



Silicon Application Increases Biomass Yield in Sunflower by Improving the Photosynthesizing Leaf Area

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Received: 30 September 2020 / Accepted: 29 October 2020

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Abstract

Sunflower is an oilseed plant important for food and biodiesel production. One cheap way to increase sunflower production is by the application of silicon (Si), which has shown to be beneficial in plants under a series of stresses. We analyzed if the application of Si in sunflower affected the CO₂ assimilation, N uptake, leaf quantity and quality, and total biomass yield. No difference was observed in the gas exchange measurements or in leaf N concentration. The total leaf area increased in plants applied with even the lowest dose of Si, with no loss to the net CO₂ assimilation rate or leaf N concentration. The higher total leaf area of the plants treated with Si resulted in an increase of the total CO₂ assimilation by the plant. Plants treated with any dose of Si had an increase of 24–39% in biomass yield. Statistically, the increase in biomass was explained by the total CO₂ assimilation in the plant. If the increment in biomass yield is proportional to the oilseed production, we estimate that Si application in sunflower can increase profit by up to 22%. Large-scale experiments are needed to better determine the proper Si dose to be applied and the oilseed production.

Keywords Biodiesel · Bioenergy · CO₂ assimilation rate · *Helianthus annuus* · Mineral nutrition · N uptake · Oilseed

1 Introduction

Sunflower (*Helianthus annuus* L.) is an oilseed plant adapted to many edaphoclimatic conditions. Its oil is well used in culinary and in the production of biodiesel. According to the FAO (<http://www.fao.org/faostat/en/#data/QC>), 52 million tons of sunflower seed was produced in 2018. In Brazil,

sunflower is used as an option for rotation and succession crop in regions where grains are produced [1]. Although Brazil is one of the greatest biodiesel producers, this country is still not among the biggest sunflower seed producers. Thus, if Brazil wants sustainability in the production of bioenergy, it is important to increase the productivity of this plant without significantly increasing the production costs.

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One simple and cheap solution is the application of silicon (Si), which has been observed to benefit many plant species subjected to a wide variety of stresses, by improving or restoring photosynthesis in plants under biotic or abiotic stress. Silicon (Si) is one of the most abundant elements on Earth, freely available in rocks and soil [2, 3]. Even though this element is presented as beneficial for most plants, it is not considered an essential nutrient to the plants, because it is not present in essential molecules or metabolites [3]. Furthermore, most plants can be easily grown in hydroponic solutions where Si is absent [2]. Many studies reported the benefits of Si on plants under stress, but whether unstressed plants can also take advantage of this element is still undetermined.

It has been shown that plants under stress alleviate or even restore CO_2 assimilation upon application of Si. The presence of Si in plants has been associated with increment in the production of antioxidants [4, 5], restoration of the water status [6, 7], disease and insect control [8], salt tolerance [3, 9], and tolerance to toxic elements such as lead [10], arsenic [11], cadmium [12] and chromium [13]. In cotton (*Gossypium* sp.), silicon deposits in the cell wall lignin, and binds to toxic elements like lead, decreasing the mobility of this toxic element in the plant [10]. Also, Si increase the activity of aquaporins, reestablishing the hydraulic conductivity in the plants, which also restore photosynthesis in rice (*Oryza sativa*) leaves intoxicated by As [11]. Furthermore, Si protects the thylakoid membrane in cucumber (*Cucumis sativus*) leaves with high concentration of Cd, and restore the activity of the enzyme nitrogen reductase (NR), responsible for the reduction of NO_3^- to NO_2^- [12].

Because Si produce many benefits in stressed plants, we hypothesize that unstressed sunflower plants can also benefit from Si application. Thus, by applying silicon to sunflower leaves we expect that this element is absorbed by the leaves and increase even further the activity of aquaporins and NR enzymes. Therefore, stomatal conductance should not be a limitation on these plants, increasing the intercellular CO_2 concentration (reflected in higher C_i/C_a) and in the chloroplast around Rubisco, which will increase this enzyme's carboxylation activity and inhibits photorespiration. If water can be freely transpired, the water use efficiency should decrease. Also, if NR activity increases, an increment the N uptake should be observed, resulting in more chlorophyll and Rubisco content in the leaf what will contribute to an increased CO_2 assimilation rate. All these benefits should result in higher biomass yield in the end of the growing period. Alternatively, unstressed plants might be already operating with its optimal hydraulic capacity and N uptake, so Si application may not have any effect on sunflower plants' physiology and productivity.

