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SILICON ACCUMULATION IN BIOENERGETIC SUNFLOWER GERMINATION

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ABSTRACT - Silicon is considered a beneficial element for several plants, especially in crops with potential for residual biodiesel, and can provide an increase in dry matter in oilseeds. The objective of this work was to evaluate the accumulative power of silicon on sunflower germination and its development when submitted to different doses. Sunflower seeds cv. Multissol were sown in polypropylene trays in substrate incorporated at different concentrations of organic silicon, in treatments: T1 - control; T2 - 5.0; T3 - 10.0; T4 - 15.0 and T5 – 20.0 g kg^{-1} (BioMarkan®, 90% SiO_2), totaling 128 repetitions per treatment. The germination percentage was evaluated in five periods (3rd, 6th, 9th, 12th and 15th day). On the 20th day, the seedlings were evaluated in terms of: number of leaves, shoot length, stem diameter, total fresh and dry mass. Subsequently, the analysis of chemical concentration of silicon in the seedlings and X-ray microanalysis in the leaves of the sunflower seedlings were carried out. The interaction between treatments and germination days was significant for the days in which germination was higher on the 6th day. The silicon incorporated into the substrate accelerates the germination of sunflower seeds cv. Multissol. The sunflower cv. Multissol is a silicon accumulator and improves development up to a concentration of 10 g kg^{-1} of silicon.
Keywords: *Helianthus annuus* L., biodiesel, oilseeds, diatomaceous earth.

ACÚMULO DE SILÍCIO NA GERMINAÇÃO DE GIRASSOL BIOENERGÉTICO

RESUMO - O silício é considerado um elemento benéfico para várias plantas, principalmente em culturas com potencial para biodiesel residual, podendo proporcionar aumento da matéria seca em oleaginosas. O trabalho teve por objetivo avaliar o poder acumulativo do silício na germinação de girassol e seu desenvolvimento submetidos a diferentes concentrações. As sementes de girassol cv. Multissol foram semeadas em bandejas de polipropileno em substrato incorporados a diferentes concentrações de silício orgânico, nos tratamentos: T1 - controle; T2 - 5,0; T3 - 10,0; T4 - 15,0 e T5 – 20,0 g kg^{-1} (BioMarkan®, 90% SiO_2). A porcentagem de germinação foi avaliada em cinco períodos (3°, 6°, 9°, 12° e 15° dia). Ao 20° dia, as plântulas foram avaliadas quanto à: número de folhas, comprimento da parte aérea, diâmetro do caule, massa fresca e seca total. Posteriormente, foram realizadas as análises de concentração química de silício nas plântulas e microanálise de raios-X nas folhas das plântulas de girassol. A interação entre tratamentos e os dias de germinação, foi significativo para dias em que obteve maior germinação ao 6° dia. O silício incorporado ao substrato acelera a germinação de sementes de girassol cv. Multissol. A cultura do girassol cv. Multissol acumula silício e melhora o desenvolvimento até a concentração de 10 g kg^{-1} de silício.
Palavras-chave: *Helianthus annuus* L., biodiesel, oleaginosas, terra diatomácea.

INTRODUCTION

Sunflower (*Helianthus annuus* L., Asteraceae) has its center of origin in Southwest Mexico, being among the five main oil-producing species in the world (NOBRE et al., 2010), due to its desirable characteristics in the agricultural sector and from an economic point of view (SILVA et al., 2013). As socioeconomic importance, its multiple uses stand out, such as in human food, as the oil from its seeds is rich in nutrients and vitamins (GRILO et al., 2014), animal feed (OLIVEIRA et al., 2015) and raw material for biodiesel production (FERRARI; SOUZA, 2009; HARRIS et al., 2016).

Most of the volume of biodiesel produced in the world uses soy as a raw material, however, all vegetable oils classified as fixed can serve this purpose (EVANGELISTA et al., 2015). In the biodiesel production chain, residual lignocellulosic material is also generated (straw, trunk, branches, bark and bagasse), from oilseed species such as sunflower, increasing the availability of residual biomass in Brazil, which plays an important role in energy production. using renewable sources (GUEDES et al., 2010).

