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SILÍCIO EM PLANTAS DE FEIJÃO CAUPI SUBMETIDAS A ESTRESSE HÍDRICO: TERMOTOLERÂNCIA E EFICIÊNCIA FOTOSSINTÉTICA

SILICIO EN PLANTAS DE FRIJOL SOMETIDAS A ESTRÉS HÍDRICO: TERMOTOLERANCIA Y EFICIENCIA FOTOSINTÉTICA

SILICON IN COWPEA PLANTS SUBMITTED TO WATER STRESS: THERMOTOLERANCE AND PHOTOSYNTHETIC EFFICIENCY

Presentation: Oral Communication

Jeane Ferreira de Jesus¹; Manderson Nunes da Silva²; Stelamaris de Oliveira Paula Marinho³ Wallace de Sousa Leite⁴; Rafael de Souza Miranda⁵

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RESUMO

As condições climáticas de altas temperaturas e altas radiações potencializam o efeito do déficit hídrico limitando a produção vegetal. A influência de vários estresses conjuntamente irá ativar mecanismos de respostas, sejam fisiológicas, bioquímicas, metabólicas e/ou moleculares, produzindo efeitos em todo o maquinário funcional da planta, que podem ser reversíveis e irreversíveis, a depender do grau de comprometimento de estruturas importantes. Nesse contexto, o silício (Si) vem sendo estudado como elemento benéfico atenuador de vários estresses, devido a sua ação eficiente em funções estruturais e de defesa das plantas, como ao evitar a perda excessiva de água pelo seu acúmulo nos órgãos de transpiração, bem como devido a seu papel sinalizador em mecanismos de resposta. Estudos reportam que a adubação com Si representa um potencial mediador dos mecanismos de defesa nas plantas em déficit hídrico, como a indução de vias de absorção e transporte de água. Diante disso, o objetivo do trabalho foi avaliar o efeito do silício no ajuste térmico, crescimento e produção de biomassa de plantas de feijão-caupi (Vigna unguiculata) sob condições de restrição hídrica. Os ensaios foram conduzidos em casa de vegetação, utilizando duas cultivares de feijão-caupi (Xique-xique e Novaera) submetidas a três níveis hídricos [controle (75% da capacidade de campo - CC), estresse moderado (60% CC) e estresse severo (45% CC)] e duas concentrações de silício [sem Si (0 mM) e com Si (2,0 mM)]). O estresse foi aplicado 28 dias após a semeadura. Os dados demostraram que o estresse hídrico reduziu a matéria seca, o número de trifólios, o crescimento e os índices de clorofila a e b das plantas, e os efeitos foram intensificados com a severidade do estresse, ao passo que as plantas apresentaram elevados valores de temperatura foliar. Em contrapartida, o silício foi eficiente em atenuar os efeitos nocivos do

¹ Mestranda em Ciências Agrárias, Universidade Federal do Piauí, <u>jeane.ferreiraj@hotmail.com</u>

² Graduando em Engenharia Florestal, Universidade Federal do Piauí,<u>mandersonnunes@ufpi.edu.br</u>

³ Pós-Doutoranda em Ciências Agrárias, Universidade Federal do Piauí, <u>maris.biologa@gmail.comr</u>

⁴ Doutorando em Agronomia, Universidade Federal do Piauí, <u>wallace.leite@ufpi.edu.br</u>

⁵Doutor em Bioquímica Vegetal, Universidade Federal do Piauí, <u>rsmiranda@ufpi.edu.br</u>

estresse sobre a temperatura foliar, promovendo aumento na produção de biomassa e no conteúdo de pigmentos fotossintéticos de plantas do genótipo Novaera, o que conferiu maior tolerância a essa cultivar.

Palavras-Chave: Tolerância a seca, Temperatura foliar, Adubação com silício.

