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Silício aumenta a clorofila e a fotossíntese e melhora altura e índice da diferença de vegetação normalizada (NDVI) em algodoeiro (*Gossypium hirsutum* L. r. *latifolium* Hutch)

Silicon increases chlorophyll and photosynthesis and improves height and NDVI of cotton (*Gossypium hirsutum* L. r. *latifolium* Hutch)

El silicio aumenta la clorofila y la fotosíntesis y mejora la altura y el NDVI en el algodón (*Gossypium hirsutum* L. r. *latifolium* Hutch)

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Resumo

O silício (Si) é um elemento que apresenta vários benefícios, em plantas é capaz de mitigar estresses bióticos e abióticos. Entretanto, plantas de algodoeiro são consideradas não acumuladoras de Si, com baixo potencial de absorção do elemento pelas raízes. O objetivo deste estudo foi avaliar o efeito de combinações de doses de Si aplicadas via pulverização foliar e solo na fisiologia, crescimento e rendimento de algodoeiro (*Gossypium hirsutum* L. r. *latifolium* Hutch). Foram utilizados blocos completos, em um esquema fatorial 3 x 4, com quatro repetições. A pulverização foliar consistiu de três doses de Si (0, 500, e 1000 mL ha⁻¹) correspondentes a 0, 100, e 200 mL ha⁻¹ de ácido monossilícico, com as pulverizações divididas em três aplicações nos estádios V4, V6 e V8. A fertilização do solo consistiu de quatro doses de Si (0, 2,5, 5, e 10 kg ha⁻¹) correspondentes a 0, 0,5, 1 e 2 kg ha⁻¹ de SiO₂. No florescimento, a fotossíntese, índice de cor verde, altura de plantas e o Índice da Diferença de Vegetação Normalizada (NDVI) foram avaliados. A aplicação de Si no sulco de plantio próximo à rizosfera aumentou o índice de cor verde, refletindo em ganhos na fotossíntese e altura de plantas, os quais aumentaram positivamente o NDVI. O uso de Si de alta solubilidade no sulco de plantio pode aumentar a concentração de ácido monossilícico na área

com maior distribuição de raízes, melhorando o efeito deste elemento em uma planta não acumuladora como o algodoeiro por melhorar o índice de cor verde, a fotossíntese e conseqüentemente refletir em ganhos na altura de plantas e área foliar da planta como demonstrado pelo NDVI.

Palavras-chave: *Gossypium hirsutum*; Elemento benéfico; Fisiologia vegetal; Nutrição de plantas.

Abstract

Silicon (Si) it is a beneficial element that relieves biotic and abiotic stresses in plants. However, cotton plants are not considered Si accumulators, with low potential for uptake the element by roots. The objective of this study was to evaluate the effect of combinations of Si rates applied by leaf spray and soil on the physiology, growth and yield of cotton (*Gossypium hirsutum* L. r. latifolium Hutch). The experimental design was a randomized complete block in a 3 x 4 factorial scheme with four replications. Leaf spraying consisted of three Si concentrations (0, 500, and 1000 mL ha⁻¹) corresponding to 0, 100, and 200 ml ha⁻¹ of monosilicic acid, with spraying split into three applications at stages V4, V6 and V8. Soil-based fertilization consisted of four Si rates in (0, 2.5, 5.0, and 10.0 kg ha⁻¹) corresponding to 0, 0.5, 1.0, and 2.0 kg ha⁻¹ of SiO₂. At flowering, photosynthesis, green color index (GCI), plant height, and NDVI were evaluated. The application of Si in the planting furrow near the rhizosphere increased the green color index, reflecting a gain in photosynthesis and plant height, which positively increased NDVI. The use of high solubility Si in the planting furrow can increase the concentration of monosilicic acid in the area with the highest root distribution, enhancing the effect of this element in a non-accumulator crop such as cotton, by improving the green color index, photosynthesis and hence reflecting on gains in plant height and plant leaf area demonstrated by NDVI.

Keywords: *Gossypium hirsutum*; Beneficial element; Plant physiology; Plant nutrition.