The goal of this study is to analyze the effects of Si application in four different concentrations and one control in sunflower plants. To achieve this, we performed gas exchange measurements, chlorophyll and nitrogen concentration quantification, morphological and biomass measurements.

2 Materials and Methods

2.1 Plant Material

The study was conducted in a greenhouse at the School of Agronomy of the Universidade Federal de Goiás (UFG), Brazil, from February to October 2017. For this experiment, 20 plants of sunflower (*Helianthus annuus*) variety Altis 99 were used. Plants were grown in 10 L pots filled with local soil (red latosol) supplemented with dolomitic limestone ($\text{CaO} = 36\%$; $\text{MgO} = 15\%$; $\text{PN} = 98\%$; $\text{PRNT} = 92.54\%$), to maintain the soil moisture. Plants were watered daily and fertilized weekly with Johnson-Hoaglands solution [14]. Irrigation was made with deionized water, using the pot weighing method to keep humidity at 60% retention capacity. The greenhouse temperature during the plant growth and the experiment was around 32 ± 3 °C during the day, and 26 ± 3 °C during the night.

2.2 Silicon Application

Plants were randomly separated into five groups of 4 plants each. Plants were assigned to receive Si application in five doses depending on the treatment: control, 0; T1, 0.84; T2, 1.68; T3, 2.52; and T4, 3.36 g L^{-1} . Silicon was applied in the form of potassium and sodium silicate stabilized with sorbitol ($\text{Si} = 62.9\%$; $\text{K}_2\text{O} = 21.3\%$; $\text{Na}_2\text{O} = 15.7\%$; $d = 1.15$ g L^{-1}). In order to maintain the same potassium fertilization for all plants, each treatment received KCl fertilization at the following concentrations: control, 1.904; T1–1.428; T2–0.952; T3–0.476; and T4–0 g L^{-1} of KCl.

Leaf Si fertilization was divided into three applications: 1/3 of the vegetative state (at 15 days after plant emergence - DAE), 2/3 of the vegetative state (30 DAE), and at the end of the vegetative state in pre-bloom (45 DAE). Silicon was applied on the leaves and was made using a sprinkler. Before fertilizing each leaf, the pots were covered with impermeable material to avoid the solution from reaching the soil.

2.3 Data Collection

The relative chlorophyll index (RCI) evaluations were performed on the third fully expanded leaf on each plant at 30, 45 and 60 DAE, using a ClorofiLOG CFL 1030 chlorophyll meter (www.falker.com.br).

At 70 DAE, gas exchange measurements were performed using a LiCor 6400 (LiCor, Lincoln-NE), at 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of illumination, ambient CO_2 concentration (400 $\mu\text{mol mol}^{-1}$), temperature of 32 °C, and vapor pressure deficit of the leaf of 1.5–2.0 kPa. Gas exchange was performed in one leaf per plant, on the youngest fully expanded leaf of each plant. Right after gas exchange measurement, the leaves were counted, the area of each leaf was measured, and the plant was harvested. The samples were

washed with a solution containing 0.1% detergent, 0.3% acidic solution and distilled water, to decontaminate possible residues from the foliar applications. The plant was then dried for 72 h at 60 °C and weighted for biomass yield. Dried leaf samples were taken for quantification of Si and N content. Silicon content was determined using the alkaline digestion method described by Komdörfer [16]. Nitrogen content was measured by the Kjeldahl method [17].

Finally, we estimated the costs of sunflower production in accordance with Dalchiavon et al. [18]. The Si application was based using the costs of the product in the local market. We transformed the currency from Brazilian Real to US Dollar using the currency exchange rate on 13 of May of 2019, which was USD\$1 = BR\$ 3.98.

2.4 Statistics

Plants were randomly positioned in the greenhouse and rotated every week to avoid within room effects. All statistical analyses were performed using R Statistics software (The R Project for Statistical Computing, www.r-project.org). On the analyzes, each plant was considered as a random effect. The mixed linear regressions were made using the “lme4” package. Corrections for heterogeneous distribution of the data was performed by adding “weights:varFixed” to the model according to Zuur et al. [19]. Models were selected based on their best fit and AIC. When significant differences were detected, sequential analysis was performed to identify differences between the treatments.

