Article in Press

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Seed priming with two types of glassy microparticles in cowpea subjected to water deficit induced by polyethylene glycol 6000

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Abstract. The relevance of cowpea (*Vigna unguiculata* (L.) Walp.) in semi-arid regions drives the search for drought tolerance strategies. Our hypothesis was that seed priming with glassy silicon microparticles (SiMPs), a low-cost recycled glass byproduct, confers comprehensive resilience, functioning as both a biostimulant under optimal conditions and a structural and metabolic protector under drought stress. Methods: The experiment was conducted in a 4x2 factorial design, with four primings: Control (water), P1 (Ψs = -0.4 MPa with PEG 6000), P2 (P1 + Blue SiMPs), and P3 (P1 + Amber SiMPs), applied to 'BRS Tapaihum' cowpea seeds and at two water replacement levels (35% and 75% of ETc), with five replicates. Results: Priming with P1 (PEG 6000) was ineffective at mitigating stress (W35), resulting in lower TDM values and higher oxidative stress (SOD and CAT). In contrast, SiMPs demonstrated bifunctional benefits, as under optimal conditions (W75), P2 acted as a biostimulant, increasing TDM by 18%. Under stress (W35), P3 and P2 conferred protection through membrane stabilization (30% reduction in electrolyte leakage (EL) and optimization of WUEi compared to P1), resulting in TDMs up to 25% higher than the control under W35. Conclusions: Priming with SiMPs (blue and amber) is an effective strategy, conferring comprehensive resilience by optimizing WUEi at W75 and structurally protecting membranes at W35, in contrast to the insufficiency of PEG 6000. The use of this recycled glass material constitutes a sustainable and

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low-cost solution with significant potential to increase productivity and food security in semi-arid regions.

Keywords: Water deficit, *Vigna unguiculata* (L.) Walp, PEG 6000, silicon.

1 First Section

1.1 Introduction

The exponential growth of the global population poses a challenge for the agricultural sector to increase food production and ensure food security (Calone et al. 2022). However, increased production must occur with minimal impact on natural resources to meet one of the goals of the 2030 Agenda for Sustainable Development (Costa et al. 2022). Furthermore, global climate change has reduced crop development and, consequently, reduced productivity across several crops, resulting in negative ecological, economic, and social impacts (Dias et al. 2025).

Water scarcity is a consequence of these changes and one of the principal abiotic stresses affecting agriculture. This type of stress has several implications in plants, including physical, biochemical, and physiological effects, such as changes in metabolic processes, reduced cell expansion, reduced leaf area, decreased CO_2 assimilation rate, stomatal closure, and increased leaf abscission. These are examples of the consequences of water deficit on plants (Melo et al. 2022; Melo et al. 2024; Cavalcante et al. 2025; Ibrahim et al. 2025). This is especially true in the semi-arid region of northeastern Brazil, characterized by low rainfall, high temperatures, and low relative humidity (Sousa et al. 2023).

In this context, cowpea [*Vigna unguiculata* (L.) Walp.] becomes a promising option, considering its socioeconomic importance as a subsistence crop, especially in the North and Northeast regions of Brazil (Alencar et al. 2024). Although hard, cowpea plants grown in these regions can be severely affected by water restriction at any phenological stage, thereby reducing productivity (Melo et al. 2022; Alencar et al. 2024). Therefore, implementing technologies that enable cowpea production under such conditions is necessary to increase the plants' tolerance to water deficit and thus ensure food security and a source of income for farmers.

Among the technologies used, seed priming stands out as a highly effective and cost-efficient technique for enhancing plant tolerance to abiotic stress. This process induces a stress memory by early activation of germination metabolism, resulting in essential physiological and metabolic modulations. These modulations include strengthening antioxidant defenses, promoting osmotic adjustment, and activating key seed enzymes, thereby conferring greater vigor and stress resistance (Alencar et al. 2024; Diya et al. 2024; Dias et al. 2025). Indeed, this technique has shown promising results in economically important crops, including cereals (Kakar et al. 2023; Al Zoubi et al. 2025), legumes (Vanitha et al. 2024; Aboellail et al. 2025), and, notably, cowpea (Nabi et al. 2020; Alencar et al. 2024). This consensus across the literature confirms that seed priming is a fundamental primary defense strategy in plants.

The choice of tolerance-inducing agents for seed priming is broad, encompassing water, hormones, osmoprotectants such as polyethylene glycol 6000 (PEG 6000), and

elements such as silicon (Si) (Ellouzi et al. 2021; Costa et al. 2022; Alencar et al. 2024). Among these, PEG 6000 is critical. Due to its high molecular weight (which prevents cell penetration) and non-toxicity, PEG 6000 is primarily used to assess water-stress-related information in plants by simulating osmotic stress (Meher Shivakrishna et al. 2018). While studies using PEG 6000 have been effective in inducing rapid plant tolerance, this approach relies fundamentally on a transient osmotic effect to trigger stress memory, which may not provide sustained protection throughout the plant's developmental cycle (Lei et al. 2021). For long-term resilience, a more durable protective mechanism, such as the gradual release of a biostimulant element, is required.

Among the promising alternatives, the use of silicon glass microparticles (SiMPs) as a Si source stands out for its strong potential to mitigate abiotic stresses, such as drought (Alencar et al. 2024). Crucially, the SiMPs used in this study were derived from recycled post-consumer glass bottles. This approach aligns directly with the Sustainable Development Goals (SDG) by transforming a significant waste stream, such as glass discarded in landfills, into a value-added agro-input. By using recycled glass, this research not only explores a novel solid-state Si delivery system but also provides an environmentally responsible, low-cost alternative to commercial fertilizers.

Furthermore, this solid-state priming approach addresses a critical research gap: soluble Si provides rapid protection, whereas SiMPs, as an innovative slow-release Si source, are expected to induce more sustained stress-tolerance memory (Costa et al. 2022). The SiMPs were synthesized from blue and amber glass, which, although both primarily consist of silicon dioxide (SiO_2), sodium oxide (Na_2O), and calcium oxide (CaO), are very similar, indicating that the glass matrix is essentially the same. However, contain trace amounts of subtly different elements (e.g., cobalt oxide for blue and iron/sulfur oxide for amber). This variation in the elemental profile may influence Si release kinetics and the resulting physiological response, warranting separate investigation in seed priming.

Given the importance of cowpea cultivation in water-scarce regions and the need for sustainable agricultural inputs, we hypothesized that seed priming with silicon glass microparticles (SiMPs) may generate seed memory and confer tolerance to water deficit in the 'BRS Tapaihum' cultivar, compared to priming with polyethylene glycol (PEG) alone.

Therefore, this study evaluated the development of the cultivar 'BRS Tapaihum' under water deficit (induced by polyethylene glycol 6000) and the effect of seed priming with blue and amber silicon glass microparticles.