Resumen

El silicio (Si) es un elemento beneficioso que atenúa el estrés biótico y abiótico en las plantas. Sin embargo, se considera que las plantas de algodón no acumulan Si, con un bajo potencial de absorción del elemento por las raíces. El objetivo de este estudio fue evaluar el efecto de las combinaciones de dosis de Si aplicadas mediante pulverización foliar y de suelo sobre la fisiología, el crecimiento y el rendimiento de los algodoneo (*Gossypium hirsutum* L. r. latifolium Hutch). El diseño experimental utilizado fue de bloques completos en un esquema

de factores 3 x 4 con cuatro repeticiones. La pulverización foliar consistió en tres dosis de Si (0, 500 y 1000 mL ha⁻¹) correspondiente a 0, 100 y 200 mL ha⁻¹ de ácido monosilícico, con las pulverizaciones divididas en tres aplicaciones en los estadios V4, V6 y V8. La fertilización del suelo consistió en cuatro dosis de Si (0, 2,5, 5 y 10 kg ha⁻¹) correspondientes a 0, 0,5, 1 y 2 kg ha⁻¹ de SiO₂. En la floración, se evaluaron la fotosíntesis, el índice de color verde, la altura de la planta y el Índice de Diferencia de Vegetación Normalizada (NDVI). La aplicación de Si en el surco de plantación cerca de la rizosfera ha aumentado el índice de color verde, lo que se refleja en ganancias en la fotosíntesis y la altura de la planta, que han aumentado positivamente el NDVI. El uso de Si de alta solubilidad en el surco de plantación puede aumentar la concentración de ácido monosilícico en el área con mayor distribución radicular, mejorando el efecto de este elemento en una planta no acumulativa como el algodónero al mejorar el índice de color verde, la fotosíntesis y, por consiguiente, reflejarse en ganancias en la altura de la planta y el área foliar, como demuestra el NDVI.

Palabras claves: *Gossypium hirsutum*; Elemento beneficioso; Fisiología de las plantas; Nutrición de las plantas.

1. Introduction

Silicon (Si) is a beneficial element that relieves multi-stresses, both biotic and abiotic (Vasanthi et al., 2014). Si is absorbed by plants as monosilicic acid and deposited on the leaf epidermis as amorphous silica, increasing its rigidity through interaction with pectin and polyphenols (Pilon-Smits et al., 2009).

In cotton (*Gossypium hirsutum* L. r. *latifolium* Hutch), there are several notes of positive effects of Si such as increased resistance to ramulose (*Colletotrichum gossypii* var. *cephalosporioides* A. S.) (Guerra et al., 2014) and *Fusarium oxysporum* f. sp. *vasinfectum* (Whan et al., 2016), increased CO₂ assimilation (Curvêlo et al., 2013) and photosynthetic rate (Barros et al., 2019; Ferraz et al., 2014), provided by increased concentration of phenolics and Si accumulation below the cuticle of the leaves forming a cuticle-silica bilayer (Schoelynck et al., 2010).

Si accumulation below the cuticle also improves leaf architecture by increasing plant height of cotton, providing a higher incidence of light on the leaf surface (Ferraz et al., 2014), increasing the light stimulus to photosynthetic pigments and hence increasing the green color index (GCI) and the normalized difference vegetation index (NDVI). NDVI is a variable that strongly correlates with several phenological variables in cotton crops, including leaf area

index, photosynthetic rate, and plant leaf mass (Silva et al., 2017).

However, cultivated species differ in their ability to accumulate Si by the presence or absence of proteins specialized in element absorption (Mitani et al., 2008). Cotton plants are considered non-accumulators (Katz, 2014), with low potential for uptake the element by roots. Thus, application in the planting furrow associated with leaf spraying could better enrich the plant, being able to reflect in improvements in the crop physiology and yield.

In this sense, the hypothesis of this study is that the use of high solubility Si in the planting furrow near the rhizosphere and its complementation by foliar spraying during vegetative development may improve the effect of this element in a non-accumulator crop such as cotton. Therefore, the objective was to evaluate the effects of Si rates, applied in the planting furrow with complementation via leaf spraying, on the photosynthesis, NDVI and cotton growth.

