

## NANOENCAPSULATED SILICON IN TRIGGERING DEFENSE RESPONSES FOR DROUGHT TOLERANCE IN SUGARCANE

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**ABSTRACT:** Developing soluble silicon (Si) sources is crucial to increase its availability and activate drought tolerance mechanisms in crops. This study evaluated the potential of nanosilica (nSi) in mitigating drought effects and promoting acclimation in two sugarcane cultivars (RB867515 and RB027040). A field experiment followed a randomized block design in a split-split plot scheme ( $2 \times 2 \times 2$ ), with four replications was carried out. Treatments involved two irrigation regimes (80% and 40% of field capacity), two nSi fertigation (with and without) and two sugarcane cultivars. After 140 days of drought imposition, parameters such as growth, gas exchange, water status, photosynthetic pigments, and relative stress tolerance were measured. Under drought, the nSi reduced stress effects in RB027040, increasing fresh and dry biomass and enhancing relative tolerance. The nSi also improved photosynthesis in both cultivars and increased stomatal conductance in RB027040. The findings indicate that nSi promotes higher photosynthetic efficiency and biomass accumulation, contributing to better acclimation and yield stability under drought. Thus, nanosilica emerges as a promising tool for sustainable sugarcane management in drought-prone regions.

**KEYWORDS:** Drought; Silicon supplementation; Nanosilica; Plant defense; *Saccharum* spp.

## SILÍCIO NANOENCAPSULADO NO ACIONAMENTO DE RESPOSTAS DE DEFESA PARA TOLERÂNCIA AO DÉFICIT HÍDRICO EM CANA-DE-AÇÚCAR

**RESUMO:** O desenvolvimento de fontes solúveis de silício (Si) é fundamental para aumentar sua disponibilidade e ativar mecanismos de tolerância à seca em culturas agrícolas. Este estudo avaliou o potencial da nanosilica (nSi) na mitigação dos efeitos do déficit hídrico e na promoção da aclimação em duas cultivares de cana-de-açúcar (RB867515 e RB027040). O experimento foi conduzido em campo, em delineamento de blocos casualizados, no esquema de parcelas subdivididas ( $2 \times 2 \times 2$ ), com quatro repetições. Os tratamentos incluíram dois regimes de irrigação (80% e 40% da capacidade de campo), fertirrigação com e sem nSi e duas cultivares. Após 140 dias sob déficit hídrico, foram avaliados parâmetros de crescimento, trocas gasosas, estado hídrico, pigmentos fotossintéticos e tolerância relativa ao estresse. Em condições de

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déficit hídrico, a nSi reduziu os efeitos negativos do estresse na cultivar RB027040, promovendo aumento na biomassa fresca e seca, além de melhorar a tolerância relativa. A aplicação de nSi também aumentou a fotossíntese em ambas as cultivares e a condutância estomática na RB027040. Esses resultados indicam que a nSi favorece maior eficiência fotossintética e acúmulo de biomassa, contribuindo para melhor aclimação e estabilidade produtiva sob estresse hídrico. Assim, a nanosílica se destaca como uma ferramenta promissora para o manejo sustentável da cana-de-açúcar em regiões suscetíveis à escassez de água.

**PALAVRAS-CHAVE:** Defesa das plantas; Nanosílica; Restrição hídrica; Suplementação de silício; *Saccharum* spp.

## INTRODUCTION

Sugarcane is one of the most important crops from an energy perspective, providing 80% of the raw material for global sugar production (Liu et al., 2021). The plant is classified as a C4 metabolism and presents four well-defined phenological stages: germination, tillering, grand growth (or stalk elongation), and maturation. The complete cycle lasts about one year, encompassing all climatic variations across the seasons (Mall et al., 2022).

In semi-arid regions, sugarcane cycle is subject to periods of water deficit, limiting crop productivity (Camargo et al., 2019). In sugarcane, drought can delay leaf elongation and reduce nitrogen absorption efficiency, ultimately causing losses in sugar production and yield (Dinh et al., 2017). The physiological alterations in drought-stressed sugarcane may include reductions in relative water content, water potential, and photosynthetic activity, as well as increased electrolyte leakage (Bezerra et al., 2025).