RESUMEN

Las condiciones climáticas de altas temperaturas y alta radiación potencian el efecto del déficit hídrico limitando la producción vegetal. La influencia de varios estreses en conjunto activará mecanismos de respuesta, ya sean fisiológicos, bioquímicos, metabólicos y/o moleculares y por lo tanto producirá efectos sobre toda la maquinaria funcional de la planta, que pueden ser reversibles e irreversibles, dependiendo del grado de compromiso de importantes estructuras En este contexto, el silicio ha sido estudiado como un elemento benéfico que mitiga diversos estreses, siendo eficiente en funciones estructurales y de defensa de las plantas, atenuando el estrés, previniendo la pérdida excesiva de agua por su acumulación en los órganos de transpiración, además de tener una acción señalizadora en los mecanismos de respuesta. La fertilización con silicio (Si) ha sido citada como un potencial mediador de defensa en las plantas en déficit de agua, lo que ayudó en la inducción de vías de absorción y transporte de agua. Por lo tanto, el objetivo de este trabajo fue evaluar el efecto del silicio en el ajuste térmico, el crecimiento y la producción de biomasa de plantas de caupí (Vigna unguiculata) en condiciones de restricción hídrica. Los experimentos se realizaron en invernadero utilizando dos cultivares de caupí (Xique-xique y Novaera) sometidos a tres niveles de agua [testigo (75% de capacidad de campo - CC), estrés moderado (60% CC)] y estrés severo (45% CC)] y dos concentraciones de silicio [sin Si (0 mmol L-1) y con Si (2,0 mmol L-1)]). El estrés se aplicó 28 días después de la siembra. Los datos mostraron que el estrés hídrico redujo Los datos mostraron que el estrés hídrico redujo la materia seca, el número de trifoliados, el crecimiento y los índices de clorofila a y b de las plantas, y los efectos se intensificaron con la severidad del estrés, las plantas presentaron valores altos de temperatura foliar y el silicio fue eficiente en atenuar los efectos nocivos del estrés sobre la temperatura foliar, favoreciendo incremento en la producción de biomasa y en el contenido de pigmentos fotosintéticos de plantas del genotipo Novaera, lo que confirió mayor tolerancia a este cultivar.

Palabras Clave: Tolerancia a la sequía, Temperatura de la hoja, Fertilización con silicio.

ABSTRACT

Climatic conditions of high temperatures and high radiation potentiate the effect of water deficit limiting plant production. The influence of several stresses together will activate response mechanisms, whether physiological, biochemical, metabolic and/or molecular and therefore will produce effects on the entire functional machinery of the plant, which can be reversible and irreversible, depending on the degree of compromise of important structures. In this context, silicon has been studied as a beneficial element that mitigates various stresses, being efficient in structural and plant defense functions, attenuating stress, preventing excessive loss of water due to its accumulation in the transpiration organs, in addition to having a signaling action in response mechanisms. Fertilization with silicon (Si) has been cited as a potential mediator of defense in plants in water deficit, which helped in the induction of water absorption and transport pathways. Therefore, the objective of this work was to evaluate the effect of silicon on thermal adjustment, growth and biomass production of cowpea (Vigna unguiculata) plants under water restriction conditions. The experiments were carried out in a greenhouse using two cowpea cultivars (Xique-xique and Novaera) submitted to three water levels [control (75% of field capacity - CC), moderate stress (60% CC)] and stress severe (45% CC)] and two concentrations of silicon [without Si (0 mmol L-1) and with Si (2.0 mmol L-1)]). Stress was applied 28 days after sowing. The data showed that water stress reduced the dry matter, the number of trifoliates, the growth and the chlorophyll a and



b indices of the plants, and the effects were intensified with the severity of the stress, the plants showed high values of leaf temperature and silicon was efficient in attenuating the harmful effects of stress on leaf temperature, promoting increase in biomass production and in the content of photosynthetic pigments of plants of the Novaera genotype, which conferred greater tolerance to this cultivar.

Keywords: Drought tolerance, Leaf temperature, Silicon fertilization.

INTRODUCTION

Agricultural production has been affected over the years as a result of climate change, mainly due to periods with low rainfall, which has become a major challenge for productivity, leading to extensive losses. However, plants have developed several mechanisms to avoid or minimize the deleterious effects of water deficit, and these mechanisms can confer drought tolerance (BIANCHI, 2016). Even so, there is no single physiological variable that, by itself, is indicative of drought tolerance (NOGUEIRA et al., 2001); demonstrating the need to evaluate a set of variables related to plant defense, such as water potential, thermal adjustment, growth, stomatal conductance, temperature and leaf transpiration, considered important parameters to evaluate the responses of plant species to water stress.