1.2 Materials and Methods

Experimental design

The experiment was carried out in a completely randomized design, in a 4x2 factorial scheme, in which the first factor consisted of four seed conditioning treatments: Control, priming 1 (Ψh - 0.4 MPa), priming 2 (Ψh -0.4 MPa + 200 mg L^{-1} blue glass microparticles (SiMPsB)), and priming 3 (Ψh -0.4 MPa + 200 mg L^{-1} amber glass mi-

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croparticles (SiMPsA)), and two levels of water replacement (35% and 75% of ETc), with five replicates and two plants per pot, totaling 40 experimental units.

Seed conditioning

Seed conditioning was performed at the Laboratory of Ecophysiology of Cultivated Plants (ECOLAB), in the Três Marias complex, belonging to the State University of Paraíba (UEPB), in Campina Grande, Paraíba, Brazil. The region has a semi-arid climate, with an average temperature of 25°C and a relative humidity of 72-91%.

The 'BRS Tapaihum' cowpea seeds used in this study were kindly donated by the germplasm collection of Embrapa Meio-Norte, located in Teresina, Piauí, Brazil (Coordinates: 5°05' S, 42°49' W, Altitude: 72 m). 'BRS Tapaihum' has black, kidney-shaped grains with an average mass of 19 grams per 100 grains. Any that were physically damaged or malformed were discarded. They were then subjected to a three-minute sterilization process with sodium hypochlorite (1%) (Carvalho and Carvalho, 2009), followed by washing and drying for osmotic conditioning. After sterilization, 200 seeds were placed in four plastic Gerbox-type boxes measuring 11 x 11 x 3.5 cm in length, width, and height, respectively. The substrate inside the boxes consisted of two layers of Germitex paper, moistened with solutions corresponding to each conditioning solution, to a volume approximately three times their dry mass (Ferreira et al., 2017).

The osmotic potential (-0.4 MPa) tested in this study was chosen based on previous research by Alencar et al. (2024) and Dias et al. (2025). These studies demonstrated that water potentials more negative than (-0.4 MPa) caused unfavorable reductions in cowpea growth and development.

To simulate the water potential, a solution of polyethylene glycol 6000 (PEG 6000, Neon, PA) in distilled water was prepared under constant stirring. The exact amount of PEG required to reach the osmotic potential of (-0.4 MPa) was calculated using the equation proposed by Michel and Kaufmann (1973):

$$\Psi s = -(1{,}18x10^{-2})C.(1{,}18x10^{-4})C^2 + (2{,}67x10^{-4})CT + (8{,}39x10^{-7})C^2T,$$

Where:

Ψs = Potential osmotic (MPa).

C = Concentration (grams of PEG 6000/1 liter of water).

T = Temperature (°C)

Silicon concentration (200 mg L^{-1}) was used in the form of glassy microparticles from amber and blue glass bottles. To obtain the glassy microparticles, amber and blue glass beverage bottles were collected from the municipal landfill of Toledo, Paraná, Brazil. Clean, dry bottles were manually ground and sieved through a 400-mesh Tyler sieve to obtain powder particles smaller than 38 μm. The microparticles were analyzed by PR-CR-098 XRF spectrometry, and their chemical composition was determined by the SENAI Institute of Technology - Ceramics, Brazil. Their composition is shown in Table 1.

	Al$_2$O$_3$ (%)	CaO (%)	Fe$_2$O$_3$ (%)	K$_2$O (%)	MgO (%)	MnO (%)	Na$_2$O (%)	P$_2$O$_5$ (%)	SiO$_2$ (%)	TiO$_2$ (%)	BaO (%)	Co$_2$O$_3$ (%)	Cr$_2$O$_3$ (%)	PbO (%)	SrO (%)	ZnO (%)	ZrO$_2$/Hf (%)	Loss due to fire (%)
SiMPsA	1.420	10.072	0.387	0.424	1.125	<0.05	13.629	<0.05	71.873	<0.05	<0.05	<0.05	<0.05	<0.05	0.093	0.138	<0.05	0.659
SiMPsB	1.365	10.817	0.113	0.545	0.406	N.D	13.984	<0.05	72.024	<0.05	<0.05	0.076	<0.05	<0.05	0.092	0.121	<0.05	0.39

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Table 1. Chemical composition of microparticles from amber (SiMPsA) and blue (SiMPsB) glass.

Subsequently, after conditioning applications, the boxes containing the seeds were placed in Biochemical Oxygen Demand B.O.D. germination chambers (MA 402, Marconi, Brazil). The conditioning application time was 18 h, the period required for seed imbibition without completing germination (Alencar et al. 2024). Subsequently, the seeds were transferred to Gerbox® boxes without lids, with two layers of dry germitest paper, and subjected to drying under the same light and temperature conditions used during conditioning for 48 h, so that the seeds returned to their initial dry weight, as established by the methodology of Masruri et al. (2023).

Conducting the Experiment and Water Replacement

The prepared seeds were sown in polyethylene pots (3.6 L capacity) filled with soil. Whose physicochemical characteristics are: sand (71.56%), silt (18.07%), clay (10.35%), textural class: Sandy loam, soil density (1.38 g cm^{-3}) particle density (2.69 g cm^{-3}), porosity (48.70%), nitrogen (0.46 g kg^{-1}), phosphorus (2.84 mg kg^{-1}), potassium (0.26 cmolc kg^{-1}), calcium (1.78 cmolc kg^{-1}), magnesium (4.18 cmolc kg^{-1}), sodium (0.55 cmolc kg^{-1}), sulfur (7.11 cmolc kg^{-1}), organic matter (1.05 g kg^{-1}), V (%): 96.88%, SB: 6.22, CTC (6.42 cmolc kg^{-1}), hydrogen (0.92 cmolc kg^{-1}), aluminum (0.20 cmolc kg^{-1}), pH H$_2$O (6.46), electrical conductivity (0.83 mmhos cm^{-1}) and available water (10.03% dry soil basis).

The seedlings were grown in a Fitotron growth chamber (SCG 120, Weiss Technik, UK) under a 12-hour photo period, with temperatures of 30 °C (day) and 28 °C (night) and a constant relative humidity of 50%. This controlled environment provided stable conditions for early plant development prior to the physiological assessments. The growth chamber is located at the Experimental Station situated at 07° 12' 42.99" S and 35° 54' 36.27''W, altitude of 521 m, belonging to the State University of Paraíba (UEPB), Campina Grande, Paraíba, Brazil.

Soil moisture management was implemented through daily irrigation shifts using the weighing method, in which water lost through evapotranspiration was replaced (Silva et al. 2020). Water deficit began 8 days after plant emergence, with replacement of 35% and 75% of water consumption, and was maintained continuously until stage V5, at which point the plants were analyzed.

Variables analyzed

The vegetative development of cowpea plants was monitored at 20 and 28 days after emergence, corresponding to phenological stages until the V4 and V5 phenological stages. The V4 stage was identified by the complete expansion of the fourth trifoliate leaf on the main stem and the complete formation of the third internode. Subsequently, the V5 stage was characterized by the complete expansion of the fifth trifoliate leaf and the visible development of axillary buds, marking the period of maximum vegetative vigor before the transition to the reproductive phase. Growth analyses, water

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status, and indicators of membrane damage, gas exchange, and activity of the non-oxidative and antioxidant mechanisms were evaluated.