Several studies have found improved performance of sugarcane plants subjected to drought under silicon nutrition compared to plants without supplementation. The main responses are associated with greater final biomass production, due to physiological and biochemical improvements in metabolic processes (Verma et al., 2021; Camargo et al., 2021; Teixeira et al., 2022; Berto et al., 2025). This phenomenon is attributed to plant metabolism as a Si-accumulating crop, capable of accumulating more than 10 g Si kg<sup>-1</sup>. The Si-accumulation ability results from the activation of specialized proteins involved in the active absorption and transport of silicon (Si) through the roots, namely the transporters *SILsi1* and *SILsi2* (Barreto & Barão, 2023; Shilpha et al., 2023). However, the main challenge in making Si available for agricultural production in field lies in the type of source, which is often insoluble or precipitates upon contact with the soil. Therefore, developing readily available and soluble Si sources with greater stability in the soil is of paramount importance, especially under water stress conditions, are crucial for agricultural production.

In recent years, studies related to nanotechnology have gained ground in agriculture, indicating that silicon-based nanofertilizers contribute positively to plant growth and production under a variety of biotic and abiotic stress conditions, as reported in grass, maize, and eggplant plants (Alves et al., 2023; Melo et al., 2023; Nadeem et al., 2025). Nanoparticles possess important properties, such as nanometric size, high surface area, and unique shapes, making them more efficient than conventional sources (Adrees et al., 2022). These characteristics help reduce nutrient losses through leaching, enhance photosynthetic responses

due to improved photoassimilation capacity, and optimize nutritional management by enabling more effective nutrient application (Verma et al., 2022; Ali et al., 2024).

Our investigative study tested the hypothesis that nSi application via fertigation modulates physiological processes and sugarcane growth under drought. To this end, two cultivars were evaluated under two irrigation levels, with and without nSi application. Growth performance, relative stress tolerance, and physiological parameters were assessed to confirm the potential of nSi as a management strategy in water-limited environments.

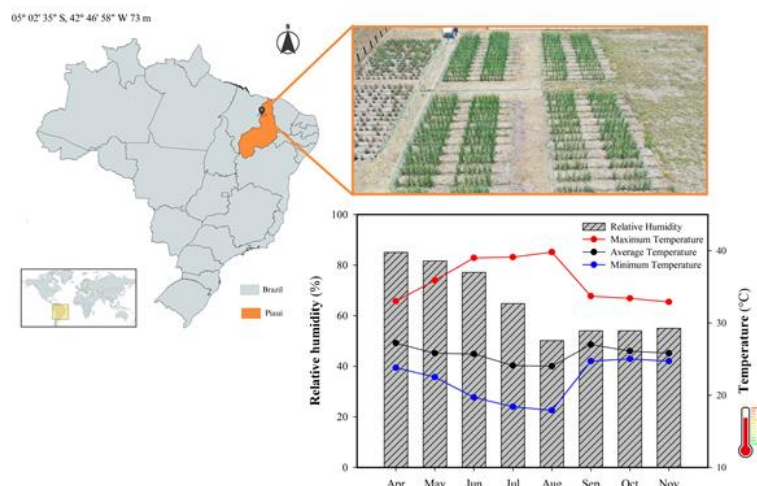
## MATERIAL AND METHODS

The experiment was carried out at the experimental station of the Sugarcane Breeding Program at UFPI, in Teresina-PI (05°05'S, 42°48'W), between April and November 2024. The local climate is tropical with a dry winter season (Aw). Temperature and relative humidity were monitored daily throughout the crop cycle (Figure 1).

Before planting, a physicochemical soil analysis was performed to assess fertility and pH, and fertilization was applied according to recommendations for Cerrado soils (Sousa & Lobato, 2004). A randomized block design was used, with a split-split-plot arrangement ( $2 \times 2 \times 2$ ), and four replications. The factors evaluated were: water regimes (80% and 40% of field capacity – FC), with (+nSi) or without silicon nanoparticles (-nSi) applying Si at 2.0 mM via fertigation every 15 days (corresponding to a dose of 8 kg ha<sup>-1</sup> of silicon), and two sugarcane cultivars (RB867515 and RB027040).

Drip irrigation was used with emitters spaced every 0.20 m. Initially, plants received full irrigation (80% FC) and drought treatment (40% FC) was imposed 42 days after transplanting. Irrigation was calculated using the Penman-Monteith method, based on data from a nearby weather station.

After 140 days of drought imposition, two representative plants were collected from each sub-subplot. Stems and leaves were weighed for fresh mass, and then oven-dried at  $65 \pm 5$  °C until constant weight for dry mass determination. Means were calculated for each treatment. Parameters such as stomatal conductance, net photosynthesis, transpiration, and internal CO<sub>2</sub> concentration were measured using an IRGA (Walz-GFS3000) on flag leaves under sunny conditions. Rubisco efficiency ( $A/C_i$ ) and water use efficiency (intrinsic and instantaneous) were also calculated.