In order to mitigate the effects of water deficit in plants, studies related to the induction of plant defense pathways become an important tool for the viability of agriculture in regions with predominance of water deficit. Therefore, in addition to proper irrigation management, the use of stress signals and differential fertilization can help increase water use efficiency and activate metabolic defense pathways, resulting in plant acclimatization to environmental changes (AGOSTINI et al., 2013; FERRAZ et al., 2014). Among the cultivation strategies, fertilization with silicon (Si) has been cited as a potential mediator of plant defense, where it support in the induction of water absorption and transport pathways in sorghum plants under drought conditions (HATTORI et al., 2007).

Despite the divergence on its essentiality, research carried out with the element silicon has shown important beneficial effects that confer an increase in the growth and production of several plants, especially when subjected to environmental stresses (WILLADINO and CAMARA, 2010; MENEGALE et al., 2015), by nutritional deficiency caused by the absence of nitrogen (BUCHELT, 2019) or water deficit (TEIXEIRA et al., 2020).

Even in the face of a wide range of studies, the role of Si in activating the defense of cowpea under water limitation is still unknown, as well as there are no reports of its beneficial



role in the semiarid region, either in water use efficiency or for production systems. In this sense, the present study aims to evaluate the effect of silicon on thermal adjustment, growth and biomass production of cowpea plants under water restriction conditions.

THEORETICAL FOUNDATION

Cowpea beans

Cowpea (*Vigna unguiculata* (L.) Walp.) is predominantly cultivated in the North and Northeast regions of Brazil. In the 2021/2022 season, the total production expected for the country (combining the production of common bean colors and black, in addition to cowpea) is 929.4 thousand tons, indicating a reduction of 4.8% in relation to the result obtained in 2020/2021 season (CONAB, 2022). Thus, the current estimate is for a national production of 189.4 thousand tons of cowpea first crop, indicating an increase of 58% compared to the amount obtained last season. The main production highlights are Piauí and Bahia, which traditionally allocate significant portions of area for the cultivation of the crop in this first cycle (CONAB, 2022).

Beans are a staple food for the population and a source of income for the small farmer. Additionally, due to its grain yield, fresh and dry mass, it is possible to use it in animal feed and also in crop rotation and green manure, aiming at the recovery of soil fertility. Despite showing good adaptation to environmental conditions of cultivation (BOYER, 1978), it has low average productivity, with only 1,033 kg/ha (CONAB, 2022), mainly due to drastic fluctuations in environmental conditions that affect the semi-arid region.

Given the importance of cowpea for the North-Northeast of Brazil, it is essential to carry out studies to evaluate the performance of cultivars developed for cultivation under rainfed conditions, in view of the imposed water limitations that occur in different stages of growth.

Water stress in plants

Plant responses to water stress conditions vary according to species, cultivar, exposure time, edaphic factors, among others. For Oliveira et al. (2005), water deficit reduces stomatal conductance and increases diffusive resistance to water vapor, by closing the stomata, reducing



transpiration and, consequently, the supply of CO_2 for photosynthesis. Water availability has been one of the limiting factors for species evolution. Thus, the ability to deal with water deficit is an important determinant of natural selection of plants and productivity of agricultural crops (OLIVEIRA et al., 2015).

Plants have developed physiological and morphological mechanisms that confer tolerance and adaptation to water stress, especially to the deficit. Overall, adaptation can be characterized as genetically acquired resistance through selection from previous generations. Thus, the term adaptation must be distinguished from the term acclimatization, which in turn is the increase in plant tolerance according to its exposure to a certain stress (TAIZ; ZEIGER; 2009). In this context, water restriction conditions can negatively affect the growth and development of cultivated plants, culminating in production losses due to low water availability (NOBRE et al., 2013). It is also noteworthy that under water deficit conditions, a reduction in leaf area occurs in some plants, although this depletion culminates in lower plant growth and development, it is a strategy to avoid expenditure of metabolic energy (TAIZ; ZEIGER, 2013).

Thermal adjustment in plants under stress

Temperature, along with solar radiation, is one of the main factors related to plant growth and development. However, excesses of temperature, along with the reduction of soil moisture below the critical limit for the crop, generally cause damage to biomass production and significantly affect the development of roots and shoots of the plant (HATFIELD and PRUEGER, 2015).

