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RESEARCH ARTICLE

Foliar application of zinc selenide enhances drought tolerance in maize

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Abstract

Drought stress severely limits maize (*Zea mays* L.) growth and grain yield. Foliar application of nanomaterials with antioxidant properties offers a promising strategy for mitigating drought effects. This study evaluated the potential of zinc-selenide quantum dots (ZnSe QDs) to improve drought tolerance in maize. A pot experiment was conducted using a factorial randomised block design (RBD). The first factor was the irrigation regime with 2 levels: (i) irrigated control, where plants were watered daily to maintain 0.9 fraction of transpirable water (FTSW) and (ii) drought-stressed, where plants experienced progressive soil drying from 0.95 to 0.05 FTSW. The second factor was foliar spray with 3 levels: (i) water spray, (ii) combined zinc sulphate (10 mg L^{-1}) and sodium selenate (10 mg L^{-1}) spray (Zn+Se) and (iii) ZnSe QDs (20 mg L^{-1}). The field trial used the same treatment structure. Under drought conditions, foliar application of ZnSe QDs at 20 mg L^{-1} during the vegetative stage significantly (p<0.05) increased photosystem II quantum yield by 10 %, leaf water content by 22 % and stomatal conductance by 28 % compared to water spray. The rise in photosynthetic rate (28 %) under drought was linked to increased tissue water content, catalase activity (47 %) and peroxidase activity (60 %). During the reproductive stage, ZnSe QDs spray enhanced the number of seeds m^{-2} and individual seed weight, leading to increased seed yield under drought stress. These findings demonstrate that foliar application of ZnSe QDs at 20 mg L^{-1} can mitigate drought-induced effects in maize.

Keywords: drought; maize; photosynthesis; stomatal conductance; zinc-selenide QDs

Introduction

Globally, 700 million people will be projected to be hungry and 600 million will be projected to be chronically undernourished in 2030, with the situation expected to worsen due to the growing global population (1). Most individuals who are hungry and undernourished reside in Asia and Sub-Saharan Africa. This increase has been linked to factors such as adverse climate events, low export commodity prices that hinder public investment and unequal access to food (2). Climate change is widely recognized as the foremost challenge to 21st century food security and environmental sustainability (3). In India, about 26 % of the total land area has been affected by drought stress conditions (4). In the 21st century, the risk of drought worldwide will further intensify due to extreme water deficits. India's annual rainfall is decreasing by 0.04 % each year and over the past 50 years, the country has experienced a mild to moderate rainfall deficit. Furthermore, it is projected that many regions in India will face future rainfall shortages (5). The plant's response to drought depends on the duration and severity of the drought, as well as the species, age and developmental stage of the plant. To withstand drought stress, many plants have developed various tolerance mechanisms like antioxidant defense, reduced transpiration and osmotic adjustment, which differ among species.

Climate change presents significant risks to agricultural production and to ensure global food security, it is essential to increase crop productivity. Studies show that rising temperatures and shifting precipitation patterns have negatively impacted food production due to climate change in recent decades (6). Under drought conditions, crop yield losses typically range from 30 % to 90 %, depending on the crop species (7). As a consequence of the global drought, wheat yields have decreased by 21 % and maize yields have declined by 40 % (8). By 2030, maize is expected to surpass wheat as the most widely cultivated crop, as trends suggest a relatively static wheat area (9).

Maize, grown on ~197 million hectares worldwide, is second only to wheat but more vulnerable to drought. It is cultivated across various agroecological zones, from wet to dry areas and from lowland to highland regions (10). Drought during vegetative stages significantly affects plant growth, architecture, ear size and kernel numbers. Research shows that drought stress during the vegetative stage can reduce grain yield by 25 %, while drought during flowering decreases yield by 50 % to 70 %. The reduction in maize grain yield, depending on the severity and stage of drought, can range from 10 % to 76 % (11-13). The adverse effects of drought stress can be mitigated through the application of nanoparticles (NPs).

Nanotechnology involves particles ranging from 1-100 nm in size, known as NPs. These particles differ significantly from bulk materials in terms of surface chemistry, morphology and their large surface area-to-volume ratio, making them more reactive (14).