Growth

Total leaf area (TLA) was determined by tracing leaflets on an A4 sheet of paper and photographing them with a smartphone camera, using a 2 cm scale. The images were correctly labeled, and measurements in centimeters were subsequently obtained using ImageJ (Martin et al. 2021; Suárez et al. 2022). Total dry matter (TDM) was obtained by collecting leaves, stems, and branches, which were packed separately in paper bags, labeled, and oven-dried with forced-air circulation at 60 °C for 72 hours. The dry plant material was then weighed on an analytical balance with an accuracy of ±0.0001 g (Santos et al. 2022).

Water Status and Membrane Damage Indicator

Relative water content (RWC%) was determined from five leaf discs, obtained by precise cuts using a 5 mm copper punch. Subsequently, the leaf discs were weighed to determine their fresh mass (FMD) and placed in aluminum capsules containing 10 mL of distilled water at room temperature, which were then sealed with plastic wrap. After 24 hours, excess water was removed with paper towels, and the material was dried for reweighing to obtain the turgid mass (TMD). The discs were placed in paper bags and heated in a forced-air circulation oven at 60 °C for an additional 48 h to measure their dry mass (DMD) (Ferraz et al. 2015). RWC (%) was quantified according to the equation.

$$RWC\ (\%) = \left(\frac{FMD-DMD}{TMD-DMD}\right) x100,$$

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

Electrolyte leakage (EL) was determined using a copper perforator, obtaining five leaf discs with an area of 113 mm^2 per experimental unit, which were washed and placed in Petri dishes containing 20 mL of deionized water. Subsequently, these discs were placed in test tubes containing 10 mL of distilled water and left sealed at rest for 24 hours. The electrical conductivity of the solution in the test tubes (Xi) was measured using a high-precision digital conductivity meter (Ec/temp Waterproof) according to the protocol proposed by Brito et al. (2011).

The tubes were then sealed with plastic film and heated to 100°C for 60 minutes in a digital water bath (NI 1217, Nova Instruments). After the tube contents had cooled to room temperature, the final conductivity (Xf) of the solution was measured. The percentage of electrolyte leakage was calculated using the following equation.

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

Where:

EL – 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 to 11:00 a.m., using an infrared gas analyzer (LI-6800 Portable Photosynthesis System, Li-Cor, Inc., The United States), equipped with a 6 cm^2 leaf area chamber (LI-6800). The leaf temperature was maintained at around 25 ± 2 °C, and the air flow rate was 500 μmol s^{-1}, under a fixed actinic photon flux density of 1200 μmol m^{-2} s^{-1}. The chamber relative humidity was maintained at 60%, and the CO_2 concentration at 400 μmol mol^{-1}. The air temperature inside the chamber was monitored using a thermoelectric sensor at the base and set to 27°C. While this irradiance level represents a high-light condition for cowpea (a C_3 species), it was chosen to evaluate the maximum photosynthetic potential under the experimental treatments, based on conditions typically observed for cowpea genotypes under semi-arid environments (Silva et al. 2019; Dias et al. 2025), ensuring that photosynthetic rates reflected the plants' maximum capacity without inducing severe photoinhibition. Evaluations were performed on the third fully expanded leaf from the apex of the main branch to obtain the photosynthetic rate (*A*) (μmol CO_2 m^{-2} s^{-1}), transpiration rate (*E*) (mmol H_2O m^{-2} s^{-1}), stomatal conductance (*gs*) (mmol m^{-2} s^{-1}), and intercellular CO_2 concentration (*Ci*) (μmol CO_2 m^{-2} s^{-1}). From these data, the instantaneous water use efficiency (WUEi) [(μmol CO_2 m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$] was quantified, and the instantaneous carboxylation efficiency (iCE) was obtained through the ratio (*A/Ci*) [(μmol CO_2 m^{-2} s^{-1}) (μmol CO_2 m^{-2} s^{-1})$^{-1}$].

Non-Oxidative and Antioxidant Mechanism Activity

Proline content was determined using the colorimetric method described by Bates et al. (1973), as modified by Bezerra Neto and Barreto (2011). Initially, 250 mg of fresh material was weighed and macerated in 5 mL of 3% sulfosalicylic acid, then centrifuged at 2000 rpm for 10 minutes. The acid ninhydrin solution was diluted in 99% glacial acetic acid and 6.0 M phosphoric acid (88%), heated to 40 °C for 5 minutes. In 2.5 mL tubes, the supernatant, ninhydrin, and glacial acetic acid were added, capped, shaken, and incubated in a water bath at 100 °C for one h. The tubes were placed in an ice bath for one hour, then 99% pure toluene was added and shaken again, and subsequently used to determine the PRO content; results were expressed as (μmol g^{-1} of fresh mass).

The activity of the antioxidant mechanism was evaluated by determining the enzymatic activities of superoxide dismutase (SOD, UA gMF^{-1}), catalase (CAT, μmol of H_2O_2 min^{-1} gMF^{-1}), and ascorbate peroxidase (APX, μmol of asc min^{-1} gMF^{-1}). 200 mg of fresh material were ground with 2 mL of 100 mM phosphate buffer (pH 7.5) associated 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 su-

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pernatant was aspirated and aliquoted into 2.5 mL plastic tubes (Eppendorf), then stored at -80 °C for enzymatic analysis.

Superoxide dismutase (SOD) activity was determined using a reaction mixture consisting of 100 µL of enzyme extract, 0.3 mL of methionine13 mM, 0.1 mL of p-nitro blue tetrazolium (NBT) 75 µM, 0.1 mL of EDTA 100 nM, 0.2 mL of riboflavin 2µM, 0.75 mL of deionized water, and 1.5 mL of sodium phosphate buffer 50 mM at pH 7.8. Then, the reaction absorbance was measured in the absence of the enzyme extract. Thus, the determination of SOD was based on the photoreduction inhibition capacity of p-nitro blue tetrazolium (NBT) (Beauchamp and Fridovich, 1971).

Catalase (CAT) activity was determined by adding 150 µL of the enzyme extract, 1950 µL of potassium phosphate buffer (100 mM at pH 7.5), and 150 µL of the extraction buffer (monobasic potassium phosphate (1000 mM), + dibasic potassium phosphate (1000 mM) + EDTA (1mM), + ascorbic acid (10 mM), + polyvinylpyrrolidone PVP (1%)), and hydrogen peroxide 50 mM. Enzyme activity was determined by measuring the decrease in absorbance at 240 nm, as described by Kar and Mishra (1976).

The activity of the enzyme ascorbate peroxidase (APX) was determined by adding 100 µL of the extract, and 2.7 mL of the determination buffer at 50 mM at pH 6.0 (constituted of monobasic potassium phosphate + dibasic potassium phosphate + ascorbic acid (0.8 mM)). The reaction was initiated by the addition of 200 µL of hydrogen peroxide (H_2O_2) (30 mM). Enzyme activity was determined by measuring the decrease in absorbance at 290 nm (Nakano and Asama, 1981).

