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Influence of CuO nanoparticles on photosystem II structural stability and functional activity of corn (*Zea mays* L.) under drought stress

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Abstract
Photoinhibition and photooxidation of photosystem II (PSII) is one of the key damages caused by drought stress. The present study aimed to assess whether or not foliar application of copper oxide nanoparticles (CuO NPs) is effective in promoting PSII stability and activity in corn plants under drought stress. Three-week old plants of corn were subjected to drought stress and varying levels of CuO nano-particles (0, 25, 50, and 100 mM) of the rooting medium. In this study, the effects of foliar application of copper oxide nanoparticles were assessed on growth, plant water status, nutrient uptake and structural stability of photosystem II of drought stressed corn plants. Drought stress impeded overall growth of the corn plants by reducing leaf relative water content, water potential, chlorophyll *a* content, and accumulation of K^+ in plant leaves and roots. Exogenous application of 25 mM CuO NPs significantly enhanced the growth of corn. Exogenous application of CuO NPs improved the dry biomass and length of roots of the corn plants under drought stress. Although the application of nano-particles did not change leaf photosynthetic pigments, relative water content, K^+ accumulation, it enhanced the accumulation of K^+ in roots. Drought stress did not affect the structural stability of PSII, but it reduced its activity (performance index, PIABS) due to changes in the reaction center density and the biochemical reaction efficiency or electron transport capability. Exogenous application of CuO NPs improved PIABS due to increase in active reaction center density and electron transport efficiency. However, 100 mM CuO NPs application caused PSII damage at the donor end and reduced active reaction center density in the corn plants under both normal and drought stress conditions. The present findings provide a baseline information that foliar application of CuO-nanoparticles in low concentrations can improve the growth of corn by improving accumulation of K^+ and increasing PSII activity. Further research is required to explore its effect on cellular redox balance and activities of PSII and PSI, and optimum dose of CuO-nanoparticles for developing formulations for their commercial applications in farmers' fields.

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Introduction

Drought stress for shorter or longer periods reduce crop yield by disrupting physiological and biochemical processes such as plant water status, uptake and accumulation of mineral nutrients, rate of photosynthesis, etc. (Ashraf et al., 2011; Webber et al., 2018). However, the adverse effects of drought on plant growth vary with the type of plant species and developmental stage (Blum, 2017; Messina et al., 2023). For example, in maize, drought stress at the tasseling stage resulted in a greater decline in yield than that at the vegetative growth stage (Bashir et al., 2016). Moreover, drought stress causes moisture deficit in leaves that interferes with photosynthetic machinery of plants (Qiao et al., 2024), maybe resulting in a subsequent poor distribution of carbohydrates across grains. Moreover, drought stress was reported to reduce maize productivity by reducing the number of kernels in each row and number of kernels per cob (Bashir et al., 2016; Bashir et al., 2021; Stefanov et al., 2023). Generally, it is believed that plants capable of maintaining plant water status, osmotic adjustment, mineral nutrient accumulation, higher photosynthetic capacity or better antioxidant capacity are tolerant to drought stress (Avramova et al., 2015; Zhan et al., 2015; Blum, 2017; Eapen et al., 2017; Danilevskaya et al., 2019; Messina et al., 2023; Qiao et al., 2024). Correction of plant physiological processes that are disturbed by drought through any chemical or physical agent is a shotgun approach to improve drought tolerance in plants (Ashraf et al., 2008). For example, foliar applications of mineral nutrients, antioxidants, plant growth regulators or nano-particles can modulate plant physiological processes thereby improving plant stress tolerance (Ashraf et al., 2011; Kaya and Ashraf, 2024).

Nanoparticles (NPs; 1-100 nm) are used in agriculture as nano-pesticides, nano-fertilizers, and nanocarriers (Paramo et al., 2020; Ogbaga et al., 2024). Nanoparticles are involved in various physiological and biochemical processes controlling plant growth and development as well as plant environmental stress responses (Paramo et al., 2020; Singh et al., 2024). Metal nanoparticles can release metal ions such as Cu^{2+} ion from CuO nanoparticles, and released Cu ions can attach to protein thiol groups, causing conformational changes in proteins (Chung et al., 2019). Plants that are exposed to high concentrations of Cu ions released from CuO nanoparticles experience toxicity and stunted growth (Feigl, 2023). Phytotoxicity of CuO nanoparticles depends on size of nanoparticles and concentration. Higher concentration of CuO nanoparticles and extremely lower size, i.e., less than 40 nm can become toxic as they can penetrate the plant itself and may cause particle stress (Velicogna et al., 2020). The main mechanisms of toxicity caused by high concentration of nanoparticles are assumed to be the production of high amounts of ROS, which cause oxidative stress and cytotoxicity (Paramo et al., 2020; Velicogna et al., 2020; Feigl, 2023). These findings suggest optimizing of the dose and size of nanoparticles for their application in agriculture. It has been observed that Cu nanoparticles improved the quality and quantity of seed yield of soybean under drought stress (Linh et al., 2020). Furthermore, foliar application of CuO nanoparticles improved the growth rate as 51% of maize (Adhikari et al., 2016). In maize, copper nanoparticles scavenged reactive oxygen species (ROS) and improved the production of photosynthetic pigments (Van Nguyen et al., 2022).

Maize (*Zea mays* L.) is one of the most important crops worldwide. This crop normally requires a large quantity of water to complete its life cycle (Molla et al., 2023). It is believed that even a short spell of water deficiency can impede its growth and overall productivity (Du and Xiong, 2024). Therefore, The primary objective of the current study was to evaluate the influence of foliar application of CuO nanoparticles on corn under drought stress and explore the potential of CuO as a promising agent in alleviation of drought stress in corn by improving nutrition and photosynthetic potential, particularly photosystem II structural stability and functional activity. Furthermore, the research is intended to reveal the possible beneficial role of CuO nanoparticles in maintaining leaf water status, chlorophyll content, and Na^+ and K^+ ions in water deficient soil.

