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Abstract

Drought stress severely limits maize growth and productivity worldwide. In this study, we examined the effects of foliar-applied carbon nanoparticles (CNPs) on morphological and physiological traits in maize plants exposed to drought stress for 25 days. Two maize hybrids one drought-tolerant (LG 36745 PRO4) and one drought-sensitive (AG 8088 PRO2) were treated with 0 or 1.0 mL L^{-1} of a CNP-based nanofertilizer and exposed to three drought levels: 0 MPa (control), -0.4 MPa (moderate stress), and -0.8 MPa (severe stress). The experiment followed a 2 × 2 × 3 factorial design with four replicates. Results indicated that drought stress adversely affected most morphological and physiological traits, particularly in the drought-sensitive hybrid. However, foliar CNP application showed strong potential to alleviate drought's adverse effects in maize under moderate and severe stress, primarily by preserving plant water status, enhancing water use efficiency, carboxylation efficiency, photosynthetic rate, and early growth in challenging environments. These findings will provide the basis for future research on management practices adopted to control drought and ensure the development of modern and sustainable agriculture.

Keywords: C-Dots; nanofertilizer; nutritional management; sustainable agriculture; *Zea mays*

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1. Introduction

Maize (*Zea mays* L.) ranks among the world's primary cereal crops, owing largely to its extensive applications in human and animal nutrition, biofuel production, and various industrial products. Although Brazil is one of the world's largest maize producers, the growth and yield of the crop have frequently been impacted by adverse environmental conditions, such as high temperature and water deficiency [1–3]. The Brazilian Cerrado region produces about two-thirds of the national maize production [4]. However, the Cerrado experiences a pronounced dry season in winter (April to September), leading farmers in many cases to sow maize under suboptimal soil moisture, potentially compromising early plant establishment in the field.

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Low soil water availability during sowing can hinder and delay seed germination by reducing imbibition, leading to suboptimal plant stands [3] and compromising crop development and yield potential [5–7]. Moreover, low soil water availability triggers various morphological, physiological, and metabolic alterations in plants [6,8,9], inhibiting leaf expansion and overall growth while adversely affecting transpiration and nutrient uptake. Thus, drought stress presents significant challenges to global maize production, making research into innovative, sustainable mitigation strategies crucial for advancing food security, plant protection, and environmental resilience amid climate change [10,11].

Nanofertilizers represent an innovative and sustainable approach to enhancing plant growth, physiology, and metabolism under drought conditions [12–15]. However, their efficacy in mitigating drought's negative effects hinges on the plant's tolerance mechanisms, genotype-specific tolerance, stress severity, and interactions among genotype, nanofertilizer, and environment. Key drought tolerance mechanisms in early maize growth involve greater photoassimilate allocation to roots, chlorophyll maintenance, leaf area reduction, and synthesis of metabolites for osmotic adjustment and turgor preservation [3,6,16]. These mechanisms collectively support optimal plant development in adverse conditions.

Carbon nanoparticle (CNP)-based nanofertilizers offer a sustainable option for alleviating drought stress in agricultural systems, as their molecules are biodegradable and environmentally benign. CNPs also possess unique structural and mechanical properties, with their use in drought management expanding recently [17–20]. Alluqmani and Alabdallah [19] demonstrated that CNP application alleviates drought stress by regulating water status, boosting chlorophyll and photosynthetic rates, and reducing oxidative stress via antioxidant activation and ROS scavenging. Foliar CNP application similarly mitigated drought damage to growth, metabolism, and physiology in bell pepper (*Capsicum annuum* L.) plants [20]. Shekhawat et al. [17] reported that CNP application enhances the antioxidant defense and abiotic stress tolerance in cowpea (*Vigna radiata* L.) Wilczek] plants. Chen et al. [18] showed that CNP application reduces drought's adverse effects on tomato (*Solanum lycopersicum* L.) growth and yield, particularly through improved water status, use efficiency, and antioxidant regulation

Thus, CNPs present an excellent tool for enhancing growth, nutrition, biochemistry, and physiology in plants under stress. This approach can lessen drought impacts, lower production costs, and promote sustainable, innovative farming systems. However, the effects of CNP-based nanofertilizers like Arbolina® on inducing drought tolerance in maize remain unexplored. This study therefore assessed foliar CNP application's effects on morphological and physiological traits in maize under varying drought levels. We evaluated CNP efficacy in enhancing drought tolerance in two common Cerrado maize hybrids: one tolerant and one sensitive.

2. Results

Analysis of variance revealed significant effects ($P \leq 0.05$) from hybrids, drought levels, and CNP application, plus interactions among them, for most traits (Table 1). Significant hybrid × drought and hybrid × CNP interactions for many traits indicate differential responses between tolerant and sensitive hybrids under stress or CNP treatment. Accordingly, we unfolded interactions and present responses for each hybrid separately.

Coefficients of variation (CVs) were <10% for all traits, indicating high precision for lab assays (Table 1). The CV is a measure of dispersion used to estimate the precision of the experimental trial, and when the values are less than 10% it indicates that the results of the experiment have excellent precision.

Table 1. Summary of analysis of variance for measurements of morphological and physiological traits of maize hybrids (*Zea mays* L.) in response to drought stress levels and foliar application of carbon nanoparticles.