Statistical Analysis

The data obtained were subjected to the Shapiro–Wilk normality test (Shapiro and Wilk, 1965). Once the assumptions were met, the data were subjected to analysis of variance using the F test ($p < 0.05$), followed by the Tukey test ($p \leq 0.05$) for the priming combinations, and by the t-student ($p \leq 0.05$) for the water replacement levels, using the statistical software SISVAR® v. 5.6 (Ferreira 2019).

2   Results

Growth

Water deficit (W35) negatively affected cowpea "BRS Tapaihum" growth, resulting in significant reductions in Total Leaf Area (TLA) and Total Dry Mass (TDM) at both phenological stages (V4 and V5) across all priming treatments (Figure 1A-D).

The total leaf area (TLA) under water deficit (W35) was consistently higher in SiMP-primed plants compared to the control (C) and PEG 6000 (P1). At the V4 stage, the amber SiMP-primed plants (P3) maintained TLA (46.39 cm²), exhibiting the smallest reduction among all primings subjected to water deficit (Figure 1A). On the other hand, under optimal conditions (W75), P3 produced the smallest leaf area. In contrast, blue SiMP-primed plants (P2) promoted the largest leaf areas at W75 in both V4 and V5, suggesting a more pronounced biostimulant effect when water deficit is not limited (Figure 1A and 1B).

Biomass accumulation (TDM) was consistent with observations verified in TLA, with an apparent reduction in TDM at both phenological stages under water deficit (W35) (Figure 1C, D). Under water deficit (W35), a dynamic change in the priming response was observed between developmental stages; at stage V4, the amber SiMP-primed plants (P3) stood out as the most efficient in mitigating biomass reduction, not differing statistically from (P1), but significantly surpassing (P2) under water deficit (W35). At stage V5, blue amber SiMP-primed plants (P2) were superior, promoting the highest TDM accumulation (0.526 g) under water deficit (W35). Under optimal irrigation conditions (W75), the blue SiMP-primed plants (P2) treatment provided the highest TDM accumulation at both stages (V4 and V5). The TDM results reinforce that SiMPs are effective but demonstrate a functional difference: blue SiMPs (P2) provide superior initial protection. In contrast, amber SiMPs (P3) indicate improved growth and long-term resilience (Figure 1C and 1D).
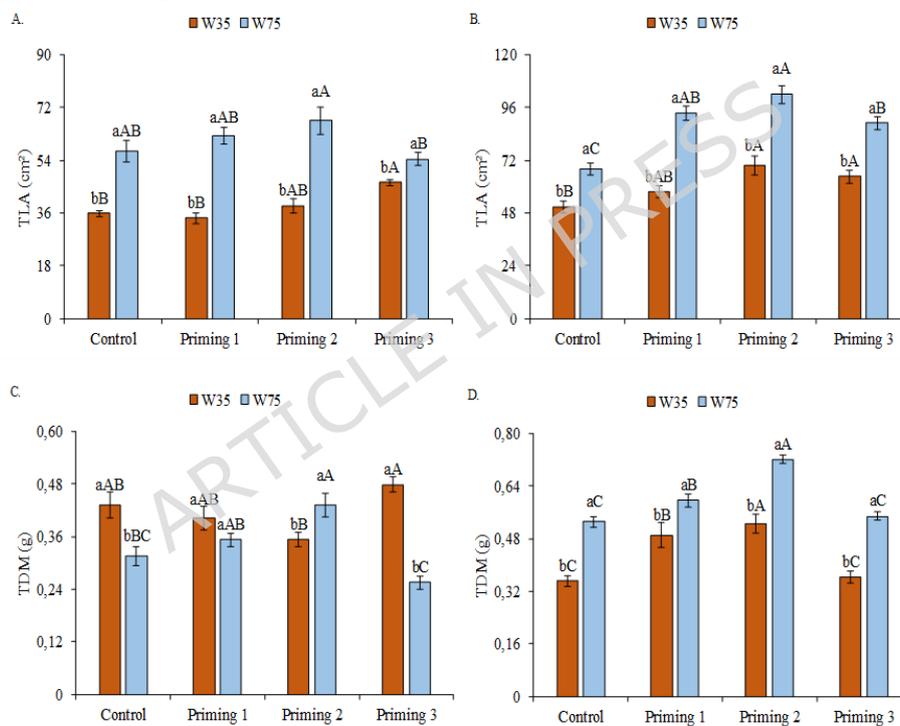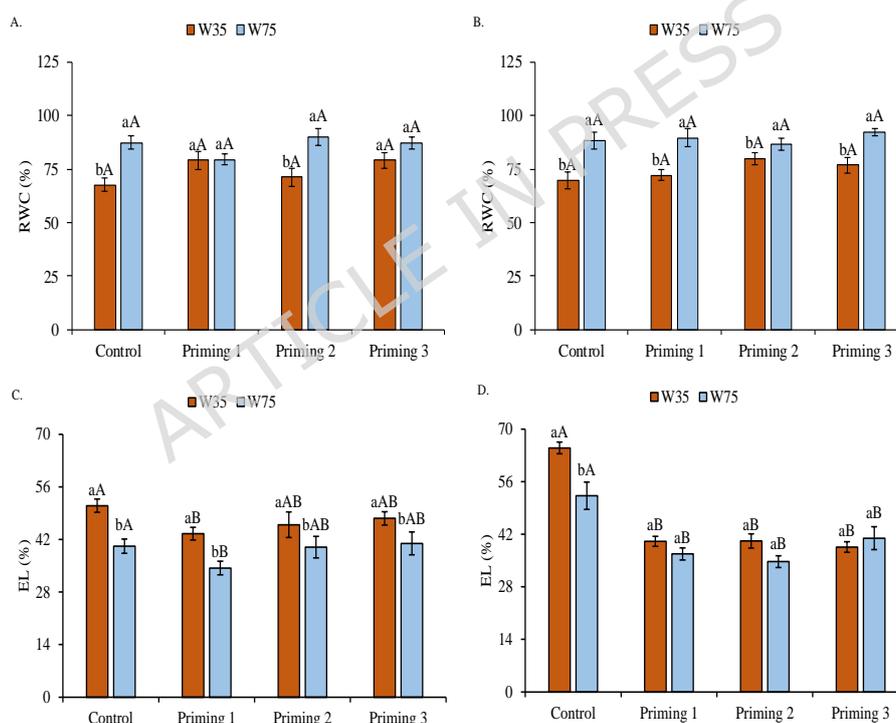

Figure 1. Total leaf area (TLA) at two phenological stages V4 (A) and V5 (B), and total dry mass (TDM) at two phenological stages V4 (C) and V5 (D) in cowpea "BRS Tapaihum" subjected to different conditioning – Control, priming 1 (Ψh - 0.4 MPa), priming 2 (Ψh -0.4 MPa + 200 mg L^{-1} blue glass microparticles (SiMPsB)), and priming 3 (Ψh -0.4 MPa + 200 mg L^{-1} amber glass microparticles (SiMPsA)), and two irrigation depths (W35 and W75). Capital letters differentiate primings within the irrigation treatment (Tukey p ≤ 0.05) and lowercase letters differentiate irrigation treatment (t-student p ≤ 0.05).