Materials and Methods

Preparation and characterization of CuO nanoparticles

One liter of 0.1 M copper sulphate pentahydrate solution was prepared. An aliquot of 20 mL of 0.5 M ascorbic acid solution was diluted up to 50 mL. In 1 L of 0.1 M copper sulphate solution, 30 mL of NaOH solution and 50 mL of ascorbic acid solution were added. This mixture was heated at 80 °C for 2 h. Then it was filtered through a Whatman filter paper. The filter paper was dried, and the material left on it was removed and further dried. The characterization of CuO NPs was performed at LUMS University Lahore, Pakistan. Scanning electron microscopy (SEM) was used with EDX and E-beam lithography (FEI Nova 450 Nano-SEM) and morphology, topography, chemical and phase composition were studied from nanometers to millimeters.

Experimental design

Grains of the corn (*Zea mays* L.) variety DK-7024 were obtained from the Maize and Millets Research Institute, Yousaf Wala, Sahiwal. The average seed germination rate was 90%. The experiment was arranged in a completely randomized design with eight treatments and four replicates. Before sowing, the seeds were immersed first in 5% hypochlorite solution for some time, then washed five times with distilled water and dried. Six seeds were sown in each pot filled with soil. After 14 days of growth, plants were thinned to 4 plants per pot. The plants were subjected to drought stress by withholding irrigation water. At the time of initiating water stress application, four concentrations (0, 25, 50, and 100 mM) of CuO NPs were applied to the foliage of the maize plants grown under normal or drought stress regimes.

Growth attributes measurements

During harvest, the plants were uprooted carefully and separated into shoots and roots. Shoot length and root length of each plant were measured. Leaf area was calculated by measuring length and width of each leaf. Shoot and root fresh biomass was measured and the samples were placed in an oven at 70 °C. When the samples were dried, their shoot and dry biomass was also recorded. Before harvesting the plants, following physiological attributes were measured:

Determination of leaf water potential

Water potential of leaves excised early morning was determined by the Scholander type pressure chamber (PMS chamber apparatus).

Determination of leaf relative water content

Leaves were excised from the plants and fresh weight of each leaf was measured. Then the leaves were dipped in distilled water for 8 h at 4 °C (Barrs and Weatherley, 1962) and turgid weight of each leaf was measured. The leaf samples were then placed in an oven at 70 °C. The samples were dried, and then dry weight of each leaf was measured. Leaf relative water content was calculated using the following formula:

$$\text{Leaf relative water content (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Estimation of chlorophyll contents

Concentrations of chlorophyll *a*, chlorophyll *b* and total chlorophyll were estimated by the formulae devised by Arnon (1949). A proportion of 0.2 g leaf was ground in 6 mL of acetone solution (80%) and filtered with a Whatman filter paper. The samples were wrapped with an aluminum foil and kept at 4 °C. Absorbance was observed at 645 nm, 652 nm and 663 nm using a spectrophotometer.

Chlorophyll *a* (mg/g leaf fresh weight) = [12.7 (O.D 663)-2.69(O.D 645] × V/1000 × W
Chlorophyll *b* (mg/g leaf fresh weight) = [22.9 (O.D 645)-4.68(O.D 663] × V/1000 × W

Chlorophyll *a* f*luorescence*

Chlorophyll *a* fluorescence was measured with the Pocket PEA (Hansatech Instruments Ltd. Narborough Road Pentney, The United Kingdom). To assess chlorophyll fluorescence emission in dark-adapted leaves, the leaf was placed in one of the leaf clips for 20 min prior to each measurement. A saturation pulse of 3,500 mmol quanta m^{-2} sec^{-1} of red light (650 nm) was applied on each leaf sample. Data of chlorophyll *a* fluorescence were recorded in the Pocket PEA using specific software Pocket PEA v-1.13. Data was transferred using a data cable and further evaluated.

Potassium (K^+) and sodium (Na^+)

Accumulation of K^+ and Na^+ in leaves was determined by the wet digestion method (Allen et al., 1986). $LiSO_4.2H_2O$ (14 g) and selenium (42 g) were mixed in 350 mL of H_2O_2. Then 450 mL concentrated H_2SO_4 were added in aliquots and the digestion mixture was prepared. During digestion, a 2 mL digestion mixture was added to 0.2 g dry leaf material and kept it overnight. The next day, the material was heated on a hot plate. One mL of perchloric acid was also added to the digesting material until it became colourless. The digested material was cooled and diluted up to 50 mL by adding distilled water. Potassium (K^+) and Na^+ were determined using a flame photometer (410, Corning).

Statistical analysis of data

The data obtained for different attributes were subjected to analysis of variance. When the interaction terms were significant, the means under control and drought conditions were compared using the LSD test worked out using the computer software COSTAT v 6.5 following Snedecor and Cochran (1989).

Results

Morphology, topography, chemical and phase composition of green synthesized nanoparticles were studied using SEM and EDX. The results showed that the synthesized particles were 40-100 nm in size. The nanoparticles were of semi-spherical shape with irregular surface. The data for EDX showed that more than 80% nanoparticles were of similar size, i.e., 40-60 nm in range. Around 20% of the nanoparticles were larger than 60 nm but less than 100 nm in size (**Figure 1**).

A marked inhibitory effect of drought stress on shoot and root fresh weight and dry weight of corn plants was observed (**Table 1**). However, foliar applications of CuO NPs significantly improved fresh and dry weights of shoots as well as roots under the stress regime (**Figure 2**). The improvement in shoot fresh and dry weights was more prominent at 25 and 50 mM CuO NPs under drought conditions. Drought stress reduced the shoot length of con plants, but root length increased significantly due to CuO NPs. Shoot length of corn plants was recorded to be reduced due to 100 mM CuO NPs applied under stress conditions, whereas the reverse was true for root length.