Sources of variation	Probability > F							
	PH	LA	LLR	TRL	RV	SDM	RDM	TDM

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Hybrid (H)	<0.001	<0.001	<0.001	<0.001	<0.001	0.012	<0.001	<0.001
Drought (D)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001
Nanofertilizer (NF)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
H × D	<0.001	<0.001	<0.001	0.001	<0.001	0.065	<0.001	0.003
H × NF	0.367	0.054	0.040	<0.001	<0.001	<0.001	<0.001	<0.001
D × NF	<0.001	0.048	<0.001	0.072	0.018	0.036	0.550	0.112
H × D × NF	0.476	0.849	0.157	0.191	0.308	<0.001	0.134	<0.001
CV (%)	4.64	4.88	4.32	6.30	5.81	4.97	5.45	4.43

	RWC	***A***	**Ci**	***E***	*g*s	**WUE**i	***A*/Ci**
Hybrid (H)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Drought (D)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Nanofertilizer (N)	<0.001	<0.001	<0.001	<0.001	0.067	<0.001	<0.001
H × D	<0.001	<0.001	<0.001	<0.001	<0.001	0.035	0.015
H × N	0.018	0.028	0.044	0.019	0.035	0.048	0.004
D × N	<0.001	0.042	0.038	0.037	0.068	0.132	0.146
H × D × N	0.012	0.214	0.359	0.403	0.609	0.544	0.799
CV (%)	3.79	6.47	4.15	3.36	8.70	4.58	9.03

PH: plant height. LA: leaf area. LLR: length of the longest root. TRL: total root system length. RV: root volume. SDM: shoot dry matter. RDM: root dry matter. TDM: total dry matter. RWC: relative water content. A: photosynthetic rate. Ci: intercellular CO2 concentration. E: transpiration rate. gS: stomatal conductance. WUEi: intrinsic water use efficiency. A/Ci: carboxylation efficiency.

Drought levels and CNP application significantly ($P < 0.05$) influenced plant height, leaf area, and relative water content in both hybrids (Figure 1). Drought restricted shoot growth in both hybrids, with the sensitive one showing greater height reductions (Figure 1A, B). Under severe stress, sensitive and tolerant hybrids exhibited average height reductions of 57% (43.2 to 18.5 cm) and 37% (44.4 to 27.9 cm), respectively, compared to controls. Foliar CNP application, however, mitigated these height reductions. CNP-treated sensitive hybrid plants had greater heights under moderate and severe stress (Figure 1A). Tolerant hybrid plants similarly showed height increases with CNPs under moderate stress (Figure 1B).

Leaf area decreased progressively with increasing drought intensity in both hybrids (Figures 1C, D). CNP fertilization, however, yielded significantly larger leaf areas under moderate and severe stress. Under non-stress, relative water content stayed consistent at 92-95% for both hybrids (Figures 1E, F). Drought reduced this to 72-84% under moderate stress and 56-68% under severe. CNP application attenuated these reductions in the sensitive hybrid under both stress levels and in the tolerant hybrid under severe stress (Figures 1E, F).

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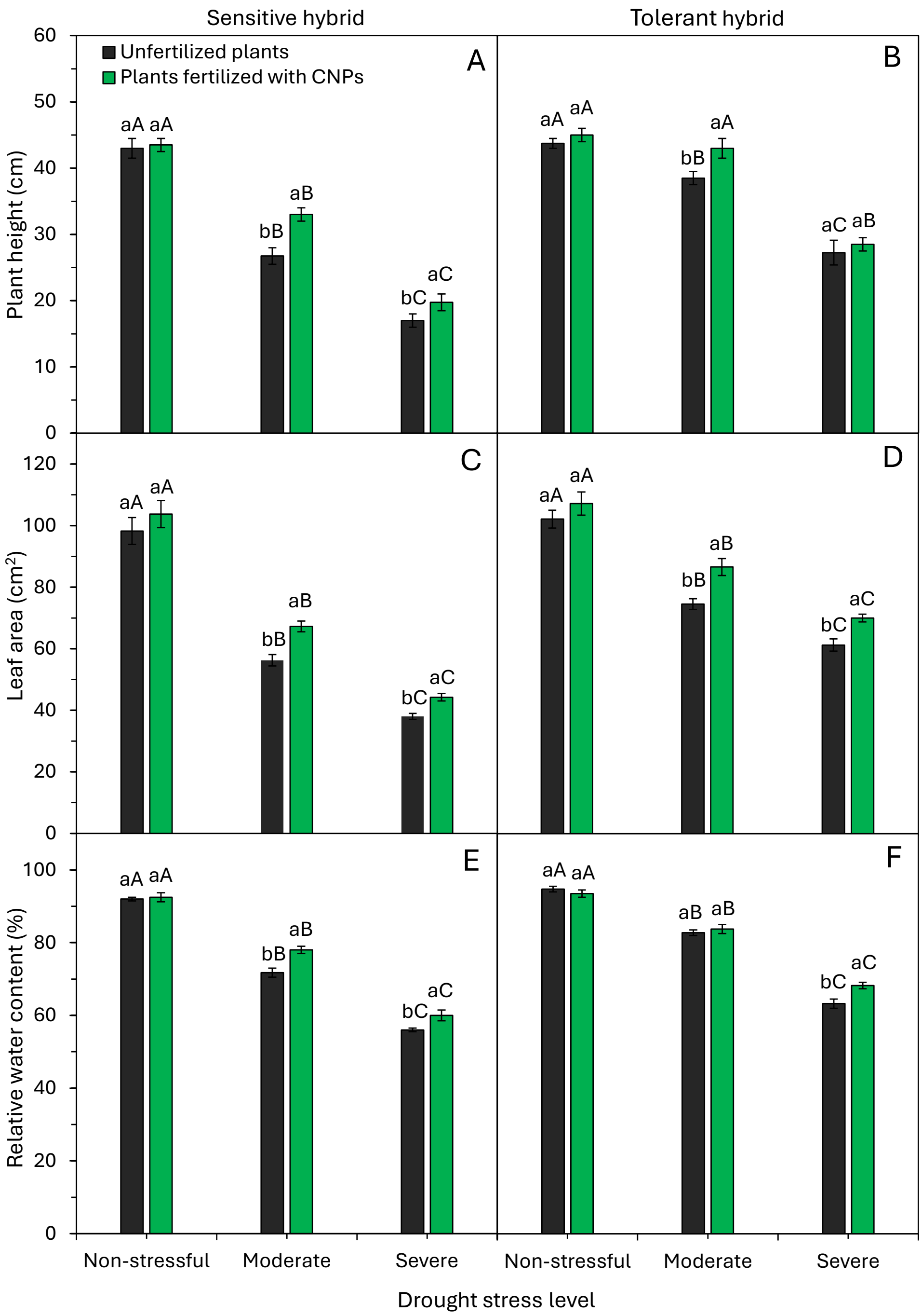

Figure 1. Effect of drought stress levels and foliar application of carbon nanoparticles (CNPs) on plant height (A and B), leaf area (C and D) and relative water content (E and F) of maize plants from a drought-sensitive hybrid (A, C, D) and another drought-tolerant hybrid (B, D, E). Bars followed by distinct lowercase letters for the foliar application of CNPs or distinct uppercase letters for the drought stress levels show significant differences by the Tukey test ($\alpha = 0.05$). Data refers to mean values (n = 20) ± standard error of the mean.

