

## Physiological quality and dry mass production of *Sorghum bicolor* following silicon (Si) foliar application

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### Abstract

The forthcoming of silicon (Si) highly soluble sources provided a suitable alternative to Si use in agroecosystems. There are many benefits associated to Si application in crops, such as improvement in feed quality. In this sense, the aim of this work was to evaluate the effect of Si foliar application on physiological quality, biomass production, and silicon accumulation in *Sorghum bicolor*. The experiment was conducted under greenhouse condition using an entirely randomized design, with five Si rates (0 as control), 0.84, 1.68, 2.52, and 3.36 g L<sup>-1</sup> of Si) applied as potassium and sodium silicate, with four repetitions. In each treatment, applied solutions were balanced in potassium in order to isolate the Si effect. The following measurements were taken: growth, biomass production, Si accumulation, and physiological quality. Supplying Si via leaves did not affect the sorghum growth rate and the relative chlorophyll index; however, leaf area increased 23% with the use of 2.36 g L<sup>-1</sup> of Si. Physiological variables are influenced by increasing Si rates, with rates close to 1.68 g L<sup>-1</sup> of Si causing the best photosynthetic rates and stomatal conductance. The use of potassium silicate as a source of silicon is an alternative for productivity increases up to 30%, but an economic study on the viability of its commercial application in the production chain of *Sorghum bicolor* is necessary.

**Keywords:** Silicon fertilization; beneficial element; mineral plant nutrition; photosynthesis; stomata conductance; relative chlorophyll index; transpiration.

**Abbreviations:** Si\_Silicon; pH\_hydrogen potential; Al\_aluminum; P\_phosphorus; K\_potassium; Mg\_magnesium; SO<sub>4</sub><sup>2-</sup>\_sulfate; Zn\_zinc; B\_boron; H\_hydrogen; CEC\_cation exchange capacity; V%\_base saturation; ERD\_randomized experimental design; MAP\_monoammonium phosphate; KCl\_potassium chloride; PRNT\_relative total neutralization power; DAE\_days after emergence; RCI\_relative chlorophyll index; DAS\_days after sowing; IRGA\_system portable infrared gas analyzer; PPFD\_density of photon flux; ABef\_nutritional indices comprising absorption efficiency; UTef\_efficiency of use of nutrients for conversion to dry matter.

### Introduction

*Sorghum* (*Sorghum bicolor*) is currently considered to be the fifth most important grain for use as food, fiber production, and energy (Zheng et al., 2011). The crop stands out for presenting satisfactory grain and biomass productivity in regions that are considered unproductive for other cereals because they are very warm and/or dry, with annual precipitation between 375 and 625 mm (Ribas, 2003). In the last decade, total production was 60 million tons in an area of 44 million hectares (Zheng et al., 2011).

*Sorghum* can be cultivated in conditions that are adverse to other crops, however the management strategies aiming reduce the deleterious effect of drying is important to guarantee a suitable production. In this sense, silicon (Si) application can be performed, since it may increase the plant's tolerance to elevated temperatures (Okamoto, 1969; Soundararajan et al., 2014), hydric deficit (Lux et al., 2002;

Hattori et al., 2005), Mg toxicity (Galvez et al., 1987) e Al toxicity (Cocker et al., 1998) and salinity (Kafi and Rahimi, 2011). Silicon is absorbed by sorghum as opaline silicon (phytolites), increasing the growth rates and, therefore, crop production. Moreover, some quality improvements and protection to abiotic and biotic stress have been associated with Si application (Tripathi et al., 2013). When absorbed, Si leads to the formation of a double layer of silica-cuticle and silica-cellulose in the cellular wall, giving to the plants physical resistance and reducing transpiration, resulting in an increase in water use efficiency (Ma and Yamaji, 2006; Barbosa Filho et al., 2001). Silicon accumulation in leaves can reduce the salt accumulation and the plasmatic membranes permeability, maintaining their integrity and functioning through a decrease in lipid peroxidation and an increase in superoxide dismutase activity (Matoh et al., 1986; Zhu et al.,