**Figure 1.** Location of the experimental area and variation of average, maximum, and minimum temperature, as well as relative air humidity during the sugarcane trials from April to November 2024.

Relative water content (RWC) and leaf succulence (LS) were determined from leaf discs, following the methodology of Catsky (1960). RWC was calculated using fresh, turgid, and dry masses. LS was estimated by the difference between fresh and dry mass of the aerial part, divided by the leaf area (Mantovani, 1999). Data were subjected to ANOVA using the F test (5% significance level) and means were compared using Tukey's test ( $P \leq 0.05$ ), with R software version 4.3.1.

## RESULTS AND DISCUSSION

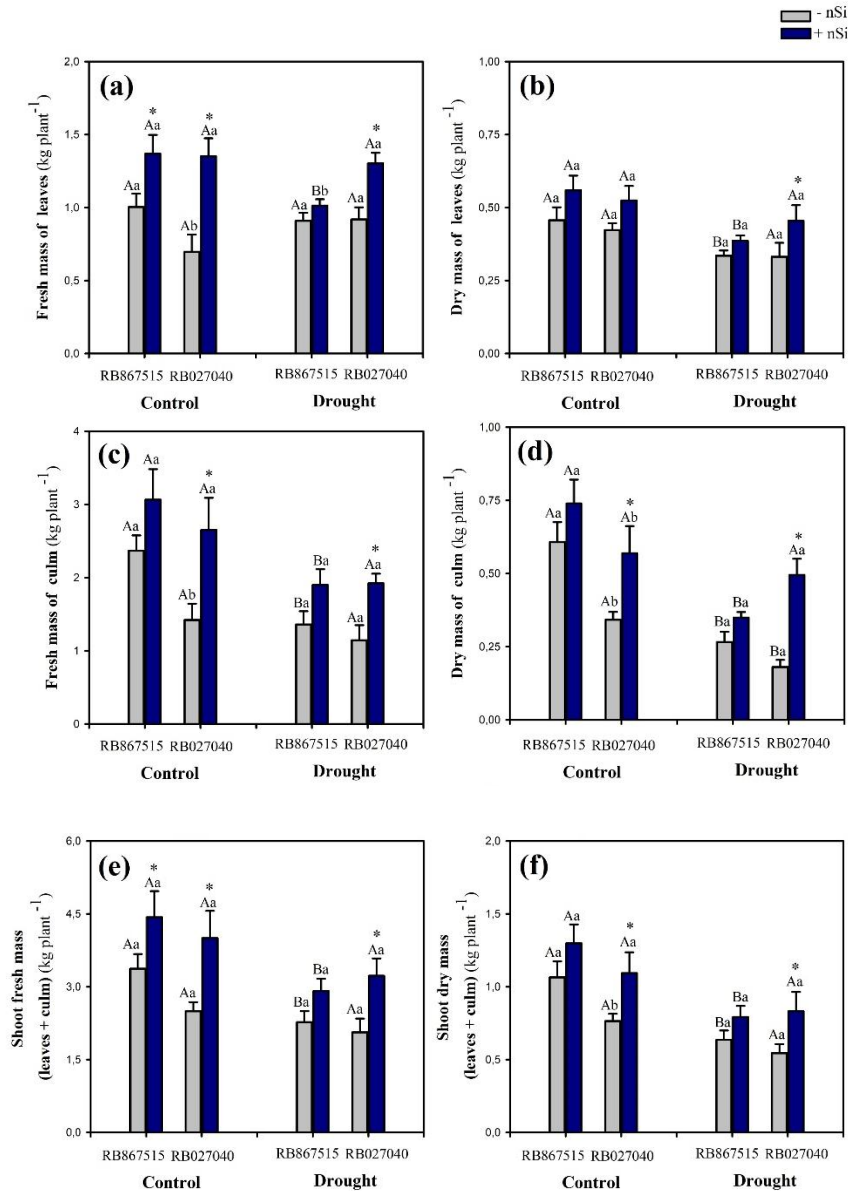
Under well-watered conditions, nSi supplementation increased the fresh and dry mass of the RB027040 cultivar compared to the control, except for leaf dry mass (Figure 2). In RB867515, the positive effects were restricted to leaf and shoot fresh mass. Drought significantly decreased the biomass of most organs, regardless of nSi, except for leaf fresh mass that remained relatively stable. In RB027040, significant decreased occurred only in stem dry mass without Si, and nSi application consistently mitigated the impacts of water restriction, increasing biomass production and the relative stress tolerance rate.