Drought and CNP application significantly ($P < 0.05$) influenced root system growth in both hybrids (Figure 2). Moderate and severe stress inhibited root development in the sensitive hybrid. Longest root length, total root length, and root volume declined progressively with stress intensity (Figures 2A, C, E). In the tolerant hybrid, root growth was inhibited only under severe stress (Figures 2B, D, and F).

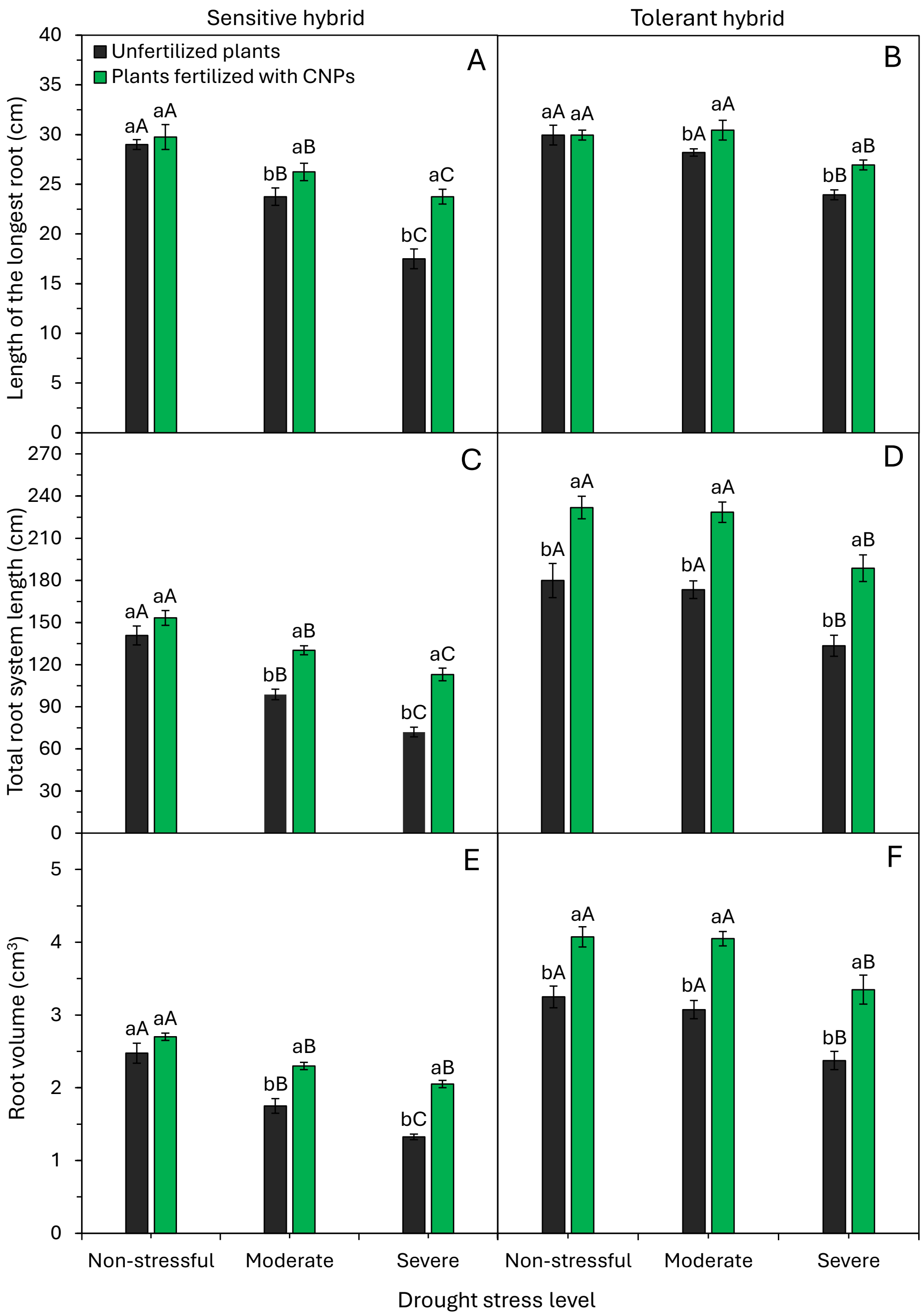

Figure 2. Effect of drought stress levels and foliar application of carbon nanoparticles (CNPs) on length of the longest root (A and B), total root system length (C and D) and root volume (E and F) of maize plants from a drought-sensitive hybrid (A, C, D) and another drought-tolerant hybrid (B, D, E). Bars followed by distinct lowercase letters for the foliar application of CNPs or distinct uppercase letters for the drought stress levels show significant differences by the Tukey test (α = 0.05). Data refers to mean values (n = 20) ± standard error of the mean.

CNP application mitigated these effects in both hybrids. CNP-treated sensitive plants had longer longest roots, total roots, and higher volumes under moderate and severe stress (Figures 2A, C, and E). Tolerant plants showed enhanced root growth with CNPs under all conditions, except longest root length in non-stress (Figures 2B, D, and F).

Drought and CNPs significantly ($P < 0.05$) affected shoot, root, and total dry mass in both hybrids (Figure 3). Severe stress reduced shoot, root, and total dry mass compared to non-stress (Figure 3). CNPs increased these masses in both hybrids versus controls, except root mass in non-stress (Figure

3D). Overall, CNPs enhanced shoot and root growth under drought, particularly in the sensitive hybrid (Figure 4).

