Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Silicon attenuates the physiological and biochemical damage induced by waterlogging in cowpea (Vigna unguiculata L. Walp)

Alberto Soares de Melo
alberto.melo@servidor.uepb.edu.br
State University of Paraíba - UEPB
Priscylla Marques de Oliveira Viana
State University of Paraíba - UEPB
Guilherme Félix Dias
State University of Paraíba - UEPB
Semako Ibrahim Bonou
State University of Paraíba - UEPB
Leticia Diniz Ribeiro
State University of Paraíba - UEPB
Yngrid Mikhaelly Lourenço de Araujo
State University of Paraíba - UEPB
Eulália Margarethe da Costa Melo
State University of Paraíba - UEPB
Igor Enéas Cavalcante
Federal University of Paraíba
Hermes Alves de Almeida
State University of Paraíba - UEPB
Liziane Maria de Lima
Brazilian Agricultural Research Corporation
Agda Malany Forte de Oliveira
State University of Paraíba - UEPB

Research Article

Keywords: Vigna unguiculata (L.) Walp., antioxidant activity, hypoxia, crop resilience, silicic acid

Posted Date: April 10th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9226074/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Silicon attenuates the physiological and biochemical damage induced by waterlogging in cowpea (*Vigna unguiculata* L. Walp)

Alberto Soares de Melo[1]*[0000-0002-4586-5388] Priscylla Marques de Oliveira Viana[1][0000-0002-7387-6404] Guilherme Félix Dias[1][0000-0002-5304-6518] Semako Ibrahim Bonou[1][0000-0001-9547-6236] Leticia Diniz Ribeiro[2][0009-0007-6852-3974] Yngrid Mikhaelly Lourenço de Araujo[2][0009-0004-5539-8359] Eulália Margarethe da Costa Melo[1][0009-0004-5539-8359] Igor Enéas Cavalcante[3][0009-0007-7075-1845] Hermes Alves de Almeida[1][0000-0001-5339-5120] Liziane Maria de Lima[4][0000-0003-2314-6881] Agda Malany Forte de Oliveira[1][0000-0002-1828-1088]

[1] Postgraduate Program in Agricultural Sciences - PPGCA, State University of Paraíba - UEPB, Campina Grande, Paraíba, Brazil.
[2]Department of Biology, State University of Paraíba - UEPB, Campina Grande, Paraíba, Brazil.
[3]Department of Plant Science and Environmental Sciences, Federal University of Paraíba - UFPB, Areia, Paraíba, Brazil.
[4]Brazilian Agricultural Research Corporation, EMBRAPA Cotton, Campina Grande, Paraíba, Brazil.
*Corresponding author: Alberto Soares de Melo, e-mail: alberto.melo@servidor.uepb.edu.br, Fone: +55 (83) 98871-3902

Abstract. Waterlogging is an environmental constraint that limits cowpea (Vigna unguiculata L. Walp.) productivity by inducing root hypoxia and oxidative stress. In this context, the objective was to evaluate the potential of exogenous silicon (Si) application to mitigate the negative impacts of waterlogging on the physiological, biochemical, and biometric performance of cowpea. The experiment was conducted in a completely randomized design, in a 2x3 factorial scheme, combining two water conditions (field capacity and waterlogging) and three silicon doses (0, 100, and 200 mg L^{-1}). Waterlogging significantly restricted gas exchange, leaf area, and biomass accumulation. However, the application of 100 mg L^{-1} Si proved effective in maintaining leaf area and optimizing dry matter accumulation under waterlogging conditions. Silicon supplementation potentiated the activity of antioxidant enzymes (SOD and CAT) and increased proline levels, reducing electrolyte leakage and preserving membrane integrity. Silicic acid acts as a critical modulator of abiotic stress, conferring greater resilience to cowpea by attenuating damage caused by root hypoxia. Silicon supplementation is a viable and efficient biotechnological strategy to enhance cowpea resilience in areas prone to seasonal waterlogging, thereby preserving physiological integrity and optimizing water-use efficiency under stress.
Keywords: *Vigna unguiculata* (L.) Walp., antioxidant activity, hypoxia, crop resilience, silicic acid.

1 First Section

1.1 Introduction

Extreme climate changes, such as prolonged droughts or intense rainfall, make plants vulnerable to these effects, harming crop growth, reducing productivity, and causing significant losses for producers (Piedra-Bonilla et al. 2025). According to the Intergovernmental Panel on Climate Change (IPCC) (2023), a United Nations scientific body responsible for systematically analyzing the impacts and future scenarios of global warming, the intensity of extreme rainfall increases by approximately 6.7% for every degree Celsius increase in the average global temperature, indicating that intense rainfall outside historical patterns tends to become increasingly frequent in the coming decades. This scenario makes agricultural systems vulnerable, as excess soil moisture leads to waterlogging that harms crop growth, drastically reduces productivity, and causes severe economic losses globally. Therefore, it is necessary to understand the effects of soil water saturation on major food crops to develop management and adaptation strategies in the face of worsening future climate conditions.

In this context, understanding the effects of hypoxia on crops that are strategic for food security is urgent. Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the main sources of protein and income for populations in tropical and semi-arid regions, and is fundamental for food security (Araújo et al. 2018; Silva et al. 2024; Chakravaram et al. 2025; Dias et al. 2025). According to data from FAOSTAT (2025), the statistical database of the Food and Agriculture Organization of the United Nations (FAO), the cultivated area increased from approximately 12,000 to 15,700 hectares, while production increased from approximately 6,000 to almost 10,000 tons, reinforcing the relevance of studies focused on the cultivation and management of this crop.

However, the productivity of this crop is frequently limited by abiotic stresses, among which soil waterlogging has gained prominence due to the intensification of extreme weather events. Excess water in the soil profile induces root hypoxia, which triggers a series of negative responses in the plant, such as impaired root respiration and nutrient absorption, leading to physiological and biochemical changes, such as reduced stomatal conductance, degradation of photosynthetic pigments, and a sharp drop in the CO_2 assimilation rate, which limit leaf expansion and total biomass and, consequently, production (Olorunwa et al. 2023; Senthamil et al. 2024).

Given this scenario, the search for strategies that increase plant resilience to water stress is essential. The use of beneficial elements, such as silicon (Si), has shown promising results in mitigating abiotic stresses in various crops. When applied as silicic acid, silicon becomes readily available to the plant, strengthening mechanical barriers and, more importantly, modulating physiological and biochemical processes. Under waterlogging conditions, silicic acid can help maintain leaf water potential, protect the photosynthetic apparatus against oxidative damage, promote osmotic adjustment by accumulating compatible solutes, such as proline, and strengthen cell membrane integrity by minimizing electrolyte leakage (Biju et al. 2023; Queiroz et al. 2025). The specific mechanisms in legumes, such as cowpea, still require further clarification. In addition

to playing a relevant role in growth and development, it contributes to the accumulation of plant biomass (Silva et al. 2024; Souza et al. 2025; Queiroz et al. 2025).

Although the benefits of silicon are well documented in mitigating abiotic stresses in some species, there is still a significant gap in knowledge about its effectiveness in mitigating waterlogging stress in legumes, particularly cowpea, in vegetative stages. Since most studies focus on tolerance to water deficit or salinity, little has been explored regarding the dynamics of silicon in protecting the photosynthetic apparatus and in enzymatic regulation under conditions of severe water saturation. In this context, it was hypothesized that the exogenous application of silicic acid to the soil attenuates the physiological damage caused by waterlogging in cowpea, promoting osmotic adjustment, preserving the integrity of the photosynthetic apparatus by strengthening the antioxidant system, and maintaining plant growth even under waterlogging. Thus, the objective was to evaluate the influence of different doses of silicic acid on the physiological, biochemical, and biometric performance of cowpea plants subjected to soil waterlogging.

1.2 Materials and Methods

Characterization of the experimental area

The experiment was conducted at the State University of Paraíba, located in the city of Campina Grande, Paraíba, Brazil, georeferenced at (07° 13' 50" S, 35° 52' 52" W) and at an altitude of 551 m above sea level. The experimental work was conducted in the university's Botanical Garden, and the biochemical analyses were performed in the Laboratory of Ecophysiology of Cultivated Plants (ECOLAB) (Figure 1A).

The climate, according to the Köppen classification, is of type 'AS', tropical with a dry season, with average annual temperatures around 22 °C, with a minimum of 19 °C and a maximum of 26 °C, average annual precipitation above 700 mm, with the highest concentration of rainfall between April and August; average annual reference evapotranspiration of 500 mm and average annual relative humidity of 80% (Alvares et al. 2013). The experiment was conducted from May to July 2025, and during the experimental period, the minimum and average temperatures and relative humidities were 23.05°C, 22.19°C, and 22.61°C, and 92.63%, 88.80%, and 90.78%, respectively, and the accumulated precipitation was 307.7 mm. The INMET meteorological station recorded these data.