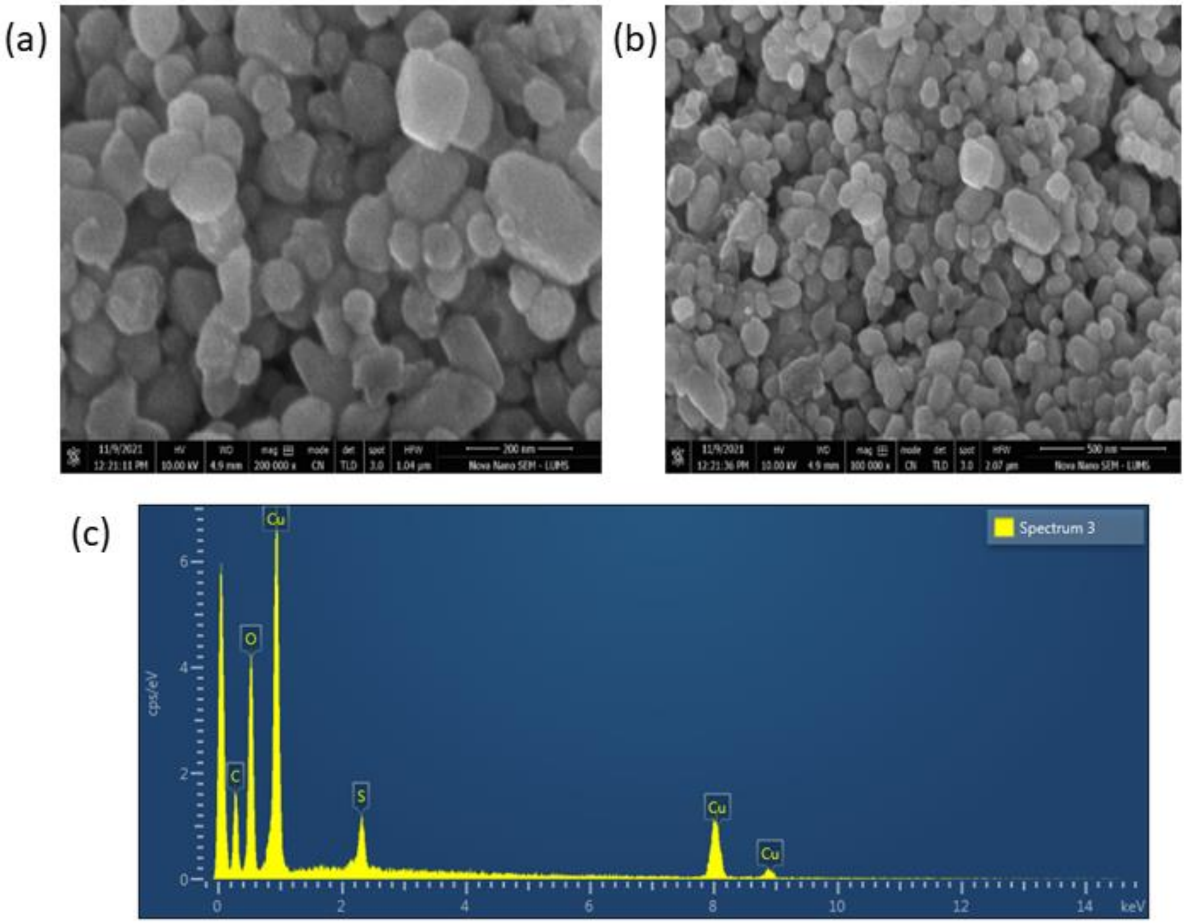

Figure 1. Characterization of synthesized copper oxide nanoparticles (CuO NPs) (a) Under 200,000X Power (200 nm) (b) Under 100,000X Power (500 nm) (c) EDX (Energy Dispersive X-Ray).

Table 1. Mean squares from analysis of variance (ANOVA) of the data for morphological growth and biomass of corn (*Zea mays* L.) when different levels of CuO NPs were exogenously applied as foliar spray to drought-stressed and non-stressed plants

Sources	df	SFW	RFW	SDW	RDW	SL	RL
Drought	1	7269.79***	562.58***	288.09***	79.28***	480.27***	269.29*
Treatment	3	73.63ns	35.25***	10.38*	3.81*	401.01***	772.2***
Interaction	3	424.56***	132.42***	10.95*	16.06***	350.15***	128.25*
Error	24	48.06	3.79	2.69	0.82	18.25	35.55

CuO, Copper oxide; NPs, Nanoparticles; SFW: Shoot fresh weight; RFW: Root fresh weight; SDW: Shoot dry weight; RDW: Root dry weight; SL: Shoot length; RL: Root length, ns, non-significant; *, **, *** significance at 0.05, 0.01 and 0.001 levels of probability, respectively.

