect does not occur, such as reported in common bean (Table 2) and soybean [45]. Results of Deus et al. [46] demonstrated different effects among Si applications with rice plants with and without N supply. In this study Si increased N accumulation per plant with gains in lignin synthesis, liquid photosynthesis index and grain production.

Silva et al. [47] tested the effects of interaction between Si and N in corn crop production and they pointed that Si can improve chlorophyll content per plant with no effects in N accumulation. Same effect was observed by Souza Junior et al. [5] which proved that sodium and potassium silicate stabilized with sorbitol performed the best situation at 0.8 g L^{-1} of Si. This concentration improved pigments production and the efficiency in photosystem II, which as the consequences favored dry matter and seed production.

The results obtained in this present study was as similar as proved by Souza Junior et al. [5] with grain yield (1.05 g L^{-1}), weight of 100 grains (1.21 g L^{-1}) (Fig. 4), and differential profit (1.17 g L^{-1}) (Fig. 5).

The effects of Si application on gas exchange are controversial in the literature. Although some research related that Si increase gas exchange [7], others indicates that Si decreases this physiologic plant parameter [48]. Chen et al. [9] pointed out that these conflicts of results are due to the variation of the species studied, with a tendency for Si to decrease transpiration in plants that received the element in high concentration. This may occur due to the polymerization of Si on the leaf surface, forming a crust, reducing gas exchange. According to Haynes [38], the increase in the concentration of this element in the solution increases the risk of formation of polysilicon acids and silica gel.

Also, Si increased Fv Fm^{-1} to 0.75 with the application of 1.38 g L^{-1} of Si after two foliar applications (60 DAS). The increase in Fv Fm^{-1} indicates a better use and conversion of