2004). In dry soil, Si acts maintaining the photosynthetic rate and stomatal conductance of plants, keeping them at high levels (Gong et al., 2008). Moreover, Si is also indicated to increase antioxidant defense capacity (Zhu et al., 2004). The element thus gives plants greater tolerance to hydric deficit. The positive effect of Si application may be related with the accumulation of large Si amounts in the endoderm tissues of sorghum, increasing the crop tolerance to the stress (Lux et al., 2002), since endoderm cells have an important role in water transportation by roots (Steudel and Peterson, 1998). Moreover, silicon increases membrane permeability in sorghum roots (Hattori et al., 2003). Silicon has fast absorption and deposition in sorghum (Lux et al., 2003), favoring water transport and/or root growth in stress condition. The silicate use incorporated into the soil requires high rates, moreover polymerization reactions may occur in the soil, decreasing its availability (Carvalho et al., 2009). The emergence of highly soluble sources can make the Si use in different crops viable and reinforce the sustainability of agriculture, especially in the case of forage grasses that produce biomass for animal feed, such as forage sorghum.

The use of highly soluble foliar Si, in stabilized form and in small rates can improve the nutritional and physiological plant's state, as well as reducing phytosanitary problems and promoting gains in productivity. The silicon application in stabilized form is an alternative in cropping systems, since it has showed some benefits to crops development and production mainly under stress conditions (Crusciol et al., 2013). In this source, silicon is applied as stabilized silicic acid, and after dissolution form orthosilicic acid, which may increase Si absorption by plants (Sorato et al., 2012).

The Si application has been show some increase in Si foliar in wheat and white oat, following an increase in roots length of aot and in seeds dry mass of wheat (Toledo et al., 2012). Assuming that foliar Si application using a source with high solubility can result in higher Si fertilization efficiency, the aim of this study was to evaluate the effect of leaf-applied silicon on physiological quality, biomass production, and silicon accumulation in *Sorghum bicolor*.

## Results

### **Height, leaf area and Relative chlorophyll index – RCI**

Increasing Si application rates did not affect height and relative chlorophyll index (RCI) of sorghum plants; however, leaf area was linearly increased (Table 1). The highest leaf area (642.71 cm<sup>2</sup>) was obtained with the rate of 3.36 g L<sup>-1</sup> of Si; that is 23% higher compared to control, which was 518.64 cm<sup>2</sup> (Fig. 1).

### **Physiological quality**

The physiological parameters of *Sorghum bicolor* plants were affected by increasing Si rates application, with exception of transpiration rates, which was not affected by Si supply, presenting an average of 0.21 mmol m<sup>-2</sup> s<sup>-1</sup> (Table 2). Stomatal conductance presented a quadratic response, with the highest value (4.71 mmol m<sup>-2</sup> s<sup>-1</sup>) obtained with the use of 1.96 g L<sup>-1</sup> of Si (Fig. 2). With the reduction in stomatal opening caused by an increased supply of Si, the internal concentration of CO<sub>2</sub> is also affected (Fig. 3), decreasing by approximately 8% (429.66 ppm) when the highest Si rate

was applied, in relation to the control treatment (467.03 ppm). Net photosynthesis rate also presented a quadratic behavior, with an increase being observed up to the rate of 2.03 g L<sup>-1</sup>, which provided a net photosynthesis rate of 3.06 μmol m<sup>-2</sup> s<sup>-1</sup> (Fig. 4).

### **Dry mass**

Increasing Si rates were observed linear increases in dry mass production of roots, aerial part and entire plant (Table 3, Fig 5). It was noted that the increase in biomass production with the highest rate (3.36 g L<sup>-1</sup>) was 19 (aerial part), 80 (roots), and 31% (entire plants) higher than observed in the control treatment (without applying Si), which produced was 14, 3.36, and 17.36 g plant<sup>-1</sup> in aboveground part, root, and entire plant, respectively (Fig. 5).

### **Accumulation and efficiency**

Silicon accumulation in all plant's part, and efficiencies of utilization and absorption were affected by increasing Si rates application (Table 4). A positive linear response was observed in Si accumulation in all plant's part, reaching 246, 314, and 257% more Si accumulation in relation to the control treatment (without applying Si) in the aboveground part, root, and entire plant, respectively (Fig. 6). The Si accumulation in roots probably indicating the Si redistribution from leaves to the roots through the phloem (Table 4). The absorption and utilization efficiencies presented contrasting behavior; that is, there was an increase in Si absorption efficiency by the plant with a greater supply of it, whereas the utilization efficiency decreased (Table 4). It was noted that absorption efficiency presented a quadratic response, reaching 12.12 mg g<sup>-1</sup> with application of 3.13 g L<sup>-1</sup> of Si. On the other hand, the utilization efficiency by sorghum plants was reduced, practically halting the rate of dry material conversion, and reaching levels close to null (Fig. 7).