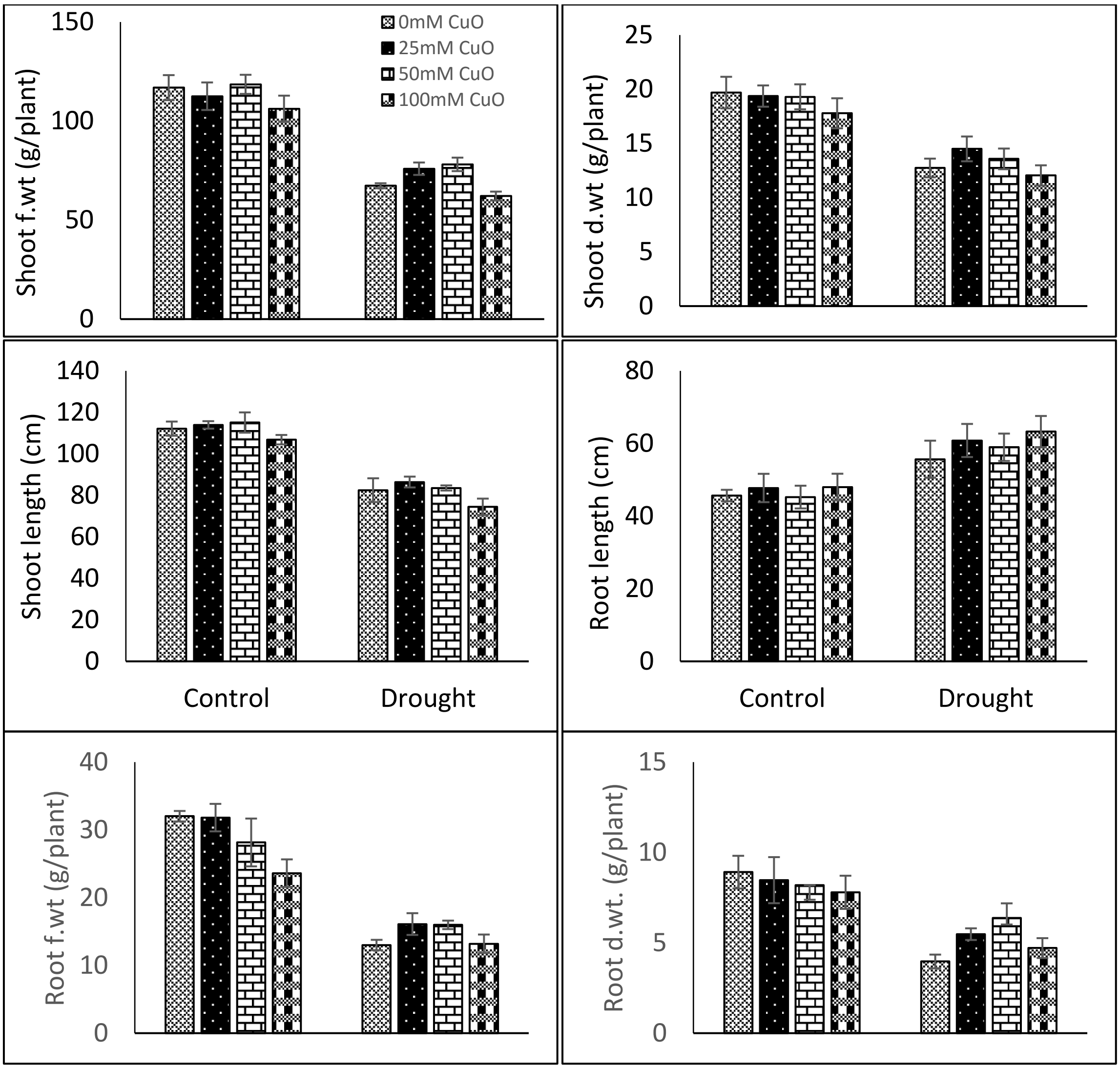

Figure 2. Fresh and dry weights of shoots and roots of drought-stressed and non-stressed corn (*Zea mays* L.) plants treated with varying concentrations of CuO nano-particles

Drought stress caused a significant reduction in chlorophyll *a,* but it did not affect the chlorophyll *b* content in the corn plants (**Table 2**; **Figure 3**). Foliar application of CuO NPs did not affect both these pigments under normal or stress conditions. The amount of chlorophyll *a* was significantly reduced in 100 mM CuO NPs fed plants under drought stress (**Figure 3**). Leaf relative water content was significantly reduced when the corn plants were exposed to drought stress (**Table 2**; **Figure 2**). Foliar application of CuO NPs to the corn plants did not affect significantly leaf relative water content under both control and drought stress conditions (**Figure 3**). Drought stress caused a significant decrease in leaf water potential (**Figure 3**). However, foliar application of CuO NPs significantly improved leaf (less -ve) water potential under drought stress conditions.

Table 2. Mean squares from analysis of variance (ANOVA) of the data for chlorophyll *a*, chlorophyll *b*, relative water content (RWC) and leaf water potential of corn (*Zea mays* L.) when different levels of CuO NPs were exogenously applied as foliar spray to drought-stressed and non-stressed plants

Source	df	Chlorophyll *a*	Chlorophyll *b*	RWC	Water potential
Drought	1	$6.66 e^{-4}$ ***	0.019 **	3.50 ***	1046.53***
Treatment	3	$2.87 e^{-4}$ ***	0.016 ***	2.37***	172.78*
Interaction	3	$3.57 e^{-4}$ ns	0.006 *	1.52 ***	23.61ns
Error	24	$1.66 e^{-5}$ ns	$1.5 e^{-3}$	0.175	48.61

CuO, Copper oxide; NPs, Nanoparticles; ns, non-significant; *, **, *** significance at 0.05, 0.01 and 0.001 probability levels, respectively