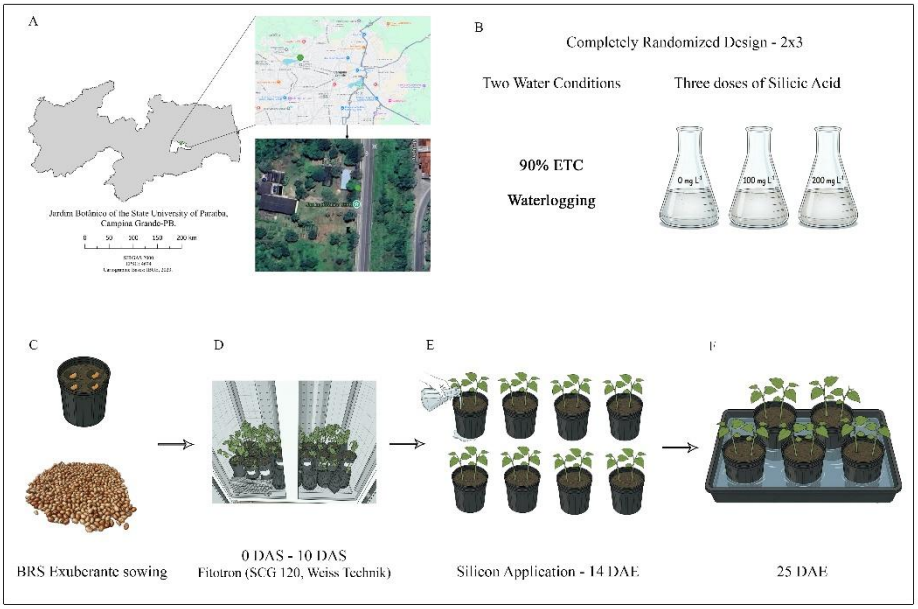


Figure 1. Diagram of the experiment and treatment application. Characterization of the experimental area (A); experimental design and treatments (B); seeds of the BRS Exuberant cultivar sowing (C); aclimatacion of pots in a Fitotron growth chamber (D); application of Si doses at 14 days after emergence (DAE) (E); the pots were subjected to water stress via waterlogging at 25 days after emergence (DAE) (F). This figure was created using Photoshop.

Experimental design and treatments

The experiment was conducted in a completely randomized design, in a 2 x 3 factorial scheme, with three concentrations of silicic acid (0, 100, and 200 mg L^{-1}) and two water conditions (90% ETc and waterlogging), with five repetitions and two plants per pot, totaling 30 experimental units (Figure 1B).

Conducting the Experiment and Waterlogging Mitigation Strategies

The 'BRS Exuberante' cowpea seeds used in this study were kindly provided by the germplasm collection of Embrapa Meio-Norte, located in Teresina, Piauí, Brazil (Coordinates: 5°05' S, 42°49' W, Altitude: 72 m), and any physically damaged or malformed grains were discarded. The experiment was conducted in polyethylene pots and in an open field. The seeds of the 'BRS Exuberante' cultivar were sown at approximately 1 cm depth in polyethylene pots (capacity of 2.6 L) filled with 3.0 kg of previously sifted soil (Figure 1C). The soil was classified as an Argisol, of the clay loam type, whose physical-chemical characteristics are: 86.04% sand, 12.05% silt, 1.91% clay, apparent density of 1.62 g cm^{-3}, particle density of 2.69 g cm^{-3}, porosity of 39.77%, calcium of 1.90 cmolc dm^{-3}, magnesium of 1.47 cmolc dm^{-3}, sodium of 0.13 cmolc dm^{-3}, potassium of 0.28 cmolc dm^{-3}, sulfur of 3.76 cmolc dm^{-3}, hydrogen of 1.07 cmolc

dm^{-3}, aluminum of 0.00 cmolc dm^{-3}, organic matter of 0.91% and pH of 6.47, determined according to Teixeira et al. (2017). Thinning was carried out 8 days after sowing, leaving 2 plants per pot.

During the first 10 days, the pots remained in a Fitotron-type growth chamber (SCG 120, Weiss Technik) at 32 °C and 28 °C, with 50% humidity, ensuring germination and establishment up to the V2 phenological stage (Figure 1D). Subsequently, the pots were transferred to the outdoor area and irrigated daily without water stress, using the pot-weighing method, with water replacement at 90% of the crop evapotranspiration (ETc) (Silva et al. 2020).

The silicon source used was Sifol® powder, containing approximately 42% silicon as silicic acid (H_4SiO_4). The silicon acid solutions were prepared in distilled water at concentrations of 100 and 200 mg L^{-1}. The application was carried out 14 days after emergence, by fertigation (Figure 1E).

25 days after emergence, to impose water stress by waterlogging, the pots were kept in plastic trays (model, brand) with the following dimensions (35.05 cm × 57.00 cm × 23.00 cm = 45.95 cm^3) and maintaining the water level at approximately 2.5 cm above the soil surface; the water in the trays was replenished to maintain the uniformity of the amount of water over 5 days (Senthamil et al. 2025a). After the scheduled waterlogging period, the pots were removed from the trays, and the plants were evaluated (Figure 1F).

Variables analyzed

The vegetative development of cowpea plants was monitored at 32 and 35 days after emergence, corresponding to the V6 phenological stage. Growth, water status determination, membrane damage indicators, osmotic adjustment, gas exchange, and photosynthetic pigments, as well as the activity of antioxidant mechanisms, were evaluated.

Growth

Total leaf area (TLA)

Total leaf area (TLA) was determined by counting the leaflets on an A4 sheet of paper and photographing them with a smartphone camera, using a 2 mm scale. The images were properly labeled, and the measurements, in millimeters, were subsequently obtained in ImageJ (Martin et al. 2020; Suarez et al. 2022; Barroso et al. 2026).

Specific leaf area (SLA)

Specific leaf area (SLA) is a measure that relates the leaf surface area to its dry mass, reflecting morphological and anatomical aspects of the leaf structure. To determine the SLA, three leaf discs were removed by means of cuts made with a copper punch. After removal, the discs were stored in paper bags and dried in an oven with controlled air circulation at 65 °C for 48 hours to determine their dry mass. The SLA was calculated according to the method described by Silva (2021) with adaptations, using equation 1.

$$SLA = \frac{DA}{DDM} \quad (1)$$

Where:

SLA – Specific leaf area, mm²/µg;
DA – Disc area (obtained from the average area of the three discs), mm²;
DDM – Disc dry mass (obtained from the average mass of the three discs), µg.

Total dry mass (TDM)

Total dry mass (TDM) was obtained by collecting leaves, stems, and branches, which were separately packaged in paper bags, labeled, and dried in a forced-air oven at 65 °C for 72 hours. After this period, the plant material was weighed on an analytical balance with a precision of 0.0001 g (Santos et al. 2022), according to equation 2.

$$TDM = \left[\frac{(FFtr \times RDM)}{SDM}\right] \quad (2)$$

Where:
TDM: Total dry mass; g
FMtr- Fresh mass, g;
RDM- Remaining dry mass, g;
SDM- Remaining shoot dry mass, g;

Root volume (RV) and number of nodules (NN)

Root volume (RV, cm³) was determined by measuring the displacement of the water column in a graduated cylinder with a known volume of water (500 mL), as described by Böhm (1979). The number of nodules (NN) was determined by manual counting of the roots.

Water Status and Membrane Damage Indicator

Relative water content (RWC%)

The relative water content in the leaf (RWC%) was determined according to the methodology described by Brito et al. (2011). Five discs of fresh leaves were obtained by cutting them with a copper punch (5 mm diameter). These discs were weighed to determine their fresh mass (FMD) and then placed in hermetically sealed aluminum capsules containing 10 mL of distilled water at room temperature. After 24 hours, the same material was surface-dried and weighed to obtain the turgid mass of the discs (TMD). Subsequently, they were packaged in paper bags, properly identified, and placed in a forced-air oven at 80 °C for 48 hours to measure the dry mass of the discs (DMD). Using this data, the RWC was calculated using equation 3.

$$\text{RWC (\%)} = \left[\left(\frac{FDM-DMD}{TDM-DMD}\right)\right] * 100 \quad (3)$$

Where:
FMD = fresh mass of discs;
TMD = turgid mass of the discs;
DMD = dry mass of discs.

Electrolyte leakage (EL%)

Electrolyte leakage (EL%), which indirectly estimates cell membrane damage, was measured according to Scotti-Campos (2013) with adaptations. For this, a copper punch was used to obtain, for each experimental unit, five leaf discs of each type, which were placed in test tubes containing 10 mL of distilled water. The test tubes were then closed and left at rest for 24 hours. After this period, the electrical conductivity of the solution in the test tube (Xi) was measured using a portable conductivity meter (Ow1387, Vomeko). The tubes were again closed and placed in a water bath at 100 °C for 60 minutes. After cooling to room temperature, the final conductivity (Xf) of the solution was measured. The percentage of electrolyte leakage was calculated based on equation 4.

$$EL\ (\%) = \frac{\mathrm{Xi}}{\mathrm{Xf}} X100 \quad (4)$$

Where:
EL – Internal Electrolyte Leakage, %,
Xi - initial electrical conductivity of the medium, dS m^{-1},
Xf - final electrical conductivity of the medium, dS m^{-1}.