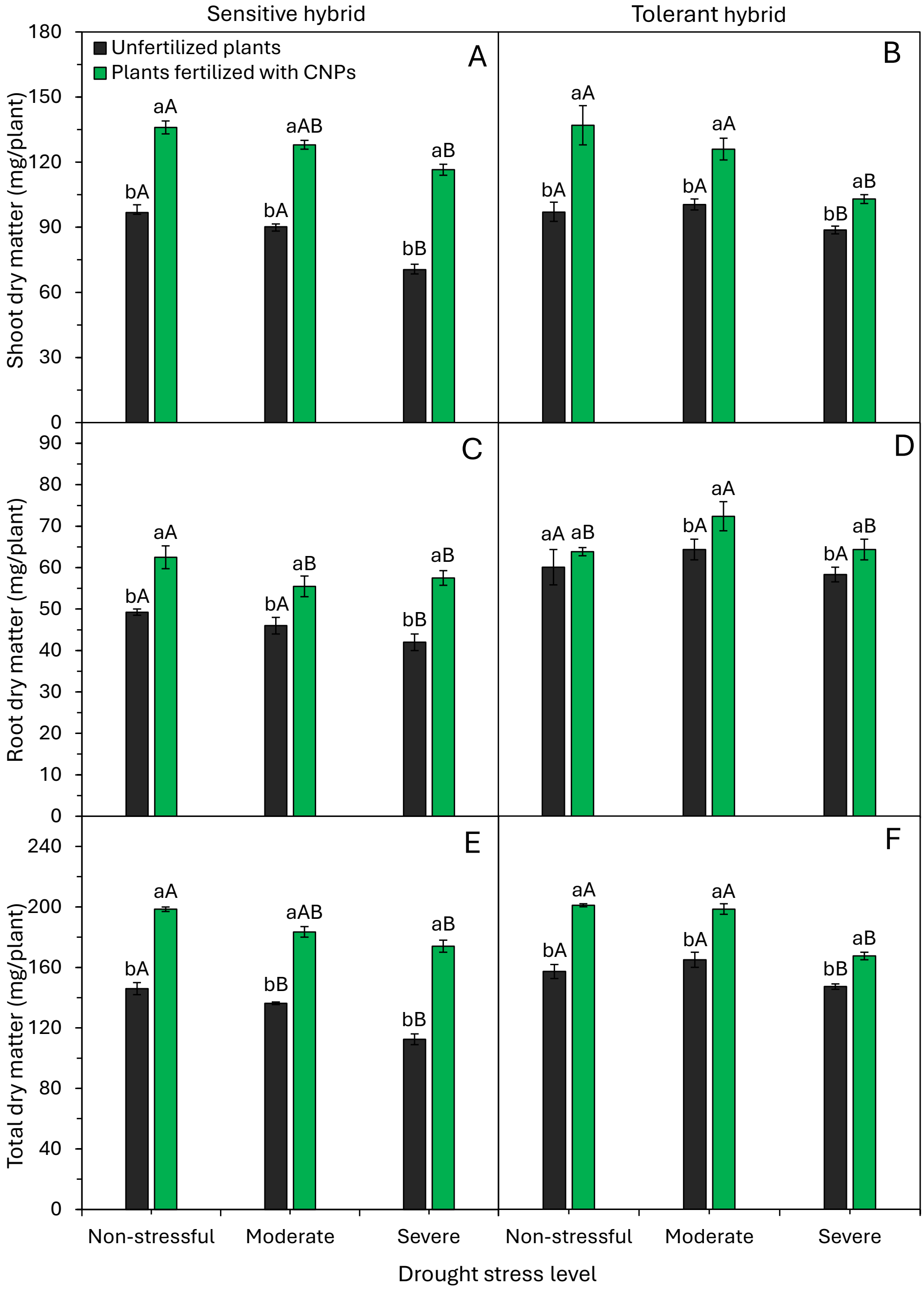

Figure 3. Effect of drought stress levels and foliar application of carbon nanoparticles (CNPs) on shoot dry matter (A and B), root dry matter (C and D) and total dry matter (E and F) of maize plants from a drought-sensitive hybrid (A, C, D) and another drought-tolerant hybrid (B, D, E). Bars followed by distinct lowercase letters for the foliar application of CNPs or distinct uppercase letters for the drought stress levels show significant differences by the Tukey test ($\alpha = 0.05$). Data refers to mean values (n = 20) ± standard error of the mean.

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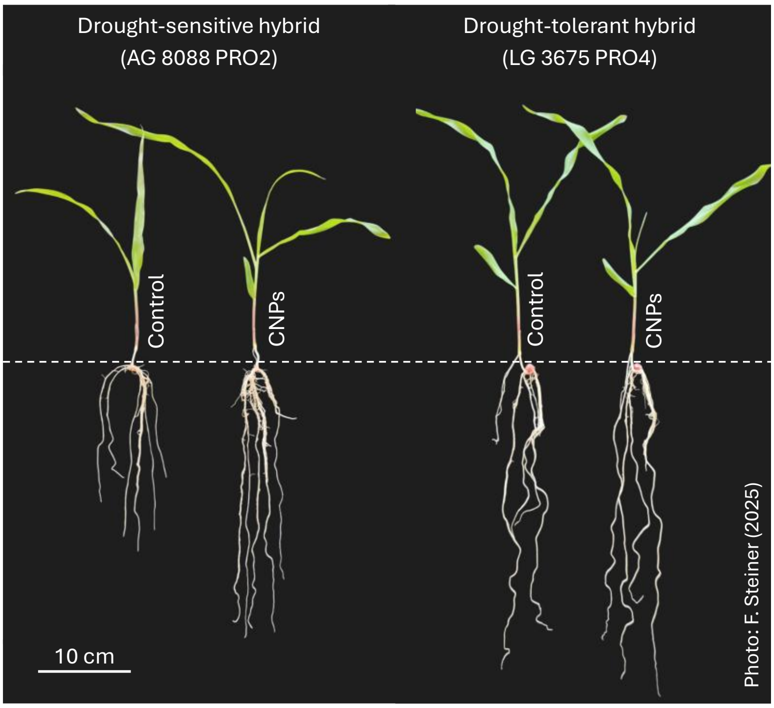

Figure 4. Drought-sensitive maize hybrid (AG 8088 PRO2) and drought-tolerant hybrid (LG 3675 PRO4) plants fertilized with carbon nanoparticles (CNPs) and grown for 25 days under severe drought stress conditions simulated with –0.80 MPa PEG-6,000 osmotic solution.