Water Status and Membrane Damage Indicator

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The relative water content (RWC) of cowpea "BRS Tapaihum" was significantly influenced only by the studied irrigation depths. At the V4 stage, only the control (C) and the blue SiMP-primed plants (P2) at W75 had the highest RWC values (Figure 2A). However, the application of the different priming agents was not the determining factor in maintaining RWC under water deficit, indicating that the protective effect of SiMP-primed was not based on maintaining absolute leaf turgor.

In contrast, cell membrane damage, measured by electrolytic leakage (EL), demonstrated SiMP-primed induced protection. Underwater deficit (W35) induced an overall increase in EL at both the V4 and V5 stages (Figures 2C and 2D). However, at both stages, control plants consistently exhibited the highest leakage rates under water deficit (W35) and under optimal conditions (W75). At stage V5, priming with amber SiMP-primed plants (P3) managed to maintain membrane integrity under water deficit (W35), providing a reduction in EL values (40.62% reduction compared to the control under water deficit W35), followed by blue SiMP-primed plants (P2) (Figures 2C and 2D).

Figure 2. Relative water content (RWC) at two phenological stages V4 (A) and V5 (B), and electrolyte leakage (EL) at two phenological stages V4 (C) and V5 (D) in cowpea "BRS Tapaihum" subjected to different conditioning – Control, priming 1 (Ψh - 0.4 MPa), priming 2 (Ψh - 0.4 MPa + 200 mg L^{-1} blue glass microparticles (SiMPsB)), and priming 3 (Ψh -0.4 MPa + 200 mg L^{-1} amber glass microparticles (SiMPsA)), and two irrigation depths (W35 and W75). Capi-

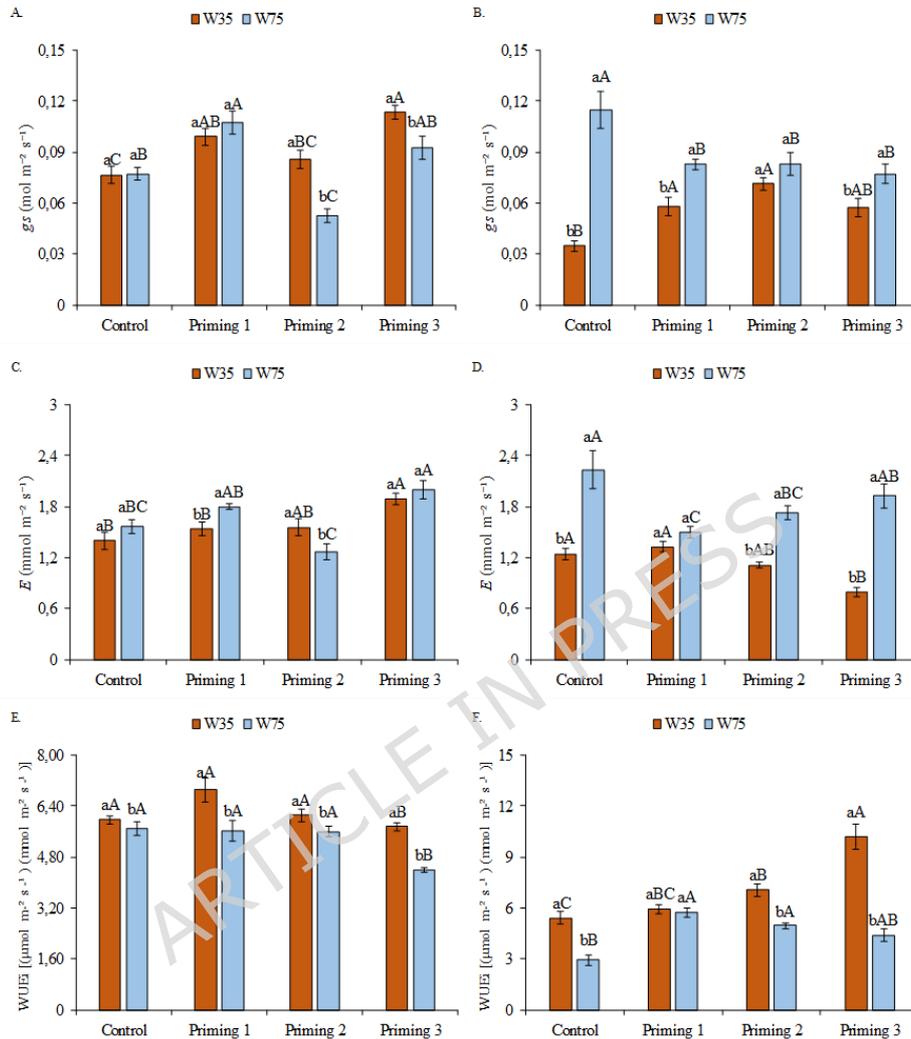tal letters differentiate primings within the irrigation treatment (Tukey $p \leq 0.05$) and lowercase letters differentiate irrigation treatment (t-student $p \leq 0.05$).

Gas Exchange

Water deficit (W35) imposed significant physiological limitations on cowpea plants "BRS Tapaihum", as evidenced by the overall decline in gas exchange parameters compared to adequate irrigation conditions (W75). Although measurements were performed under high fixed irradiance (1200 μmol m^{-2} s^{-1}), which represents a demanding photochemical condition for this C$_3$ species, relatively distinct responses were observed between treatments (Figures 3A–F and 4A–F).

At the V4 stage under water deficit (W35), in SiMP-primed plants (P3), the highest stomatal conductances (gs) and transpiration rates (E) (Figure 3A and 3C). Conversely, in SiMP-primed plants (P2), reduced gs was compared to P3, which was still superior to the control. At stage V5, P2 and P1 showed the highest gs under water deficit, while the control showed lower gs (Figure 3B).

While absolute values of water-use efficiency (WUEi) were influenced by the measurement conditions with IRGA, the SiMP-primed seedlings showed the highest water-use efficiency (WUEi), equivalent to an increase of 46.85% compared to the control with water deficit (W35) in the two stages (V4 and V5) (Figure 3E and 3F).

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Figure 3. gs at two phenological stages V4 (A) and V5 (B), E at two phenological stages V4 (C) and V5 (D), and WUEi at two phenological stages V4 (E) and V5 (F) in cowpea "BRS Tapaihum" subjected to different conditioning – Control, priming 1 (Ψh - 0.4 MPa), priming 2 (Ψh -0.4 MPa + 200 mg L^{-1} blue glass microparticles (SiMPsB)), and priming 3 (Ψh -0.4 MPa + 200 mg L^{-1} amber glass microparticles (SiMPsA)), and two irrigation depths (W35 and W75). Capital letters differentiate primings within the irrigation treatment (Tukey p ≤ 0.05) and lowercase letters differentiate irrigation treatment (t-student p ≤ 0.05).