Gas Exchange

Gas exchange was quantified during the morning period, from 7:00 AM to 11:00 AM, using an infrared gas analyzer (LI-6800 Portable Photosynthesis System, Li-Cor, Inc., United States) equipped with a 6 cm² leaf-area chamber. Leaf temperature was maintained at 25 ± 2 °C, and the air flow rate was 500 μmol s^{-1}. Throughout the evaluations, a constant actin photon flux density of 1200 μmol m^{-2} s^{-1} was maintained. The relative humidity of the chamber was maintained at 60% and the CO_2 concentration at 400 μmol mol^{-1}. The air temperature in the chamber was monitored using a thermoelectric sensor at the base and maintained at 27 °C. The assessments were carried out on the third fully expanded leaf, from the apex of the main branch, to obtain the photosynthetic rate (A_N) (μmol CO_2 m^{-2} s^{-1}), the transpiration rate (E) (mmol H_2O m^{-2} s^{-1}), the stomatal conductance (gs) (mmol m^{-2} s^{-1}) and the intercellular CO_2 concentration (Ci) (μmol CO_2 m^{-2} s^{-1}). Based on this data, instantaneous water-use efficiency (WUE) – the ratio of photosynthesis to transpiration (A_N/E)- was quantified.

Photosynthetic pigments

Chlorophyll *a*, Chlorophyll *b*, and Total Chlorophyll

The chlorophyll content index was determined using an electronic chlorophyll content meter (ClorofiLOG®, CFL 2030; Falker, Porto Alegre, Brazil). Evaluations were performed on the third fully expanded leaf from the apex of the main branch in all plants of the experimental unit. In each plant, young, fully expanded leaves were selected from the upper middle third, showing no signs of pest or disease attack, for measurement at three equidistant points on the adaxial surface of each leaf. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll values were obtained.

Antioxidant Mechanism Activity

Antioxidant activity was evaluated by measuring the enzymatic activities of superoxide dismutase (SOD, UA g FM^{-1}), catalase (CAT, μmol H_2O_2 min^{-1} g FM^{-1}), and ascorbate

peroxidase (APX, µmol ascorbate min^{-1} g FM^{-1}). 200 mg of fresh material were ground with 2 mL of 100 mM phosphate buffer (pH 7.5), combined with ascorbic acid (10 mM), EDTA (1 mM), and polyvinylpyrrolidone (1%). The extracts were centrifuged at 20,000 G for 20 minutes at 4 °C, and the supernatant was aspirated; the aliquot was placed in 2.5 mL plastic tubes (Eppendorf) and stored at -80 °C for enzymatic analysis.

Superoxide Dismutase Activity (SOD)

The determination of superoxide dismutase (SOD) activity was performed using the method proposed by) (Beauchamp and Fridovich, 1971), in which 100 µL of the enzymatic extract were added to the reaction mixture composed of 0.3 mL of 130 µM methionine, 0.1 mL of 2250 µM p-nitrotetrazolium blue (NBT), 0.1 mL of 3 µM EDTA, 0.2 mL of riboflavin, 0.75 mL of distilled water, and 1.5 mL of 50 mM sodium phosphate buffer (pH 7.8). The absorbance was then measured at 560 nm and subtracted from the reading of the reaction mixture without the enzymatic extract (blank). Under these conditions, one SOD unit was defined as the amount of enzyme required to inhibit the photoreduction of NBT by 50%, with the activity expressed in UA g FM^{-1}.

Catalase Activity (CAT)

Catalase (CAT) enzyme activity was quantified according to the method of Kar and Mishra (1976) by measuring the consumption of hydrogen peroxide (H_2O_2) by the enzymatic extract. The reaction was initiated by adding 150 µL of the enzymatic extract to a quartz cuvette containing 2.85 mL of the reaction medium, composed of potassium phosphate buffer (50 mM, pH 7) with 20 mM H_2O_2. After stirring, the solution was analyzed using a spectrophotometer at 240 nm, with absorbance measured every 10 seconds for 2 minutes. The Lambert-Beer equation ($A = \varepsilon.b.c$) was used for the calculation, where A represents the decrease in absorbance (averaged in triplicate), ε is the molar extinction coefficient (39.4 mol^{-1} cm^{-1}), b is the optical path length (1 cm), and c is the enzyme concentration in mol L^{-1}. CAT activity was expressed in µM of H_2O_2 min^{-1} g FM^{-1}.

Ascorbate Peroxidase Activity (APX)

Ascorbate peroxidase (APX) was determined by monitoring the decrease in absorbance at 290 nm as ascorbate was consumed in a quartz cuvette (Nakano and Asada, 1981). For the reaction, 300 µL of enzymatic extract was mixed with the reaction medium (2.5 mL), composed of potassium phosphate buffer (50 mM, pH 6.0) and ascorbic acid (0.8 mM). The reaction was initiated by adding 200 µL of hydrogen peroxide (2 mM) and monitored by the reduction in absorbance over 1 minute, with readings every 10 seconds. APX activity calculations followed the Lambert-Beer equation, using the molar extinction coefficient of ascorbate (2.8 mM^{-1} cm^{-1}). The final activity was expressed in µM of ascorbate min^{-1} g of FM^{-1}.

Osmotic adjustment indicators

Proline

The proline (PRL) content was quantified using the colorimetric method proposed by Bates et al. (1973) and modified by Bezerra Neto and Barreto (2011). 250 mg of fresh

leaf tissue was macerated in 5 mL of 3% sulfosalicylic acid and centrifuged at 2000 rpm for 10 minutes. The supernatant was removed and stored in 2.5 mL centrifuge tubes for subsequent determination of proline content. Screw-top test tubes containing the mixture: extract + acid ninhydrin + glacial acetic acid, in a 1:1:1 ratio (total volume of 3 mL), were placed in a water bath regulated at 100 °C for one hour. After this period, the tubes were cooled in an ice bath to stabilize the reaction, and subsequently, 2 mL of toluene reagent was added. The samples were homogenized in a Vortex mixer for 20 seconds, followed by a 10-minute resting period, which allowed the solution to separate into two phases: the chromophore and the translucent phase. The supernatant was read on a spectrophotometer (Nova 2000 UV, Akso Brasil) at 520 nm, using pure toluene as the blank to zero the instrument. The concentration of free proline was quantified based on the L-proline standard curve and expressed in μmol PRL g of FM^{-1}.

Total soluble sugars

The extract for quantifying total soluble sugars (TSS) content was obtained from 200 mg of fresh leaf mass, macerated in 2 mL of 80% (v/v) ethanol. After maceration, 2 mL centrifuge tubes were placed in a water bath at 60 °C for 30 minutes and then transferred to a centrifuge at 2,000 g to obtain the supernatant. After removing the supernatant, an additional 2 mL of 80% ethanol was added for a second extraction, then the sample was heated in a water bath and transferred to the centrifuge. The supernatants from the two extractions were mixed in Falcon-type tubes and stored in two 2 mL centrifuge tubes, totaling 4 mL of extract per sample. The total soluble sugar (TSS) content was determined by the anthrone colorimetric method described by Yemm and Willis (1954). 50 μL aliquots of the sample were added to test tubes, completed with 1950 μL of distilled water and 2000 μL of anthrone, and shaken. After the addition, the tubes were shaken again to homogenize the reaction. Then, the tubes were placed in a water bath at 98 °C for 3 minutes, then in an ice bath, and subsequently read in a spectrophotometer at 620 nm. The TSS content was quantified using a glucose standard curve, and the results were expressed as mg TSS g FM^{-1}.

Statistical Analysis

The data obtained were subjected to the Shapiro-Wilk test (Shapiro and Wilk, 1965) for normality. Once the assumptions were met, the data were subjected to analysis of variance using the F-test ($p \leq 0.05$), followed by Tukey's test ($p \leq 0.05$) for silicon acid concentrations and Student's t-test ($p \leq 0.05$) for water conditions, in the statistical software SISVAR® v. 5.6 (Ferreira, 2019).

2 Results

Growth

The leaf expansion of cowpea BRS Exuberante was significantly influenced by the interaction between waterlogging and silicic acid (Si) supplementation. In plants maintained at field capacity, the absence of Si and a Si dose of 200 mg L^{-1} promoted total leaf area (TLA) that was 22.20% and 16.36% higher, respectively, compared to plants under waterlogging. The application of 100 mg L^{-1} of Si promoted stability in TLA,

with no statistically significant difference between water conditions at this dosage, suggesting a protective effect of the element in maintaining the leaf apparatus under stress (Figure 2A).

