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RESEARCH ARTICLE

Zinc-Selenium nanocomposite enhances drought tolerance in pearl millet by improving photosynthesis

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Abstract

Pearl millet (*Pennisetum glaucum* L.) is grown in arid and semi-arid regions of the world as a staple food crop owing to its better adaptability to different climatic conditions and nutrition. Due to climate change, occurrence of drought is becoming more frequent and in pearl millet, this drought stress at critical growth stages significantly reduces the yield. This generates the need to produce an appropriate technology for enhancing drought tolerance. Research shows that nanotechnology can provide an effective solution in the form of nanoparticle application. Previous studies have shown that foliar application of zinc-selenium quantum dot in maize under drought stress has shown to significantly enhance its drought tolerance. The pot culture experiment was conducted by adopting Factorial Completely Randomized Design (FCRD) design to evaluate the effect of zinc-selenium nanocomposite foliar application in mitigating the ill effects of drought stress in pearl millet at its peak vegetative stage. The zinc-selenium (Zn-Se) nanocomposite was applied at different doses which included 0 mg L^{-1}(water sprayed) as control, 5 mg L^{-1}, 10 mg L^{-1}, 15 mg L^{-1} and 20 mg L^{-1}. Foliar application significantly reduced the impact of drought stress on growth and physiology of plant. It enhanced photosynthetic rate, transpiration rate, stomatal conductance, F_v/F_m ratio, plant height and plant girth compared to control plants. Also it reduces leaf temperature and F_0 values in sprayed plants as compared to control. The study indicates that zinc-selenium nanocomposite at 20 mg L^{-1} was most effective in mitigating drought stress. Hence it can be concluded that foliar application of zinc-selenium nanocomposite at 20 mg L^{-1} can effectively enhance drought tolerance by stabilizing the photosynthesis associated traits thereby reducing the effect of drought stress in pearl millet at its peak vegetative stage.

Keywords: drought; nanoparticle; pearl millet

Introduction

Drought inhibits crop growth and development and reduces crop yield, thus severely threatening the stability of global food stocks (1). Since 2000, the number and duration of the occurrence of droughts has risen 29 % from 1970 to 2019 and will continue to rise (2). Drought inhibits crop's growth by affecting plants at multiple levels. It increases the ROS generation within plant system thus leading to membrane damages and lowering antioxidants (3). It reduces the chlorophyll content thus impairing its photosynthetic machinery (4). In addition, drought also reduces the relative water content in crop plants and thus making them to lose their turgidity and become wilted (5). This overall destabilizes the cellular homeostasis and reduce the growth of plant (6). Thus drought remains detrimental for crop production.

Pearl millet, also known as bajra (*Pennisetum glaucum*), is mainly grown in tropical semi-arid regions of Africa and Asia (7). It serves as a staple food for around 90 million poor people in these regions, with about 30 million hectares of land used for its cultivation. In India, pearl millet is the fourth most widely grown food crop after rice, wheat and maize. About 46 % of pearl millet produced in India is used for human consumption, while 38 % is used as animal feed. Its high fiber content slows down the digestion process, by prolonging its retention in the stomach for longer time and, thus reducing frequent food intake and risk of obesity (8). This makes it one of the most important nutricereal. Even though pearl millet is better adapted to hot and dry conditions as compared to cereals such as maize, rice and wheat, with adaptive mechanisms such as leaf rolling, reduced canopy leaf area and reducing transpiration yet the crop is susceptible to drought at critical growth stages such as seedling emergence, establishment and reproductive stage (9). A study showed that mid-season drought may reduce yield of pearl millet by more than 50 % (10). Exposure to drought stress for more than 21 days had shown to increase ROS production and MDA content in leaves and root tissues (11).