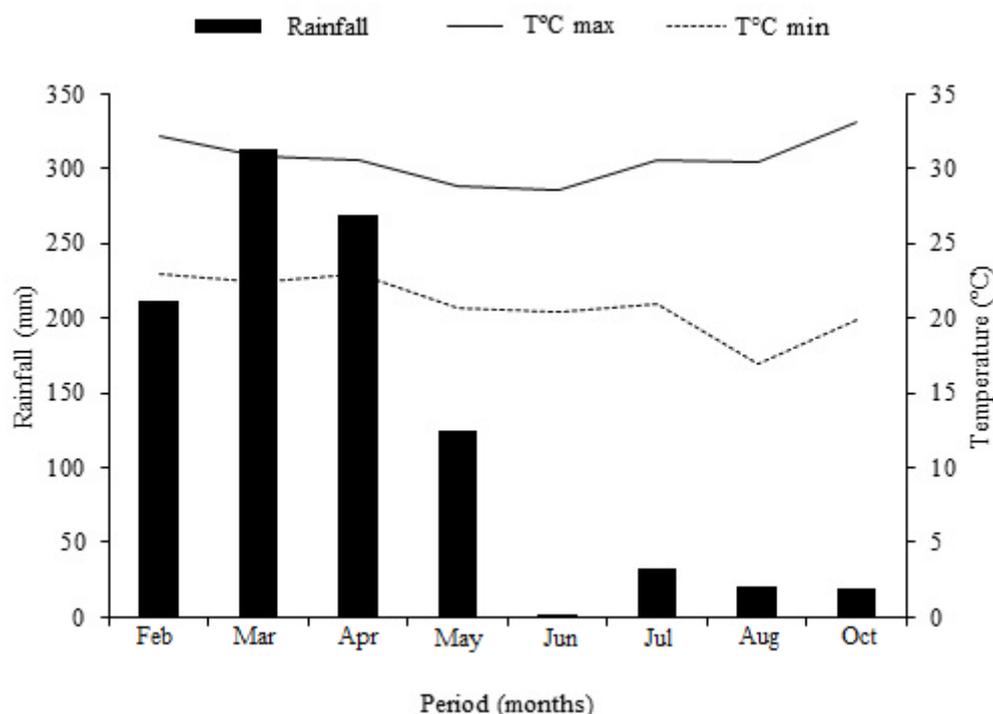
2. Material and Methods

2.1. Plant material and growing conditions

The study was carried out in the period from February to September 2015, using the cotton cultivar FM 975 WS Bayer ®, long cycle and technology Wide Strike (resistance to Lepidoptera) with an approximate final population of 100,000 plants ha⁻¹. The experiment was installed an area of the Chapadão Agricultural Research Support Foundation, in Chapadão do Sul, State of Mato Grosso do Sul, located at 18°41'33" S and 52°40'45" O, with 840 m of altitude.

The region climate according to the Köppen classification is Aw, defined as humid tropical (Kotttek et al., 2006). Rainfall (mm) and maximum and minimum temperature (°C) data over the experiment period are shown in Figure 1. Temperature and rainfall ranges in the experimental area were favorable to cotton crop growth, with average temperatures of 30°C during the day and 22°C at night, close to those considered suitable to crop (Lamas and Yamaoka, 2012), and rainfall between 500 mm and 1,500 mm year⁻¹.

Figure 1 - Monthly accumulated rainfall and maximum and minimum temperature during the cotton-growing period.



Source: Data from Campo Bom Farm, 2015.

The soil of the experimental area is classified as Latossolo Vermelho distroférrico (Santos et al., 2018). A sample was previously collected for chemical analysis, according to the method described by Raij et al. (2001) and for particle size analysis (Embrapa, 1997) (Table 1).

Table 1 - Analysis of chemical and physical soil attributes in the 0-20 and 20-40 cm depth layers.

Depth	pH (CaCl ₂)	Ca	Mg	Al	H+Al	K	P (res)	S	OM	CEC	V	M
cm		cmol _c dm ⁻³					mg dm ⁻³		g dm ⁻³	cmol _c dm ⁻³	%	
0-20	4.9	3.0	0.7	0.05	5.6	61	15.2	13.3	26.7	9.4	40.7	1.3
20-40	4.9	2.3	0.5	0.07	5.8	46	10.3	9.8	19.9	8.7	33.5	2.3
Depth	B	Cu	Fe	Mn	Zn	Clay	Sand	Silt				
cm		mg dm ⁻³						%				
0-20	0.29	0.8	58	19.6	4.2							
20-40						60	35	5.0				

Source: Elaborated by the authors.

The fertilization was performed by applying 250 kg ha⁻¹ of MAP (11-52-00) before sowing. Topdressing was performed by applying 150 kg ha⁻¹ of KCl (00-00-60) at pre-sowing and 260 kg ha⁻¹ of urea (46-00-00) divided into stages B1 (first visible flower bud) and F1 (first floral bud at the first branch transformed into a flower), with expected seed yield higher than 6 t ha⁻¹ (Sousa & Lobato, 2004).