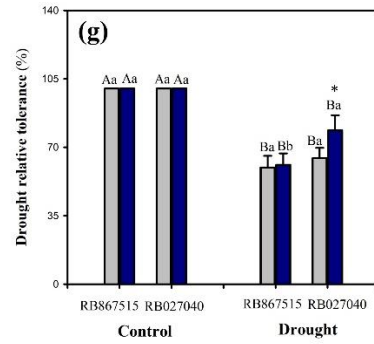
Under well-watered conditions, gas exchange parameters were not significantly affected by nSi (Figure 3). Under water deficit, supplementation increased net photosynthesis in both cultivars and stomatal conductance in RB027040, being associated with increases in Rubisco carboxylation efficiency and instantaneous water use efficiency (Figure 4).

Changes in relative water content and leaf succulence were minimal, suggesting that the benefits of nSi are more related to the optimization of gas exchange than to adjustments in water status (Figure 5).

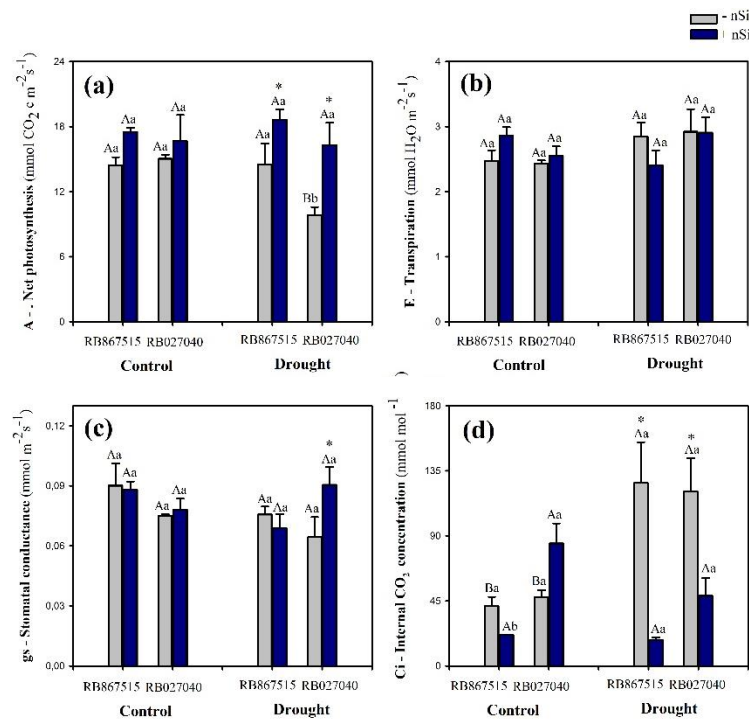
The RB027040 cultivar proved to be more responsive to Si, possibly due to its higher susceptibility to stress, whereas the rusticity of RB867515 reduced the impact of the treatment, as reported for other species in which sensitive genotypes respond more strongly to tolerance inducers (Silva et al., 2023; Santos et al., 2023).

The beneficial effects of nSi may be associated with the activation of pathways related to photosynthetic metabolism, antioxidant defense, and the synthesis of phytohormones such as ABA, JA, and GA, which act synergistically in regulating metabolism and growth under stress (Deng et al., 2021). Studies in maize have already shown that nSi increases the activity of key photosynthetic enzymes such as Rubisco, PEPcase, Malase, NADP-MD, and PPDK, optimizing CO<sub>2</sub> fixation and promoting greater biomass accumulation (Bhardwaj & Kapoor, 2021; Sirisuntornlak et al., 2021; Hao et al., 2023).