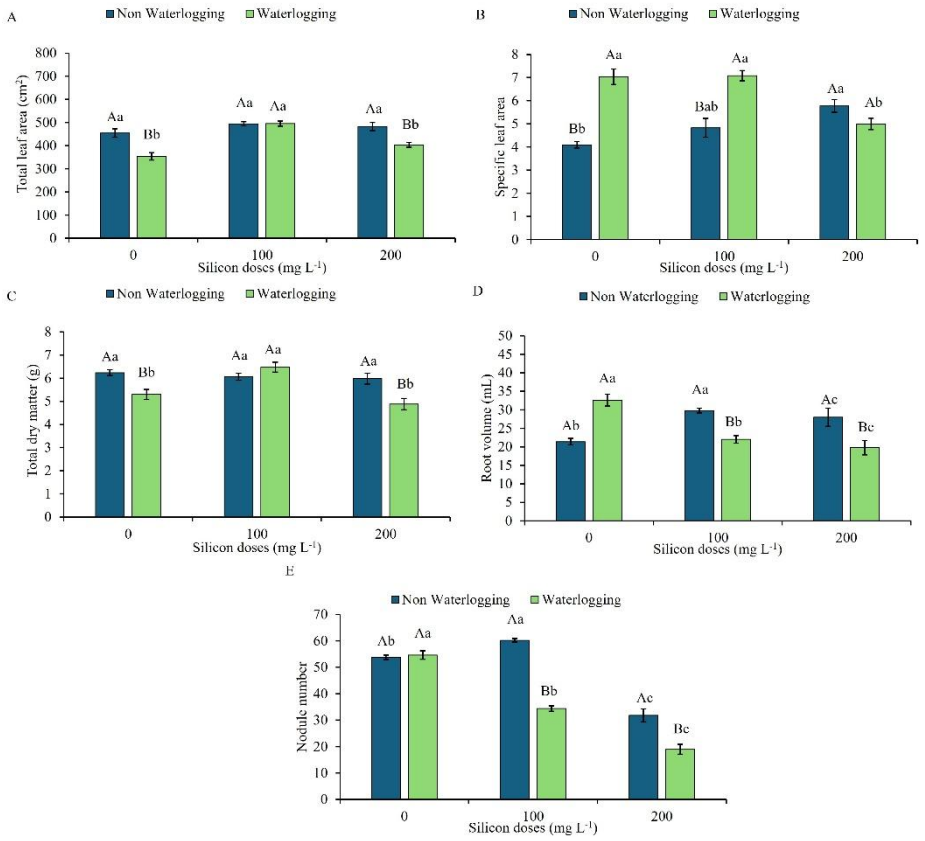


Figure 2. Total leaf area (TLA) (A), specific leaf area (SLA) (B), total dry mass (TDM) (C), root volume (RV) (D), and number of nodules (NN) (E) in 'BRS Exuberante' cowpea plants subjected to different doses of silicic acid and different soil water conditions. Uppercase letters differentiate different soil water conditions at each silicic acid dose (Student's t-test, $P \leq 0.05$), and lowercase letters differentiate silicic acid doses at each soil water condition (Tukey's test, $P \leq 0.05$).

Regarding specific leaf area (SLA), waterlogging resulted in increases of 41.74% and 31.85% in plants grown at 0 and 100 mg L^{-1} Si, respectively. This increase in SLA under stress indicates a change in leaf morphology, resulting in less-dense tissue. In contrast, at the highest silicon dosage (200 mg L^{-1}), a reversal of this behavior was observed, with non-waterlogging plants showing a 13.67% higher SLA (Figure 2B).

Biomass accumulation and root system architecture were also significantly influenced by the interaction between waterlogging and silicic acid supplementation. In plants maintained at field capacity, the absence of Si and a dose of 200 mg L^{-1} resulted in increases of 15.07% and 18.49% in total dry mass (TDM), respectively, compared

to the waterlogging treatment. However, a unique behavior was observed with the application of 100 mg L^{-1} of Si; at this dosage, waterlogging plants outperformed non-waterlogging plants by 6.42% in TDM accumulation (Figures 2C and 3), suggesting that this concentration may have optimized the energy metabolism of cowpea under hypoxia.

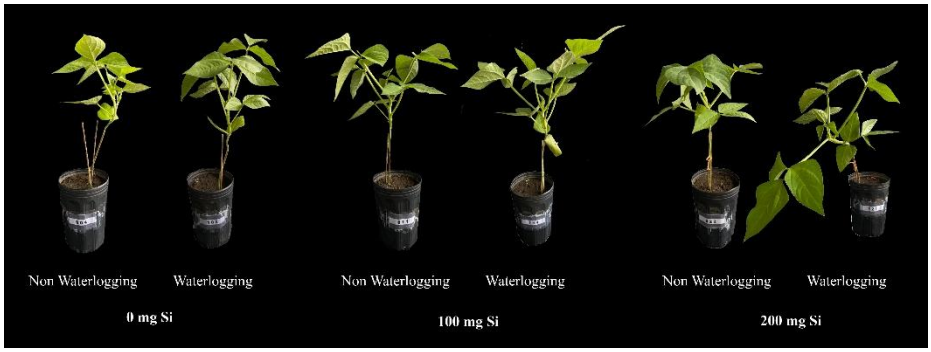


Figure 3. Growth in 'BRS Exuberante' cowpea plants subjected to different doses of silicic acid and different soil water conditions.

Root volume (RV) showed contrasting responses under waterlogging; plants that did not receive silicon showed the highest RV among all treatments, which may be associated with the induction of structures adapted to excess water. In contrast, in plants maintained at field capacity, silicon acted as a stimulant for root growth, promoting increases of 26.17% and 29.28% at doses of 100 and 200 mg L^{-1}, respectively (Figures 2D and 4).

Nodulation proved highly sensitive to both waterlogging stress and silicon supplementation. In plants maintained at field capacity, Si significantly favored biological fixation, with increases of 42.86% and 40.25% in the number of nodules at silicon doses of 100 and 200 mg L^{-1}, respectively. However, under waterlogging, the presence of silicon led to a marked reduction in nodulation compared with flooded plants without silicon. This result indicates that, under hypoxic conditions, the application of Si may have prioritized other metabolic survival pathways over symbiosis with rhizobia, thereby evidencing a complex redistribution of resources in the plant (Figures 2E and 4).

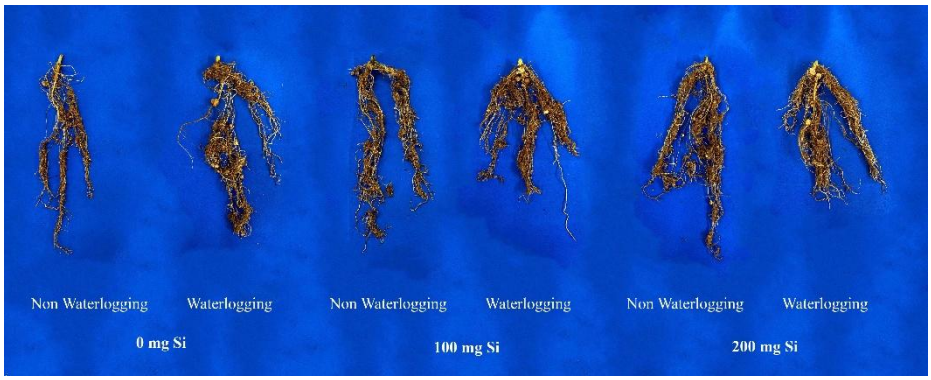


Figure 4. Root systems in 'BRS Exuberante' cowpea plants are subjected to different doses of silicic acid and different soil water conditions.

Water Status and Membrane Damage Indicator

The interaction between waterlogging and silicon supplementation significantly influenced the relative water content (RWC). In the absence of silicon, waterlogging plants showed an RWC of 13.39%, higher than that of control plants, suggesting a potential for accumulation of osmotically active solutes as a response to stress. However, silicon supplementation at 100 and 200 mg L^{-1} reduced the RWC of waterlogging plants by 22.41% and 12.85%, respectively, compared to plants at field capacity (Figure 5A).

Electrolyte leakage (EL) was predominantly influenced by waterlogging, regardless of the silicon dose. Plants subjected to waterlogging showed higher electrolyte loss across all silicon treatments, with increases ranging from 13.75% to 28.39% relative to the control (Figure 5B).

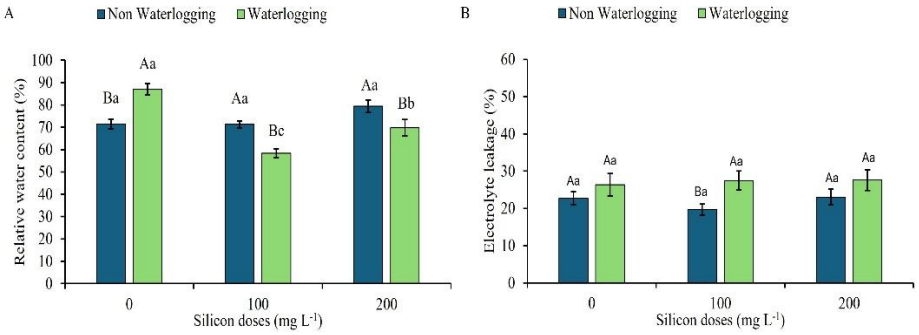


Figure 5. Relative water content (RWC) (A), electrolyte leakage (EL) (B) in 'BRS Exuberante' cowpea plants subjected to different doses of silicic acid and different soil water conditions. Uppercase letters differentiate different soil water conditions at each silicic acid dose (Student's t-test, $P \leq 0.05$), and lowercase letters differentiate silicic acid doses at each soil water condition (Tukey's test, $P \leq 0.05$).

Gas Exchange

Gas exchange was severely restricted by waterlogging, highlighting the impact of hypoxia on leaf metabolism. The transpiration rate (*E*) was consistently lower in waterlogging plants at all Si dosages. Under field capacity conditions, the application of 200 mg L^{-1} of Si increased the transpiration rate. In contrast, under waterlogging conditions, a progressive reduction in *E* was observed with increasing Si doses, reaching its lowest values at the 200 mg L^{-1} dose (Figure 6A).