Recent research applying NPs to crops such as maize, wheat, soybean, tomato and cucumber has shown improvements in seedling growth, germination, nitrogen metabolism, photosynthetic activity and protein content, indicating their potential to enhance crop productivity (15). Studies also suggest that nanomaterials can help reduce abiotic stress in crops. For example, the use of zinc oxide NPs as seed priming agents has been shown to promote uniform seedling emergence and improve rice growth, partly by increasing antioxidant enzyme activity, which protects against damage from reactive oxygen species (ROS) (16). Some research also indicates that nanoselenium (n-Se) exhibits improved antioxidant properties under high-temperature stress (17). The role of zinc-selenide quantum dots (ZnSe QDs) remains unclear; before this, no data were available on their effects under water stress during early vegetative stages or their influence on maize yield components. Consequently, it is hypothesized that foliar application of ZnSe QDs to maize plants subjected to vegetative stage drought stress could enhance maize grain yield by modulating the antioxidant defence system.

Materials and Methods

Synthesis of zinc-selenide quantum dots

The synthesis of ZnSe QDs was carried out by the hydrothermal method. In brief, 2 g of polyvinyl pyrrolidine was dissolved in 100 mL of double-distilled water. To this solution, 1.2 g of zinc sulphate and 0.85 g of sodium selenate were added and dissolved. Following this, 2.5 g of sodium hydroxide was added to the solution. Ten mL of hydrazine hydrate was added to the solution and transferred to a Teflon-lined hot air oven maintained at 100 °C for 24 hr. After 24 hr, the solution was kept at room temperature, the content was filtered and washed with double-distilled water 5 times. After the sediment was kept in a hot air oven at 60 °C for 1 hr. The formed product was collected and used for foliar spray. The average particle size ranged from 10 to 20 nm, which was measured using the dynamic light scattering method. Our earlier study indicated that ZnSe does not exhibit ecotoxicity up to 25 mg L^{-1} (18).

Effect of ZnSe QDs under drought stress during the vegetative stage – pot culture experiment

Crop husbandry

A controlled environment experiment was carried out at the Department of Crop Physiology, Tamil Nadu Agricultural University, Tamil Nadu. Co(H)M 8 variety of maize was used in this study. The seeds were surface sterilized using sodium hypochlorite (10 % v/v) for 5 min. After washing in deionized water 5 times, the seed was sown in a plastic pot (25.7 x 25.5 x 22.1 cm; L x B x H) containing 27 kg of sandy clay loam soil. The hole was created at the bottom level for proper drainage. To each pot, 3 seeds were sown. After seedling emergence, the seedling was thinned to 1 per pot. To each pot, 2 g urea, 1 g diammonium phosphate and 1 g potash were added and repeated every 7 days. The irrigation was provided on alternative days till complete emergence of the 5th leaf (30 days after emergence; DAE). After the 30th day, all pots were well irrigated until they reached field capacity and then they were covered with a polyvinyl chloride sheet to prevent evaporation. The experiment was conducted in a factorial randomized block design with 4 replications. Factor 1 was soil moisture regime [irrigated control (fraction transpirable soil water, i.e., 0.9 FTSW) and drought stress (0.2 FTSW)]. Factor 2 was foliar spray [water, ZnSe QDs (20 mg L^{-1}), or bulk material (10 mg L^{-1} of $ZnSO_4$ and 10 mg L^{-1} of sodium selenate) or zinc ethylene diamine tetra acetic acid (Zn-EDTA) (20 mg L^{-1})]. The methodology involved spraying 8 pots with water, 8 pots with ZnSe QDs, 8 pots with bulk zinc-selenium and 8 pots with Zn-EDTA using a hand sprayer. Among the 8 pots, 4 were maintained under drought stress (0.2 FTSW). Four were kept at the irrigated control (field capacity, i.e., 0.9 FTSW). The maintenance of FTSW in each pot was carried out as described elsewhere (18). In brief, the irrigated control and drought stress pots were weighed at 7.00 AM. The amount of water required to maintain 0.9 FTSW and 0.2 FTSW was added. The plant was maintained under drought stress for 13 days and during the stress period, various physiological and biochemical traits were recorded.