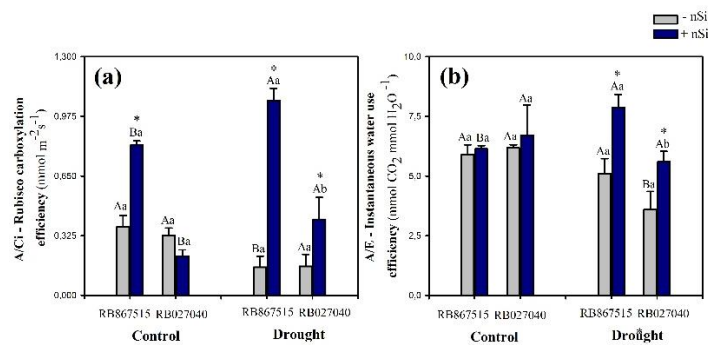


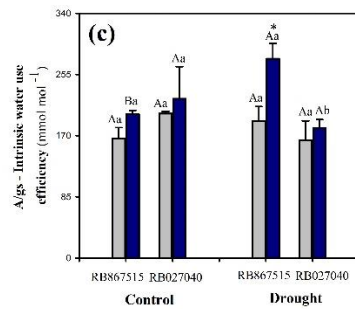


**Figure 2.** Fresh and dry mass of the leaf (a, b), stalk (c, d), total (e, f), and relative stress tolerance (g) of two sugarcane cultivars grown under water regimes of 80% FC and 40% FC, in the absence and presence of silicon nanoparticles (2 mM Si). Different uppercase letters denote significant differences as a function of irrigation level (control × water deficit) within the same cultivar and Si level; lowercase letters represent differences between cultivars (RB867515 × RB027040) at the same Si level and irrigation regime; and the presence of asterisks (\*) indicates significant differences due to Si nutrition (-nSi × +nSi) at the same water regime and cultivar, according to Tukey’s test at a 5% probability level ( $p \leq 0.05$ ).

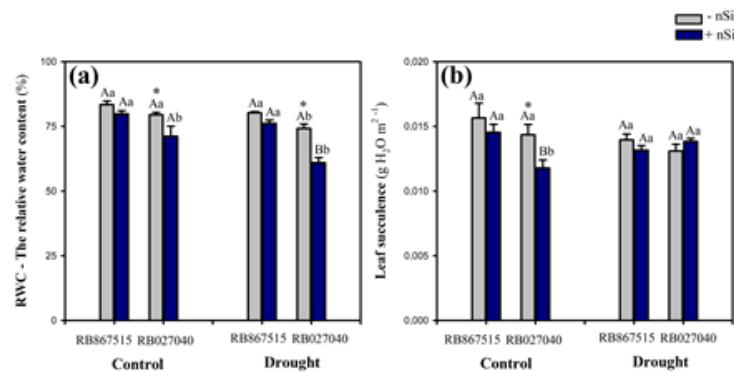


**Figure 3.** Net photosynthesis (a), transpiration (b), stomatal conductance (c), and internal CO<sub>2</sub> concentration (d) of two sugarcane cultivars grown under water regimes of 80% FC and 40% FC, in the absence and presence of silicon nanoparticles (2 mM Si). Statistical information as presented in Figure 2.





**Figure 4.** Rubisco carboxylation efficiency (a), instantaneous water use efficiency (b), and intrinsic water use efficiency (c) of two sugarcane cultivars grown under water regimes of 80% FC and 40% FC, in the absence and presence of silicon nanoparticles (2 mM Si). Statistical information as presented in Figure 2.