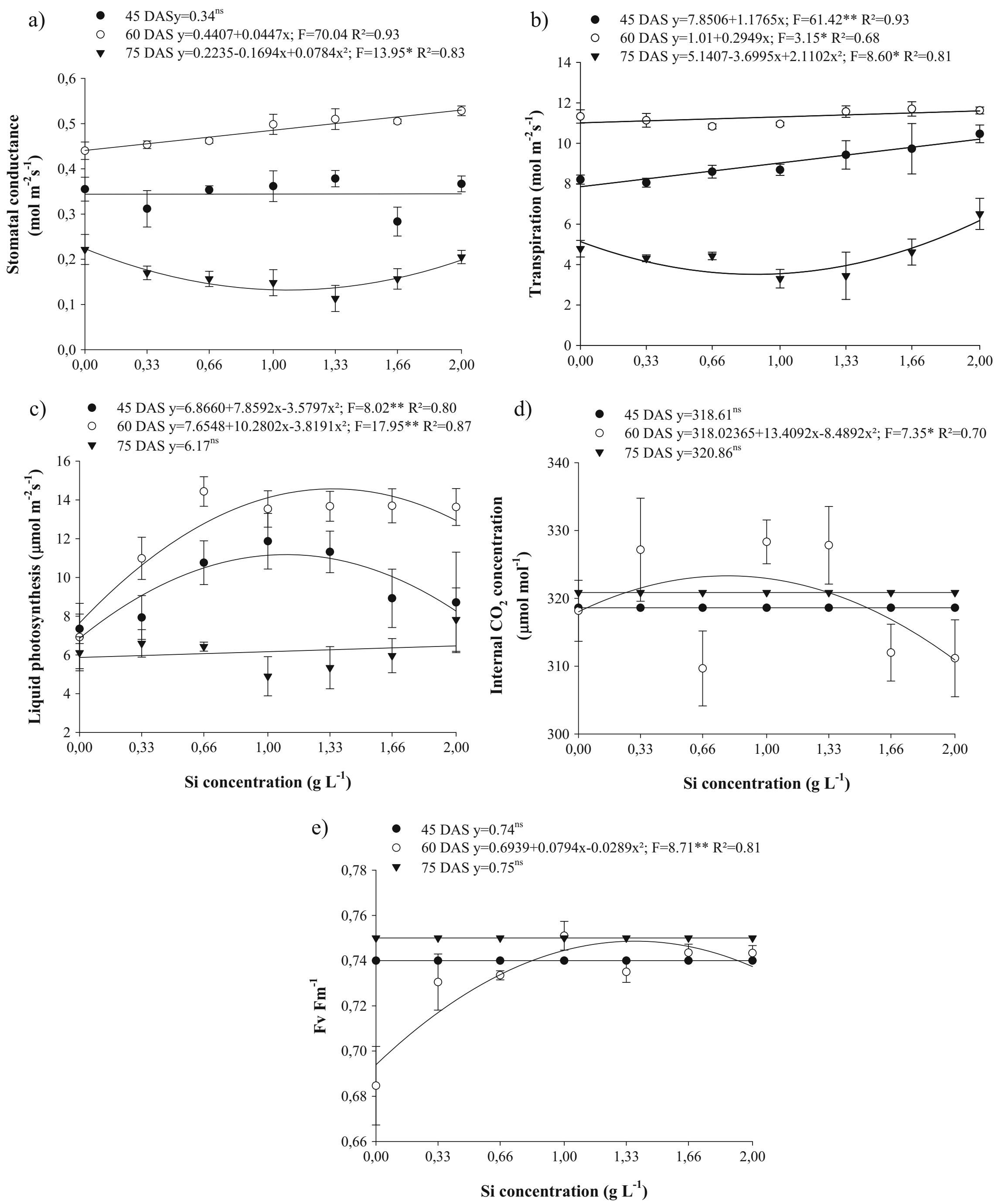


Fig. 3 Stomatal conductance (**a**), transpiration (**b**), liquid photosynthesis (**c**), internal CO_2 concentration (**d**), and quantum efficiency of photosystem II (Fv Fm^{-1}) (**e**) of the bean plants in function of Si-concentrations applied via leaf at 45, 60 and 75 DAS, respectively. ^{ns} and ** - non-significant by the F-test at 5 % probability, and significant by the F-test at 1 % probability

light energy [49]. Si can, directly and indirectly, favor photosynthetic reaction centers [50] and increase Fv Fm^{-1}. Directly, the element is related to greater cell wall stiffness, forming a double layer of Si in the epidermis of leaves, thereby improving leaf architecture and light absorption capacity [51], with consequent smaller energy loss through fluorescence. Indirectly, silicon acts as a protector to stress, such as high temperature [52] and production of reactive oxygen species [53], which can impair the functioning of the photosynthetic system.

It is important to highlight that the absence of beneficial effect of Si foliar application on liquid photosynthesis (Fig. 3c), internal CO_2 concentration (Fig. 3d) and on Fv Fm^{-1} (Fig. 3e) at 45 and 75 DAS was observed. This result can be attributed to the fact one application of Si (45 DAS) is not enough to increase the photosynthetic parameter, and, at the end of the cycle (75 DAS),