Plants can respond to heat stress and water deficit through physiological, metabolic and morphological adjustments to cope with imposed conditions. Thermal adjustment mainly involves mechanisms aimed at controlling stomatal conductance, transpiration and leaf temperature. Such responses are activated to reverse disturbances in plant characteristics, which delay growth and development and, consequently, affect productivity (GERGANOVA et al., 2016).

Role of Si as a mitigator of the effects of water deficit

Silicon (Si) is the second most abundant element on the Earth's surface, only surpassed



by oxygen (EPSTEIN, 2009). Its use is considered beneficial for most cultivated plants and its absorption capacity varies between species (BAKHAT et al., 2018). As a general attenuator of abiotic stresses, silicon is associated with mechanisms linked to the elimination of Reactive Oxygen Species (ROS), with the activation of antioxidant defense responses and phytohormonal signaling (SOURI et al., 2021). In crops under water deficit, silicon acts as an attenuator, due to its accumulation in the transpiration organs, preventing excessive water loss, as well improves leaf architecture and photosynthetic rate (BIANCHINI et al., 2019; COELHO et al., 2019). In general, Si accumulation in plants can increase drought tolerance capacity, maintaining leaf water potential, inducing osmotic adjustment, modifying gas exchange attributes such as CO₂ assimilation and transpiration rates, and reducing oxidative stress (FAROOQ and DIETZ, 2015).

METHODOLOGY

The experiment was carried out in a greenhouse at the Federal University of Piauí, Campus Professora Cinobelina Elvas (CPCE/UFPI), in Bom Jesus, Piauí, Brazil, between July and October 2021. According to the Köppen climate classification (1948), the climate of the region is Aw, hot and semi-humid. During the experiments, the environmental conditions included a maximum temperature of 35 °C and a minimum of 22 °C, and an average air humidity of approximately 56%.

Two cowpea genotypes were evaluated: Xique-xique and Novaera, subjected to three water stress regimes [control (75% of field capacity - FC), moderate stress (60% FC) and severe stress (45% FC)], and two concentrations of silicon [without Si (0 mM) and with Si (2.0 mM)]), combined in a $2 \times 3 \times 2$ factorial scheme, in a randomized design with four repetitions. Supplementation with Si was performed via foliar spraying at the V3 phenological stage (second trifoliate leaf), approximately 21 days after sowing, prior to the application of water deficit treatments. The water stress treatments were applied at the end of the phenological stage (third trifoliate leaf), approximately 28 days after sowing.

Biometric parameters (plant height, stem diameter, number of trifoliates) were estimated every 7 days, as well as photosynthetic pigments (chlorophyll a and b) were also estimated weekly, non-destructively, using a SPAD meter. At the end of the vegetative phase,



28 days after the imposition of stress, a group of plants was collected and used to measure the parameters: leaf area, fresh mass (MF) and dry mass (DM) of the shoot (leaves + stems).

Throughout the experiment, leaf temperature was recorded through images with the aid of a portable infrared thermal camera FLIRT6 - 267, with image monitoring campaigns every 7 days. After 28 days of water stress, thermal images of a representative plant of each treatment were recorded, as well as the temperature of the first fully expanded leaf of the plants in each treatment.

The analyzed data were submitted to ANOVA, and the means were compared by the Tukey test at 5% of probability, using the SISVAR program. Graphs were constructed using SigmaPlot11 software.

RESULTS AND DISCUSSION

Leaf area (LA), shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) data are shown in figure 01. In general, water stress reduced all analyzed parameters, and the effects were intensified with the severity of stress. Under moderate stress, the biomass data in the absence of Si (-Si) clearly evidence the higher tolerance of the Xique-xique genotype compared to Novaera. However, even without statistical evidence, the addition of silicon (+Si) promoted an increase in LA, SDM, RDM and TDM of plants of the Novaera genotype under moderate stress compared to plants not treated with Si. This growth recovery was quite evident, as Novaera plants (considered sensitive to water deficit) supplemented with Si showed similar biomass production data to those of the Xique-xique genotype under moderate stress. Under severe stress, the variables did not show differences between the treatments studied (Figure 01).