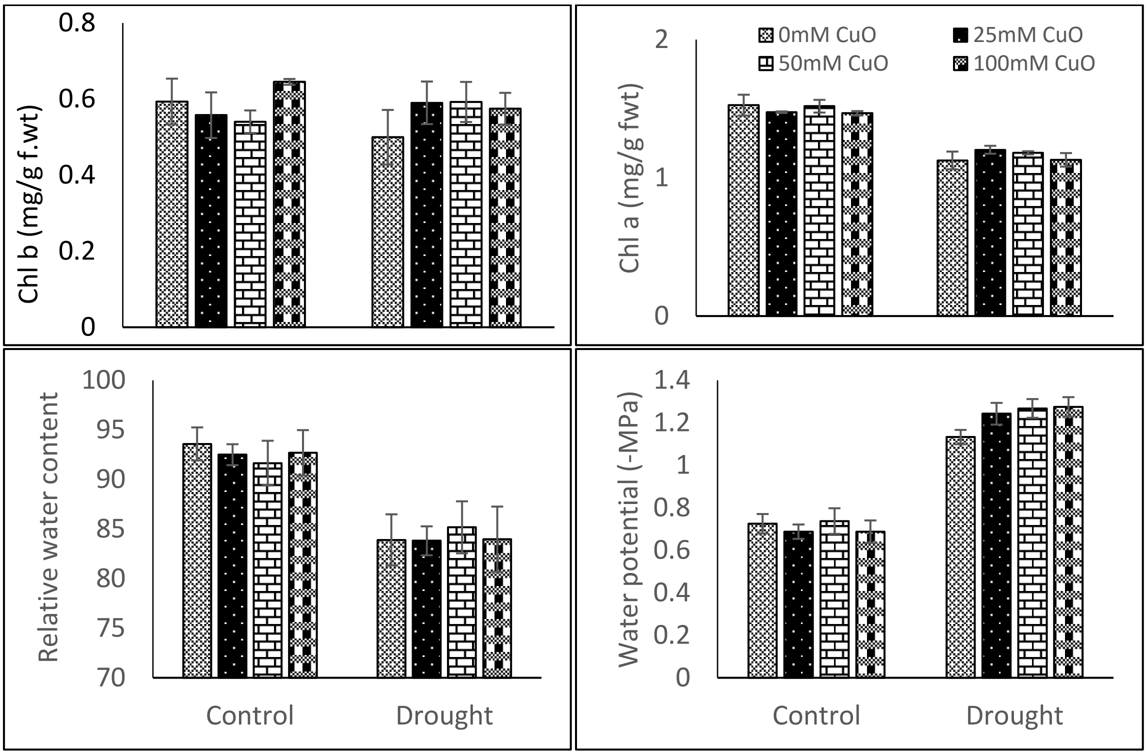

Figure 3. Chlorophyll a, chlorophyll b, relative water content and leaf water potential of drought-stressed and non-stressed corn (*Zea mays* L.) plants treated with varying concentrations of CuO nano-particles

Drought stress reduced the accumulation of K^+ in the leaves and roots of the corn plants (**Table 3**; **Figure 4**). Foliar application of CuO NPs significantly improved root K^+ particularly at 25 mM CuO NPs. Drought did not change the accumulation of Na^+ in the leaves, but it increased that in the roots (**Table 3**; **Figure 4**). In addition, foliar application of 25 and/or 100 mM CuO NPs increased the accumulation of Na^+ in the leaves. However, foliar application of CuO NPs boosted Na^+ accumulation in the roots under control conditions. Application of 50 mM CuO reduced the Na^+ content in the roots under drought stress (**Figure 4**).

Table 3. Mean squares from analysis of variance (ANOVA) of the data for K^+ and Na^+ in shoot and root of corn (*Zea mays* L.) plants when different levels of CuO NPs were exogenously applied as foliar spray to drought-stressed and non-stressed plants

Sources	df	K^+ in shoot	K^+ in root	Na^+ in shoot	Na^+ in root
Drought	1	9.29ns	0.15 ns	0.27ns	7.03 ***
Treatment	3	21.86*	86.13 ***	0.47ns	2.65 ***
Interaction	3	5.65ns	13.85 ns	0.47ns	0.061 ns
Error	24	4.78	6.40	0.47	0.254

ns, non-significant; CuO, Copper oxide; NPs, Nanoparticles; ns, non-significant; *, **, *** significance at 0.05, 0.01 and 0.001 probability levels, respectively

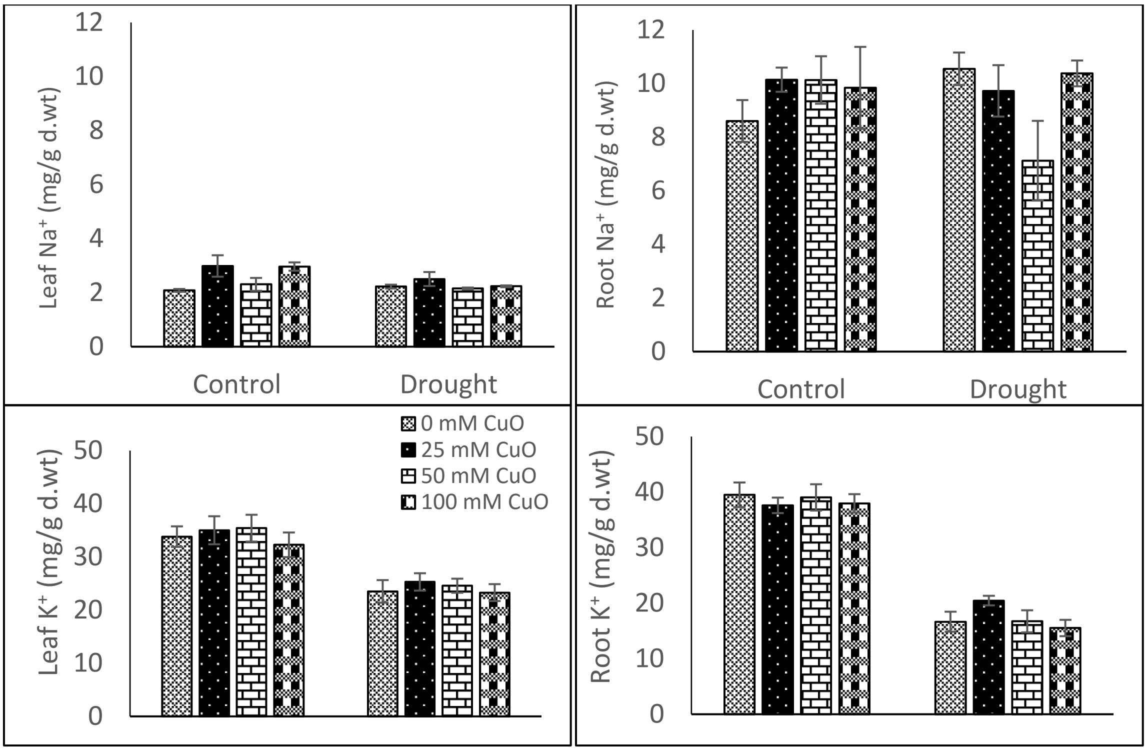

Figure 4. Accumulation of potassium and sodium in leaves and roots of drought-stressed and non-stressed corn (*Zea mays* L.) plants treated with varying concentrations of CuO nano-particles