In recent years, to combat the ill effects of abiotic stress in crop plants nano-technology based interventions is gaining momentum and becoming an important emerging solution (12). Nanoparticles are modified forms of basic elements derived by altering their atomic as well as molecular properties. Because of their small size(< 100 nm), high surface area and special physical and chemical characters like color, dispersion and thermodynamics, they behave very differently and possess unique properties compared to their larger counterparts (13). Application of nanoceria helped to combat drought stress in sorghum by reducing ROS and enhancing the antioxidant enzyme activity (14). Also zinc oxide nanoparticles was found beneficial for brinjal to mitigate drought stress by enhancing Photosystem (PS) II efficiency and RWC resulting in better fruit yield (15). In tomato, similar results were observed by application of nano selenium (16). Nanosilicon treatment in chickpea under drought stress have shown to enhance drought tolerance by altering its metabolites (17). Also upregulation of stress related genes (*ABC1, Wdhn13, CHP* and *EXP*) was seen in wheat when nano silica was applied under drought stress conditions (18). Application of copper oxide nanoparticles at germination stage in pearl millet under artificially induced drought conditions showed to enhance its germination percentage and morphological features of treated seeds along with an increase in chlorophyll a and b and carotenoid content (19). Similarly silver nanoparticle have shown to enhance osmotic and Reactive Oxygen Species (ROS) scavenging mechanisms in pearl millet under salinity stress conditions (20). Also its application showed to increase transcripts related to stress resistance mechanism (21). Altogether, these results indicate that nanoparticles possess bio-stimulating properties and can be used to mitigate drought stress in plants. However, there is lack of knowledge regarding the effect of nanoparticle application under vegetative stage drought stress in pearl millet. So, this study attempts to unravel the potential of nano Zn-Se in counteracting the drought stress in pearl millet by analyzing the photosynthesis and growth-related attributes in relation to drought tolerance. Thus, the nano Zn-Se concentration is optimized to promote drought tolerance in pearl millet.

Materials and methods

Nano Zn-Se was synthesized using standard procedure and characterized as quasi-spherical in shape with an average size of 10 nm (22).

Plant material and growth conditions

A pot culture experiment was carried out in protected net house facility at Department of crop physiology, TNAU, Coimbatore using factorial randomized block design with set of four replications. The factor 1 comprised of soil moisture regime with two levels [Irrigated control (I): watered every evening (after 25 days of emergence) to maintain field capacity; Drought stress (D): by withholding irrigation for 15 days] and factor 2 comprised of five levels of foliar spray of nano Zn-Se concentrations [T_1: water (control), T_2: 5 mg L^{-1} nano Zn-Se, T_3: 10 mg L^{-1} nano Zn-Se, T_4: 15 mg L^{-1} nano Zn-Se, T_5: 20 mg L^{-1} nano Zn-Se]. The plastic pots of 15 L capacity were filled with air dried and sieved red soil. Each pot was provided with a drainage hole to remove excess water and 14 kg of red soil was added to each pot. Seeds of pearl millet (variety CO-10) were sown at a depth of 3 cm. A single plant was maintained in each pot until harvest and the required amount of fertilizer was applied at seedling stage. The daytime maximum temperature, inside net house, during the experiment ranged between 37° C and 39° C.

The pots were watered at two days interval until the eight leaves half emerged (25 days after emergence; DAE). On the 26th DAE, the whole set of pots were divided into two groups: group 1 (irrigated control) and group 2 (drought stress). Before initiating the drought stress experiment, all pots were saturated to reach 100 % field capacity. Next morning, a polyvinyl chloride sheet was used to cover all the pots for preventing soil evaporation, with a small slit made to fit the plant. On the fourth day, four pots from each group were sprayed with the treatments mentioned under factor 2. The various physio-logical traits were recorded after 4 days of spraying.

Traits recorded

On the eighth day of drought stress, the following physiological characteristics were measured on the third leaf from the top in all four replications: Chlorophyll index (SPAD units), canopy temperature (°C), minimum fluore-scence yield (F_0), maximum quantum yield of PS II (F_v/F_m ratio) [F_v: variable fluorescence, F_m: maximum fluore-scence], stomatal conductance (m mol m^{-2} s^{-1}), photo-synthetic rate (m mol $m^{-2} s^{-1}$) and transpiration rate (m mol $m^{-2} s^{-1}$). Using an infrared camera (Raytek, Wilmington, NC, USA), the temperature of the leaf was determined and reported in (°C). The chlorophyll meter [Soil Plant Analytical Device (SPAD), Model 502, Spectrum Tech-nologies, Plainfield, IL, USA] was used to measure chlorophyll index and the values were expressed in SPAD units. A modulated fluorometer (OS5p, Optisciences, Hudson, NH, USA) was used to assess the fluorescence characteristics of chlorophyll in a 30 min dark-adapted leaves. portable photosynthesis device LICOR 6400XT (LI-COR, Lincoln, NE, USA) was used for measuring transpiration rate, photosynthetic rate and stomatal conductance (23). The growth traits such as plant height and stem girth were recorded on the last day of stress. The plant height was measured, using a standard scale, from ground to the top of ear head. The stem girth was measured near the first node above the ground. The yield traits such as ear-head length, number of seeds per ear-head and 100 grain weight were recorded after harvest.