Physiological traits

The physiological traits were recorded in the fully expanded top leaf. The traits were chlorophyll index, maximum quantum yield of photosystem II, relative water content and gas exchange traits, which were collected from 10.00 AM to 2.00 PM on 4, 8 and 12 days after stress imposition. Leaf chlorophyll index was recorded non-destructively using a chlorophyll meter and chlorophyll A fluorescence trait, was measured using a modulated fluorometer (OS5p+, Opti Sciences, Hudson, NH) (19). Gas exchange traits were measured with an infrared gas analyzer (CI-340 portable photosynthesis system, CID Inc., Camas, WA, USA) (20). Relative water content (RWC) was estimated as described elsewhere (21) and expressed as a percentage. Leaf water potential was measured on the 6th day after stress imposition. Leaf water potential was measured using a pressure bomb apparatus (ARIMAD 3000, MRC lab, Essex, UK).

Biochemical traits

A second leaf from the top was collected on the 4th, 8th and 12th days after stress imposition and immediately frozen in liquid nitrogen from 4 replications for each treatment. The frozen leaf sample was used to measure the antioxidant enzyme activities (catalase (CAT) and peroxidase (POX)) and oxidative stress markers (hydrogen peroxide (H_2O_2) and malondialdehyde (MDA)). The H_2O_2 was quantified by adopting the procedure described earlier (22) and expressed as nmol g^{-1}. Lipid peroxidation (MDA) was quantified as described earlier (23). The CAT enzyme activity was quantified (24) and expressed as µmol H_2O_2 destroyed $min^{-1} g^{-1}$. The POX enzyme activity was quantified (25) and expressed as µmol tetra guaiacol formed $min^{-1} g^{-1}$.

Effect of ZnSe QDs on reproductive stage drought stress in maize – field experiment

Crop husbandry

The experiment was conducted in a split-plot design with 4 replications. The maize variety CoH(M) 8 was sown at 60 x 30 cm spacing and the pre-emergence herbicide atrazine was sprayed. Following this, life irrigation was given on the 3rd day after sowing and thinning was done on the 10th day after sowing. The plants were fertilized with 135:62.5:50 NPK kg ha^{-1} and the plants were maintained under irrigated conditions up to the tasseling stage. The main plot treatment was soil moisture regime 1. irrigated control (irrigation once in 7 days) and 2. drought stress (skipped irrigation for 30 days). The subplot treatment was foliar sprays 1. water (control), 2. bulk material (foliar application of 10 mg L^{-1} of $ZnSO_4$ + 10 mg L^{-1} of sodium selenate)] and 3. ZnSe QDs (foliar application of 20 mg L^{-1} of the ZnSe QDs). At the tasseling stage, the sub-plot treatments were imposed and the irrigated control plants were irrigated every 7 days, while the drought-stressed plants were not irrigated for 30 days. After the stress period, all the plants were irrigated weekly until maturity. After maturity, the plant was harvested and the yield components were recorded.

Traits recorded

At harvest, the plants were harvested, separated into plant parts and dried at 60 °C for 48 hr. After that, dry weight was measured and expressed as total dry matter production (kg ha^{-1}). Then, the cob was cleaned, the seeds were collected and expressed as the number of seeds m^{-2}. The individual seed weight was calculated by dividing the total seed weight by the number of seeds and expressed in milligrams (mg). The seed weight was expressed as seed yield (kg ha^{-1}). The harvest index was calculated as a ratio between grain yield and total dry matter production and expressed in percent (%).

Statistical analysis

The data were analyzed using statistical analysis system version 9.3. Tukey's test was used to compare treatment means at a *p*-value of 0.05.

Results

Effects of ZnSe QDs in alleviating the vegetative stage drought stress

Chlorophyll index and chlorophyll a fluorescence traits

The effect of irrigation regimes, foliar sprays and their interactions was significant ($p<0.05$) for chlorophyll index (Fig. 1a) and maximum quantum yield of PSII (Fig. 1b). Drought stress decreased the chlorophyll content (5.5 %) and F_v/F_m ratio (3.5 %) compared to the irrigated control (Fig. 1a, b). Under drought stress, foliar application of ZnSe QDs (20 mg L^{-1}) significantly ($p<0.05$) increased the chlorophyll content (27 %) and F_v/F_m ratio (10 %) compared to water spray.

Leaf tissue water status

A significant difference ($p<0.05$) was observed in leaf water potential (Fig. 2a) and RWC (Fig. 2b) for the effect of irrigated regimes, foliar sprays and their interaction. Drought stress significantly ($p<0.05$) decreased the leaf water potential (60 %) and RWC (15 %) compared to the irrigated control. Under drought stress, foliar application of ZnSe QDs (20 mg L^{-1}) significantly ($p<0.05$) improved the leaf water potential (32 %) and RWC (22 %) compared to water spray (Fig. 2a, b).