Semi-quantitative analyses of OJIP curves showed that drought stress mainly affected the I-P steps. However, double-normalized differential curves of OP showed that drought stress changed the fluorescence at J, I and P steps (**Figure 5**). Double normalized (*F*o and *F*m) differential curves of foliar applied plants with CuO NPs under normal and drought stress conditions were separately presented to monitor the changes due to varying concentrations of CuO NPs; it showed that only 100 mM CuO NPs negatively affected the J step under control and drought stress conditions (**Figure 5**). In addition, 50 mM CuO NPs application significantly changed the fluorescence at I step. Drought and CuO NPs both did not change the ∆OK (Energetic connectivity of antenna and reaction center) except 100 mM CuO NPs treatment. However, application of CuO NPs increased the amplitude of ∆OJ (stability of oxygen evolving complex), particularly at 100 mM CuO NPs (**Table 4**; **Figure 5**).

Table 4. Mean squares from analysis of variance (ANOVA) of the data for chlorophyll fluorescence of corn (*Zea mays* L.) when different levels of CuO NPs were exogenously applied as foliar spray to drought-stressed and non-stressed plants

Sources	df	*Fo*	*Fm*	*Fv/Fm*	*Fv/Fo*
Drought	1	0.098 ns	0.0893 ns	0.0099 **	0.0196 *
Treatment	3	0.175 ns	0.0009 ***	0.0016 **	0.0008 ***
Interaction	3	0.001 **	0.9787 ns	0.0000***	0.0000 ***
Error	24	4.36	0.61	0.007	1.15

ns, non-significant; CuO, Copper oxide; NPs, Nanoparticles; ns, non-significant; *, **, *** significance at 0.05, 0.01 and 0.001 probability levels, respectively

Drought stress did not affect the basic fluorescence attributes (*F*o, *F*m) and ratios of basic fluorescence attributes (*F*v/*F*m, *F*v/*F*o). However, application of 100 mM CuO NPs increased the *F*o and decreased the *F*m, *F*v/*F*m and *F*v/*F*o values (**Figure 6**). The JIP-test attributes were also calculated. Drought stress reduced the performance index (PI_{ABS}) of the PSII activity of the corn plants. However, application of 25 and 100 mM CuO NPs reduced the PI_{ABS} of the corn plants under normal conditions. In contrast, under drought stress, application of 25 mM CuO NPs improved the PI_{ABS}. These changes in PI_{ABS} could be due to any one or combination of its components, i.e., changes in reaction center density, efficiency of primary photochemistry or efficiency of electron transport beyond Q_B. The results showed that the decline in PI_{ABS} due to 100 mM CuO was mainly attributed to decrease in active reaction center density and primary photochemistry. However, increase in PI_{ABS} due to 25 mM CuO NPs was attributed to increase in active reaction center density and efficiency of biochemical reaction or electron transport efficiency beyond Q_B (**Figure 6**).