Sunflower is a demanding plant in terms of fertility, presenting a drastic reduction in growth when subjected to adverse conditions (BISCARO et al., 2008). Thus, coating seeds with micronutrients becomes a promising alternative, contributing to germination, emergence and establishment of seedlings in the field (VIEIRA et al., 2011). Despite being considered non-accumulators of Si, they benefit from the application of

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this element, and positive effects have also been reported on ornamental and vegetable crops. The effects of silicon in agriculture have been researched, such as in the cultivation of sunflower, since this element minimizes several stress factors suffered by the plant, both biotic and abiotic during its production (CARVALHO et al., 2009), it is important to increase beneficial elements, such as the micronutrient silicon (Si).

Plants that grow in an environment rich in silicon differ from those present in conditions of deficiency, mainly in terms of chemical composition, mechanical resistance of cells, leaf surface characteristics, tolerance to various types of abiotic stresses, attack by pests and pathogens (RODRIGUES, 2010), tolerance to heavy metals and improvement in the quality of agricultural crops (MA; YAMAJI, 2006), as well as in the production, quality and morphophysiology of potted sunflowers (CARVALHO et al., 2009; ZANÃO JÚNIOR et al., 2017).

The absorption of Si may occur due to some form of plant defense, such as biotic or abiotic stress, which absorbs more of this element under these conditions and, therefore, better resists this disturbance (DALLAGNOL et al., 2009). In sunflower, the processes of absorption of monosilicic acid (H_4SiO_4), a neutrally charged molecule that is transported to the shoot by the xylem, can occur both actively and passively. The determining factor for each type of transport is the concentration of the mineral applied, which, at low concentrations, leads to a reduction in transport by mass flow (GUNES et al., 2008).

Considering the benefits of silicon and the importance of the sunflower crop, it is possible to obtain answers in germination and, consequently, the optimization of plant development in the field. In this context, the objective of this work was to evaluate the accumulative power of silicon incorporated into the substrate on sunflower germination and plant development in the field.

MATERIAL AND METHODS

The work was carried out in a greenhouse belonging to the Department of Plant Science, located in the Didactic Garden of the Federal University of Ceará (UFC), Pici Campus, in Fortaleza-CE (3º 44' 22" S and 38º 34' 35" W, altitude of 21 m). The experiment was conducted in a protected environment, covered with a monofilament screen that provides 30% of shading to the environment. The structure's right foot was approximately 1.8 m high, with the length and width of approximately 8.0 m each. Sunflower seeds cv. Multissol were sown in polypropylene trays, containing one seed per cell, and the concentrations of silicon incorporated into the Vermicomposto® substrate in the concentrations of the following treatments: T1 - control; T2 - 5.0; T3 - 10.0; T4 - 15.0 and T5 – 20.0 g kg^{-1} (BioMarkan®, 90% SiO_2), in a completely randomized design, where each tray constituted a treatment with 50 repetitions. Irrigation was performed in two periods (morning and afternoon) daily, by a permanent sprinkler system suspended for 10 to 15 min, according to the needs of the culture and the measurement of temperature and relative humidity monitored by a Thermo-hygrometer (Supermedy® model HCT-2).

The germination percentage was evaluated in five periods (3rd, 6th, 9th, 12th and 15th day). On the 20th day, the seedlings were evaluated for the number of leaves, shoot length (cm), stem diameter (mm), with the aid of a millimeter ruler and digital caliper, respectively, and later, total fresh biomass (mg) and total dry biomass (shoot and root), in the Olericulture Laboratory of UFC. Total dry biomass samples were obtained after drying the plant material (shoots and roots) in a forced circulation oven at 60ºC for 72 h, until constant weight. The samples were collected from 25 seedlings, dried, crushed and separated into 6 repetitions with 1.0 g each sample and, later, sent to the Fertilizer Laboratory (LAFER), at the Federal University of Uberlândia (UFU). The determination of silicon was carried out by the molybdenum blue colorimetric method, according to the methodology proposed by Gallo and Furlani (1978).

For quantification and mapping of nutrients by X-ray microanalysis, leaves were collected from three different seedlings of the five treatments, on the 20th day. These fragments were fixed in stubs with the help of double-sided carbon tape and properly identified. They were then analyzed using a scanning electron microscope (XL30 Philips, Cambridge, UK), coupled to the X-ray microanalysis detection system: FEI Quanta 450 FEG (Bruker, Berlin, Germany), following the protocol described by Alves and Perina (2012).

The data obtained were submitted to analysis of variance, with those qualitative analyzed by the Tukey test, at 5% error probability and quantitative by polynomial regression. For the statistical analysis, the Assistant 7.7 software was used (SILVA; AZEVEDO, 2016).