2.2. Treatments and experimental design

The crop was sown in February, 2015. The experimental design was factorial (3 x 4) based on a randomized block design with four replications. The treatments consisted of combinations of Si rates applied to soil and by foliar spray, being: three Si rates applied by foliar spray (0, 500 and 1000 mL ha⁻¹) corresponding to 0; 100 and 200 ml ha⁻¹ of monosilicic acid using as source the ZumSil™ (79.3 g L⁻¹ of Si and density of 1.25); and four Si rates applied to soil (0; 2.5; 5.0 and 10.0 kg ha⁻¹) corresponding to 0; 0.5; 1.0 and 2.0 kg ha⁻¹ of SiO₂ using as source the Silcoat® (200 g kg⁻¹ of Si) (Table 2), where Si coating was performed on the base fertilizer formulation.

Table 2 - Description of the tested treatments demonstrating the combinations of Si rates applied via leaf spray and soil fertilization.

Treatments	Si by leaf spray mL ha ⁻¹	Si by soil fertilization kg ha ⁻¹	Total Si g
1	0	0	0
2	0	2.5	21.65
3	0	5.0	43.31
4	0	10.0	86.62
5	500	0	
6	500	2.5	
7	500	5.0	
8	500	10.0	
9	1000	0	
10	1000	2.5	
11	1000	5.0	
12	1000	10.0	

Source: Elaborated by the authors.

The experimental unit consisted of seven rows spaced 0.45 m long and 5.50 m long, totaling an area of 17.32 m² per plot. Useful area was composed of three central rows 0.45 m wide and 3.00 m in length, totaling an area of 4.05 m².

Leaf spraying was performed at the phenological stages V4 (from the end of V3 until when the central vein of the fifth leaf reached 2.5 cm), V6 (from the end of V5 until when the central vein of the fifth leaf reached 2.5 cm) and V8 (from the end of V7 until when the central vein of the fifth leaf reached 2.5 cm), by using a backpack sprayer pressurized by CO₂ Herbicat.

The sprayer used has a six-nozzle spray boom, spaced 0.50 m and calibrated to the 150 L ha⁻¹ application rate, using 2.5 MPa pressure and tips Teejet XR 110015 (green), which produced fine drops (148 µm). The application speed was 1 m s⁻¹.

Phytosanitary crop management was performed by glyphosate desiccation with 792.5 g kg⁻¹ (2.5 kg ha⁻¹), application of S-Metolachlor 960 g L⁻¹ (1.2 L ha⁻¹) in pre-emergency and Glufosinate-Ammonium Salt 200 g L⁻¹ (1.2 L ha⁻¹) at 15 and 25 days after emergency (DAE). The insecticides used were Acefato 750 g kg⁻¹ (1.5 kg ha⁻¹), Flubendiamida 480 g L⁻¹ (150 mL ha⁻¹), Etiprole 200 g L⁻¹ (1 L ha⁻¹), Espiromesifeno 240 g L⁻¹ (600 mL ha⁻¹), Malathion (1,5 L ha⁻¹), Clorfenapir 240 g L⁻¹ (1 L ha⁻¹) and Beta-Ciflutrina 125 g L⁻¹ (0.3 L ha⁻¹).

Fungicide treatment was also performed. Rifloxistrobina 150 g L⁻¹ and Protiocanazole 175 g L⁻¹ (0.5 L ha⁻¹), Fluxaproxade 167 g L⁻¹ and Piraclostrobin 333 g L⁻¹ (500 mL ha⁻¹) and Fentina Hydroxide 400 g L⁻¹ (0.4 L ha⁻¹). The growth regulators Mepiquat chloride 250 g L⁻¹ and Chloroquate Chloride 100 g L⁻¹ were applied at a rate of 50 mL ha⁻¹ and 1.5 L ha⁻¹, respectively.

2.3. Evaluated variables

When the plants emitted the first flower bud in the first flowering reproductive branch (F1), photosynthesis (mmol CO₂ s⁻¹ m⁻²) assessments were performed by means of an infrared gas analyzer (LICOR, Inc., LI- 6400), under ambient CO₂ concentrations (372 ± 10 mol s⁻¹ m⁻²), with photosynthetically active photon flux of 1000 mol s⁻¹ m⁻². Measurements were performed on the fifth leaf from the main stem apex in four plants per treatment, between 9:00 and 12:00 am.

NDVI measurement was performed in the three central rows of the experiment with the sensor positioned 0.80 m above the crop canopy. The equipment used was the Crop Circle[®] ACS-470 (Holland Scientific, Lincoln, USA), at the red (670 nm) and NIR (730 nm) wavelengths on the sensor to calculate the NDVI value.

The green color index (GCI) was performed on the fifth leaf from the apex to the base of the plants, carrying four measurements per plot using Falker[®] Clorofilog chlorophyll

meter. Plant height was measured using a continuous plant sample from the plot useful area using measuring tape, measuring from the base of the stem to the beginning of the last fully developed leaf.