Gas exchange traits

A statistically significant difference ($p<0.05$) was found in the photosynthetic rate (Fig. 2c) and stomatal conductance (Fig. 2d) due to the effects of irrigation regimes, foliar sprays and their interactions. Drought stress decreased the photosynthetic rate (28 %) and stomatal conductance (28 %) compared to the irrigated control. The foliar application of zinc-selenide QDs at 20 mg L^{-1} sprayed plants significantly ($p<0.05$) increased photosynthetic rate (17 %) and stomatal conductance (8.9 %) over water spray.

Oxidative stress markers (H_2O_2 and MDA)

The effect of irrigation regime, foliar spray and their interaction was significant ($p<0.05$) for H_2O_2 content (Fig. 3a) and MDA (Fig. 3b). Drought stress increased the H_2O_2 content and MDA content compared to the irrigated control. The foliar application of ZnSe QDs (20 mg L^{-1}) sprayed plants had a lower H_2O_2 content (50 %) and MDA (33 %) than water and bulk material sprayed plants under drought stress. Overall, the variations in H_2O_2content and MDA content did not vary under irrigated conditions (Fig. 3a, b).

Antioxidant enzyme activity

The antioxidant enzymes, viz., CAT (Fig. 3c) and POD (Fig. 3d) activity were significantly ($p<0.05$) varied for irrigation regime, spray and their interactions. Drought stress decreased the CAT (30 %) and POD (50 %) compared to the irrigated control. Under drought stress, foliar application of ZnSe QDs (20 mg L^{-1}) significantly ($p<0.05$) increased the CAT (47 %) and POD (60 %) compared to water spray.

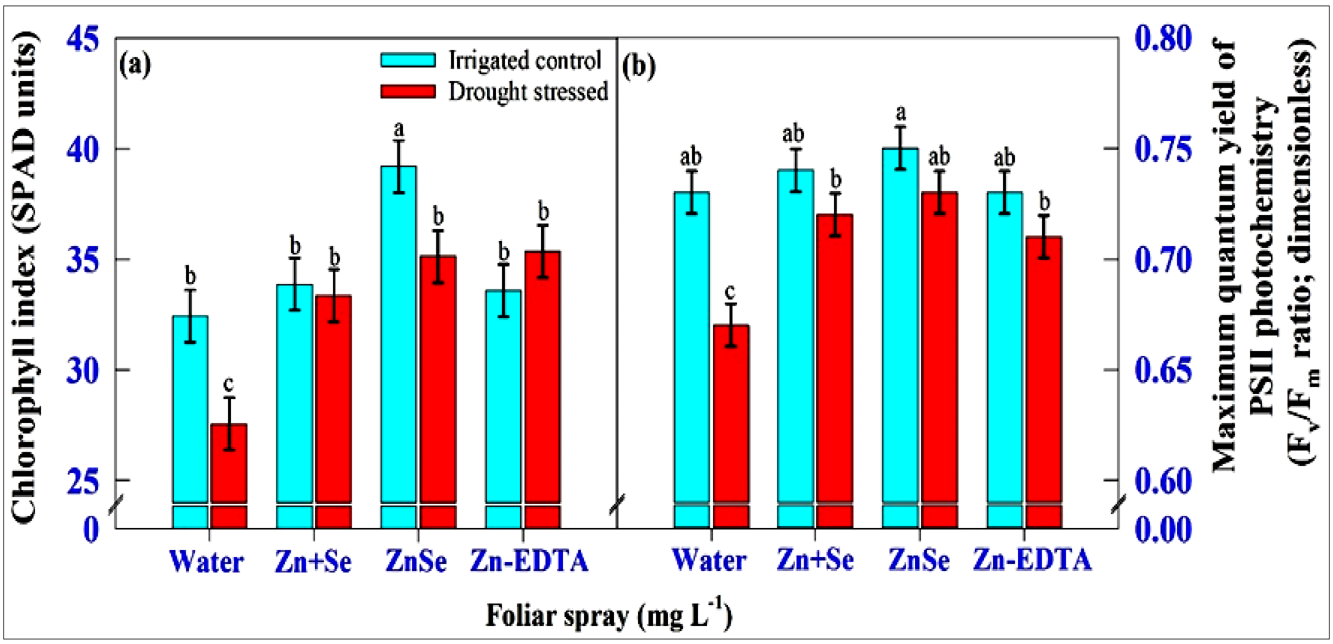

Fig. 1. Interaction of effects of irrigation regime and foliar spray of ZnSe QDs on chlorophyll index (**a**); and maximum quantum yield of PS II photochemistry (**b**), of maize.