RESULTS AND DISCUSSION

For the phytotechnical characteristics (number of leaves, shoot length, stem diameter, total fresh biomass and total dry biomass), no significant differences were observed between the germination days and the concentrations of silicon (Si) used in the substrate, corroborating others researchers, who also reported a negative correlation between Si accumulation and plant biomass (JOHNSON; HARTLEY 2018; TOMBEUR et al., 2021).

According to Thorne et al. (2022), this negative correlation is possibly due to a substantial fraction of Si that is translocated to the shoot by mass flow through the xylem and, perhaps, that transpiration fluxes are relatively large in slow-growing plants, related to the seedling development stage. Another hypothesis is that there may be an energy cost associated with the high absorption of Si (SIMPSON et al., 2017), justifying the indifference in the accumulation of plant biomass in the seedlings.

Evangelista et al. (2015), however, observed positive effects at 60 days on the development of sunflower plants subjected to Si concentrations, in shoot height, stem diameter, dry mass of the leaf, stem and inflorescence. This positive effect may be due to the plant stage, where the supply of nutrients is greater according to its development, compared to the initial stage of a seedling, such as the one in this work. The germination percentage showed a significant interaction between the germination days and the Si concentrations (Table 1), reaching the germination peak on the 6th day, with up to 94% of germinated seeds, in a non-accumulative count. According to Caldeira et al. (2015), in a commercial sunflower seed germination test, the germination peak occurs on the 7th day after sowing, in substrate and sand, with different percentages in relation to the cultivars used (89% for cv. Hélio 253 and 93% for cv. Hélio 251).

Oliveira et al. (2016), studying the physiological quality of seeds, did not observe significant interaction in the germination of rice seeds, between sources and concentrations (30, 60, 90, and 120 g) of Si, verifying the main effect only in relation to concentrations. The same was observed by Santos et al. (2020), studying saline stress, where Si (30 and 60 g) had a positive effect on pepper germination, in addition to the decreasing sensitivity to salinity, according to the same authors, the use of silicon favored physiological and metabolic processes, allowing conditions for its development to the plant.

TABLE 1 - Non-accumulated germination (%) of sunflower seeds at different concentrations of organic silicon (SiO_2), evaluated from the 3rd to the 15th day.

Si concentrations	Sunflower germination days				
(g kg^{-1})	3°	6°	9°	12°	15°
0.0	1.33 aB*	20.67 aA	3.00 aB	0.00 aB	0.00 aB
5.0	6.67 aB	16.00 aA	2.33 aB	0.00 aB	0.00 aB
10.0	6.67 aB	14.67 aA	2.00 aB	1.33 aB	0.33 aB
15.0	7.00 aB	17.67 aA	0.33 aB	0.00 aB	0.00 aB
20.0	4.00 aB	16.33 aA	4.67 aB	0.00 aB	0.00 aB

*Means followed by the same lowercase letter in the column and uppercase in the row, do not differ statistically from each other, by the Tukey test, at 5% error probability.

In the presence of Si, there was also better initial germination until the 3rd day, compared to the control, possibly due to water retention caused by the presence of this element in the substrate. According to Oliveira et al. (2016), seeds that germinate first are considered to have greater vigor, as they require less time for membrane reorganization, during the imbibition process, to initiate germination. Seeds and seedlings in the presence of Si acquire resistance to adverse environmental conditions, perhaps by increasing their biological capacity, thus increasing their vigor (FERRAZ et al., 2017).

Figure 1 shows the chemical concentration of Si, the percentage of element Si by X-rays in leaves, where the levels of Si obtained by the two quantification methods were comparable (Figures 1A and B). There was a significant interaction between Si concentrations in determining the content present in sunflower plants, capable of incorporating it as the concentration increased, presenting values higher than those observed in the control treatment (1.09 % and 0.5 %, according to Figure A and B). The presence of Si in the control can be explained by the abundance of the element in nature, where it is ubiquitous and can even be found in water (LUZ et al., 2006). It is also considered a mineral nutrient absorbed by the soil and translocated in the plant together with water (MA et al., 2001).