2.4. Statistical analysis

Data were subjected to analysis of variance and, subsequently, regression analysis. When there was significance between the contrast check versus factors, the t-test was applied using the Rbio statistical software (Bhering, 2017). In all analyses, a significance at 5% probability level was adopted. Subsequently, the correlations between the variables and the result were estimated, expressed graphically by the correlation network. Finally, principal component analysis was performed to identify the association between the treatments and the evaluated variables.

3. Results

The application of Si via soil in the planting furrow provided effects on GCI, photosynthesis (Photo), plant height (PH) and plant leaf mass (indicated by NDVI). Si supply by leaf spraying on cotton plants did not influence ($p>0.05$) any of the evaluated variables. The combination of Si rates with soil supply and foliar spray complementation did not promote significant interaction in any of the evaluated variables (Table 3).

Table 3 - F values calculated for green color index (GCI), photosynthesis (Photo, $\text{mmol CO}_2 \text{ s}^{-1} \text{ m}^{-2}$), plant height (PH, m) and normalized difference vegetation index (NDVI) in cotton plants under combination of Si rates applied by soil and foliar spraying.

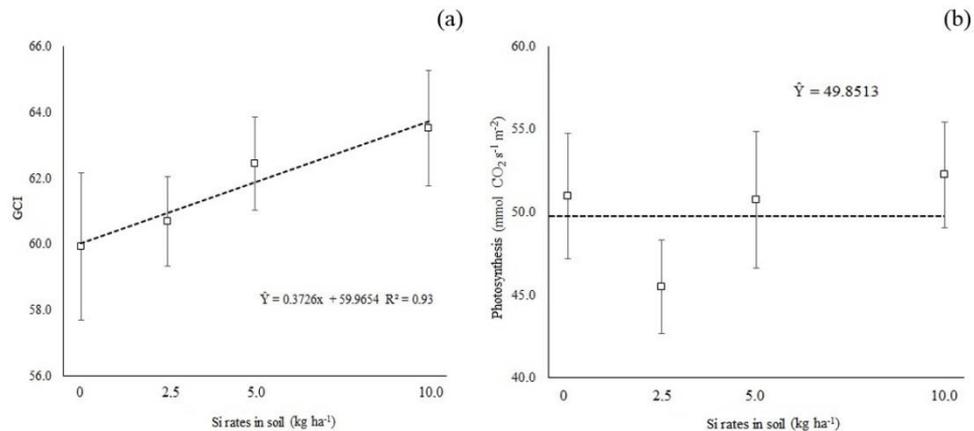
SV	GCI	Photo	PH	NDVI
Leaf (L)	0.73 ^{ns}	0.32 ^{ns}	0.36 ^{ns}	1.74 ^{ns}
Soil (S)	10.44*	6.69*	15.89*	7.67*
L x S	1.25 ^{ns}	0.59 ^{ns}	0.32 ^{ns}	0.80 ^{ns}
CV (%)	2.83	8.00	14.39	2.96
Mean	61.64	49.85	0.92	0.84

^{ns} and *: not significant and significant at 5% probability by the F test, respectively; SV: sources of variation; CV: coefficient of variation. Source: Elaborated by the authors.

The GCI showed a linear response to Si application by soil in the planting furrow, increasing 0.37 units per 1 kg ha^{-1} of Si applied to the soil (Figure 2a). Although the F test

detected differences between Si rates in soil for photosynthesis, there was no adjustment for the tested models (linear and quadratic) regarding this variable (Figure 2b).

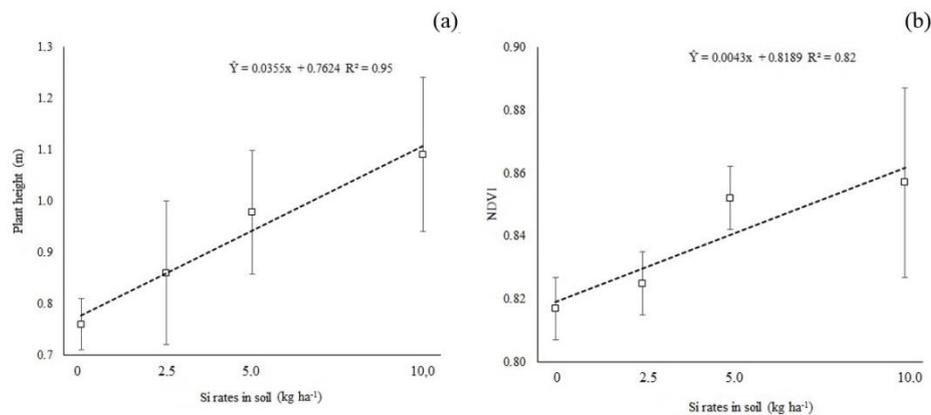
Figure 2 - Green color index (a) and photosynthesis (b) in cotton plants under Si rates applied to the soil using Silcoat® source. Bars show the mean standard error, n=4.