Drought and CNPs significantly ($P < 0.05$) affected photosynthetic rate, intercellular CO_2 concentration, and transpiration rate (Figure 5). Photosynthetic and transpiration rates declined progressively with stress intensity in both hybrids. Severe stress reduced photosynthetic rates by 64% (35.2 to 12.8 μmol CO_2 m^{-2} s^{-1}) in sensitive and 57% (36.8 to 15.7 μmol CO_2 m^{-2} s^{-1}) in tolerant hybrids versus controls (Figures 5A and B). Transpiration rates dropped by 41% (5.1 to 3.0 mmol H_2O m^{-2} s^{-1}) and 46% (6.2 to 3.3 mmol H_2O m^{-2} s^{-1}), respectively (Figures 5E, F). CNPs, however, improved photosynthetic rates. Sensitive plants had higher rates with CNPs under non-stress and moderate stress (Figure 5A). Tolerant plants showed increases under all conditions (Figure 5B). CNPs did not affect transpiration in sensitive plants (Figure 5E). Tolerant plants had higher rates with CNPs under non-stress and moderate (Figure 5F).

The intercellular CO_2 concentration of both maize hybrids was significantly higher in plants grown under severe stress compared to plants grown under moderate or non-stressful conditions (Figures 5C,D). Foliar application of CNPs resulted in the lowest intercellular CO_2 concentration in maize plants, except for the sensitive hybrid under non-stress conditions (Figures 5C,D).

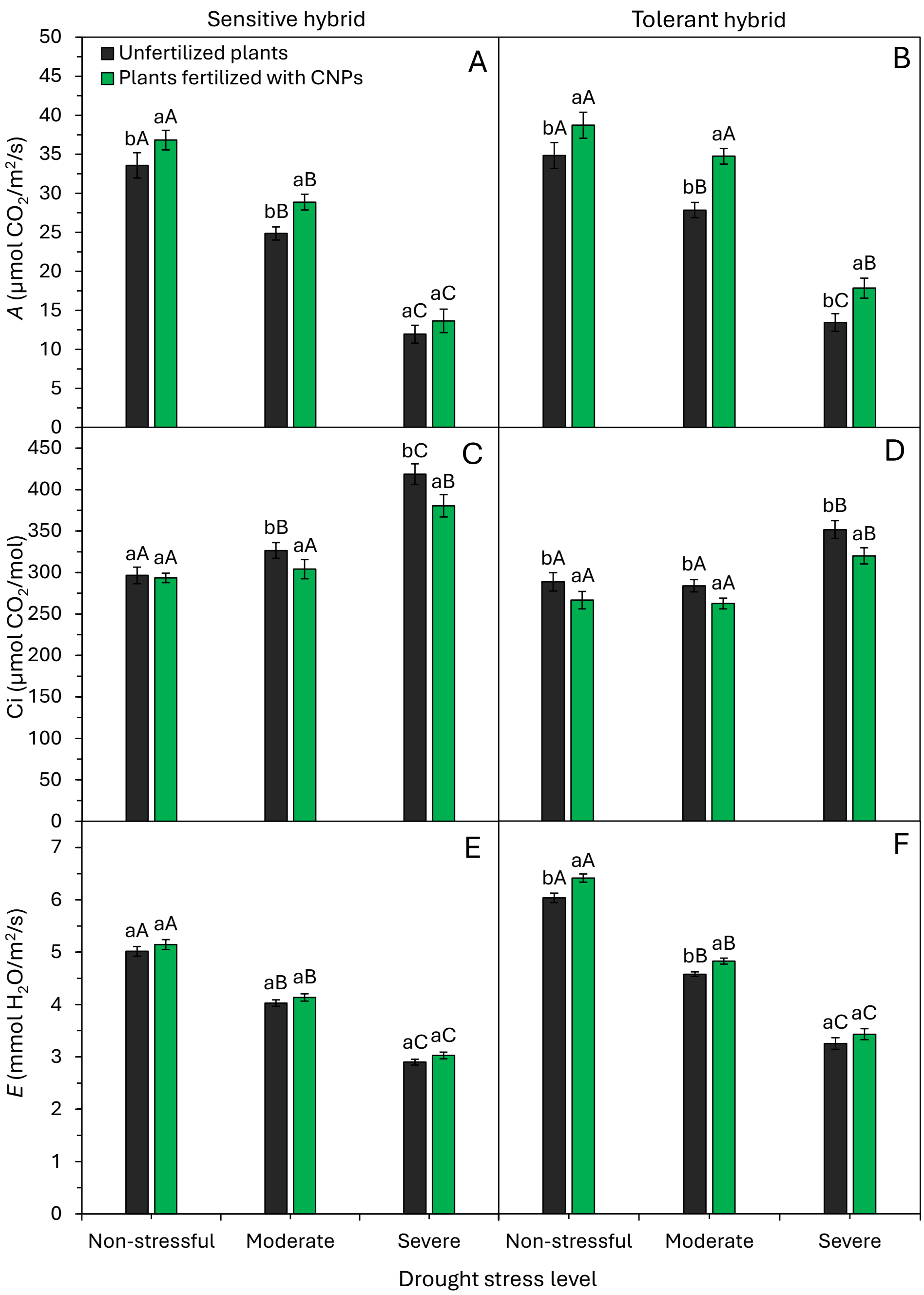

Figure 5. Effect of drought stress levels and foliar application of carbon nanoparticles (CNPs) on the photosynthetic rate – A (A and B), intercellular CO2 concentration – Ci (C and D) and transpiration rate – E (E and F) of maize plants from a drought-sensitive hybrid (A, C, D) and another drought-tolerant hybrid (B, D, E). Bars followed by distinct lowercase letters for the foliar application of CNPs or distinct uppercase letters for the drought stress levels show significant differences by the Tukey test ($\alpha = 0.05$). Data refers to mean values (n = 20) ± standard error of the mean.