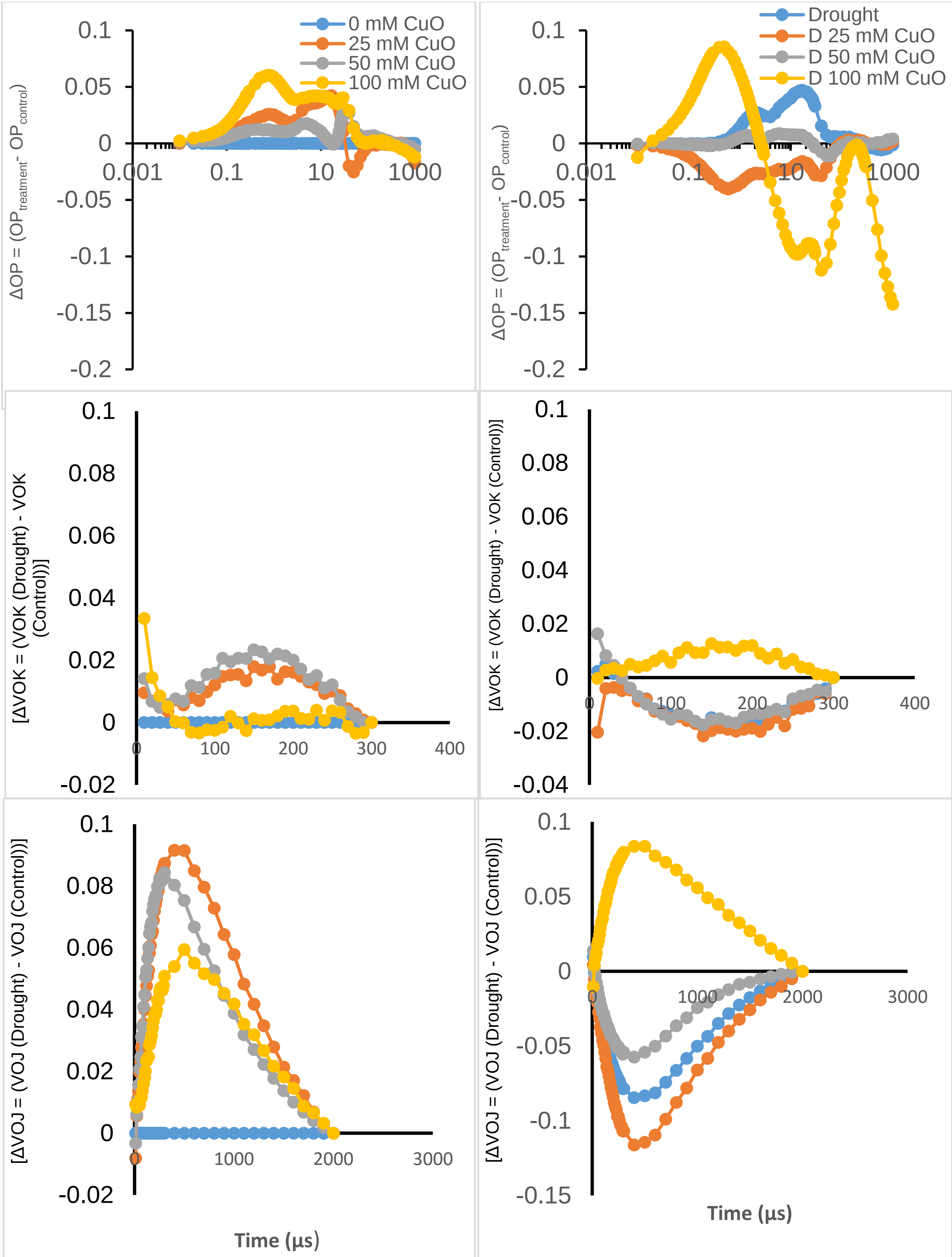

Figure 5. Semiquantitative analysis of structural stability of photosystem II at the donor end (oxygen evolving complex) and energetic connectivity of antenna and reaction centre of drought-stressed and non-stressed corn (*Zea mays* L.) plants treated with varying concentrations of CuO nano-particles

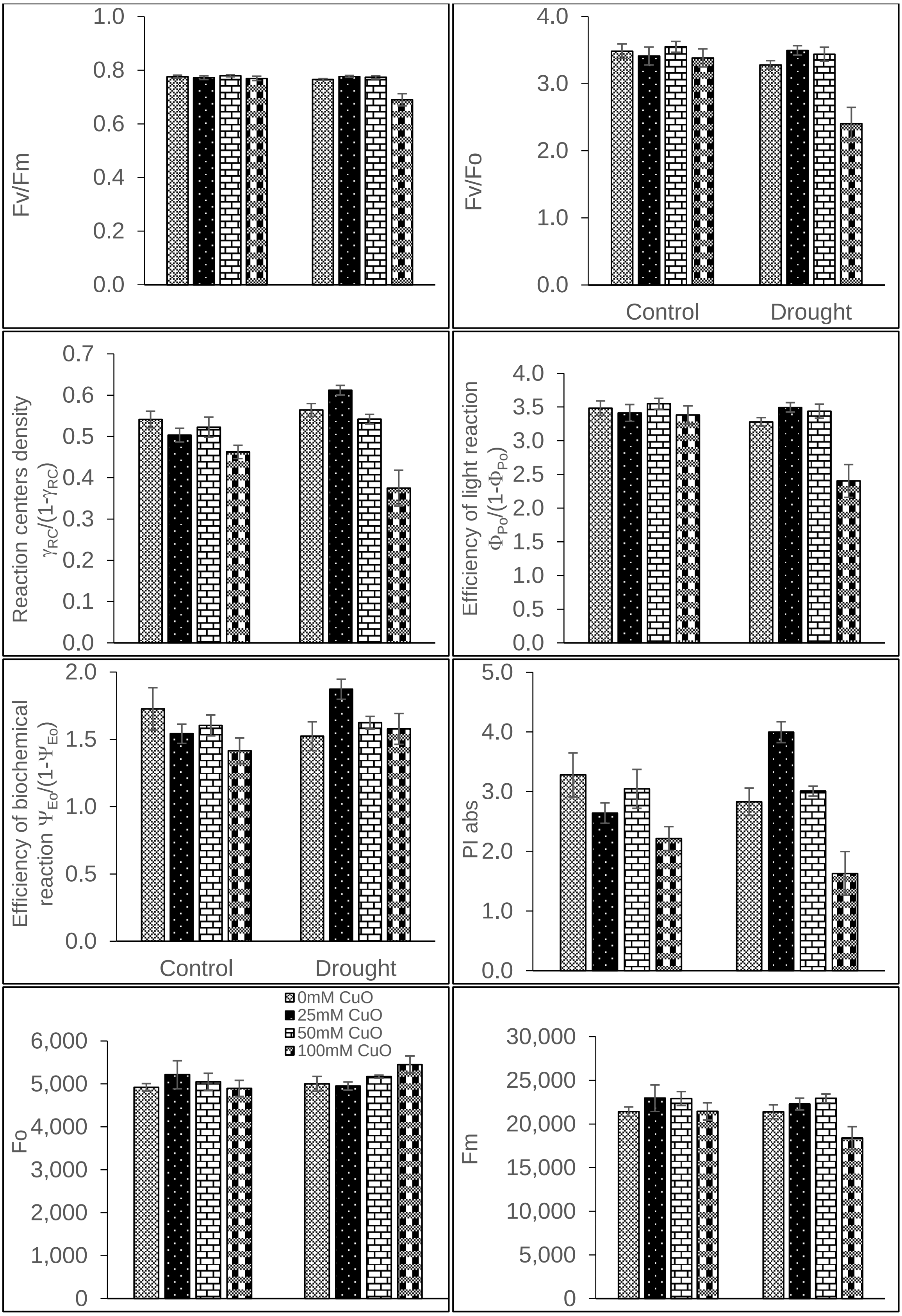

Figure 6. Semiquantitative analysis of structural stability of photosystem II at the donor end (oxygen evolving complex) and energetic connectivity of antenna and reaction centre of drought-stressed and non-stressed corn (*Zea mays* L.) plants treated with varying concentrations of CuO nano-particles

Discussion

The current study found that drought stress reduced the growth of corn plants which is a well-documented phenomenon in the literature. However, exogenous application of CuO NPs improved the growth of the corn plants. It is argued that the liberated copper ions can be employed as trace elements to promote plant growth and increase plant biomass when the concentration of NPs used in the experiment is low. A little amount of copper-based nanoparticles can help plants grow, according to some previous studies, e.g., 10 mg/L CuO NPs enhanced the root biomass of both traditional and transgenic cotton (Le Van et al., 2016). López-Luna et al. (2016) also reported improvement in fresh biomass and rate of photosynthesis on supplementation of Cu NPs in *Spinacia oleracea* L. The development of maize seedlings improved when nano-copper dioxide particles were present in low concentrations (Adhikari et al., 2016). In our present study, application of CuO NPs in lower concentration, i.e., 25 mM enhanced plant growth under drought stress (**Figure 2**). This enhancement in growth can be related to increase in root length for greater water and mineral nutrient uptake. CuO NPs are believed to stimulate proliferation and elongation of root cells as well as root hairs that improve the root length along with better absorption of nutrients from soil, leading to prominent boost in biomass production (Yang et al., 2018). In support of these findings, soybean plants treated with nano-CuO under drought stress kept more water than control plants hold (Xiong et al., 2021).