Source: Elaborated by the authors.

Plant height increased linearly as a function of Si rates via soil in the planting furrow, with an increase of 0.035 cm for every 1 kg ha⁻¹ of Si applied to the soil using the Silcoat® source (Figure 3a). The data obtained in the NDVI analysis fit to linear regression with an increase of 0.0043 units for each 1 kg ha⁻¹ of Si applied via soil (Figure 3b).

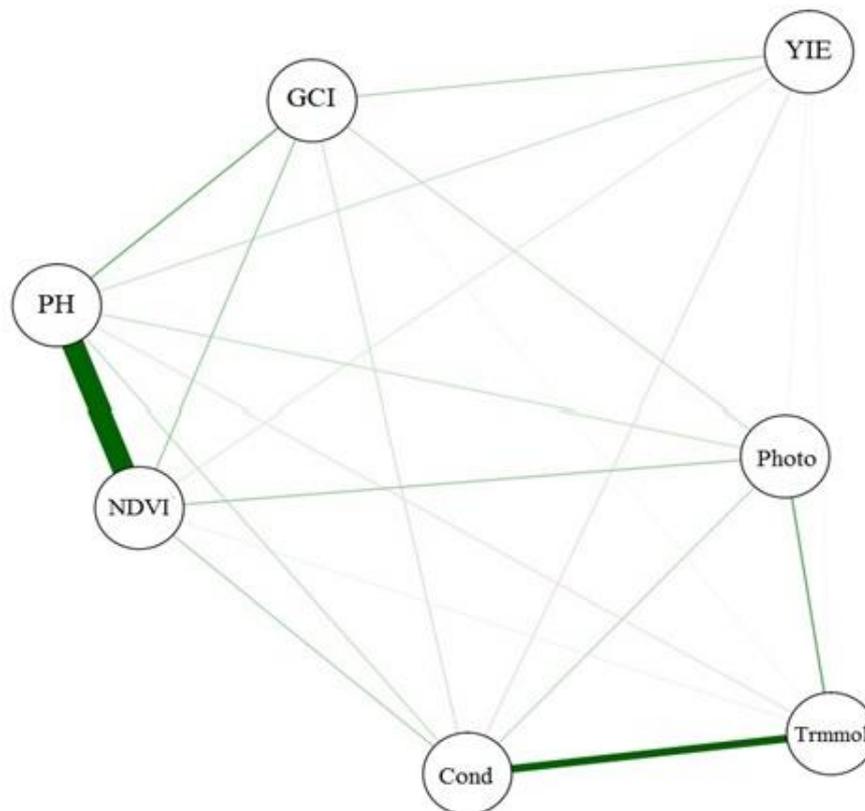
Figure 3 - Plant height (a) and normalized difference vegetation index (NDVI) (b) in cotton plants under Si rates applied to the soil using Silcoat®. Bars show the mean standard error, n=4.



Source: Elaborated by the authors.

There was a positive and significant correlation between plant height (PH) and NDVI, and between conductance (Cond) and transpiration (Trmmol) (data not shown) (Figure 4). Moderate magnitude correlations were observed between NDVI and photosynthesis (Photo) and yield (YIE) (data not shown). The other variables presented low correlation estimates (Figure 4).