The net photosynthetic rate (A_N) followed a similar trend, with significantly lower values in waterlogged plants than in the control. In plants under field-capacity conditions, silicon promoted photosynthesis, with higher values observed at doses of 100 and 200 mg L^{-1}. Although the supply of Si was insufficient to raise A_N rates to the control level during the waterlogging period, its presence was important for regulating other associated physiological parameters and for maintaining biomass accumulation (Figure 6B, 3).

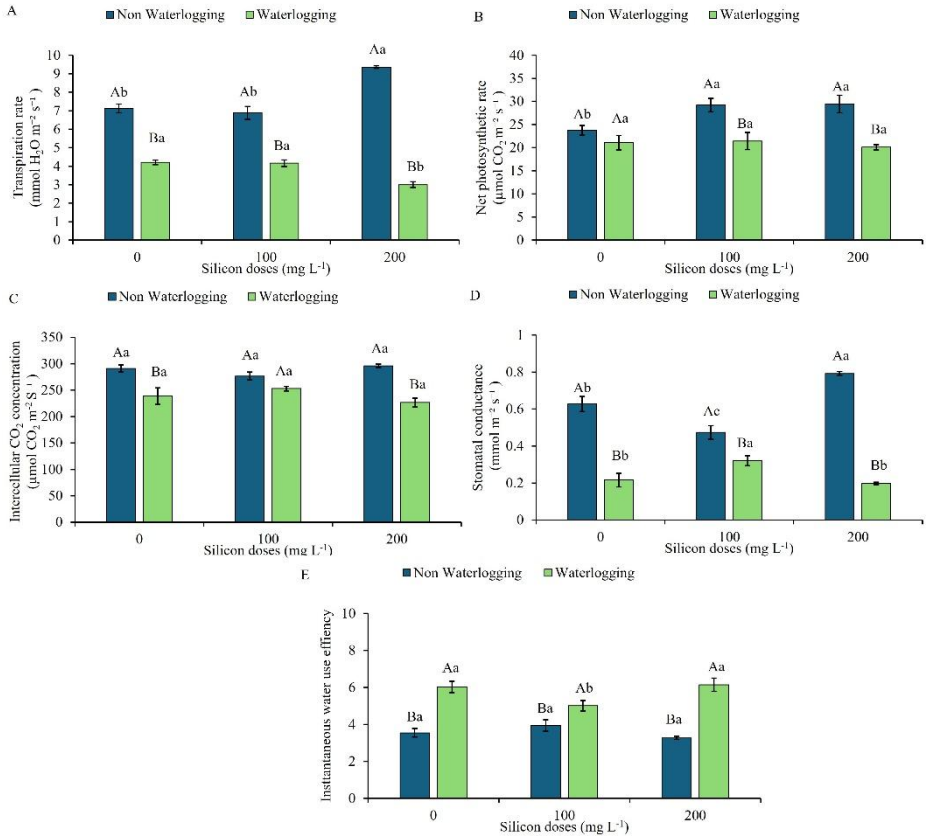


Figure 6. Transpiration rate (*E*) (A), net photosynthesis rate (A_N) (B), intercellular CO_2 concentration (*Ci*) (C), stomatal conductance (*gs*) (D), and instantaneous water use efficiency (WUE) in 'BRS Exuberante' cowpea plants subjected to different doses of silicic acid and different soil water conditions. Uppercase letters differentiate different soil water conditions at each silicic acid

dose (Student's t-test, $P \leq 0.05$), and lowercase letters differentiate silicic acid doses at each soil water condition (Tukey's test, $P \leq 0.05$).

The intercellular CO_2 concentration (*Ci*) decreased across all waterlogging treatments, regardless of Si dose, reflecting the stomatal limitations imposed by stress (Figure 6C). This limitation is confirmed by stomatal conductance (*gs*), which is drastically reduced by waterlogging. In plants under field capacity conditions, *gs* was 65.55%, 32.28%, and 75.03% higher than that of plants under stress at doses of 0, 100, and 200 mg L^{-1}, respectively. The fact that the 100 mg L^{-1} dose showed the smallest percentage difference between water conditions reinforces the hypothesis that this concentration promotes greater stomatal stability of cowpea under adverse conditions (Figure 6D).

The instantaneous water use efficiency (WUE) was significantly optimized by waterlogging, since, at all silicon doses, plants subjected to waterlogging obtained higher WUE than control plants, providing increases of 21.36% and 46.67% in WUE, at doses of 100 and 200 mg L^{-1} of silicon, respectively, compared to non-waterlogging plants (Figure 6E).

Photosynthetic pigments

Photosynthetic pigments showed a differentiated response in cowpea to silicon supplementation under waterlogging stress. Chlorophyll *a* (Chl *a*) content remained stable across treatments, with no significant effect of waterlogging or Si application observed (Figure 7A). On the other hand, chlorophyll *b* (Chl *b*) content was significantly influenced by the interaction between waterlogging and silicon supplementation. In plants under field-capacity conditions, Chl *b* content increased with silicon availability, with the highest level observed at 200 mg L^{-1} of silicon (22.04), resulting in a 16.52% increase compared to waterlogging plants. Under waterlogging, Chl *b* modulation was variable; however, silicon supplementation preserved pigment levels, mitigating the losses frequently associated with hypoxia stress. Of particular note is the 100 mg L^{-1} silicon dose, which resulted in a 32.36% increase in the chl *b* content of plants subjected to waterlogging compared to plants under field-capacity conditions (Figure 7B).

The interaction between waterlogging and silicon supplementation significantly influenced total chlorophyll (Chl *t*). In plants subjected to waterlogging, the 100 mg L^{-1} Si dose stood out by promoting a Chl *t* content of 18.73%, higher than that observed in non-waterlogging plants. The 200 mg L^{-1} dose conferred pigment homeostasis, with no statistically significant difference between waterlogging and non-waterlogging conditions (Figure 7C).

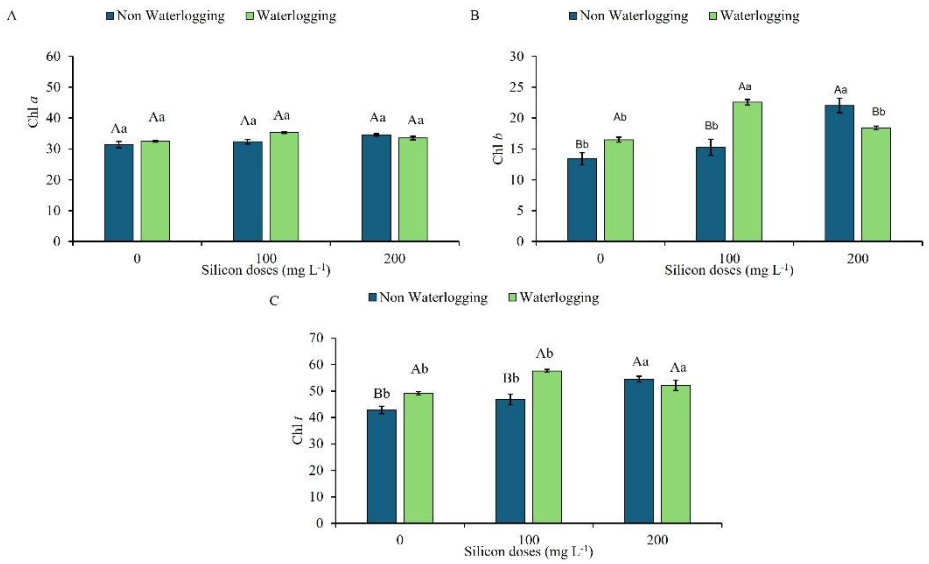


Figure 7. Chlorophyll *a* (Chl *a*) (A), chlorophyll *b* (Chl *b*) (B), and total chlorophyll (Chl *t*) (C) in 'BRS Exuberante' cowpea plants subjected to different doses of silicic acid and different soil water conditions. Uppercase letters differentiate different soil water conditions at each silicic acid dose (Student's t-test, $P \leq 0.05$), and lowercase letters differentiate silicic acid doses at each soil water condition (Tukey's test, $P \leq 0.05$).

Antioxidant mechanism activity

Oxidative stress resulting from waterlogging triggered an activation of the enzymatic apparatus in cowpea plants, a response modulated by supplementation with silicic acid (Si). Superoxide Dismutase (SOD) activity increased under waterlogging, reaching its maximum at a Si concentration of 100 mg L^{-1}, representing a 64% increase compared to non-supplemented plants under waterlogging (Figure 8A).

Catalase (CAT) activity proved highly sensitive to stress severity, remaining consistently higher in waterlogging plants compared to plants at field capacity. Silicon supplementation further enhanced CAT activity, with a 49.84% increase at the 100 mg L^{-1} dose, significantly exceeding the control (Figure 8B). Ascorbate Peroxidase (APX) demonstrated significant metabolic plasticity; in the absence of Si, the 200 mg L^{-1} dose prioritized APX under normal conditions; the intermediate dosage of 100 mg L^{-1} promoted a 15.94% increase in APX activity, especially under waterlogging, reinforcing the superiority of this dose in mitigating oxidative stress (Figure 8C).