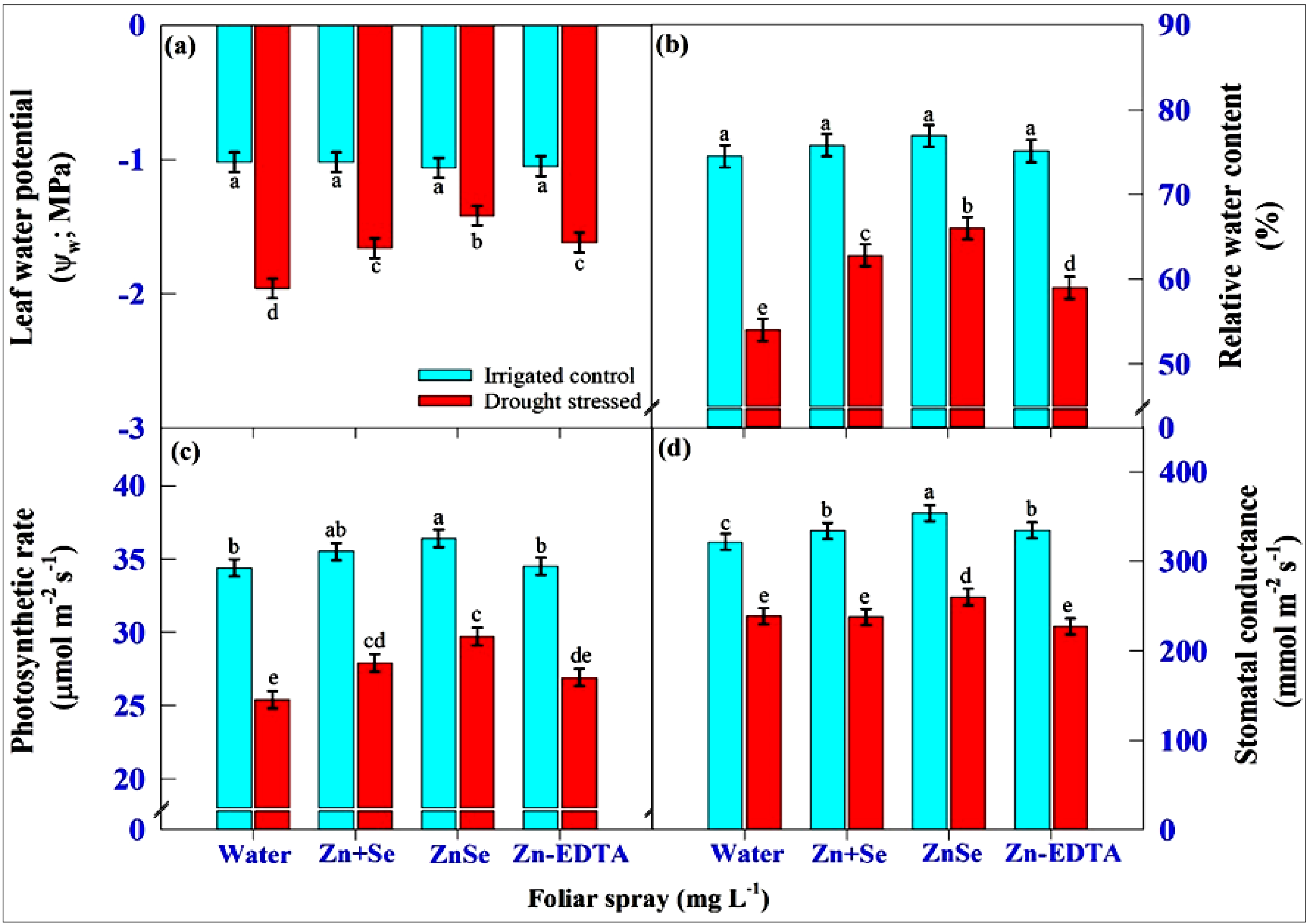

Fig. 2. Interaction of effects of irrigation regime and foliar spray of ZnSe QDs on water status and gas exchange of maize; (**a**) leaf water potential, (**b**) relative water content, (**c**) photosynthetic rate and (**d**) stomatal conductance.