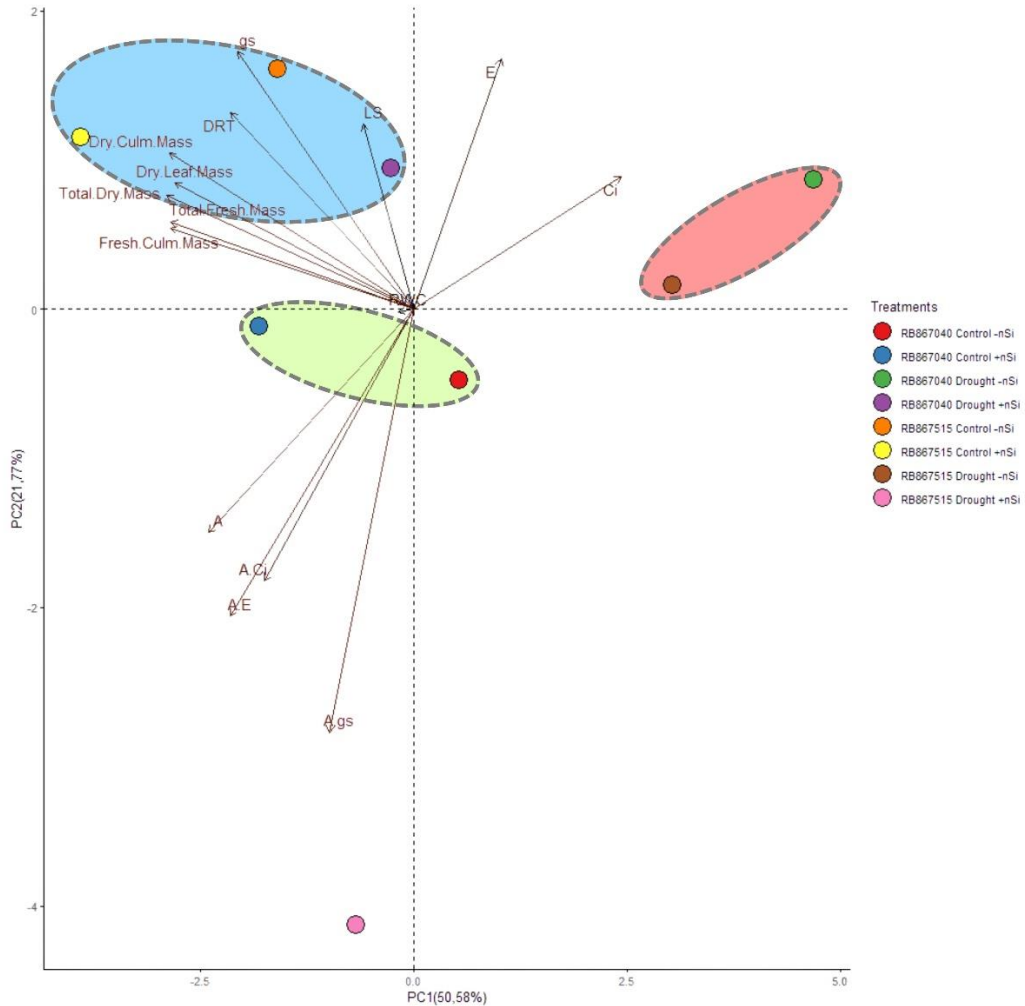
**Figure 5.** Relative water content (a) and leaf succulence (b) of two sugarcane cultivars grown under water regimes of 80% FC and 40% FC, in the absence and presence of silicon nanoparticles (2 mM Si). Statistical information as presented in Figure 2.

To investigate the influence of silicon supplementation on the evaluated parameters and the interrelation among components under different water levels, a Principal Component Analysis (PCA) was performed (Figure 6). The data indicated that the evaluated parameters were sufficient to identify correlations and the multiple effects of the interaction between nSi supplementation, cultivar, and water regime. The PCA explained 72.35% of the total variability, with 50.58% in Principal Component 1 (PC1) and 21.77% in Principal Component 2 (PC2). The distribution of treatments revealed a beneficial effect of nSi supplementation, especially in the RB867040 cultivar under water stress. In this case, the nSi treatment was positioned closer to variables related to better water status (RWC, LS, DRT), indicating greater drought tolerance.

In the RB867515 cultivar, silicon also showed a positive effect under control conditions, as indicated by the proximity of the RB867515 control + nSi treatment to vectors associated with biomass. However, the RB867515 stress + nSi treatment showed lower performance, suggesting that the benefits of silicon for this cultivar are more evident under non-limiting water conditions.

The vectors indicated a positive correlation between fresh and dry biomass variables and attributes related to water status (RWC, LS, DRT), while photosynthetic efficiencies (A/gs, A/E, A/Ci) showed a negative correlation. These results indicate that silicon supplementation contributed to maintaining plant water status and growth, with more pronounced effects in the RB867040 cultivar under stress and in RB867515 under control conditions, highlighting the interaction between cultivar, environment, and nanosilica supplementation.

Thus, the observed benefits of nSi reflect a predominantly physiological and biochemical action, and further studies are needed to elucidate the antioxidant and molecular mechanisms involved in mitigating water stress through nSi under field conditions.



**Figure 6.** Principal Component Analysis - Total dry mass, Total fresh mass, Dry culm mass, Fresh culm mass, Dry leaf mass, Fresh leaf mass, Drought relative tolerance (DRT), Leaf succulence (LS), Relative water content (RWC), Internal CO<sub>2</sub> concentration (C<sub>i</sub>), Intrinsic water use efficiency (A/g<sub>s</sub>), Rubisco carboxylation efficiency (A/C<sub>i</sub>), Instantaneous water use efficiency (A/E), Net photosynthesis (A), Transpiration (E), and stomatal conductance (g<sub>s</sub>).

## CONCLUSION

The application of nanosilica (nSi) enhances growth, gas exchange, and drought tolerance in the RB027040 sugarcane cultivar under field conditions. These findings highlight the potential of nSi as an effective nutritional management strategy under water-limited conditions, offering important benefits for the sugar-energy sector in semiarid regions.

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