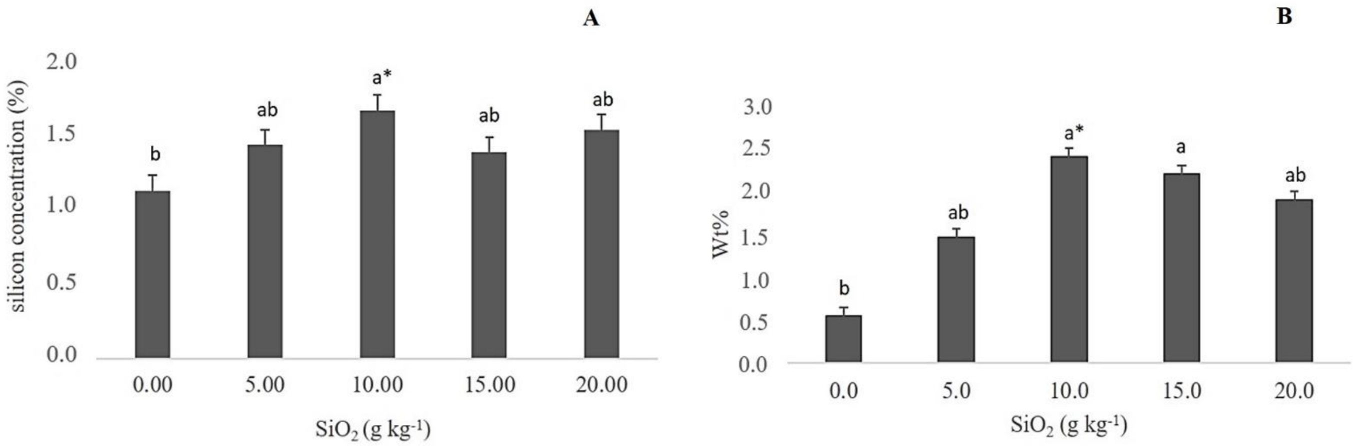

FIGURE 1 - Si concentration in sunflower seedlings, resulting from germination in substrate with different concentrations of organic silicon (SiO_2). (A) Colorimetric method on the seedling, (B) X-ray mapping on leaf fragments, *Averages followed by the same lowercase letter between columns, do not differ statistically from each other, by Tukey test, at 5% error probability.

The use of Si at 10 g kg^{-1} resulted in higher levels in both methods (1.88% and 2.5%, Figure 1A and B), with 35% and 24% higher when compared to the control treatment, respectively. In the present study, it was observed that sunflower seedlings are potential Si accumulators, since - according to Ma et al. (2001) - in ratios above 1.0% plants are considered accumulators, between 1.0 and 0.5% they are intermediate and below 0.5% they are not accumulators.

In the X-ray microanalysis images, the element Si was detected in all treatments, including the control, which showed low staining (Figure 2A, F and L). Thus, the evolutionary distribution of Si was directly related to the concentration applied to the substrate, confirming, through the mapping, its absorption in the sunflower leaf fragments (Figure 2A, B, C, D and E). Martins et al. (2018) and Dias et al. (2017) also noticed in the mapping of bromeliad and anthurium leaves, respectively, an evolution in the detection of the element Si, according to the concentration used. As for Cruz et al. (2014), working with beans cv. Pérola, observed that the silicon was detected in higher levels close to the veins of the leaves of the plants, realizing a greater mechanical resistance in the region. It may be related to the Si rate and the number of silicified cells that are formed in the epidermis of the treated leaves (RODRIGUES; DATNOFF, 2015).