## Discussion

### **Biometric variables**

Sorghum plants height and the relative chlorophyll index (RCI) were not affected by Si application rates. These results support Yin et al. (2013) in studies involving the Si application to mitigate the detrimental effect of salinity in sorghum, and Cessa et al. (2011) in studies involving different P and Si rates in sorghum. In these studies, both authors verified that Si did not affect the plants' vegetative growth under normal cultivation conditions. Regarding leaf area, there was a linear increase after increasing Si rates application. Similarly, Gong et al. (2003) verified that even in adequate cultivation conditions, Si resulted in an increase in leaf area for wheat.

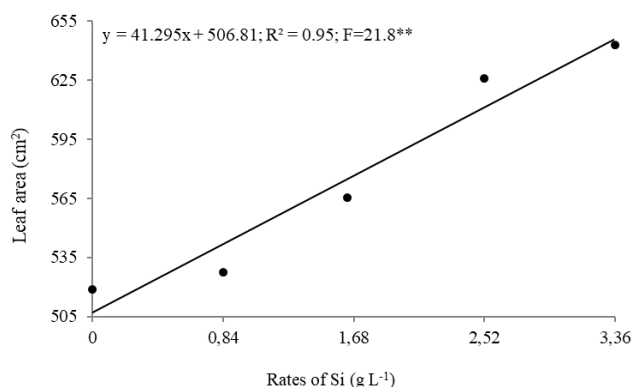
### **Physiological quality**

Silicon rates affected stomatal conductance, which is due to the fact that Si accumulates in large quantities in the stomas and guard cells, forming a silica double layer, resulting in a

**Table 1.** Leaf area, height, relative chlorophyll index (RCI), of *Sorghum bicolor* fertilized with increasing silicon rates.

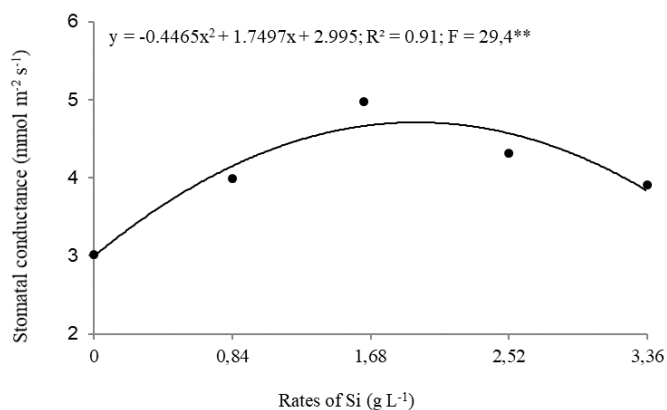
Rates	Leaf area	Height	RCI <sup>1</sup>	RCI <sup>2</sup>	RCI <sup>3</sup>
g L <sup>-1</sup>	cm <sup>2</sup>	cm	μg cm <sup>-2</sup>		
0	518.64	88.00	33.14	46.04	38.34
0.84	527.27	89.75	34.19	44.86	40.55
1.64	565.09	90.25	33.89	43.34	39.95
2.52	625.56	97.62	34.43	46.51	40.40
3.36	642.71	97.75	32.31	45.45	38.76
Test F	**	ns	ns	ns	ns
C.V. (%)	8.15	8.34	6.65	6.68	5.56

RCI<sup>1</sup>, RCI<sup>2</sup> and RCI<sup>3</sup> – evaluations of relative chlorophyll index at 15, 30 and 45 days after the emergency (DAE). C.V. - Coefficient of variation. \*\* and <sup>ns</sup> - significant at 1% and not significant at 5% of probability by the F test, respectively.