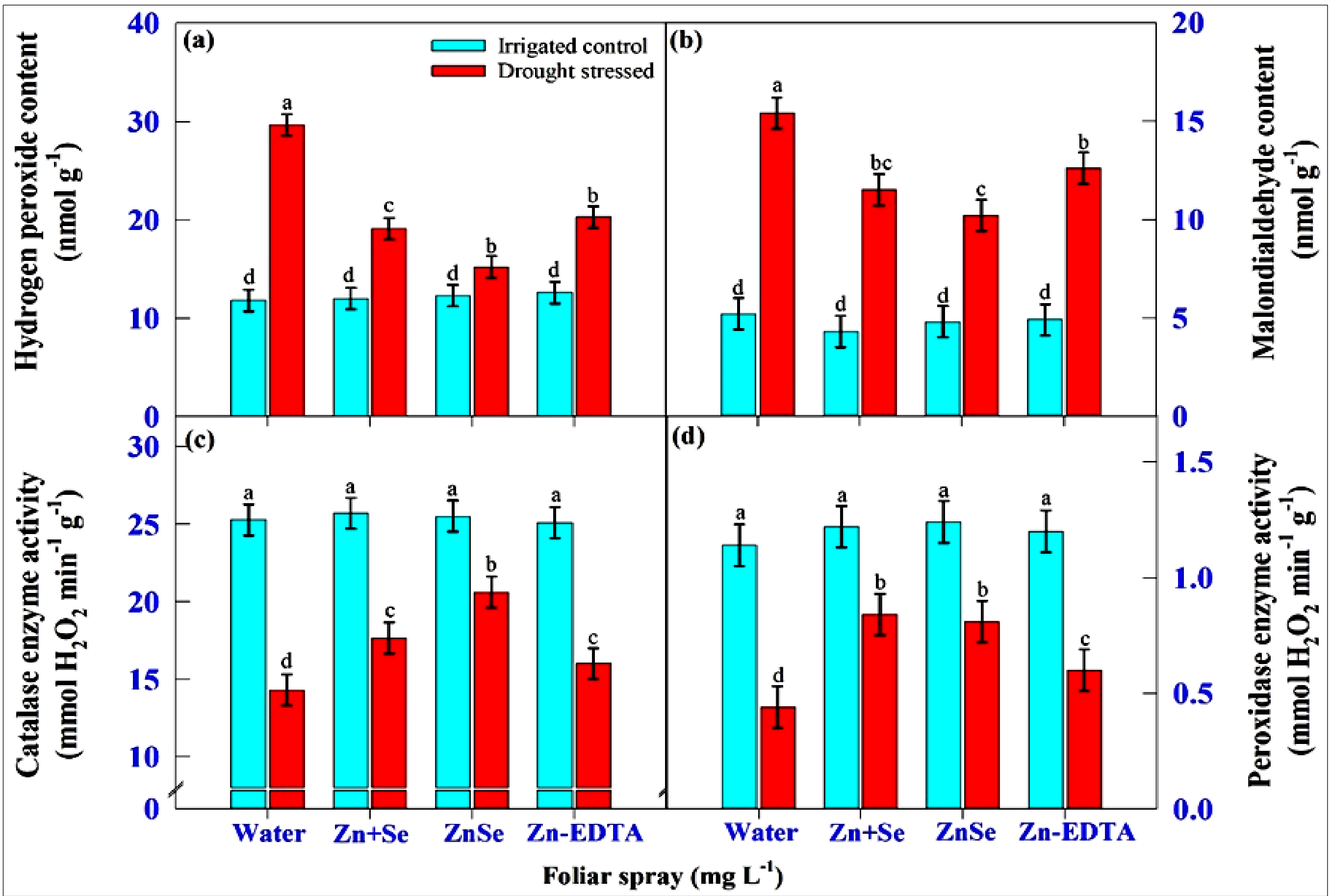

Fig. 3. Interaction of effects of irrigation regime and foliar spray of ZnSe QDs on antioxidant defense in maize; (**a**) H_2O_2 content, (**b**) MDA content, (**c**) CAT activity and (**d**) POD activity.