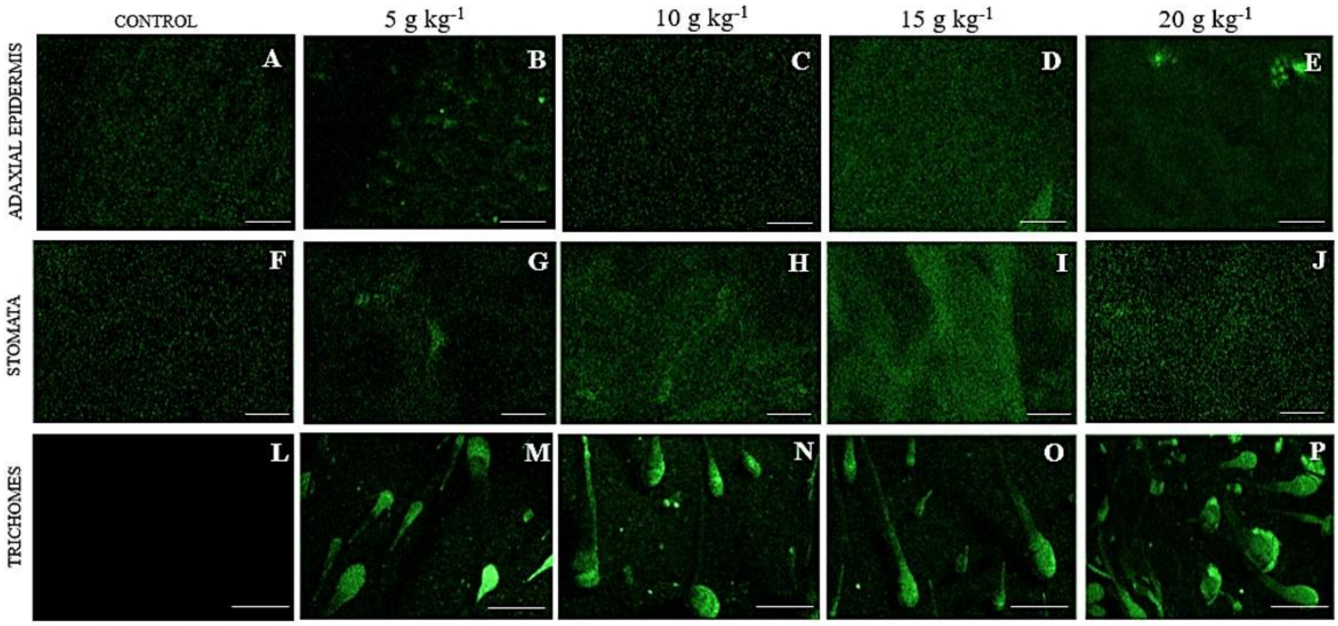

FIGURE 2 - Detection of the Si element by X-ray image, mapping in the adaxial epidermis, stomata and trichomes of the surface of sunflower leaves cv. Multissol, subjected to different concentrations of organic silicon (SiO_2). Bar = 10, 50 and 250 µm.

At the base of the trichomes (Figures 2M, N, O and P) and in the stomata (Figures 2G, H, I and J) there is an intense presence of the element Si, possibly because part of the absorbed Si remains in the soluble form. However, most of it is incorporated into the cell wall of the epidermis, stomata and leaf trichomes, and a small part forms amorphous deposits (EPSTEIN, 2001). The deposition of the element in the tissues is influenced by several factors, such asthe age of the plant, type and location of the tissues involved, and absorption through the roots and transpiration (KORNDÖRFER, 2014).

In the ultrastructural analysis by scanning microscopy of the abaxial part of the leaves, originating from sunflower germination in a substrate containing concentrations of Si, it was observed the presence of structures that, probably, are of accumulation of Si, as well as stomata with less turgid guard cells. (Figures 3H, I and J). In the treatments that had the presence of Si, the closing of the stomata was verified.

The stomata are related to the entry and exit of air inside the organs in which they are located, or even with the exit of water (APPEZZATO-DA-GLÓRIA; CARMELLO-GUERREIRO, 2012). The smaller stomatal opening reduces water losses from the stomatal transpiration, but does not compromise the concentration of CO_2 inside the leaves (WALTER et al., 2015). For Costa et al. (2018), stomata in passion fruit seedlings may be more functional in the presence of Si, as plants, in the presence of this element, have higher photosynthetic rates compared to non-application.

Regarding the trichomes, it can be observed that, in the treatments in which Si was used, they were apparently more rigid (Figures 3M, N, O and P) and well formed, when compared to the control treatment (Figure 3L). Trichomes are structures composed mainly of Si (SAMELS et al., 1994), and their high availability in the substrate may contribute to the greater formation and accumulation of this defense structure. Zanão Júnior et al. (2017), when analyzing the sunflower cv. 'Sunbright', observed a greater accumulation of silicon in the leaf trichomes without changing the thickness of the epidermis. The same authors also mention that this possible relationship is a pattern in dicotyledonous plants.

According to Castro et al. (2009), trichomes can be used to prevent excessive transpiration, and, for that, they offer a barrier against winds, which can decrease the humidity on the leaf surface and, thus, cause an increase in transpiration. Another way of reducing transpiration through trichomes is the reflection of solar radiation, which can increase leaf temperature. The large coverage by trichomes, as well as the presence of chemical substances in the glandular trichomes are characteristics that, combined with the characteristics present in other plant organs, classify the plant as invasive (LIMA et al. 2009).