**Fig 1.** Leaf area of *Sorghum bicolor* fertilized with increasing silicon rates. \*\*Significant at 1% of probability by the F test.**Table 2.** Physiological variables of *Sorghum bicolor* fertilized with increasing silicon rates.

Rates	Stomatal conductance	Liquid photosynthesis	Transpiration	Internal concentration of CO <sub>2</sub>
g L <sup>-1</sup>	mmol m <sup>-2</sup> s <sup>-1</sup>	μmol m <sup>-2</sup> s <sup>-1</sup>	mmol m <sup>-2</sup> s <sup>-1</sup>	ppm
0	3.02	0.25	0.25	463.52
0.84	3.99	1.51	0.22	461.17
1.64	4.97	4.01	0.20	452.80
2.52	4.32	2.06	0.21	439.77
3.36	3.91	2.07	0.18	430.26
Test F	**	**	ns	**
C.V. (%)	10.70	32.43	28.94	2.03

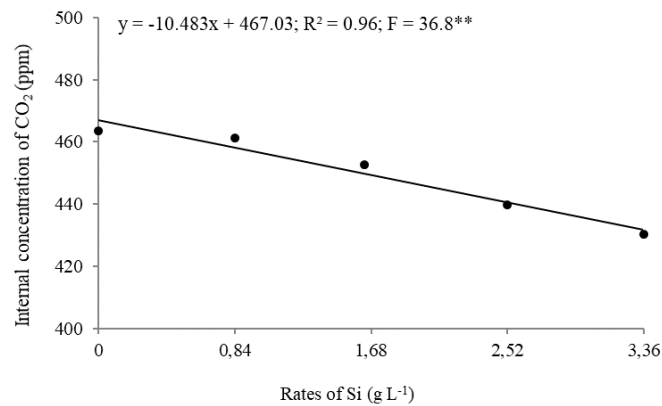
C.V. - Coefficient of variation. \*\* and <sup>ns</sup> Significant at 1% and not significant at 5% of probability by the F test, respectively.

**Fig 2.** Stomatal conductance of *Sorghum bicolor* fertilized with increasing silicon rates. \*\* Significant at 1% of probability by the F test.

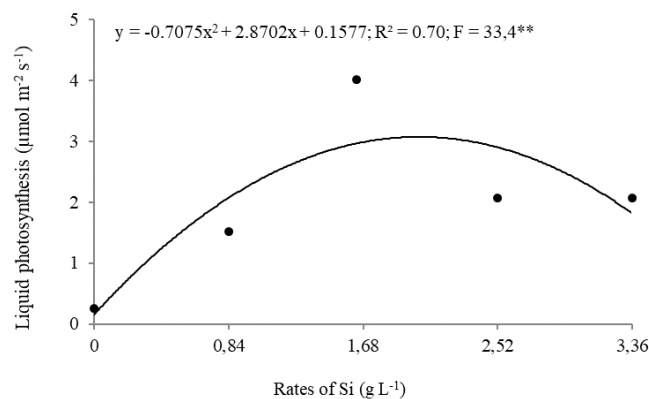
**Table 3.** Dry mass of aboveground part (DMAP), roots (DMR) and entire plant (DMEP) of *Sorghum bicolor* fertilized with increasing silicon rates.

Rates	DMAP	DMR	DMEP
g L <sup>-1</sup>	-----g plant <sup>-1</sup> -----		
0	14.00	3.50	17.50
0.84	14.75	4.00	18.75
1.64	15.25	4.50	19.75
2.52	15.75	5.25	21.00
3.36	16.75	6.25	23.00
Test F	**	**	**
C.V. (%)	4.46	12.88	3.29

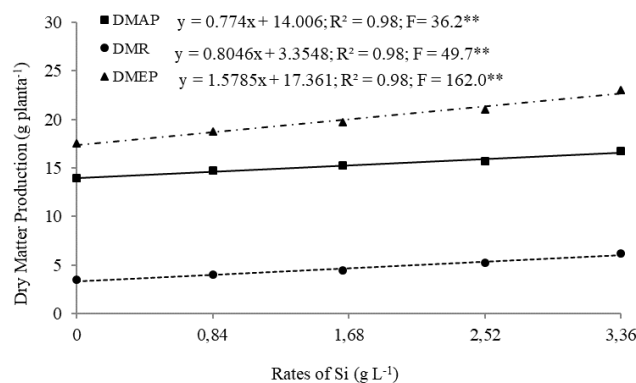
C.V. - Coefficient of variation. \*\* Significant at 1% of probability by the F test.