Drought and CNPs significantly ($P < 0.05$) influenced stomatal conductance, water use efficiency, and carboxylation efficiency (Figure 6). These parameters declined progressively with stress intensity in both hybrids. Severe stress reduced stomatal conductance by 54% (0.28 to 0.13 mol H_2O m^{-2} s^{-1}) in sensitive and 50% (0.30 to 0.15 mol H_2O m^{-2} s^{-1}) in tolerant hybrids (Figures 6A, B). Intrinsic water use efficiency fell by 68% (5.9 to 1.9 µmol CO_2 mol^{-1} H_2O) and 60% (6.2 to 2.5 µmol CO_2 mol^{-1} H_2O), respectively (Figures 6C, D). Carboxylation efficiency decreased by 75% (0.12 to 0.03) and 62% (0.13 to 0.05), respectively (Figures 6E, F).

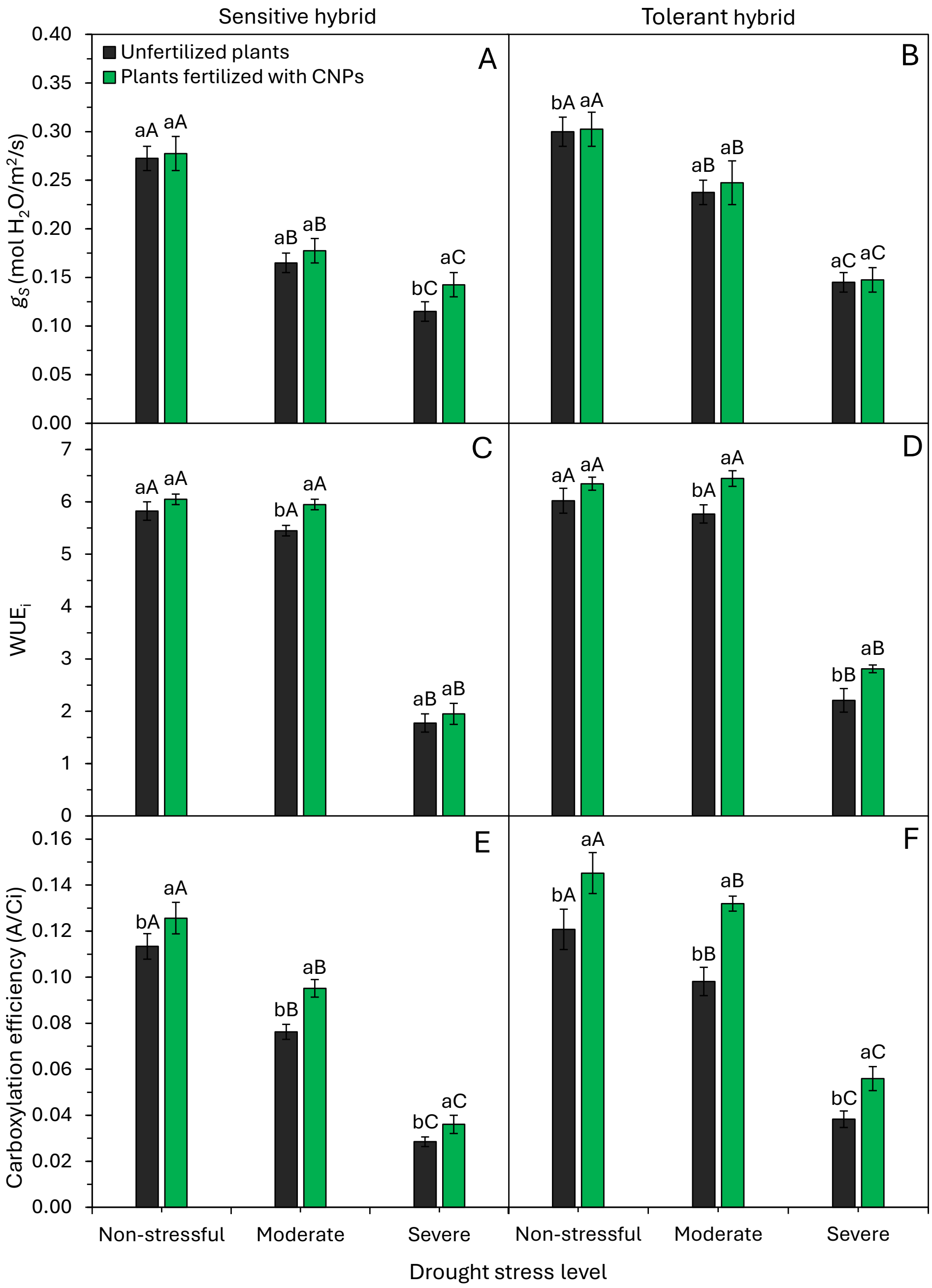

Figure 6. Effect of drought stress levels and foliar application of carbon nanoparticles (CNPs) on the stomatal conductance – g_s (A and B), intrinsic water use efficiency – WUE_i (C and D) and carboxylation efficiency – A/Ci (E and F) of maize plants from a drought-sensitive hybrid (A, C, D) and another drought-tolerant hybrid (B, D, E). Bars followed by distinct lowercase letters for the foliar application of CNPs or distinct uppercase letters for the drought stress levels show significant differences by the Tukey test (α = 0.05). Data refers to mean values (n = 20) ± standard error of the mean.

Drought, particularly severe, drastically lowered most physiological traits (Figures 5, 6). Severe stress reduced A by 61%, E by 45%, gs by 52%, WUEi by 64%, and A/Ci by 69% versus non-stress. These impairments in A, WUEi, and A/Ci led to reduced growth and dry mass, especially in sensitive hybrids (Figures 3, 4). Moderate stress decreased shoot and root dry mass by ~5% and 19%, severe by 5% and 11%, versus non-stress (Figure 3).