3 Results

The application of silicon (Si) in sunflower leaves caused an accumulation of this element in shoot (Table 1). This accumulation was proportional to the dose applied, and differences were significant for all treatments ($p < 0.01$). Compared to the control, the average of leaf area was highest in plants treated with 1.68 g of Si L⁻¹ with an increment of 22% ($p = 0.01$). At 2.52 g L⁻¹ the increment was only of 16%, and at 3.36 g L⁻¹ the leaf area was 2% smaller than the control. Increase in the leaf number was also observed in the treated plants, but this difference was not significant ($p = 0.17$). Considering the average leaf area multiplied by the number of leaves, the total leaf area for each plant increased significantly compared to the control ($p = 0.02$), but there was no difference between the Si fertilized plants. No differences were observed in the relative chlorophyll index between treated plants and the control ($p = 0.77$), nor changes in chlorophyll content was observed with time ($p = 0.30$; Table 1). There were also no significant differences between the treatments and the control for N concentration ($p = 0.10$), even though we observed a slight increase of this element in plants treated with Si.

The net CO₂ assimilation rate ($p = 0.53$), stomatal conductance ($p = 0.89$), C_i/C_a ($p = 0.18$), and water use efficiency ($p = 0.14$) were not altered by Si application (Fig. 1). At 32 °C, photosynthesis ranged between 25 and 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$, stomatal conductance varied between 1.10–1.44 mol of H₂O m⁻² s⁻¹, and C_i/C_a was between 0.79–0.83. However, when we take into consideration the total leaf area of the plant and estimate the total CO₂ assimilation by the plant, we observe that there is an increase in the total CO₂ assimilation of the plants treated with Si at 1.68 g L⁻¹ and above concentrations ($p = 0.02$; Fig. 1e). Biomass increased with Si application and the maximum yield was achieved at 1.68 g L⁻¹ and higher concentrations ($p < 0.01$). While control plants had the dried mas of 24 g per plant in average, plants treated with Si had the dried mass of 32–39 g. That represents an increment of 24–38% in the biomass. We then tested if the increase in biomass on plants treated with Si was due to photosynthesis. The total CO₂ assimilation per plant did cause a significant increase of biomass ($p = 0.03$).

Finally, the estimation of the cost of sunflower crop was made based on the numbers of Dalchiavon et al. [18]. According to their estimates a sunflower crop costs about USD\$336.08 per ha (Table 2). Based on the cost of the products on the local market, Si application increases the costs of crop by 7.4% at the lowest dose to 30% at the highest dose used.

4 Discussion

In the present study, silicon (Si) application in unstressed sunflower plants did not increase stomatal conductivity (g_s), intercellular CO₂ concentration relative to ambient CO₂ concentration (C_i/C_a) or CO₂ assimilation rate. It also did not increase the chlorophyll content or N concentration in the leaves. However, the leaf area in the plant increased, with no loss to the CO₂ assimilation, water status or N concentration. Thus, the total CO₂ assimilated by the plant increased, resulting in higher biomass production.

When the total leaf area of a plant increases, it is generally expected that the limited nitrogen stock of the leaf is distributed over a greater area, decreasing the production of the photosynthetic tissue per m² of leaf. The enzyme Rubisco (1, 5 ribulose biphosphate carboxylase/oxygenase) is the major limitation in photosynthesis in a series of temperatures and conditions, and it is the largest N cost to the leaf construction, compromising 20–30% of the leaf N investment [15, 20]. Even tough plants treated with Si had larger leaf area, no differences in net CO₂ assimilation or N concentration between the control and the plants treated with Si were observed. That is an indication that even though leaf area increased, more N was assimilated maintaining the leaf N concentration and the net CO₂ assimilation rate. Moreover, the net CO₂ assimilation rates observed in the present study (25–30 $\mu\text{mol m}^{-2} \text{s}^{-1}$) are higher than the 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$

Table 1 Changes in sunflower plants due to application of soluble Si in the leaves. Si was not applied in the control, and four different doses were applied in the treatments

Si applied (g L ⁻¹)	Average leaf area (cm ² leaf ⁻¹)	Leaf number	Total leaf area (m ² plant ⁻¹)	Accumulated Si in leaf (g plant ⁻¹)	Chlorophyll content index (µg cm ⁻²)			Leaf N (g kg ⁻¹)
					30 DAE	45 DAE	60 DAE	
Control	141 ^c ± 06	16 ± 0.8	0.228 ^b ± 0.009	39.7 ^c ± 6.0	42.5 ± 1.6	43.22.0±	41.3 ± 1.8	23.8 ± 1.1
0.84	149 ^{bc} ± 11	18 ± 1.1	0.270 ^a ± 0.018	158.1 ^d ± 13.8	41.2 ± 1.8	44.1 ± 2.7	40.2 ± 2.4	25.0 ± 3.0
1.68	183 ^a ± 13	17 ± 2.3	0.284 ^a ± 0.003	204.0 ^e ± 18.9	41.9 ± 1.5	42.4 ± 1.2	42.3 ± 1.0	26.6 ± 1.1
2.52	168 ^b ± 07	17 ± 1.1	0.280 ^a ± 0.009	243.3 ^b ± 21.3	42.4 ± 1.7	43.4 ± 1.6	42.0 ± 1.4	29.3 ± 1.5
3.36	137 ^c ± 01	20 ± 0.4	0.276 ^a ± 0.005	344.0 ^a ± 17.9	42.6 ± 1.9	41.1 ± 1.2	40.3 ± 1.2	26.5 ± 1.9