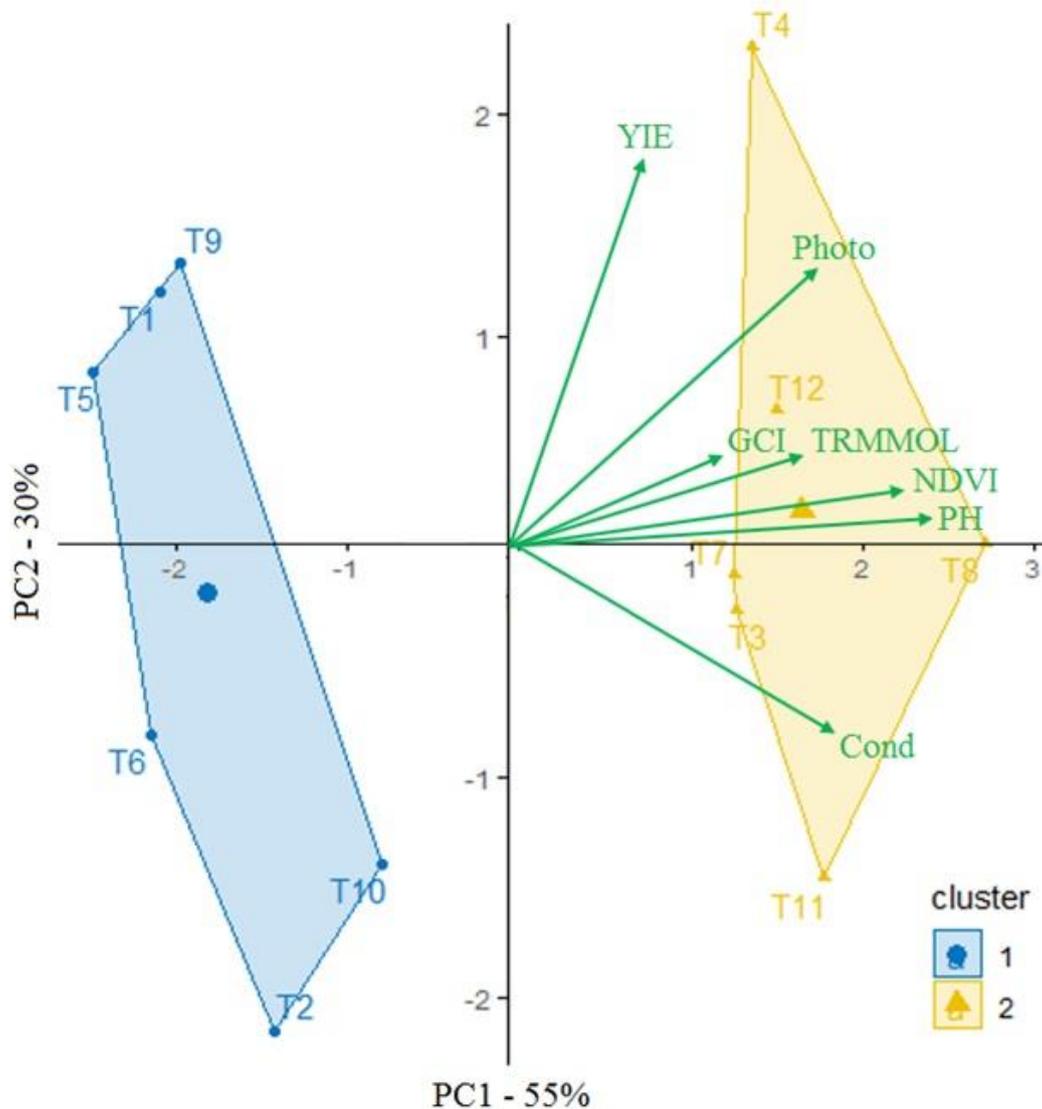
Figure 4 - Correlation network between the variables plant height (PH), NDVI, GCI, photosynthesis (Photo), stomatal conductance (Cond), transpiration (Trmmol) and seed cotton yield (PROD).



Source: Elaborated by the authors.

Principal component analysis demonstrating the relationship between treatments and evaluated variables is shown in Figure 5. Treatment 4 (0 mL ha⁻¹ by leaf spraying + 10 kg ha⁻¹ by soil) is the one closest to the yield vector, being the most effective for increasing its average yield (298.31 @ ha⁻¹) (Figure 5).

Figure 5 - Principal component analysis between the variables plant height (PH), NDVI, GCI, photosynthesis (Photo), stomatal conductance (Cond), transpiration (Trmmol) and seed cotton yield (YIE) and the rates and modes of silicon application.



Source: Elaborated by the authors.

The proximity of NDVI and PH vectors to treatment 8 (10 kg ha^{-1} by soil and 500 mL ha^{-1} by leaf spraying), demonstrates that it was effective in increasing the mean of this variable (Figure 5).

4. Discussion

Leaf spraying of Si did not affect the development of cotton plants (Table 3). Si is an element with low phloem mobility and tends to remain where it was absorbed by leaf limb

(Mitani et al., 2008). Thus, it is believed that the leaf spraying rate was insufficient to promote relevant increase in the analyzed variables.

The periodic enrichment of new leaves with weekly applications in cotton plants in the cultivars "BRS Topázio", "Sapphire" and "Ruby", using potassium silicate at concentrations 0, 50, 100, 150 and 200 mg L⁻¹ positively influenced plant height, stomatal conductance, transpiration and chlorophyll content (Ferraz et al., 2014).