Figure 01 – Leaf area (A), shoot dry mass (MSPA, B), root dry mass (MSR, C) and total dry mass (MST, D) of cowpea plants, Xique-xique and Novaera genotypes, after 28 days of imposition of water stress treatments [control (75% of field capacity – FC); moderate stress (60% FC) and severe stress (45% FC)] in a greenhouse, in the absence (-Si) and presence of silicon (+Si) at 2.0 mM. Data represent the mean of four replicates and standard error. Different capital letters indicate significant differences due to water stress within the same genotype and Si level; different lowercase letters denote significant changes between genotypes within the same level of Si and water stress; and asterisk (*) indicates difference when comparing Si doses within the same genotype and water regime, according to Tukey's test (p < 0.05).</p>

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The effects of water stress, as well as the addition of Si, were observed in the morphological aspects of cowpea plants at the end of the experiment (Figure 02). The images clearly show that water stress restricted plant growth, resulting in plants with abnormal aspects and leaf aspects different from the control treatments. These results reinforce the growth data presented in Figure 01, emphasizing that, under moderate stress, the plants of the Novaera genotype treated with Si stood out for presenting larger and morphologically well-developed plants compared to those cultivated without Si. Under severe stress, even without showing

INSTITUTO INTERNACIONAL DESPERTANDO VOCACÕES variations in the growth data (Figure 01), the plants of both genotypes treated with Si were more developed and with a more greenish appearance than the plants without Si (Figure 02). This result is probably related to regulation of the accumulation of photosynthetic pigments mediated by Si, which was fundamental for the greater growth of plants under water deficit.

Figure 02 – Visual appearance of cowpea plants, Xique-xique and Novaera genotypes, after 28 days under water stress [control (75% of field capacity – FC); moderate stress (60% FC) and severe stress (45% FC)] in a greenhouse, in the absence (Without Si) and presence of silicon (with Si) at 2.0 mm To record the images, a representative plant of each treatment was used. Without Si With Si Control Xiquexique Novaera Novaera Xiquexique Moderate Stress Xiquexique Novaera Novaera Xiquexique Severe Stress Xiquexique Novaera Novaera Xiquexique Source: Own (2022).



In order to relate biomass accumulation with fluctuations in thermal adjustment, thermal images and the temperature of the first fully expanded leaf of a representative plant of each treatment were registered after 28 days under water stress (Figure 03). Temperature fluctuations varied depended on the stress and genotype. Regardless of fertilization with Si, moderate water stress promoted increases in leaf temperature of cowpea plants compared to the respective controls, being the highest leaf temperature (50.4 $^{\circ}$ C) recorded in the Xique-xique genotype grown under moderate stress and in the absence of Si (Figure 03).

Figure 03 – Infrared thermal images and temperature of the first fully expanded leaf of cowpea plants, Xiquexique and Novaera genotypes, after 28 days of imposition of water stress treatments [control (75% of field capacity – FC); moderate stress (60% FC) and severe stress (45% FC)] in a greenhouse, in the absence (Without Si) and presence of silicon (with Si) at 2.0 mMSource: Own (2022).





With Si



Moderate Stress

> Severe Stress





However, the increases in temperature were smaller when the plants were treated with Si, with the lowest values recorded in plants of the Novaera genotype under moderate stress. These results support the beneficial role of Si in the physiological adjustment of Novaera plants under moderate water deficit, which may involve the regulation of stomatal opening and transpiration rates, resulting in efficient thermal adjustment and better growth rates (Figures 01, 02 and 03). This temperature regulation was distinct when plants were exposed to severe stress and, regardless of genotype, Si application seems not to have played a crucial role in thermal adjustment under such stressful conditions.

The effects of water treatments and the influence of Si on the photosynthetic pigments of cowpea plants at 28 days after application of stress are shown in figure 04.

Figure 04 – Accumulation of chlorophyll a (Chl a) and b (Chl b) in cowpea plants, Xique-xique and Novaera genotypes, after 28 days of imposition of water stress treatments [control (75% of the field capacity – FC); moderate stress (60% FC) and severe stress (45% FC)] in a greenhouse, in the absence (-Si) and presence of silicon (+Si) at 2.0 mM. Data represent the mean of four replicates and standard error. Different capital letters indicate significant differences due to water stress within the same genotype and Si level; different lowercase letters denote significant changes between genotypes within the same level of Si and water stress; and asterisk (*) indicates difference when comparing Si doses within the same genotype and water regime, according to Tukey's test (p < 0.05).