Superscript letters represent that differences in treatment was significant to $p < 0.05$. DAP, days after plant emergence. Data represent means ± standard error. N = 4

observed for sunflower elsewhere [21, 22]. Thus, the photosynthetic rate in unstressed sunflower plants was operating at its maximum capacity and probably physical space constrains limited the increment of N concentration per leaf area, thus the production of more photosynthetic tissue per leaf area.

Such high photosynthetic rates observed were achieved because stomata conductance (g_s) and intercellular CO₂ concentration (C_i) were not limiting, delivering a high CO₂ concentration around Rubisco. Unstressed C₃ plants, like sunflower, generally maintain C_i around 70–80% of the ambient

CO₂ concentration [23]. Here, we observed that unstressed sunflower plants are already in the upper limit (C_i/C_a of 79–83%). The high intercellular CO₂ concentration exert a strong control on the stomatal aperture, making it impossible to increase g_s , even if the activity of aquaporins is greater.

Even though the N concentration and CO₂ assimilation rate per m² of leaf did not increase, the total CO₂ assimilated per plant increased in plants treated with Si. The higher total CO₂ assimilation in the plants treated with Si was followed by an increase in biomass of these plants. Statistically, the biomass

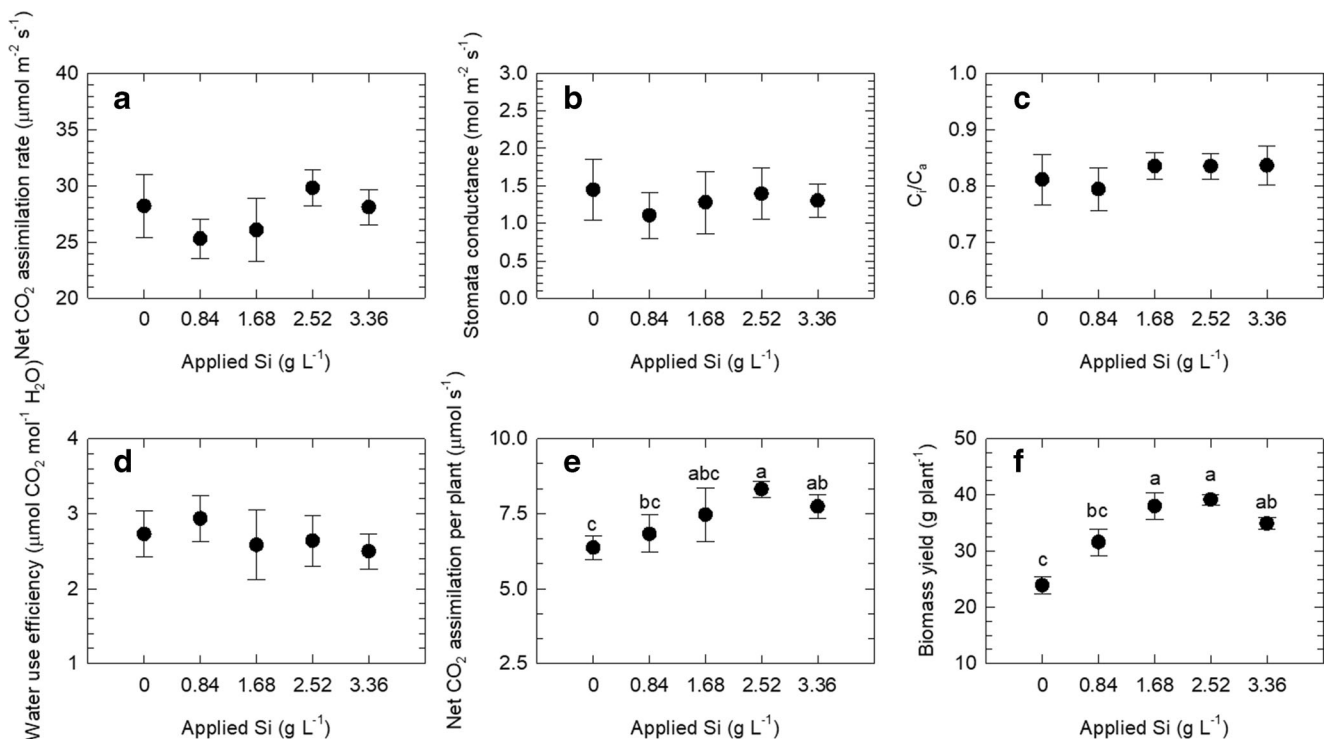


Fig. 1 Gas exchange parameters and total biomass yield in sunflower leaves after application of soluble Si in the leaves. No Si was applied in the control, and in the treatments four doses of Si was applied (0.84, 1.68, 2.52, and 3.36 g L⁻¹). **d**, the water use efficiency was estimated by dividing the net CO₂ assimilation by g_s times the vapour pressure

deficit in molar fraction. **e**, the net CO₂ assimilation per plant was estimated multiplying the net CO₂ assimilation rate times the total leaf area of the plant (see Table 1). Superscript letters represent that differences in treatment was significant to $p < 0.05$. Data represent means ± standard error. N = 4

Table 2 Estimate costs of a sunflower crop in Center-West Brazil and the cost of Silicon application. According to Dalchiavon et al. [18] the average cost of a sunflower crop is presented in the control total crop cost, based in a currency rate of 1 USD = 3.98 Brazilian Real

Si treatment (g L ⁻¹)	Cost of Si application (USD\$ ha ⁻¹)	Total crop cost (USD\$ ha ⁻¹)	Increment cost by Si application
0	0	366.08	0%
0.84	27.23	393.31	7.4%
1.68	54.46	420.54	14.8%
2.52	81.69	447.78	22.3%
3.36	108.92	475.01	29.7%

yield is explained by the total CO₂ assimilated by the plants. Thus, the greater biomass yield observed in sunflower plants treated with Si was due to higher leaf area, which increased the capture of sunlight and CO₂.