3. Discussion

Drought inhibited maize shoot growth). Inhibition levels varied between hybrids. Sensitive plants under severe stress had reductions of 57% in height, 59% in leaf area, and 22% in shoot dry mass versus controls. Tolerant plants showed 37%, 36%, and 16% reductions, respectively. These findings indicate drought inhibited elongation and photoassimilate accumulation in both hybrids. Vilas Boas et al. [3] likewise found drought limited elongation and dry mass in 15 hybrids. Kim and Lee [5] observed similar reductions. Reduced growth under drought stems from cell cycle disruptions [21]. Drought perception triggers signaling that activates checkpoints, impairing G1-S transition, slowing DNA replication, or delaying mitosis [22,23], thereby prolonging the cell cycle and restricting growth and dry mass. Drought also impairs cell wall extensibility and turgor [24]. Limited water uptake reduces turgor, slowing elongation [6]. Maize growth rates dropped from 165 to 56 mm day^{-1} under drought [25]. Cell expansion, reliant on turgor, is among the first processes affected, limiting height, leaf area, and dry mass.

Lower shoot dry mass under drought arises from reduced photosynthesis, limiting photoassimilate allocation to shoots [6]. This reduces leaf production and area. These serve as tolerance mechanisms to minimize transpiration and optimize water use efficiency's. Drought often limits cereal growth, including wheat [26], sorghum [27], and maize [3,6,16]. Therefore, the response mechanisms of plants exposed to drought stress have become a crucial topic of environmental research in drought-prone regions.

Key maize responses include smaller leaf area, enhanced root growth, lower water content, and reduced photosynthesis, transpiration, and conductance. These align with reports from other studies [5,6,26]. Roots encounter drought first, and their length and architecture critically affect water and nutrient uptake under low moisture (Walne et al. 2024). Vilas Boas et al. [3] reported that stronger root growth under low water indicates higher tolerance. Similar results were reported by Walne et al. [6], which showed that drought-tolerant maize hybrids have greater root length growth (35%) when compared to stable hybrids. Under drought, photoassimilates are preferentially routed to roots via vascular tissues [9,28]. This increased allocation of photoassimilates to the roots strengthens the growth of the root system and water uptake in the soil profile. Therefore, current plant breeding programs should select genotypes with longer roots for drought tolerance.

Higher intercellular CO_2 under severe drought relates to partial stomatal closure for water conservation, retaining CO_2 inside leaves [29]. This CO_2 increase, paired with lower conductance, reduces transpiration. Such effects lower photosynthesis via higher stomatal resistance and reduced carboxylation [9]. Under low water, this CO_2 rise may stem from reduced CO_2-fixing enzyme activity, impairing water use efficiency [30]. In non-stress, plants avoid reducing leaf area or transpiration or raising CO_2, leading to higher photosynthesis and growth, as reported in this study.

Photosynthesis, central to plant energy, can suffer greatly under drought. Drought curbs photosynthetic efficiency through biochemical and hydric constraints that diminish components and induce stomatal closure [9]. Our data show CNPs key in countering drought's photosynthetic impacts. Balanced photosynthesis is vital for growth, biomass, and yield [9,26]. CNPs can thus boost crop performance under stress.

CNP-fertilized maize had higher water content, photosynthesis, leaf area, and early growth than controls. Alluqmani and Alabdallah [19] reported similar benefits in bell pepper, including higher water content, chlorophyll, photosynthesis, and tolerance. Chen et al. [18] reported CNPs increased water content, efficiency, and photosynthesis, enhancing tomato growth and yield under drought. Shekhawat et al. [17] showed CNPs improved chlorophyll, protein, growth, and dry mass in cowpea. Ahmadi et al. [20] also reported that CNPs application alleviated the damage caused by drought stress on the growth and metabolic and physiological functions of bell pepper plants.

Here, foliar CNPs effectively boosted physiological activity in both sensitive and tolerant maize under drought. CNP application to stressed plants further improved water status, use efficiency, carboxylation, and photosynthesis. These metabolic enhancements led to better growth under stress, notably in sensitive hybrids. Many studies confirm CNPs' role in enhancing plant and crop performance under stress. Key benefits include water status regulation [18–20], elevated chlorophyll

[17,19,20], better water use efficiency [18], and antioxidant activation [17–20]. In summary, CNPs induce morphological, physiological, and biochemical changes that heighten drought tolerance, mainly via improved water uptake and synthesis of compounds for stress signaling and ROS elimination [31].

Overall, our data suggest foliar CNPs substantially lessen drought impacts, aiding maize adaptation under stress. These emphasize CNPs as nanofertilizers that boost growth and tolerance. Foliar CNPs thus offer a practical drought management tool for sustainable maize production. These findings will provide the basis for further research on the interactions between CNPs and plants and help ensure their safe and sustainable use in global agricultural production.

4. Materials and Methods

4.1. Plant Material and Treatments

Seeds of two commercial maize hybrids one drought-tolerant (LG 36745 PRO4) and one drought-sensitive (AG 8088 PRO2) were obtained from the seed market in Chapadão do Sul, Mato Grosso do Sul, Brazil, to examine foliar carbon nanoparticle effects on early growth and physiological traits under stress. The main characteristics of maize seeds and hybrids are shown in Table 1.

Table 1. Characteristics of maize hybrids (*Zea mays* L.) grown under non-stressful and drought stress conditions.

Maize hybrid	Origin	Maturation cycle (days)	Yield Potential	1,000-SW (g)	GR (%)	Drought stress response†
LG 36745 PRO4	LG® Seeds	136	High	330	98	Tolerant
AG 8088 PRO2	Agroceres® Seeds	135	High	380	94	Sensitive

1,000-SW: 1,000-seed weight. GR: germination rate. † Reference: Vilas Boas et al. [3].