In general, water deficit levels did not cause significant changes in chlorophyll levels, regardless of the presence of Si. However, the Chl a content of Novaera plants under moderate stress was changed by the treatment with Si. Regarding chlorophyll b, the Novaera genotype showed higher chlorophyll content in the control and moderate stress conditions, when it was

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submitted to Si application. In study developed by De Andrade et al. (2022) evaluating bean grain yield using different silicon doses, the Si applications improved bean yield. Similar results were found in bean crop under Water deficit (Andrade et al., 2021), corn and corn (Teixeira et al., 2022).

Moderate and severe water stress caused significant reductions in plant height (AP) as well as in the number of trifoliates (NTF) only for the Xique-xique genotype, while for Novaera, there were only reductions in the NTF (Figure 05). Under well-irrigated conditions (control), it was observed that Xique-xique genotype had higher average plant heights compared to Novaera, regardless of Si supplementation. Our results are in accordance with earlier findings by Sousa et al. (2014), studying cowpea under water deficit. The authors reported that restriction of plant growth is linked to increased stress in the environment and the consequent reduction in water availability, which affect cell division and elongation, mainly by compromising maintenance of cell turgor and nutrient absorption.

Figure 05– Number of trifoliates (NFT) and plant height (AP) of cowpea plants, Xique-xique and Novaera genotypes, after 28 days of imposition of water stress treatments [control (75% of field capacity – FC); moderate stress (60% FC) and severe stress (45% FC)] in a greenhouse, in the absence (-Si) and presence of silicon (+Si) at 2.0 mM. Data represent the mean of four replicates and standard error. Different capital letters indicate significant differences due to water stress within the same genotype and Si level; different lowercase letters denote significant changes between genotypes within the same level of Si and water stress; and asterisk (*) indicates difference when comparing Si doses within the same genotype and water regime, according to Tukey's test (p < 0.05).





INSTITUTO INTERNACIONAL DESPERTANDO VOCACÕES The water deficit is responsible for causing a reduction in the water content in the plants, leading to physiological changes and affecting the growth of the crop (Teixeira et al., 2020). After 28 days of submission to water regimes, some changes in stem diameter (DC) were observed, mainly in Xique-xique plants sprayed with Si, whereby moderate and severe stress reduced these variables compared to control conditions (Figure 06). In this context, water restriction conditions can negatively affect the growth and development of cultivated plants, culminating in production losses due to low water availability (NOBRE et al., 2013). It is also noteworthy that under drought conditions, a reduction in leaf area occurs in some plants, although this depletion culminates in lower plant growth and development, it is a strategy to avoid expenditure of metabolic energy (TAIZ; ZEIGER, 2013).

Figure 06 – Stem diameter (DC) of cowpea plants, Xique-xique and Novaera genotypes, after 28 days of imposition of water stress treatments [control (75% of field capacity – FC); moderate stress (60% FC) and severe stress (45% FC)] in a greenhouse, in the absence (-Si) and presence of silicon (+Si) at 2.0 mM. Data represent the mean of four replicates and standard error. Different capital letters indicate significant differences due to water stress within the same genotype and Si level; different lowercase letters denote significant changes between genotypes within the same level of Si and water stress; and asterisk (*) indicates difference when comparing Si doses within the same genotype and water regime, according to Tukey's test (p < 0.05).



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observed, mainly in Xiquexique plants sprayed with Si, in which moderate and severe stress reduced these variables compared to control conditions.

CONCLUSIONS

- \checkmark The water deficit severely limits the growth and accumulation of biomass of cowpea, varying depending on the genotype and time of exposure to stress;
- ✓ Plants cultivated under water restriction exhibit high values of leaf temperature, which seem to be associated with low transpiration rate and inefficiency in leaf cooling;

 \checkmark Silicon attenuates the harmful effects of stress on leaf temperature, promoting an increase in biomass production and in the content of photosynthetic pigments in plants of the Novaera genotype, which gives more tolerance to water stress conditions;

✓ The Xique-xique genotype exhibits moderate stress tolerance responses, which were not dependent on Si treatment.

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