The net CO_2 assimilation rate (A) was reduced by water deficit; however, SiMP-primed plants (both blue and amber) maintained higher relative rates of A compared to non-primed plants under water deficit (W35) (Figure 4E). Despite the high excitation pressure imposed by the IRGA light source, the internal CO_2 concentration (Ci)

tended to be lower or more stable in SiMP-primed plants, suggesting a better relative capacity for carboxylation under the combined pressure of water deficit and high light (Figures 4A and 4B). Despite the high excitation pressure potentially imposed by the IRGA light source, the SiMP-primed plants showed a more efficient instantaneous carboxylation (iCE) under water deficit (W35) compared to the control, suggesting that priming partially mitigated the non-stomatal limitations exacerbated by the combined pressure of water deficit and measurement irradiance (Figure 3C and 3D).
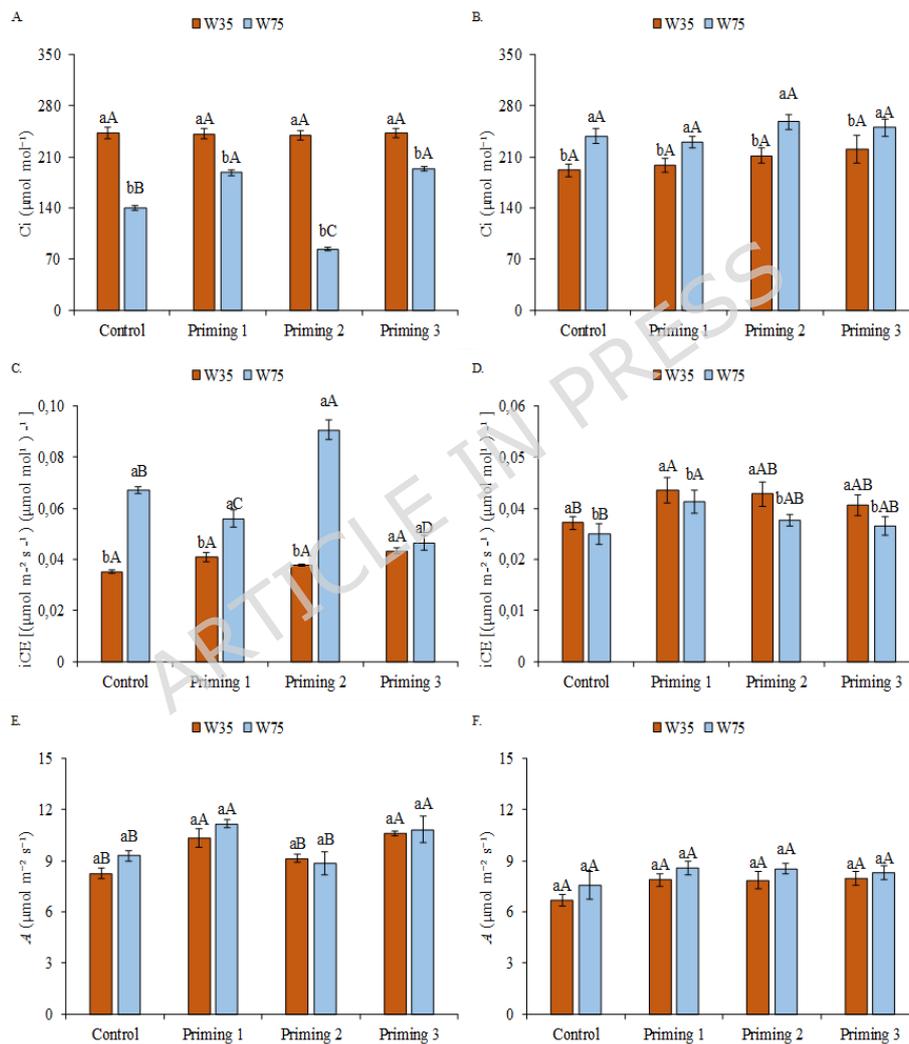
Figure 4. C_i at two phenological stages V4 (A) and V5 (B), iCE at two phenological stages V4 (C) and V5 (D), and A at two phenological stages V4 (E) and V5 (F) in cowpea "BRS Tapai-hum" subjected to different conditioning – Control, priming 1 (Ψh - 0.4 MPa), priming 2 (Ψh - 0.4 MPa + 200 mg L^{-1} blue glass microparticles (SiMPsB)), and priming 3 (Ψh -0.4 MPa + 200

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mg L^{-1} amber glass microparticles (SiMPsA)), and two irrigation depths (W35 and W75). Capital letters differentiate primings within the irrigation treatment (Tukey p ≤ 0.05) and lowercase letters differentiate irrigation treatment (t-student p ≤ 0.05).

Non-Oxidative and Antioxidant Mechanism Activity

Water deficit (W35) induced a robust response in the antioxidant defense system and the accumulation of proline (PRO). Given the high irradiance during prior physiological assessments, these biochemical responses likely reflect a synergistic defense against both water deficit and measurement-induced light stress (Figures 5A-I).

The PRO content significantly increased under water deficit (W35) in both stages (Figure 5A and 5B). However, under water deficit (W35), SiMP-primed plants (P2 and P3) exhibited lower PRO accumulation compared to PEG-primed plants (P1). This relative difference suggests that SiMP-primed may have enhanced structural protection and membrane stability (as evidenced by EL results), potentially reducing the absolute requirement for osmotic adjustment even under the demanding light conditions of the study.

The activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) was similar. At the V4 stage, under water deficit (W35), the highest SOD and CAT activities were observed in treatment P1. On the other hand, plants treated with SiMP-primed plants (P2 and P3) showed comparatively lower activities (reductions of 20.17% and 23.51%, respectively, compared to P1 (Figure 5C-E)).

However, APX activity showed a distinct pattern, with blue SiMP-primed plants (P2) exhibiting the highest activity at the V4 stage under water deficit (W35) (Figure 5G).