According to Marin (2003), the presence of silicon on plants can induce direct benefits, such as better structural development of plant organs, and indirect ones, such as increased photosynthetic rate, due to these well-formed structures, such as stomata and trichomes. It is also known that fertilization with Si leads to a better plant architecture, due to morphological and physiological adaptations, leading to more erect leaves and more efficient light interception, which is reflected in an increase in photosynthetic rate (DEREN et al., 1994).

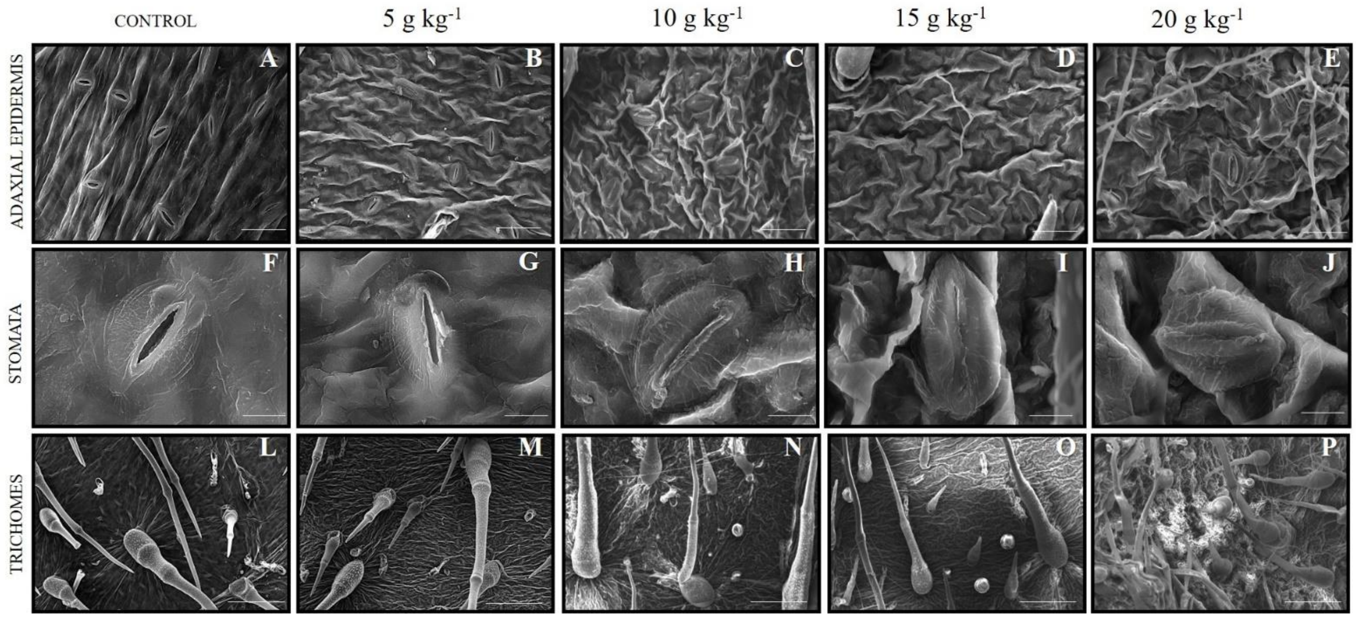

FIGURE 3 - Scanning electron micrographs of adaxial epidermis, stomata and trichomes on the surface of sunflower leaves cv. Multissol, subjected to different concentrations of organic silicon (SiO_2). Bar = 20 and 50 µm.

Simpson et al. (2017) found greater Si accumulation in cereals, associated with a lower growth rate, especially for larger plants. In this work, the sunflower, in the seedling formation phase, showed a tendency to accumulate Si, regardless of the concentration available and with no difference in terms of size. Probably, this accumulation of Si in the seedlings provided a greater survival, after its transplant to the field. The increase in Si availability has resulted in increases in growth and productivity, since the element can act indirectly on some photosynthetic and biochemical aspects (GUAZINA et al., 2019).

The morphology of sunflower seedlings was not significantly modified in the addition of silicon to the substrate, but it provided ultrastructural changes in their leaves, such as stomata and trichomes, with deposition of possibly silicate waxes, allowing better development at the transplant stage of these seedlings, with greater resistance to the weather in the countryside. These results are a possible starting point for the development of other researches aiming to increase germination efficiency with the use of silicon incorporated into the substrate in sunflower.

CONCLUSIONS

The silicon incorporated into the substrate accelerates the germination of sunflower seeds cv. multissol.

The sunflower cv. Multissol is a silicon accumulator and improves development up to a concentration of 10 g kg^{-1} of silicon.

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