Discussion

With the global population projected to reach 10 billion by 2050, food security is a pressing challenge, especially under environmental stresses like drought (26). However, environmental stresses, particularly drought, have a detrimental impact on plant growth and productivity, leading to significant crop yield losses. Plants activate various mechanisms related to growth and development under drought stress. Therefore, exploring agricultural technologies to enhance crop productivity under drought stress is crucial for sustaining food security.

In this study, we demonstrated that foliar application of ZnSe QDs improved drought tolerance in maize, as evidenced by improved chlorophyll index (SPAD units) (Fig. 1a), maximum quantum yield of PS II photochemistry (F_v/F_m; dimensionless) (Fig. 1b) and better leaf water status (Fig. 2a, b), enhanced the carbon assimilation rate and stomatal conductance (Fig. 2c, d) and elevated CAT and POX activities to neutralize excess ROS (H_2O_2) (Fig. 3c, d). These factors contributed to maintaining photosynthesis and protective mechanisms, ultimately supporting plant growth, biomass and grain yield under drought stress (Table 1).

Drought stress substantially reduced the efficiency of PS II and chlorophyll contents. Reduced photosynthesis is one of the main effects of drought, which results from decreased leaf growth, damaged photosynthetic machinery, early leaf senescence and a corresponding decline in food production. Chlorophyll content is closely linked to the rate of biomass production through photosynthesis and plays a crucial role in determining plant productivity (27). The chlorophyll index was higher in the irrigated control compared to the drought stress. Foliar application of ZnSe QDs has been shown to increase the chlorophyll index compared to water spray (Fig. 1a), with this index being directly related to photosynthesis. ZnSe QDs may enhance chlorophyll synthesis by raising endogenous cytokinin levels (28). Similarly, the application of nZnO enhanced the photosynthetic efficiency of maize leaves under drought conditions by alleviating oxidative stress and maintaining chloroplast structural integrity (29). Additionally, the increased F_v/F_m ratio indicates that the improved photosynthetic rate induced by ZnSe or selenium foliar spray under drought stress can reduce the photooxidative damage to PS II (Fig. 1b). This is achieved by effectively scavenging ROS generated in the chloroplast during the light reaction, with zinc (30) and selenium (31).

Drought-stressed plants showed decreased net photosynthesis and stomatal conductance, aligning with findings in barley (32). The photosynthetic rate was higher in the irrigated control than drought-stressed plants. The application of NPs possessing antioxidant properties can help to stabilize the ultrastructure of chloroplasts and mitochondria, allowing plants to maintain their photosynthetic efficiency during drought (33). Plants sprayed with ZnSe QDs or Se exhibited increased photosynthetic rate and stomatal conductance compared to water-sprayed plants (Fig. 2c, d). This may be due to stomatal and non-stomatal limitations on photosynthesis under drought conditions (34). Drought stress also triggers the abscisic acid (ABA) signaling pathway, leading to stomatal closure. However, applying NPs downregulates H_2O_2-mediated stomatal closure and preserves better CO_2 intake (35). The higher stomatal conductance in ZnSe QDs -treated plants under drought suggests that ZnSe alleviates drought-induced stomatal limitations, possibly through the direct effects of nano-metal oxides on stomata. Furthermore, ZnSe QDs regulate RuBisCO (Ribulose-1, bisphosphate carboxylase/oxygenase) expression, which contributes to improved gas exchange efficiency in stressed plants (36).

The lowest water potential (MPa) values were observed in stressed plants, while the application of ZnSe QDs alleviated the effects of drought stress on water potential (Fig. 2a, b). ZnSe QDs could change in xylem hydraulic resistance. Foliar application of nanosilica decreased the xylem water potential of wild pear seedlings (37). RWC is used alongside xylem water potential to assess plant water status. In the present study, among the foliar sprays, applying ZnSe QDs increased the water content and RWC compared to water spray, indicating that ZnSe QDs can alleviate drought stress by maintaining higher tissue water content through increased water potential. The higher leaf water status of plants sprayed with ZnSe QDS could help sustain photosynthesis under drought conditions (18), potentially influencing plant recovery and productivity.