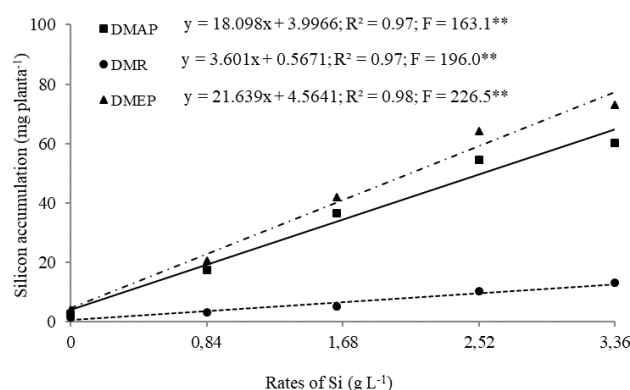
**Fig 3.** Internal concentration of CO<sub>2</sub> de *Sorghum bicolor* fertilized with increasing silicon rates. \*\* Significant at 1% of probability by the F test.



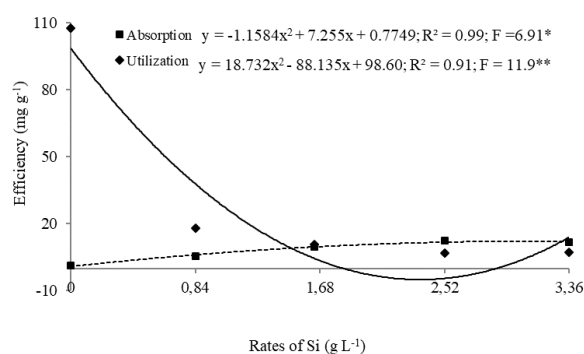
**Fig 4.** Liquid photosynthesis of *Sorghum bicolor* fertilized with increasing silicon rates. \*\* Significant at 1% of probability by the F test.



**Fig 5.** Dry mass of aboveground part (DMAP), roots (DMR) and entire plant (DMEP) of *Sorghum bicolor* fertilized with increasing silicon rates. \*\* Significant at 1% of probability by the F test.



**Fig 6.** Silicon accumulation of aboveground part (DMAP), roots (DMR) and entire plant (DMEP) of *Sorghum bicolor* fertilized with increasing silicon rates. \*\* Significant at 1% of probability by the F test.



**Fig 7.** Efficiency of absorption ( $AB_{ef}$ ) and utilization ( $UT_{ef}$ ) of silicon of *Sorghum bicolor* fertilized with increasing silicon rates. \*\* and \* Significant at 1 and 5% of probability by the F test, respectively.

smaller stomatal opening (Oliveira and Castro, 2002), and as a consequence reducing stomatal conductance. The increase in net photosynthesis rate with the Si supply may be associated with the more efficient use of water, which results in a higher photosynthetic rate (Ma and Yamaji, 2006). However, one possible explanation for the fall in the net photosynthesis rate with increased Si rates is that accumulation in the guard cells results in decreased stomatal conductance. The internal  $CO_2$  concentration, which is the substrate for photosynthesis, thus decreases in the leaf.

#### Dry mass

Following Si application, sorghum plants under hydric stress showed an increase in the leaf area and in specific leaves weight. Increases in chlorophyll and aboveground, roots and entire plant's dry mass were also observed, suggesting an increase in sorghum stress resistance (Ahmed, Qadeer and Aslan, 2011). This fact crop is mainly important in tropical regions, where crops show higher evapotranspiration. In recent literature, some papers report increases in biomass production in grasses after increasing Si supply (Korndorfer et al., 2001; Fonseca et al., 2009; Sávio et al., 2011).

The dry mass increase has no correlation with potassium supply, since the same amount of K was applied in all treatments (potassium chlorite was used to equilibrate K concentration after application of increasing doses of potassium silicate), as explained in material and methods section. The use of potassium silicate as a source of silica is

an alternative to increase productivity up to 30%, but an economic study of the economic viability of commercial application in the production chain of *Sorghum bicolor* is necessary.