The highest biomass yield was achieved with the application of 1.68 g L⁻¹ of Si, representing 37% of biomass increase compared to the control. At this dose, the cost of production of sunflower increases only in 15% in comparison to the control. If the oilseed production increases in the same proportion as biomass yield, we estimate that applying this dose of Si will provide farmers a profit of around 22%.

In the present study, we show that Si application is beneficial even to unstressed sunflower plants. Silicon application to unstressed sunflower leaves promoted an increase in the total leaf area, increasing the total plant CO₂ assimilation, resulting in higher biomass yield. Further studies are necessary to establish the increment in sunflower seed production caused by Si application. In orchid, for example, Si application improved the quality and the quantity of the flowers [24]. In ornamental sunflowers, Si application increased the size and weight of the flowers [25]. Also, a large-scale production test is also necessary to quantify whether the increase in oilseed production offsets the costs of Si application. Our current estimation is that if oilseed production increase by 15% it will already pay the cost of Si application. Here, the biomass increased 24–39%. Thus, if oilseed production increase in the same proportion, it will result in a profit up to 22%.

Acknowledgements The authors would like to thank the funding provided by the Coordination of Superior Level Staff Improvement (CAPES) and National Council for Science and Technology (CNPq). MMP would like to thank CAPES for the funds provided from the PNPd (National Program of Post-Doctorate). RAF would like to thank CNPq for the PQ 291 funds process number 306329/2019-0 and DGR process number 310070/2015-5.

Availability of Data and Material Data is available upon request to the correspondence author.

Authors' Contributions MMP led the data analysis and led the writing with input of all co-authors. CAC, RAF and RMP designed the experiment and provided overall project leadership. CAC and JCN grew the plants, applied the treatments and collected data. JSJ was responsible for the lab analysis. HDNP collected photosynthesis data under supervision of MMP and DGR. DGR provided equipment for photosynthesis data collection. RAF and RMP provided all structure for the experiment.

Funding This project was funded by the Coordination of Superior Level Staff Improvement (CAPES) and National Council for Science and Technology (CNPq) under the PQ funds process number 306329/2019–0 granted to RAF. MMP was hired using the Postdoctoral grant from the PNPd (National Program of Post-Doctorate) provided by CAPES.

Compliance with Ethical Standards

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

Ethics Approval All experiments were conducted ethically and no issues regarding ethical issues arose 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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