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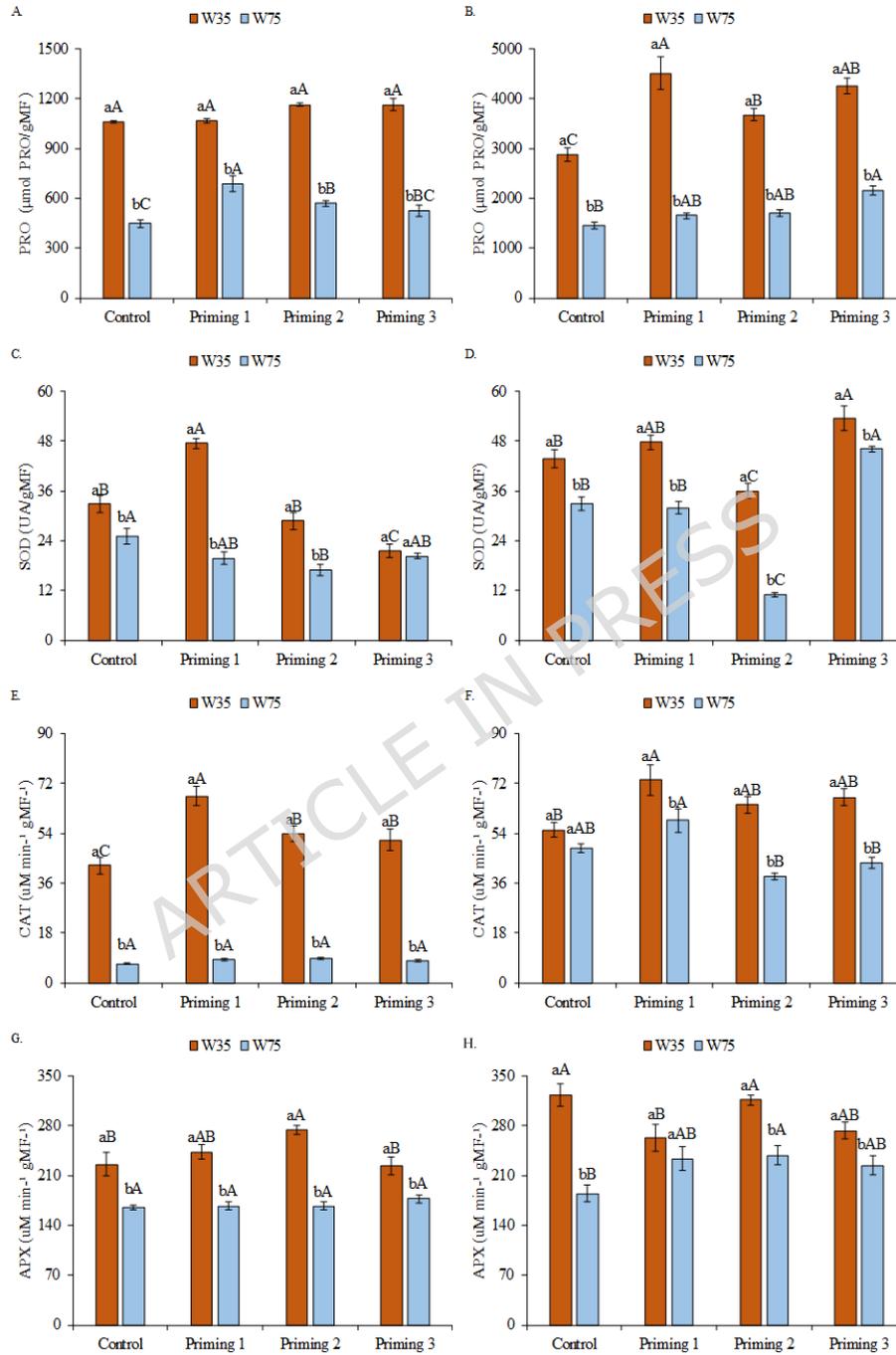
Figure 5. PRO at two phenological stages V4 (A) and V5 (B), SOD at two phenological stages V4 (C) and V5 (D), CAT at two phenological stages V4 (E) and V5 (F), and APX at two phe-

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nological stages V4 (G) and V5 (H) in cowpea "BRS Tapaihum" subjected to different conditioning – Control, priming 1 (Ψh - 0.4 MPa), priming 2 (Ψh -0.4 MPa + 200 mg L^{-1} blue glass microparticles (SiMPsB)), and priming 3 (Ψh -0.4 MPa + 200 mg L^{-1} amber glass microparticles (SiMPsA)), and two irrigation depths (W35 and W75). Capital letters differentiate primings within the irrigation treatment (Tukey p $\leq$ 0.05) and lowercase letters differentiate irrigation treatment (t-student p $\leq$ 0.05).

3   Discussion

The comparative physiological advantage observed in SiMP-priming plants (both blue and amber) under water deficit (W35) can be attributed to a synergistic mechanism involving stomatal regulation, membrane stability, and antioxidant response. It is essential to recognize that the high irradiance (1200 µmol m^{-2} s^{-1}) used during gas exchange measurements likely imposed a high excitation pressure on the photosynthetic apparatus of cowpea (C3 species). Consequently, the marked induction of antioxidant enzymes (SOD, CAT, and APX) in SiMP-primed plants could be interpreted as a coordinated defense against the combined stress of water deficit and the photooxidative pressure induced by the measurement. Rather than a simple mitigation of water deficit, the SiMP-primed treatment appears to have functioned as a 'physiological buffer'. By maintaining better membrane integrity (as shown by lower EL) and optimizing carboxylation efficiency (iCE), the microparticles allowed the plants to manage ROS generation more effectively than the control group. This integrated response explains why SiMP-primed plants maintained a superior relative performance even under the demanding light conditions of the study, protecting the photosynthetic machinery from irreversible damage (Figures 3, 4, and 5).

Under low-substrate water potential conditions, such as -0.4 MPa, ROS production can have serious consequences, including decreased cell turgor, altered membrane permeability and stability, and interference with normal plant functions, primarily due to osmotic and redox imbalances (Silva et al. 2020). These disturbances typically result in the loss of developing organs during the growth phase, limiting seedling height and, consequently, reducing biomass accumulation in the aerial and root tissues of cowpea (Melo et al. 2022).

The effectiveness of conditioning with blue (SiMPsB) and amber (SiMPsA) SiMP-priming in mitigating the adverse effects of water deficit (W35) is closely linked to their unique chemical properties. As shown in Table 1, both the SiMP-priming treatments share a standard matrix rich in silicon oxide (SiO_2), sodium oxide (Na_2O), and calcium oxide (CaO); however, the subtle variations in their elemental composition, such as trace amounts of Cobalt oxide in SiMPsB and Iron/Sulfur compounds in SiMPsA, conferred distinct functional profiles during seed priming. This chemical divergence is critical, as the presence of these metal and non-metal oxides likely alters the kinetics of silicon release from the glass matrix and modulates downstream physiological responses, ultimately reflecting the observed increases in total leaf area (TLA) and biomass accumulation (TDM) (Figures 1 and 2).

In this context, the presence of iron and manganese is particularly relevant, as they are essential cofactors for enzymes involved in the water-splitting complex of Photosystem II and serve as prosthetic groups for Fe-SOD and Mn-SOD isoforms (Alejandro et al. 2020). This enhanced micronutrient availability, facilitated by the specific composition of Amber SiMP-priming treatments (Table 1), likely bolstered the antioxidant defense system. Such reinforcement may have mitigated oxidative damage specifically within guard cells, thereby supporting the maintenance of stomatal aperture and CO_2 assimilation even under the W35 water deficit and the high excitation pressure (Guo et al. 2025).

These differences in composition can affect the solubility of the vitreous microparticles and the subsequent availability of Si to the plants, as well as potential synergistic effects of the micronutrients present in the glass matrix, which may favor better stomatal control and osmoprotection in one treatment over the other. It may help mitigate the harmful effects of water deficit across cowpea cultivars, thereby increasing water potential and promoting species growth (Silva et al. 2020; Alencar et al. 2024). Similar results were observed in a study by Alencar et al. (2024) using the cowpea cultivar "BRS Itaim".