Statistical analysis

The experiment followed a Factorial Completely Randomized Design (FCRD) having four replications. The statistical analysis of data was done by using GRAPES software (version 1.1.0). The interaction effects were studied by performing a two-way ANOVA. The Least Significant Difference (LSD) test was used to evaluate differences between the group means and the Critical Difference (CD) was calculated at a significance level of 0.05 ($p < 0.05$). Microsoft excel was used to visualize the graphs.

Results

Effect of nano Zn-Se foliar spray on physiological parameters

Chlorophyll fluorescence

The interaction effect of drought stress and foliar spray of nano Zn-Se on pearl millet at peak vegetative stage was found significant ($P < 0.05$) for minimum chlorophyll fluorescence level (Fig. 1(A), F_o: dimensionless) and maximum quantum yield of PS II photochemistry (Fig. 1(B), F_v/F_m: dimensionless). The drought stress significantly increased the F_0value by 40 % in drought control plants as compared to irrigated control plants. The foliar application of nano Zn-Se @ 20 mg L^{-1}at peak vegetative stage helped to decrease the $F_o$value by 28.33 % in comparison with drought control plant. Also, the drought stress significantly decreased the F_v/F_mvalue by 9.8 % in drought control plants as compared to irrigated control plants. The foliar application of nano Zn-Se @ 20 mg L^{-1}under drought stress at peak vegetative stage helped to increase the F_v/F_m value by 7.96 % as compared to drought control plant.

Chlorophyll index

The interaction effect of drought stress and foliar spray of nano Zn-Se on pearl millet at peak vegetative stage was found significant ($P < 0.05$) for chlorophyll index (Fig. 1(C), SPAD units), The drought stress significantly decreased the SPAD value by 18.16 % in drought control plants as compared to irrigated control plants. And the foliar application of nano Zn-Se @ 20 mg L^{-1}at peak vegetative stage helped to increase SPADvalue by 14.48 % as compared to drought control plant.