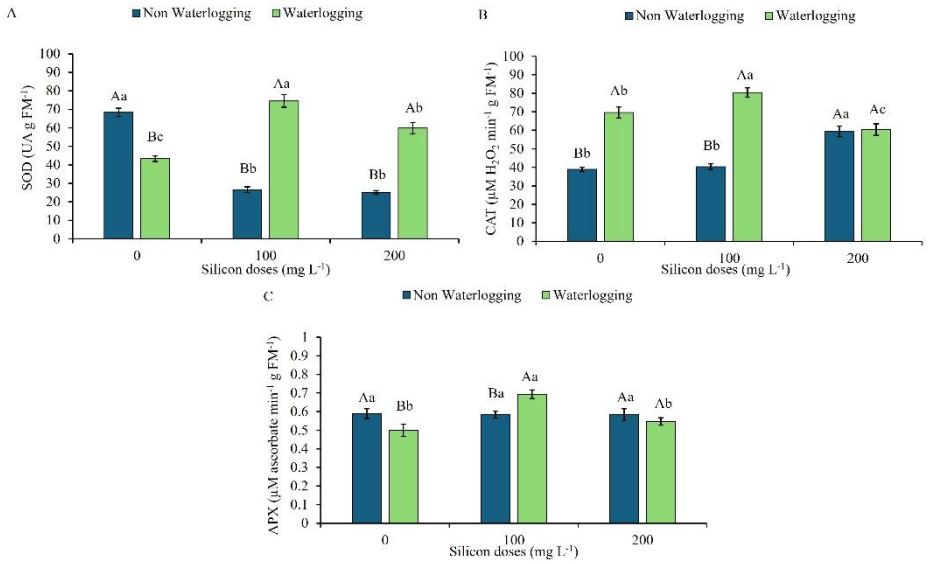


Figure 8. Superoxide dismutase (SOD) (A), catalase (CAT) (B), and ascorbate peroxidase (APX) (C) in 'BRS Exuberante' cowpea plants subjected to different doses of silicic acid and different soil water conditions. Uppercase letters differentiate different soil water conditions at each silicic acid dose (Student's t-test, $P \leq 0.05$), and lowercase letters differentiate silicic acid doses at each soil water condition (Tukey's test, $P \leq 0.05$).

Osmotic adjustment indicators

The accumulation of solutes revealed distinct osmotic adjustment strategies in cowpea under waterlogging stress. Proline levels remained stable at silicon dosages of 0 and 100 mg L^{-1}, regardless of the water regime. However, the application of 200 mg L^{-1} Si under waterlogging conditions increased proline levels by 28.57% compared to plants maintained at field capacity (Figure 9A).

Conversely, the total soluble sugar (TSS) content showed a differentiated accumulation pattern, since, in the absence of Si supplementation, waterlogging plants showed the highest TSS accumulation, with an increase of 24.46% compared to the control, which may indicate an immediate survival response, through starch hydrolysis, to maintain glycolytic metabolism under low oxygen availability. However, the application of silicon significantly reduced TSS levels under waterlogging, especially at doses of 100 and 200 mg L^{-1} (Figure 9B).

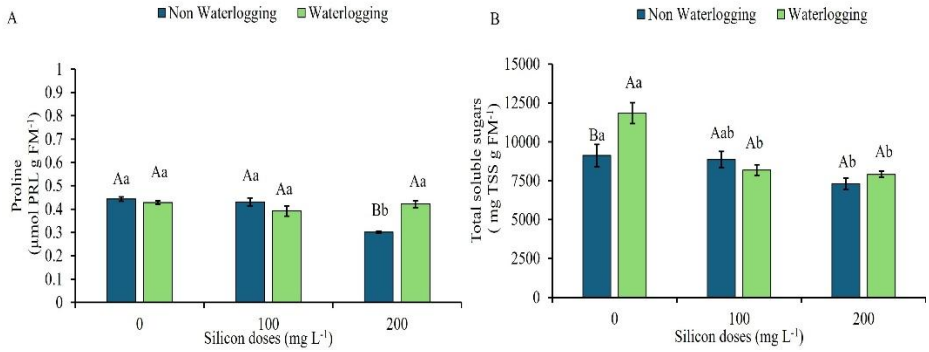


Figure 9. Proline (PRL) (A) and total soluble sugars (TSS) in 'BRS Exuberante' cowpea plants subjected to different doses of silicic acid and different soil water conditions. Uppercase letters differentiate different soil water conditions at each silicic acid dose (Student's t-test, $P \leq 0.05$), and lowercase letters differentiate silicic acid doses at each soil water condition (Tukey's test, $P \leq 0.05$).

3 Discussion

Given global climate change, waterlogging severity is projected to increase by up to 30% by 2030, representing a significant threat to agricultural production and global food security (Senthamil et al. 2025a). Therefore, there is a need to develop studies that understand crop responses and effective management strategies to mitigate the adverse effects of waterlogging. Despite few studies on the response to this phenomenon, strategies for mitigating waterlogging stress in cowpea are still poorly explored. In this context, we evaluated the physiological, biochemical, and biometric performance of cowpea plants subjected to different concentrations of silicic acid and soil waterlogging.

The results of this study demonstrate that soil waterlogging imposes severe constraints on cowpea, leading to an imbalance between root absorption and transpiration demand and inducing oxidative stress. However, supplementation with silicic acid (Si) emerged as an effective mitigation strategy, acting through a complex network of morphological and physiological responses. Silicon not only stabilized leaf area expansion and biomass accumulation but also modulated stomatal conductance, optimizing momentary water use efficiency under hypoxia. The ability of Si to enhance the antioxidant defense system, particularly the enzymatic activities of SOD and CAT, and to promote osmotic adjustment through proline accumulation is noteworthy. Our results suggest that Si confers resilience to cowpea, in which the plant prioritizes membrane stability and photosynthetic apparatus homeostasis, at the expense of morphological changes, such as the excessive increase in root volume observed in non-supplemented plants.

The reductions observed in leaf area and biomass accumulation under waterlogging (Figures 2A and 3) are direct reflections of root hypoxia, which limits cellular respiration and ATP production, compromising the energy metabolism of cowpea (Olorunwa et al. 2023). However, the behavior of root volume (RV) (Figures 2D and 4) reveals a distinct adaptive strategy: the increase in RV in waterlogging plants, in the absence of

Si, suggests high morphological plasticity, possibly directed towards the formation of aerenchyma and adventitious roots to facilitate oxygen diffusion (Senthamil et al. 2025b). Although this response is a survival strategy, it imposes a high metabolic cost on the plant, which is reflected in the lower total dry mass of these plants.

In contrast, the application of Si appears to have conferred structural efficiency, through the maintenance of biomass (especially at a dose of 100 mg L^{-1}) and a more controlled root volume, indicating that Si minimized the deleterious impact of oxygen deficiency, possibly through the fortification of cell walls and/or Si deposition in epidermal cells forming a physical barrier that not only increases leaf rigidity (improving light interception and architecture), or the modulation of hormonal signaling molecules, such as ethylene, reducing the need for drastic energy investments in root morphological alterations, in addition to conferring greater tolerance to biotic and abiotic stresses inherent to the semi-arid environment (Oliveira et al. 2026; Barroso et al. 2026). This protective effect is important because it allows the plant to allocate its carbon resources to maintaining existing tissues and the photosynthetic apparatus, rather than depleting its reserves in an emergency morphological response (Hartman et al. 2021; Oliveira et al. 2026).

The reduction in stomatal conductance (*gs*) and transpiration rate (*E*) under waterlogging (Figure 6D and 6A) is a typical physiological response to hypoxic stress, in which the plant attempts to minimize water loss amidst reduced root absorption capacity (Olorunwa et al., 2022). However, the application of Si strategically modulated this response; in waterlogging plants supplemented with Si, especially at a dose of 200 mg L^{-1}, the reduction in *E* was accompanied by a more efficient maintenance of relative water content (RWC) (Figure 5A) than in the waterlogging control without Si. This behavior suggests that Si regulates stomatal opening, possibly through silica deposition in the leaf cuticle or modulation of hormonal signals, such as abscisic acid (ABA), thereby providing an additional barrier against physiological desiccation induced by excessive water stress (Pereira et al. 2025).

As a direct consequence of this modulation, a significant increase in instantaneous water use efficiency (WUE) (Figure 6E) was observed. The fact that plants treated with 200 mg L^{-1} of Si showed the highest WUE under waterlogging indicates that silicon optimized the cost-benefit of carbon fixation; thus, the plant maintains minimum photosynthetic rates necessary for survival, while drastically reducing water loss through transpiration. This conservative strategy can be considered a resilience mechanism, allowing cowpea to withstand prolonged periods of water saturation without reaching an irreversible wilting point or suffering severe damage from leaf desiccation (Aslam et al. 2023).

The stability of chlorophyll a levels and the maintenance of total chlorophyll (Chl *t*) under waterlogging, when supplemented with 200 mg L^{-1} of Si (Figure 7C), demonstrate the protective role of this element on the photosynthetic apparatus. Soil waterlogging generally induces premature senescence and pigment degradation due to the accumulation of reactive oxygen species (ROS) and the activity of the chlorophyllase enzyme (Das et al. 2025). However, the homeostasis observed in the presence of Si suggests that the mineral helps preserve chloroplast integrity.