Under stress conditions, silicon (Si) is known to regulate specific metabolic processes (Ahanger et al. 2020; Alencar et al. 2024; Boucelha et al. 2025; Dias et al. 2025). However, beyond water deficit mitigation, the beneficial effects of Si were evident even under well-watered conditions (W75), where SiMP-priming, particularly with blue SiMP-priming (P2), exhibited a biostimulatory impact. The superior values of TDM and TLA in SiMP-priming plants (P2) (Figure 1) suggest that the presence of cobalt, alongside silicon, may have optimized basal metabolism and water-use efficiency (Boucelha et al. 2025). This enhancement is likely supported by improved leaf architecture through silica deposition and higher instantaneous carboxylation efficiency (iCE), which reinforces the role of Si as a beneficial element that optimizes plant performance regardless of the stress level (Ali and Bijay-Singh, 2025).

In contrast, the Amber SiMP-priming (P3) demonstrated a more specialized role in structural protection under water deficit (W35). At the stage of V5, SiMP-priming (P3) was the most effective treatment in maintaining cell membrane integrity, significantly reducing electrolyte leakage by 40.62% compared to the control (Figure 2D). This robust protection under stress can be attributed to the specific enrichment of Fe_2O_3 and MnO in the Amber matrix (Table 1). As previously noted, these elements are crucial for the stability of the photosynthetic apparatus and ROS scavenging, suggesting that the Amber SiMP-priming provides a more targeted structural shield when the plant is subjected to the combination of water deficit and high measurement irradiance.

The physiological resilience of cowpea was further supported by the secondary elements present in the glass matrix (Table 1). The higher concentrations of CaO (10.072%) and Na_2O (13.629%) in the microparticles (Table 1) likely acted in synergy with silicon, where calcium served as a structural component and secondary messenger in stress signaling, while micro-doses of sodium potentially aided in osmotic adjustment. This is reflected in the maintenance of higher relative water content (RWC) in SiMP-treated plants compared to the control under water deficit (W35).

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In the plants (P1), the high PRO levels were not associated with improved RWC or biomass (TDM), indicating that in the absence of SiMP-priming, PRO accumulation may be a symptom of metabolic stress rather than effective osmoregulation. In contrast, the balanced metabolic profile of SiMP-primed plants, particularly P3 at the V5 stage, suggests a more efficient protective strategy, where PRO works alongside the 'physiological buffer' provided by the glass microparticles to stabilize macromolecules without the metabolic cost seen in non-primed plants (Costa et al. 2022; Zhao et al. 2025). Studies with similar results were reported by Silva et al. (2020) and Alencar et al. (2024), indicating that SiMPs increase proline content.

This strategy is also reflected in the temporal dynamics of the antioxidant network (SOD, CAT, and APX). At the V4 stage, the reduced enzyme activity in SiMP-treated plants suggests that the initial structural protection, acting as a physical barrier, is sufficient to minimize ROS formation. However, under prolonged stress at the V5 stage, the sustained or increased enzyme activity indicates that the slow, continuous release of Si from the glass matrix (Table 1) provided essential metabolic support (Raza et al. 2023; Boucelha et al. 2025; Dias et al. 2025). This dual action, characterized by initial structural prevention followed by long-term biochemical support, conferred a distinct resilience to cowpea, allowing it to navigate the combined pressure of water deficit and high measurement irradiance more effectively than non-primed plants.

Some authors have reported that exogenous Si application increases the activity of antioxidant enzymes in the leaves of several crop species, particularly under stressful conditions (Kim et al. 2017; Mushtaq et al. 2020; Costa et al. 2022; Ellouzi et al. 2023; Alencar et al. 2024). In studies involving corn and wheat plants, priming with SiMPs enhanced the activity of antioxidant enzymes under water deficit (Sattar et al. 2017; Parveen et al. 2019; Hameed et al. 2021). In addition to these studies, it is noteworthy that cowpea plants "BRS Itaim" conditioned with SiMPs under W50 also showed increased SOD activity, which is considered the first line of defense against damage induced by reactive oxygen species (ROS) (Alencar et al. 2024).

In this context, the use of glassy silicon microparticles (SiMPs) offers significant appeal for agricultural sustainability and long-term use. First, SiMPs are produced from recycled glass, thereby transforming a waste product into a low-cost agricultural input that aligns with the UN Sustainable Development Goals (SDGs). Second, with respect to the soil-plant system, the accumulation of SiMPs (primarily SiO_2) in the soil is considered inert and ecologically safe, unlike chemical fertilizers, which can cause leaching, salinization, or contamination.

Therefore, it is believed that, in the long term, the presence of these microparticles in the soil can function as a slow-release silicon reservoir, providing residual Si for subsequent crops. This gradual release has the potential to increase cropland resilience and soil water- and nutrient-retention capacity in semi-arid regions, making it a promising and sustainable strategy for food security (Leite et al. 2023).

Despite the promising results demonstrated in mitigating drought stress, it is essential to recognize the limitations inherent in this study. The tolerance assessment was restricted to the vegetative phases (V4 and V5), and the stress condition was simulated with PEG 6000 in a controlled environment (predominantly osmotic stress). There-

fore, future research should prioritize validating the efficacy of SiMPs under field drought conditions, which involve both osmotic and matrix stress, throughout the crop cycle, with a focus on evaluating final yield.

Furthermore, regarding the gas exchange assessments, it is essential to note that measurements were conducted under a constant actinic photon flux density of 1200 μmol m^{-2} s^{-1} and ambient CO_2 (400 ppm). While these conditions reflect the high-irradiance levels typical of the Brazilian semi-arid region to which cowpea is naturally acclimated, we acknowledge that for C_3 species, this irradiance may approach or exceed the light saturation point under certain stress levels, potentially increasing photorespiratory pressure. However, the consistent differences observed between treatments, where SiMPs-primed plants showed enough results even under the challenging light conditions typical of semi-arid environments, reinforce that the observed responses were likely a result of the treatments' systemic effect on stress memory rather than an artifact of the measurement environment.

Additionally, future detailed studies on the release kinetics of SiMPs (blue and amber) in soil and the correlation between their availability and plant uptake are crucial for improving agronomic recommendations. Therefore, investigating the precise molecular mechanisms by which priming with SiMPs induces stress memory and modulates gene expression, compared to priming with PEG, could provide insights into the genetic basis of induced tolerance in plants.

4   Conclusion

The application of PEG 6000 had the least mitigating effects on water deficit, resulting in limitations in water status and significant damage to the 'BRS Tapaihum' cowpea defense mechanisms and growth.

Seed priming with silicon glass microparticles (SiMPs) has proven to be an effective bifunctional strategy for 'BRS Tapaihum' cowpea. The SiMPs-priming confers comprehensive resilience, acting as biostimulants by optimizing WUEi under optimal conditions (W75) and as structural protectants by stabilizing membranes under water deficit (W35).

The use of this recycled glass material represents a sustainable, low-cost solution with considerable potential to increase productivity and food security in semi-arid agricultural systems.

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.

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