Seeds were sterilized with 2% (w/v) sodium hypochlorite (NaOCl) for 8 minutes before germination under control (non-stress) and drought stress conditions.. Seeds were exposed to osmotic potentials of 0.0, -0.40, and -0.80 MPa using polyethylene glycol (PEG-6000) solutions. The amount of PEG-6,000 required to prepare the solutions with distinct osmotic potentials was calculated using the equation of Michel & Kaufmann [32]: $\Psi_s = [-(1.18 \times 10^{-2}) \times C - (1.18 \times 10^{-4}) \times C^2 + (2.67 \times 10^{-4}) \times C \times T + (8.39 \times 10^{-7}) \times C^2 \times T]/10$, where Ψ_s is the osmotic potential (MPa), C is the concentration (g L^{-1} of PEG-6,000) and T is the temperature (°C). Distilled water (0.00 MPa) served as the control.

Treatments were arranged in a completely randomized design with a 2 × 2 × 3 factorial: two hybrids (drought-tolerant LG 36745 PRO4 and drought-sensitive AG 8088 PRO2), two CNP foliar levels (0 and 1.0 mL L^{-1} of CNP-based nanofertilizer), and three drought levels (0 MPa control, -0.4 MPa moderate, -0.8 MPa severe), with four replicates.

Foliar CNP application occurred at the V2 stage (second fully expanded leaf) using Arbolina® (Krilltech Nano Agtech, Brasília, DF, Brazil), which contains 10% organic carbon (w/w) and 4% nitrogen (w/w). A 1.0 mL L^{-1} Arbolina® solution was applied to maize leaves via a CO_2-pressurized sprayer at 210 kPa, fitted with three ATR 4.0 cone nozzles and calibrated for 180 L ha^{-1} spray volume.

4.2. Plant Growth Condition

Seeds were sown 2.0 cm deep in plastic containers (44 × 30 × 7.5 cm) filled with sterilized quartz sand. Sand was sieved (0.05-0.8 mm mesh) and moistened to 70% water retention capacity [32] equivalent to 185 mL kg^{-1} with distilled water (control) or PEG-6000 solutions (stress). Containers were maintained in a laboratory with LED-supplemented light: red (620-630 nm) and blue (455-475 nm) at 85:15 ratio, 250 ± 80 µmol m^{-2} s^{-1} intensity, 12/12-h photoperiod, and 25.4 ± 2.1 °C temperature, for 25 days. Each treatment had four replicates of 50 seeds.

Plants received nutrient solution at 10, 15, and 20 days: 180 mg L^{-1} N, 80 mg L^{-1} P, 300 mg L^{-1} K, 150 mg L^{-1} Ca, 30 mg L^{-1} Mg, 30 mg L^{-1} S, 1.4 mg L^{-1} Fe, 0.4 mg L^{-1} B, 0.4 mg L^{-1} Mn, 0.3 mg L^{-1} Cu, 0.2 mg L^{-1} Zn, 0.01 mg L^{-1} Mo, 0.06 mg L^{-1} Ni, and 0.02 mg L^{-1} Co.. Nutrient sources included calcium nitrate, potassium nitrate, magnesium nitrate, monoammonium phosphate, potassium sulfate, magnesium sulfate, iron chelate, manganese chelate, copper chelate, zinc chelate, boric acid, sodium molybdate, nickel sulfate, and cobalt sulfate. Fertilizers were dissolved in the corresponding osmotic solutions for each treatment. Substrate moisture was monitored daily at 2:00 p.m. gravimetrically by weighing containers. Moisture was maintained at 70% sand retention capacity via daily irrigation.

4.3. Quantification of Morphological Traits

After 25 days of exposure to drought stress, plant height (PH), leaf area (LA), length of the longest root (LLR), total root system length (TRL), root volume (RV), shoot dry matter (SDM), root dry matter (RDM) and total dry matter (TDM) were measured. The PH and LLR were measured using a ruler. The LA was determined using an automatic leaf area meter (Li-Cor®, model LI-3100, Lincoln, Nebraska, USA). The SDM, RDM and TDM were recorded on an analytical balance (±0.0001 g) after drying in an oven at 85 °C for 48 h. For the determination of TRL and RV, individual plant roots were scanned using an optical scanner (Scanjet 4C/T, HP) at 300 dpi resolution, and the digitized images were analyzed with WinRhizo program version 3.8-b (Regent Instrument Inc., Quebec, Canada). All measurements were performed on five random plants per replicate.

4.4. Quantification of Physiological Traits

After 25 days of exposure to drought stress, the relative water content (RWC) also was calculated based on the weighing of fresh matter (FM) and dry matter (DM) of five randomly chosen seedlings per replicate, using the following equation proposed by Weatherley [33]: RWC (%) = [(FM – DM)/FM)] × 100.

The photosynthetic rate (A), intercellular CO_2 concentration (Ci), transpiration rate (E), stomatal conductance (g_s), and intrinsic water use efficiency (WUE_i) were recorded on the second uppermost fully expanded leaf using a portable infrared gas analyzer (IRGA, Li-Cor, Li-6400XT model) at 25 days. Measurements were taken between 9:00 and 10:00 a.m. in plants exposed to artificial supplemental light radiation, with a chamber CO_2 concentration of 380 μmol mol^{-1} and photon flux density of 1,000 μmol m^{-2} s^{-1}.

4.5. Statistical Analysis

Data were tested for variance homoscedasticity (Levene's test, $P > 0.05$) and residual normality (Kolmogorov-Smirnov test, $P > 0.05$) before analysis of variance (F-test, $\alpha = 0.05$). Means were compared using Tukey's test at $\alpha = 0.05$. Analyses were conducted with Rbio v. 140 software (Rbio Software, UFV, Viçosa, MG, Brazil).

5. Conclusions

Foliar application of carbon nanoparticles has high potential to mitigate the detrimental impacts of drought on maize plants grown under moderate and severe stress conditions, especially through maintaining plant water status and improving water use efficiency, carboxylation efficiency, photosynthesis rate, and early plant growth under adverse environmental conditions.

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