#### Accumulation and Efficiency

Similarly to observed in the present work, there are various reports in the literature that Si accumulation increases when plants are supplied with Si, starting with accumulation in the roots and passing through the leaf sheaths to the leaf lamina (Kahn et al., 2016); however, when Si is applied on soil.

With increased rates of Si, an increase in the accumulation of Si occurs in the roots, probably indicating the redistribution of Si applied to the leaf via phloem to the roots (Table 4). Redistribution is defined as the movement of an element from one place to any other organ (Malavolta, 2006) and is directly linked to the element mobility in phloem (Hocking, 1980). Si is considered an immobile element since Si redistribution in the plant is not significant (Santos et al. 2012; Alves et al., 2014); however, the presence of sorbitol may have resulted in a soluble compound with greater mobility in phloem. Sorbitol is a polyol with a moistening action, which delays the solution from drying by lowering the point of eliquescence in the formulation of the leaf, prolonging the absorption process (Will et al., 2011). Studies have also shown that the interaction between sorbitol and some nutrients, such as B,

can result in greater mobility of it within the phloem in plants that produce polyols naturally (Brown and Hu, 1998).

## Materials and methods

### Experimental area

The study was conducted under greenhouse condition at the School of Agronomy of the Federal University of Goiás, in Goiás State, Brazil, at the coordinates 16° 35" south latitude and 49° 21" west longitude. The region's climate is the Aw (Megathermal) or savanna tropical climatic type, with dry winters and rainy summers, according to the Köppen classification. The altitude is approximately 730 m and the average annual precipitation is 1600 mm.

Each experimental unit was comprised of a 4 dm<sup>3</sup> capacity pot, filled with 3.5 dm<sup>3</sup> of samples from a 0-20 cm deep superficial layer of Rhodic Hapludox (Soil Survey Staff, 2014). The initial chemical analysis of the soil presented the following attributes: pH: 4.4 (CaCl<sub>2</sub>); Al<sup>3+</sup>: 0.5 cmol<sub>c</sub> dm<sup>-3</sup>; organic material: 2.3 g dm<sup>-3</sup>; P: 0.8 mg dm<sup>-3</sup>; K<sup>+</sup>: 25 mg dm<sup>-3</sup>; Ca<sup>2+</sup>: 0.4 cmol<sub>c</sub> dm<sup>-3</sup>; Mg<sup>2+</sup>: 0.3 cmol<sub>c</sub> dm<sup>-3</sup>; SO<sub>4</sub><sup>2-</sup>: 5.6 mg dm<sup>-3</sup>; Zn<sup>2+</sup>: 1.4 mg dm<sup>-3</sup>; B: 0.14 mg dm<sup>-3</sup>; (H+Al<sup>3+</sup>): 1.27 cmol<sub>c</sub> dm<sup>-3</sup>; cation exchange capacity (CEC): 2.03 cmol<sub>c</sub> dm<sup>-3</sup>; base saturation (V%): 37.4%. The granulometric analysis presented 530, 180, and 135 g kg<sup>-1</sup> of clay, silt, and sand, respectively.

### Experimental design

The treatments were arranged in an entirely randomized experimental design, with five foliar Si rates: T1 – (0 (control); T2 – 0.84; T3 – 1.68, T4 – 2.52, and T5 – 3.36 g L<sup>-1</sup> of Si), applied as potassium and sodium silicate (Si=124 g L<sup>-1</sup>; 42 g K<sub>2</sub>O L<sup>-1</sup>; 31 g Na<sub>2</sub>O L<sup>-1</sup>; d=1.15 g L<sup>-1</sup>), with four repetitions. In each treatment containing silicon, the potassium content was balanced using potassium chloride, with T1 – 1.904; T2 – 1.428; T3 – 0.952; T4 – 0.476 de g L<sup>-1</sup> of KCl, and T5 – without applying KCl.

### Experiment development

Lime was applied as dolomitic limestone (CaO=36%; MgO=15%; PN=98%; PRNT=92.54%) to raise base saturation to 60%. Soil humidity was corrected to 60% of the soil maximum capacity to water retention-MCWR) and pots were incubated for 30 days.