Table 1. Interaction of effects of irrigation regime and foliar spray of ZnSe QDs on yield and yield components of maize

Treatment	Number of seeds m^{-2}	Individual seed size (g)	Total dry matter production ($kg\ ha^{-1}$)	Seed yield ($kg\ ha^{-1}$)	Harvest index (%)
Main plot (M) - Irrigation regime					
M_1 – Irrigated control (Watered once in 7 days)	2317.7^{a}	0.180^{a}	10403^{a}	4228.2^{a}	40.5^{a}
M_2 - Drought stressed (Withholding water for 30 days)	2039.7^{b}	0.170^{b}	9497^{b}	3499.1^{b}	36.8^{b}
C. D.	53.4	0.005	432	116	1.40
Sub-plot (S) - Foliar spray					
S_1 - Water	2077.2^{b}	0.172^{b}	10003^{a}	3654.8^{b}	36.1^{c}
S_2 - Bulk material ($ZnSO_4$ at 10 mg L^{-1} and sodium selenate at 10 mg L^{-1})	2228.6^{a}	0.175^{ab}	10172^{a}	3934.8^{a}	38.6^{b}
S_3 – ZnSe QDs at 20 mg L^{-1}	2230.3^{a}	0.178^{a}	9675^{a}	4001.3^{a}	41.3^{a}
C. D.	79.9	0.002	644	173	2.10
Interaction (M x S)					
M_1S_1 - Irrigated and water spray	2267.3^{ab}	0.180^{ab}	10375^{ab}	4177.4^{a}	39.7^{ab}
M_1S_2 – Irrigated and bulk material spray	2396.1^{a}	0.180^{ab}	10823^{a}	4322.0^{a}	32.5^{c}
M_1S_3 - Irrigated and ZnSe QDs spray	2289.8^{ab}	0.182^{a}	10011^{ab}	4185.0^{a}	41.7^{a}
M_2S_1 - Drought and water spray	1887.1^{d}	0.165^{c}	9630^{b}	3132.1^{c}	32.5^{c}
M_2S_2 - Drought and bulk material spray	2061.1^{c}	0.170^{bc}	9521^{b}	3547.5^{b}	37.2^{b}
M_2S_3 - Drought and ZnSe QDs spray	2170.9^{bc}	0.175^{abc}	9339^{b}	3817.5^{b}	40.8^{ab}
C. D.	79.9	0.002	644	173	2.10

Mean values within column that have different superscript letters are significantly different from one another under the Tukey's test at 0.05 probability level.

Drought stress-induced ROS production damages cell membranes, causes lipid peroxidation and increases MDA accumulation (38). Drought stress can damage the thylakoid membrane, reducing photosynthetic electron flow and increasing ROS production (39). This study confirmed this, as evidenced by lower F_v/F_m ratio values (Fig. 1b) and higher levels of H_2O_2 and MDA (Fig. 3a, b) compared to irrigated controls. Foliar application of ZnSe and a combined Zn and Se treatment reduced H_2O_2 and MDA levels (Fig. 3a, b) compared to water spray. Conversely, enzyme activities of CAT and POX increased in ZnSe QDs-sprayed drought-stressed plants. Based on this, it can be inferred that ZnSe QDs and Se act as antioxidants by enhancing antioxidant enzyme activities (40). Drought stress during the tasseling stage in maize severely impacts yield by reducing silk and grain development (41). In this study, drought during the reproductive stage decreased the number of seeds m^{-2}, resulting in a decline in grain yield (Table 1). The irrigated control exhibited higher seed number m^{-2} and greater grain yield than drought-stressed plants. However, foliar application of ZnSe QDs increased seed number, grain yield and harvest index relative to other treatments (42).

Conclusion

Our results showed that drought stress reduced the growth and photosynthetic performance of maize by enhancing oxidative stress, by increasing the accumulation of ROS. The foliar application of ZnSe QDs (20 mg L^{-1}) at the vegetative stage increased the photosynthetic rate and activated the antioxidant defense system, which delayed senescence. ZnSe QDs play a significant role in regulating ROS levels in maize under water stress by enhancing the activity of ROS-scavenging enzymes and antioxidants. Apart from this, foliar spray at the flowering stage increased the number of seeds m^{-2} and individual seed weight, directly related to grain yield and finally improved the harvest index. On this basis, it can be concluded that foliar application of ZnSe QDs at 20 mg L^{-1} is an effective strategy to alleviate drought stress in maize. Understanding the molecular mechanism of action of ZnSe QDs may help us in developing a novel antitranspirant material.

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Authors' contributions

MD and VKK conducted the pot and field experiments. MD performed the statistical analysis and wrote the manuscript. VKK assisted in editing the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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