This effect can be attributed to Si's ability to strengthen thylakoid membranes and modulate the antioxidant system, preventing excess light energy, unused due to low stomatal conductance, from resulting in photoinhibition. Furthermore, the increase in chlorophyll *b* observed in plants flooded with 100 mg L^{-1} of Si may indicate a compensatory adjustment to maximize light capture under metabolic stress conditions (Ali et al. 2025; Dias et al. 2026). Thus, Si supplementation ensures that, even under waterlogging, the plant maintains its photon capture machinery functional, facilitating physiological recovery after stress.

Waterlogging induces root hypoxia, disrupting the electron transport chain and leading to the accumulation of reactive oxygen species (ROS), which cause severe oxidative damage (Zhang et al. 2025). Our results demonstrate that foliar application of Si potentiated the enzymatic defense machinery of cowpea (Figure 8). The increase in SOD activity, especially at a dose of 100 mg L^{-1} under waterlogging, can be considered an indicator of tolerance, since SOD is the first line of defense; its activation, mediated by Si, promotes the rapid dismutation of the superoxide radical (O_2-) into hydrogen peroxide (H_2O_2) (Bakirova et al. 2025).

However, the accumulation of H2O2 is equally toxic and requires the coordinated action of Catalase (CAT) and Ascorbate Peroxidase (APX) (Khan et al. 2025). In our study, we found that Si supplementation not only increased CAT activity under stress but also increased APX at the intermediate dose (100 mg L^{-1}). This enzymatic coordination suggests that Si acts as a modulator of redox homeostasis, preventing lipid peroxidation and maintaining membrane integrity, as confirmed by the lower rates of electrolyte leakage observed in supplemented plants (Hussain et al. 2025). Thus, Si acts not only as a physical barrier but also as a biostimulant that refines the plant's biochemical response to abiotic stress.

The accumulation of organically compatible solutes constitutes an important strategy for maintaining cell turgor and protecting macromolecules under stress conditions (Saccone et al. 2026). Our results indicate that silicon (Si) plays a key role in the metabolic signaling of osmotic adjustment in cowpea (Figure 9). The increase in proline levels observed in flooded plants treated with 200 mg L^{-1} of Si suggests that Si potentiates proline synthesis, which acts not only as an osmolyte but also as a potent scavenger of hydroxyl radicals and a stabilizer of membranes and proteins (Khursheed et al. 2025). Its accumulation contributes to the maintenance of leaf turgor (Dias et al. 2026), to the protection of membranes, and to the preservation of cell volume, functions consistent with the responses observed in our study.

However, the greater accumulation of total soluble sugars (TSS) in waterlogging plants lacking silicon can be interpreted as a symptom of metabolic stress, resulting from starch hydrolysis to meet the energy demand of the glycolytic pathway in an anoxic environment (Ma et al. 2025). The reduction in TSS levels promoted by Si supplementation, especially at doses of 100 and 200 mg L^{-1}, indicates that Si favors a more balanced physiological state, attenuating oxidative damage and improving photosynthetic efficiency. This may reduce the need for excessive mobilization of carbon reserves, allowing the plant to maintain more efficient partitioning of photoassimilates for survival and post-stress recovery (Ali et al. 2025). This change in the profile of

compatible solutes, prioritizing proline over the disordered accumulation of sugars, reinforces the thesis that Si acts as a refiner of cowpea's metabolic response to waterlogging.

Although the results demonstrate the mitigating potential of silicic acid for physiological and biochemical homeostasis in cowpea under waterlogging, some limitations should be considered. The experiment was conducted under controlled conditions (pots), which, while allowing for the precise isolation of variables, do not fully reproduce the complexity of the field environment, where factors such as edaphic variability, the dynamics of rhizospheric microorganisms, and temperature fluctuations influence silicon bioavailability and the severity of hypoxia. Additionally, this study focused on a specific time window at the V6 stage, which did not allow evaluation of Si effects throughout the crop's phenological cycle, especially in critical phases such as grain filling, where metabolic demand is distinct. Therefore, future investigations are essential to evaluate different genotypes under field conditions and to assess the impact of supplementation on reproductive phases, thereby consolidating the applicability of silicic acid as a sustainable management strategy for cowpea resilience.

4 Conclusion

Soil waterlogging affected the growth and photosynthetic apparatus of cowpea, triggering oxidative stress and alterations in solute metabolism. Foliar application of 100 mg L^{-1} of silicon promoted greater plant resilience, acting on the modulation of the antioxidant system, maintenance of pigment homeostasis, and osmotic adjustment. Silicon supplementation is a viable and efficient biotechnological strategy to enhance cowpea resilience in areas prone to seasonal waterlogging, thereby preserving physiological integrity and optimizing water-use efficiency in water-stressed environments.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors have no competing interests to declare.

Funding: This study was financed in part by Paraíba State University, grant #01/2026.

Clinical trial: Not applicable. This study does not involve a clinical trial.

Authors' contributions: Conceptualization: ASM; HAA; LML. Methodology: PMVO; GFD; SIB; LDR; YMLA; EMCM; IEC; AMFO. Formal analysis and investigation: PMVO; GFD; SIB; LDR; YMLA; EMCM; IEC; AMFO. Writing - original draft preparation: ASM; AMFO; GFD; PMVO; SIB. Writing - review and editing: ASM; AMFO; SIB; HAA; LML. Funding acquisition: ASM Resources: AMS Supervision: ASM.

Acknowledgements: The authors would like to extend their sincere appreciation to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), financial code 001, and the Programa Institucional de Pós-Doutorado (PIPD – CAPES) (Proc. 88887.081429/2024-00). The Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the granting of financial aid (Proc. 408952/2021-0 e 307559/2022-0). The Fundação de Apoio à Pesquisa do Estado da Paraíba (FAPESP/PB) (Edital FAPESP/PB/CNPq nº 77/2022). The INCT em Agricultura Sustentável no Semiárido Tropical - INCTAGriS (CNPq/Funcap/Capes). The Universidade Estadual da Paraíba (UEPB) and the Laboratório de Ecofisiologia de Plantas Cultivadas (Ecolab) for awarding grants to the researchers.
This study was financed in part by Paraíba State University, grant #01/2026.

Corresponding author: Alberto Soares de Melo, e-mail: alberto.melo@servidor.uepb.edu.br, Fone: +55 (83) 98871-3902

5 References

1. Ali A, Zafar S, Mehmood K, Hasnain Z, Nawaz M, Gilani SMJJ, et al. Silicic acid seed pre-treatment modulates growth and antioxidant responses in maize under drought stress. Sci Nat. 2025; 112:70. https://doi.org/10.1007/s00114-025-02021-y
2. Alvares CA, Stape JL, Sentelhas PC, Gonçalves JDM, Sparovek G. Köppen's climate classification map for Brazil. Meteorol Z. 2013; 22:711–728. https://doi.org/10.1127/0941-2948/2013/0507
3. Araújo EDD, Melo ASD, Rocha MDS, Carneiro RF, Rocha MDM. Germination and initial growth of cowpea cultivars under osmotic stress and salicylic acid. Rev Caatinga. 2018; 31:80–89. https://doi.org/10.1590/1983-21252018v31n110rc
4. Aslam A, Mahmood A, Ur-Rehman H, Li C, Liang X, Shao J, et al. Plant adaptation to flooding stress under changing climate conditions: ongoing breakthroughs and future challenges. Plants. 2023;12:3824. https://doi.org/10.3390/plants12223824
5. Bakirova A, Kiremit MS, Arslan H. Augmentative role of salicylic acid and proline in overcoming waterlogging stress in sweet corn (Zea mays var. saccharata). J Soil Sci Plant Nutr. 2025;25:8375–8395. https://doi.org/10.1007/s42729-025-02683-7
6. Barroso VSF, Dias GF, de Alencar RS, Bonou SI, de Oliveira Viana PM, Cavalcante IE, et al. Seed priming with two types of glassy microparticles in cowpea subjected to water deficit induced by polyethylene glycol 6000. BMC Plant Biol. 2026. https://doi.org/10.1186/s12870-026-08374-4

7. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil. 1973;39:205–207. https://doi.org/10.1007/BF00018060
8. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem. 1971;44:276–287. https://doi.org/10.1016/0003-2697(71)90370-8
9. Bezerra Neto E, Barreto LP. Análises químicas e bioquímicas em plantas. Recife: Universidade Federal Rural de Pernambuco; 2011.
10. Biju S, Fuentes S, Gupta D. Novel insights into the mechanisms of silicon-induced drought stress tolerance in lentil plants revealed by RNA sequencing analysis. BMC Plant Biol. 2023;23:498. https://doi.org/10.1186/s12870-023-04492-5
11. Böhm W. Root parameters and their measurement. In: Methods of studying root systems. Berlin: Springer; 1979. p. 125–138.
12. Brito GG, Sofiatti V, Lima MMA, Carvalho LP, Silva Filho JL. Physiological traits for drought phenotyping in cotton. Acta Sci Agron. 2011;33:117–125. https://doi.org/10.4025/actasciagron.v33i1.9839
13. Chakravaram A, Sankarapillai LV, Poudel S, Bheemanahalli R. Interactive effects of drought and high night temperature on physiology and yield components of cowpea (Vigna unguiculata (L.) Walp.). J Agric Food Res. 2025;21:101844. https://doi.org/10.1016/j.jafr.2025.101844
14. Das AK, Lee DS, Woo YJ, Sultana S, Mahmud A, Yun BW. The impact of flooding on soil microbial communities and their functions: a review. Stresses. 2025;5:30. https://doi.org/10.3390/stresses5020030
15. de Ávila Silva L, Omena-Garcia RP, Condori-Apfata JA, Costa PMA, Silva NM, DaMatta FM, et al. Specific leaf area is modulated by nitrogen via changes in primary metabolism and parenchymal thickness in pepper. Planta. 2021;253:16. https://doi.org/10.1007/s00425-020-03519-7
16. Dias GF, Alencar RSD, Viana PMDO, Cavalcante IE, Farias ESDD, Bonou SI, et al. Seed priming with PEG 6000 and silicic acid enhances drought tolerance in cowpea by modulating physiological responses. Horticulturae. 2025;11:438. https://doi.org/10.3390/horticulturae11040438
17. Dias GF, Bonou SI, de Oliveira Viana PM, de Alencar RS, da Silva Gonçalves TW, Cavalcante IE, et al. Silicon and methionine enhance cowpea water stress tolerance. Sci Rep. 2026;16:6800. https://doi.org/10.1038/s41598-026-37795-2
18. FAO. FAOSTAT: Food and Agriculture Data. Rome: Food and Agriculture Organization of the United Nations; 2025.
19. Ferreira DF. SISVAR: a computer analysis system for fixed effects split-plot type designs. Braz J Biometrics. 2019;37:529–535. https://doi.org/10.28951/rbb.v37i4.450
20. Hartman S, Sasidharan R, Voesenek LACJ. The role of ethylene in metabolic acclimations to low oxygen. New Phytol. 2021;229:64–70. https://doi.org/10.1111/nph.16378
21. Hussain R, Shaheen MR, Rashid S, Iqbal MAR, Ali Q, Akram A, et al. Silicon as a stress alleviator in horticultural crops: a review. Acta Agric Sci. 2025;1:1–19.
22. IPCC. Climate change 2023: synthesis report. Geneva: IPCC; 2023.
23. Kar M, Mishra D. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant Physiol. 1976;57:315–319. https://doi.org/10.1104/pp.57.2.315

24. Khan TA, Saeed T, Karumannil S, Kappachery S, Yusuf M, Tran LSP, et al. Melatonin and hydrogen peroxide alleviate cadmium toxicity in tomato via regulation of transporter genes, antioxidant activity, and photosynthetic efficiency. Environ Sci Pollut Res. 2025;32:23129–23147. https://doi.org/10.1007/s11356-025-37017-8
25. Khursheed S, Bashir Surma S, Rahman MAT, Shah MD, Padder BA, Afroza B, et al. Silicon's defensive role against biotic and abiotic stress: a review. Phosphorus Sulfur Silicon Relat Elem. 2025;200:325–344. https://doi.org/10.1080/10426507.2025.2482090
26. Ma Y, Zhou Z, Xu X, Shen Y, Huang X, Shi Y, et al. Carbohydrate metabolic responses to water stress in plants: implications for yield and quality. Phytochem Rev. 2025. https://doi.org/10.1007/s11101-025-10181-y
27. Martin TN, Fipke GM, Minussi Winck JE, Márchese JA. ImageJ software as an alternative method for estimating leaf area in oats. Acta Agron. 2020;69:162–169. https://doi.org/10.15446/acag.v69n3.69401
28. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidases in spinach chloroplasts. Plant Cell Physiol. 1981;22:867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232
29. Oliveira AMF, Mendonça V, Amorim PEC, da Silva Freire RI, da Silva LRB, Pinheiro ABD, et al. Photosynthetic, nutritional efficiency, and productivity of fig trees under pruning management and fertilization with potassium silicate in the semi-arid region. Silicon. 2026. https://doi.org/10.1007/s12633-025-03622-y
30. Olorunwa OJ, Adhikari B, Brazel S, Bheemanahalli R, Barickman TC, Reddy KR. Waterlogging stress reduces cowpea (Vigna unguiculata L.) genotypes growth, seed yield, and quality at different growth stages: implications for developing tolerant cultivars under field conditions. Agric Water Manag. 2023;284:108336. https://doi.org/10.1016/j.agwat.2023.108336
31. Olorunwa OJ, Adhikari B, Brazel S, Shi A, Popescu SC, Popescu GV, et al. Growth and photosynthetic responses of cowpea genotypes under waterlogging at the reproductive stage. Plants. 2022;11:2315. https://doi.org/10.3390/plants11172315
32. Pereira S, Monteiro A, Baltazar M, Maia C, Pereira S, Oliveira MJ, et al. Modulating grapevine performance and hormonal dynamics under summer stress by the synergistic effects of kaolin and silicon. Front Plant Sci. 2025;16:1639169. https://doi.org/10.3389/fpls.2025.1639169
33. Piedra-Bonilla EB, Da Cunha DA, Braga MJ, Oliveira LR. Extreme weather events and crop diversification: climate change adaptation in Brazil. Mitig Adapt Strateg Glob Change. 2025;30:28. https://doi.org/10.1007/s11027-025-10211-2
34. Queiroz LLG, Mesquita EFD, Sousa CDS, Pereira RF, Diniz JPC, Melo ASD, et al. Foliar silicon alleviates water deficit in cowpea by enhancing nutrient uptake, proline accumulation, and antioxidant activity. Plants. 2025;14:1241. https://doi.org/10.3390/plants14081241
35. Saccone R, Fascella G, Bonfante G, Salvagno E, Montoneri E, Baglieri A, et al. The role of bio-based products in plant responses to salt and drought stress. Horticulturae. 2026;12:95. https://doi.org/10.3390/horticulturae12010095
36. Santos AR, Melo YL, de Oliveira LF, Cavalcante IE, de Souza Ferraz RLS, da Silva Sá FVS, et al. Exogenous silicon and proline modulate osmoprotection and antioxidant

activity in cowpea under drought stress. J Soil Sci Plant Nutr. 2022;22:1692–1699. https://doi.org/10.1007/s42729-022-00764-5

37. Scotti Campos P, Thu Phan Thi A. Effect of abscisic acid pretreatment on membrane leakage and lipid composition of Vigna unguiculata leaf discs subject to osmotic stress. Plant Sci. 1997;130:11–18.
38. Senthamil E, Angadi SS, Halli HM, Salakinkop SR, Doddamani MB, Gundlur SS, et al. Waterlogging stress mitigation in cowpea: leveraging the potential of foliar spray of nitrogen and phytohormones for improved resilience. Plant Soil. 2025;514:3119–3140. https://doi.org/10.1007/s11104-025-07571-8
39. Senthamil E, Angadi SS, Halli HM, Salakinkop SR, Doddamani MB, Gundlur SS. Effect of transient waterlogging stress on growth, physiology and yield of cowpea (Vigna unguiculata L.) in semi-arid region. Int J Plant Soil Sci. 2024;36:45–56. https://doi.org/10.9734/ijpss/2024/v36i84834
40. Senthamil E, Halli HM, Basavaraj PS, Angadi SS, Gangana Gowdra VM, Harisha CB, et al. Waterlogging effects on root morphology, yield, and stress tolerance in cowpea (Vigna unguiculata L. Walp.) grown on semi-arid vertisols. J Agron Crop Sci. 2025;211:e70014. https://doi.org/10.1111/jac.70014
41. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). Biometrika. 1965;52:591–611.
42. Silva AE, de Souza Ferraz RLS, da Silva PJ, da Silva PC, Viegas PRA, Brito Neto JF, et al. Microclimate changes, photomorphogenesis and water consumption of Moringa oleifera cuttings under different light spectrums and exogenous phytohormone concentrations. Aust J Crop Sci. 2020;14:751–760. https://doi.org/10.21475/ajcs.20.14.05.p2096
43. Silva FA, Silva PVB, de Meireles DA, da Costa CRG, de Oliveira MRT, da Silva Fraga V, et al. Avaliação de caracteres agronômicos de cultivares de feijão-caupi (Vigna unguiculata L. Walp.) cultivados em Esperança-PB. J Soc Technol Environ Sci. 2024;13:203–209. https://doi.org/10.21664/2238-8869.2024v13i2.p203-209
44. Souza EMBD, Paciullo DSC, Gomide CADM, Rocha WSDD, Silva RBD, Lana ÂMQ, et al. Productive and morphological responses of Megathyrsus maximus hybrids subjected to water deficit and waterlogging. Rev Bras Zootec. 2025;54:e20240111. https://doi.org/10.37496/rbz5420240111
45. Suárez JC, Casanoves F, Di Rienzo J. Non-destructive estimation of leaf weight and leaf area in common bean. Agronomy. 2022;12:711. https://doi.org/10.3390/agronomy12030711
46. Teixeira PC, Donagemma GK, Fontana A, Teixeira WG. Manual de métodos de análise de solo. 3rd ed. Brasília: Embrapa; 2017.
47. Yemm EW, Willis A. The estimation of carbohydrates in plant extracts by anthrone. Biochem J. 1954;57:508. https://doi.org/10.1042/bj0570508
48. Zhang Y, Chen X, Geng S, Zhang X. A review of soil waterlogging impacts, mechanisms, and adaptive strategies. Front Plant Sci. 2025;16:1545912.