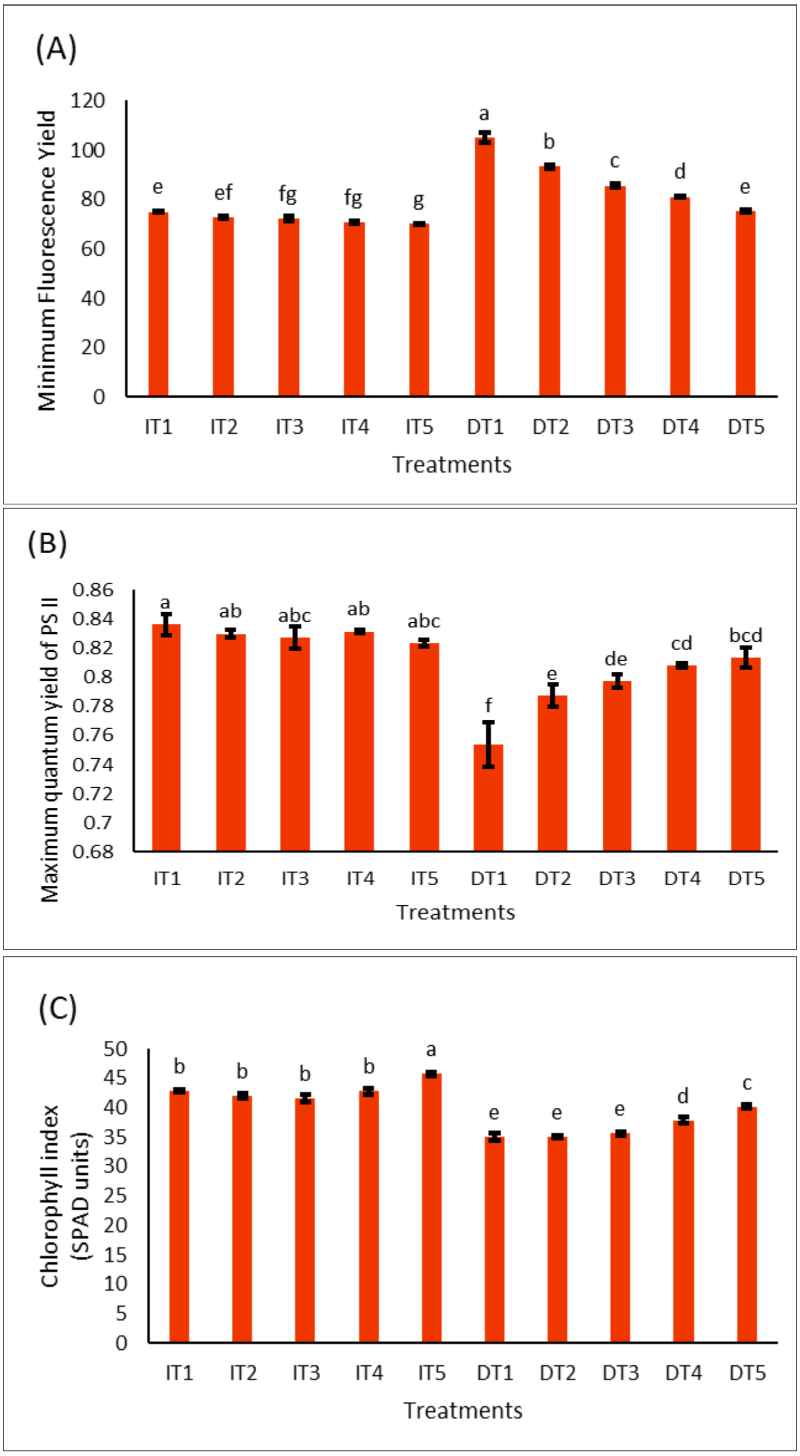

Fig. 1. Interaction effects of the moisture levels [Irrigated control (**I**): watered every evening to maintain field capacity; Drought stress (**D**): by withholding irrigation for 15 days] and foliar spray of nano Zn-Se concentrations [$\mathbf{T_1}$: water (control), $\mathbf{T_2}$: 5 mg L^{-1} nano Zn-Se, $\mathbf{T_3}$: 10 mg L^{-1} nano Zn-Se, $\mathbf{T_4}$: 15 mg L^{-1} nano Zn-Se, $\mathbf{T_5}$: 20 mg L^{-1} nano Zn-Se] on (**A**) minimum chlorophyll fluorescence level ($\mathbf{F_o}$: dimensionless), (**B**) maximum quantum yield of PS II photochemistry ($\mathbf{F_v/F_m}$: dimensionless) and (**C**) chlorophyll index (SPAD units). Each datum was the mean ± standard error of the mean (n=4). Means with different letters are significantly different at $P < 0.05$.

Gas exchange parameters

The interaction effect of drought stress and foliar spray of nano Zn-Se on pearl millet at peak vegetative stage was found significant ($P < 0.05$) for photosynthetic rate (Fig. 2(A), m mol $m^{-2}s^{-1}$), transpiration rate (Fig. 2(B), m mol $m^{-2}s^{-1}$) and stomatal conductance (Fig. 2(C), m mol $m^{-2}s^{-1}$). Drought stress significantly decreased the stomatal conductance by 29.96 % in drought control plants as compared to irrigated control plants. Foliar application of nano Zn-Se @ 20 mg L^{-1}at peak vegetative stage helped to increase stomatal conductance by 13.35 % in drought stressed plants as compared to drought control plants. The drought stress significantly decreased the transpiration rate by 61.03 % in drought control plants as compared to irrigated control plants. Whereas the foliar application of nano Zn-Se @ 20 mg L^{-1}in drought plants showed reduction of transpiration rate by 20.61 % as compared to irrigated control. The drought stress significantly decreased the photosynthetic rate by 41.09 % in drought control plants as compared to irrigated control plants and the foliar application of nano Zn-Se @ 20 mg L^{-1}at peak vegetative stage helped to increase photosynthetic rate by 39.51 % as compared to drought control plant.