Copper oxide (CuO) nanoparticles have been documented to display phytotoxic effects by inducing various physiological changes. Smaller size NPs may have greater toxicity by penetrating leaf surface and cell wall (Du et al., 2011; Servin et al., 2012; Naz et al., 2020; Feigl, 2023). However, mechanism of uptake of NPs and their translocation within the plant body is exactly not known (Ma and Yan, 2018). Similarly, lower concentrations of NPs are less toxic than the higher concentrations of NPs (Feigl, 2023). For instance, copper oxide nanoparticles have the capacity to impede the growth of both roots and shoots in *Brassica juncea*, with the extent of inhibition correlating to the dosage (Nair and Chung, 2014). These findings are parallel to our current findings (**Figure 3**) that biomass of shoot and root of corn plants declined significantly at 100 mM CuO NPs application. Research has revealed that copper oxide nanoparticles at a concentration of 500 mg/L can hinder the growth of maize (Sui et al., 2014), whereas concentrations exceeding 10 mg/L significantly curtail cotton biomass accumulation (Le Van et al., 2016). Moreover, a study indicates that CuO nanoparticles negatively impact *Pisum sativum* (Ochoa et al., 2017). It has been observed that Cu ions are released from copper oxide nanoparticles, which might lead to Cu toxicity in plants. Nevertheless, smaller quantities of CuO nanomaterials can trigger a specific stimulative response in plants, promoting their growth, development, and resilience against challenging environmental conditions. When employing a low concentration of copper oxide NPs in experimental settings, the liberated copper ions can serve as trace elements, improving plant growth and biomass (Wang et al., 2022).

High reactive oxygen species (ROS) concentrations under drought stress can harm chlorophyll pigments, thylakoid membranes, and photosynthetic activities (Bano et al., 2021b). In the present study, reduction in chlorophyll concentration and relative water content (**Figure 3**), could have been because of reduced plant water status affecting the chlorophyll biosynthesis in the mesophyll cells (Bano et al., 2021a). Nanoparticles (NPs) have been shown to enhance the photosynthetic activity of plants experiencing drought conditions. Many studies focusing on abiotic-stressed plants have demonstrated that a majority of available NPs can enhance photosynthesis by increasing the content of photosynthetic pigments (Alabdallah and Alzahrani, 2020). This enhancement is achieved through various mechanisms, including the promotion of root growth, accumulation of compatible solutes, and maintenance of ionic balance (Naz et al., 2020).

The decline in accumulation of K^+ in the leaves and roots of the corn plants under drought stress might have been due to reduction in root biomass. It has been observed that drought stress reduced K^+ content that caused poor root growth in *Panicum* grass species (Javed et al., 2022). CuO NPs in low concentrations improved nutrient uptake (Ambrosini et al., 2018), as has been observed an increase in accumulation of K^+ in roots in the current study. Moreover, Pérez-Labrada et al. (2019) also reported that foliar-applied Cu nanoparticles enhanced growth and K^+ content in tomato plants. This improved availability of K^+ could have been due to enhanced root length and branching that provides more surface area for better nutrient uptake. It is possible that CuO nanoparticles affect the expression of ion transport channels and pumps in plant roots (Juarez-Maldonado et al., 2019). These channels are responsible for the selective uptake of ions from the soil (Ward et al., 2009).

Plant photosynthesis is a vital physiological process of growth and yield of plants. Various factors affecting this vital physiological process include light and light absorbing pigments, efficiency of plant to convert light into biochemical energy, and CO_2 uptake through stomata with its subsequent fixation in the form of carbohydrates (Ashraf and Harris, 2013). In this study, photosynthetic pigment 'Chl a' was reduced due to drought stress, but it was not changed due to CuO NPs. Changes in Chl b either due to drought or application of NPs were almost negligible. Drought stress did not affect the photosystem II (PSII) structural stability but reduced the functional activity. Both semi-quantitative analysis and JIP-test parameters indicated that drought stress caused damages to functional activity of PSII at the donor end (Changes in K-band, *Fv/Fo*, PI_{ABS}). These results are similar to those of Bano et al. (2021a) who reported that mild drought stress did not affect PSII structural stability in mung bean. They further documented that more than two-week drought stress during summer season reduced the quantum yield of PSII.

Overall, application of CuO NPs improved the PSII functional activity (PI_{ABS}) which is associated with enhancement in active reaction center density and efficiency of electron transport beyond QB, mainly due to increased downstream biochemical processes.

Author(s), Editor(s) and Publisher's declarations

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Conflict of interest
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Contribution of authors
Conceptualization and designing of the study: SA, AR, IU. Conduction of experiments: SA, AR. Data collection, visualization, and interpretation: SA, AR, IU, BK, MJ. Formal statistical analysis: SA, AR, IU. Writing of first draft: SA, AR, IU, BK, MJ, MI. Proof reading and approval of the final version: SA, AR, IU, BK, MJ, MI.

Supplementary material
No supplementary material is included with this manuscript.

Ethical approval
This study does not involve human/animal subjects, and thus no ethical approval is needed.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of the experiment, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Declaration of generative AI and AI-assisted technologies in the writing process
It is declared that we the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent
All contributors have critically read this manuscript and agreed to publish in IJAaEB.

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