After this period, before planting, basic fertilization performed as a nutrient solution in rates equivalent to 80 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, using monoammonium phosphate (MAP) as P source; 60 kg ha<sup>-1</sup> of K<sub>2</sub>O as potassium chloride (KCl); and 80 kg ha<sup>-1</sup> using urea as a source, 20 kg ha<sup>-1</sup> applied during planting and the rest (60 kg ha<sup>-1</sup>) 30 days after planting, in accordance with Sousa and Lobato (2004).

The BRS655 hybrid forage sorghum (*Sorghum bicolor*) was used for planting. Sowing was carried out on 29<sup>th</sup> June, 2015. Ten days after seedlings emergence (DAE), thinning was performed and 2 plants were maintained per pot. Pots were irrigated daily with deionized water using the pot weighing method, keeping humidity at 60% of the MCWR.

Silicon application on plants was performed three times, being first of the Si rate applied at 15 DAE, second at 30 DAE, and third at the end of the vegetative state in pre-bloom (45

DAE). The volume of each application was calculated to cover the whole leaf area of the plant. Before each Si application, pots were covered with impermeable material to avoid the solution sprinkled onto the leaves seeping into the soil.

### Physiological parameters

The relative chlorophyll index (RCI) evaluations were performed 7 days after each Si application, using the third expanded leaf on each plant. A chlorophyll meter was used, ClorofiLOG CFL1030 model. When the forage grass was cut, 60 days after sowing (DAS), the shoots height and leaf area were taken, using a LI-3100 Area Meter equipment. The net photosynthesis rate and stomatal conductance were measured using an open system portable infrared gas analyzer (IRGA) model LICOR 6400, such as described by Santos Junior et al. (2006). The measurements were taken between 8 am and 3 pm in totally extended leaves in good phytosanitary conditions. The photosynthetic variables response to the luminous intensity were determined for photon flux density (PPFD) of 550 μmol m<sup>-2</sup> s<sup>-1</sup> with the foliar chamber adjusted to operate with a CO<sub>2</sub> concentration of 380 ± 5 μmol mol<sup>-1</sup>, temperature of 31 ± 1 °C and water vapor of 21 ± 1 mmol mol<sup>-1</sup> with leaf area of 1 cm<sup>2</sup>.

Thus, biomass production was evaluated, separating the aerial part from the roots. The vegetal tissue samples were washed with 0.1% detergent solution, 0.3% acidic solution, and distilled water, to decontaminate possible residues from the foliar applications. After that, plant materials were oven drying (65°C, 48hs) and determining the aerial part and roots dry mass. In plant's tissues, the silicon level was determined in accordance with Korndörfer et al. (2004). Then the Si accumulation in the aerial part was calculated using leaf tissue Si content data, as well as the increase in dry mass.

Using the dry matter and nutrients content in plants data were performed the calculation of the nutritional indices comprising absorption efficiency (AB<sub>ef</sub>) and efficiency of use of nutrients for conversion to dry matter (UT<sub>ef</sub>) (Prado, 2008). These indices calculation were proceeded as following:

Equation Swiader et al. (1994):

$$AB_{ef} = \frac{\text{total nutrient content in plant}}{\text{root dry matter}}$$

Equation Siddiqi and Glass (1981):

$$UT_{ef} = \frac{(\text{total dry matter produced})^2}{\text{total nutrient content in the plant}}$$

### Statistical analyses

Results were subjected to the variance analysis using software Sisvar Inc., Brazil (Ferreira, 2014). When it was significant, a polynomial regression analysis was proceeded. Linear and quadratic mathematical models were tested to select the one that provided the best data adjustment, based on the magnitude of the regression coefficients significance at 5% probability by the t test. The maximum points were calculated by deriving the significant equations.

### Conclusion

The growth rate and the relative chlorophyll index in sorghum are not affected by increasing silicon rates

application on leaves; however, leaf area can increase by up to 23% with the use of 3.36 g L<sup>-1</sup> of Si. Physiological parameters are influenced by silicon application, with rates close to 1.68 g L<sup>-1</sup> of Si leading to the best photosynthetic and stomatal conductance rates. Foliar application of potassium silicate in rates up to 3.36 g L<sup>-1</sup> of Si leads to increases of around 30% in sorghum biomass production.

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