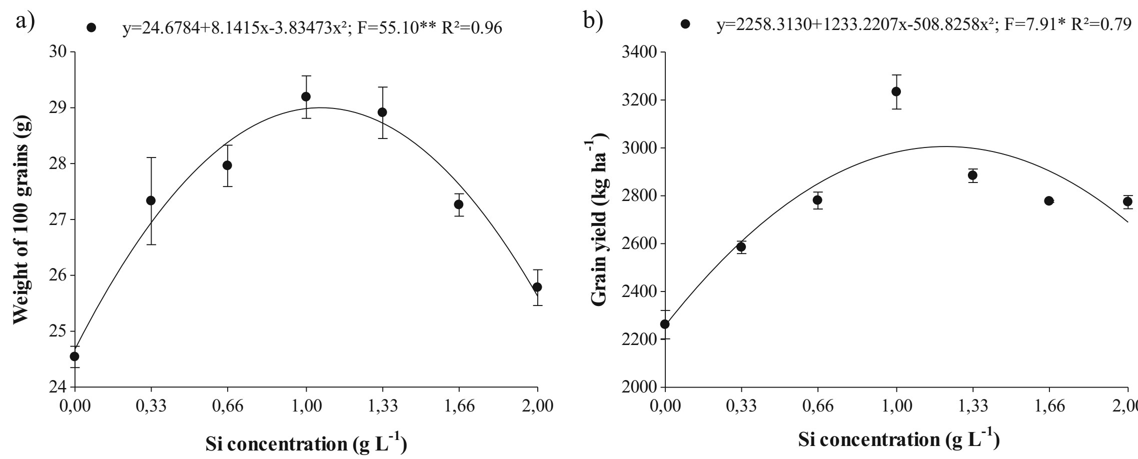
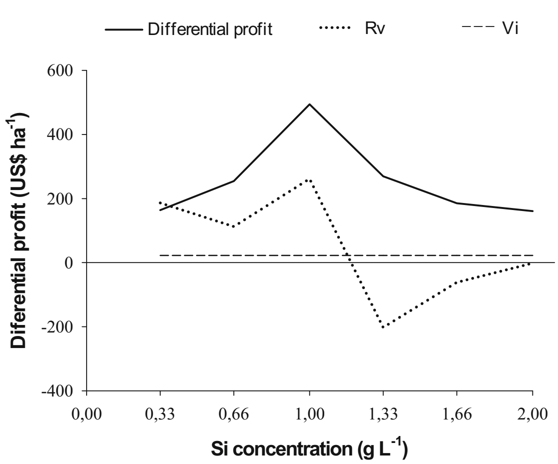


Fig. 4 Weight of 100 grains (**a**) and grain yield (**b**), of bean plants in function of Si-concentrations applied via leaf. ** - significant by the F-test at 1 % probability

the lower photosynthetic rates is a natural phenomenon attributed to senescence process itself, in which chlorophyll degradation and loss of chloroplast functionality occur.

The beneficial effect of Si on the physiological quality of beans provided an increase in crop yield, as also observed by Naiverth and Simonetti [54] for common bean crop. The authors observed that the use of potassium silicate via a foliar application provided an increase in the 1000-grain weight, reduction in the incidence of insects, a significant increase in the number of pods per plant and grains per pod. Abou-Baker et al. [55] evaluated the effect of Si application in beans production on soils with salinity stress. They observed seed increments with potassium silicate and magnesium silicate, both application of 52 and 41 % respectively over the no Si input. Still, there is a consensus in the literature that Si promotes an increase in the yield of various crops, such as soy and peanuts [35], sunflower [7, 8, 56], sorghum [57], cotton [15], rice [58–60], and beans [55, 61].



	0 g L^{-1}	0.33 g L^{-1}	0.66 g L^{-1}	1.00 g L^{-1}	1.33 g L^{-1}	1.66 g L^{-1}	2.00 g L^{-1}
Differential profit	-	164.12	254.68	494.28	269.58	185.46	161.06
Rv	-	186.54	112.99	262.01	-202,21	-61.65	-1.95
Vi	-	22.45	22.45	22.45	22.45	22.45	22.45

Fig. 5 Differential profit, revenue variance (Rv) and variation of the input (Vi) of the bean plants in function of Si-concentrations applied via leaf

5 Conclusions

The results conclude that the foliar supply of Si to the bean plants is efficient in increasing the foliar content of the beneficial element, increasing the photosynthesis and the crop yield. However, increasing the Si concentration above 1.17 g L^{-1} is not economically viable.

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Author Contributions AFA and AMB led the data analysis and led the writing with input of all co-authors. RAF, KOA and RMP designed the experiment and provided overall project leadership. AFA, AMB, ASC, MLL and JPSJ grew the plants, applied the treatments and collected data. AFA, AMB and JPSJ was responsible for the lab analysis. AFA and AMB collected photosynthesis data under supervision of RAF. RAF provided equipment for photosynthesis data collection. RAF and RMP provided all structure for the experiment.

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Data Availability Data is available upon request to the correspondence 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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