Despite the low potential of Si uptake by cotton plant roots (Katz, 2014), the supply of the element by soil in the planting furrow provided greater contact with the roots, resulting in positive effects on plant physiology and growth. This demonstrates that the application efficiency is a fundamental aspect to manage the use of Si in cotton.

The green color index is an indirect measure of chlorophyll content. In this study, the application of Si by soil in the planting furrow resulted in an increase of this parameter (Figure 2a). With the improvement photosynthesis apparatus, plants that received Si may have improved CO₂ assimilation and increased carbohydrate content, providing greater growth, as observed in plant height (Figure 3a).

The increase in plant height provided by Si can result in improved leaf architecture, making the plants upright. This effect may be justified because Si is deposited on the leaf epidermis in form of amorphous silica, increasing its rigidity through its interaction with pectin and polyphenols (Pilon-Smits et al., 2009).

Upright plants may have a larger light reception area and maintain wavelength uptake by photosynthetic pigments, besides a most efficient energy transference to the photosystem II and hence increasing photosynthesis (Streit et al., 2005). Increased photosynthesis with Si leaf spraying was also demonstrated by Barros et al. (2019), however, the authors attribute this effect to the association of Si with salicylic acid.

The maintenance of the green color intensity in the plants may also have been contributed by the preservation of the photosynthetic pigment integrity caused by the attenuating effect of Si on the oxidative damage reduction, since the excessive accumulation of reactive oxygen species may cause cell membrane disintegration (Bokhtiar et al., 2012), as was also demonstrated in cotton crop under copper toxicity with Si addition in the nutrient solution (Ali et al., 2016).

With the increased morpho-physiological components, NDVI also showed increase with Si supply by soil in the planting furrow (Figure 3b) and showed a strong correlation with plant height (Figure 4). NDVI is the difference between the emission and reflection of the electromagnetic spectrum waves in two lengths, estimating the closure of the rows promoted

by the plant canopy and the green color index (Babolim et al., 2014), a characteristic that can indirectly define yield.

The improved plant mass demonstrated by NDVI is resulting from the Si effects on increasing green color index, photosynthesis and plant height, since NDVI variability is related to several phenological variables in cotton crop, among them the leaf area index, photosynthetic rate and plant leaf mass (Silva et al., 2017).

There are no studies demonstrating the effect provided by Si on plant mass represented by NDVI, which emphasizes the relevance of this study. NDVI has a positive correlation with plant height (Motomiyia et al., 2009) and chlorophyll content (Souza et al., 2017), demonstrating that Si stimulates crop development by improving morpho-physiological parameters.

The high correlation between stomatal conductance and transpiration is significant because the plant increases the loss of water to the environment when the stomata are open. However, as these factors increase, photosynthesis is also favored, since stomata opening causes CO₂ diffusion into the leaf, increasing the internal concentration, which will be used for photosynthesis (Ferraz et al., 2014).

Thus, we can accept the hypothesis that the use of high solubility Si in the planting furrow near the rhizosphere during vegetative development may increase the concentration of monosilicic acid in the area of greater root distribution, enhancing the effect of this element in a non-accumulator crop such as cotton. PCA analysis revealed the existence of an association between the Si rate of 10 kg ha⁻¹ by soil and yield, which suggests that higher yields can be obtained by using Si in soil. By improving the green color index, photosynthesis and reflecting on gains in plant height and leaf area, as demonstrated by NDVI, the use of Si in the soil can be fundamental to obtain greater gains in the cotton yield, which is the main characteristic desired by the growers.

The results provided by our study contribute to the decision making about alternatives to the fertilization management of the cotton, such as Si, which is a technique still little explored in the crop. The use of Si is an easy and low-cost technique that can be associated with the management of soil fertilization, which usually uses only macronutrients. Our findings reveal that the application of Si in the planting furrow benefits the plant by improving its photosynthetic traits and by being associated with an increase in yield. However, further studies should be carried out with Si fertilization in the crop evaluating other application techniques and/or other physiological traits in order to provide more information on the effects of this element on cotton.

5. Conclusion

The application of Si in the planting furrow near the rhizosphere has a beneficial effect on cotton plant growth by improving green color index, photosynthesis, plant height and NDVI. The results provided by our study contribute to the decision making about alternatives to the fertilization management of the cotton. Thus, the use of Si can be associated with the management of soil fertilization, contributing to yield gains and consequent increase in the profitability of the cotton. However, further studies addressing different Si application techniques and evaluating other physiological traits should be carried to better elucidate the effects of this element in cotton.

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