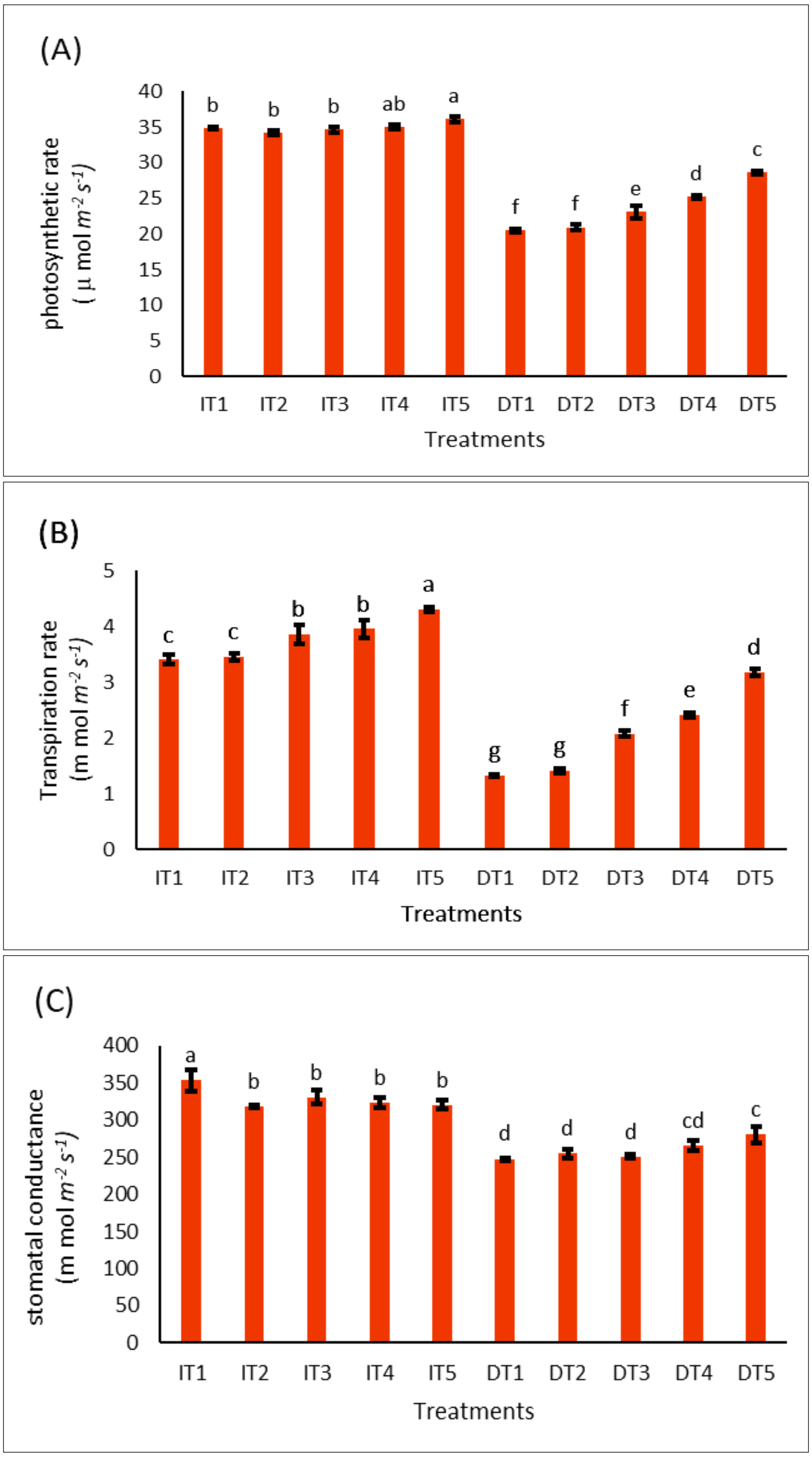

Fig. 2. Interaction effects of the moisture levels [Irrigated control (**I**): watered every evening to maintain field capacity; Drought stress (**D**): by withholding irrigation for 15 days] and foliar spray of nano Zn-Se concentrations [**T_1**: water (control), **T_2**: 5 mg L^{-1}nano Zn-Se, **T_3**: 10 mg L^{-1}nano Zn-Se, **T_4**: 15 mg L^{-1}nano Zn-Se, **T_5**: 20 mg L^{-1}nano Zn-Se] on (**A**) photosynthetic rate (μ mol $m^{-2}s^{-1}$), (**B**) transpiration rate (m mol $m^{-2}s^{-1}$) and (**C**) stomatal conductance (m mol $m^{-2}s^{-1}$). Each datum was the mean ± standard error of the mean (n=4). Means with different letters are significantly different at $P < 0.05$.

Effect of nano Zn-Se foliar spray on leaf temperature

The interaction effect of drought stress and foliar spray of nano Zn-Se on pearl millet at peak vegetative stage was found significant ($P < 0.05$) for leaf temperature (Fig. 3(A), °C), The drought stress significantly increased the leaf temperature by 16.98 % in drought control plants as compared to irrigated control plants. Foliar application of nano Zn-Se @ 20 mg L^{-1} at peak vegetative stage help to reduce leaf temperature by 13.05 % as compared to drought control plant.

Effect of nano Zn-Se foliar spray on growth parameters

The interaction effect of drought stress and foliar spray of nano Zn-Se on pearl millet at peak vegetative stage was found significant ($P <0.05$) for plant height (Fig. 3(B), cm) and stem girth (Fig. 3(C), cm). The drought stress significantly decreased plant height by 13.9 % in drought control plants as compared to irrigated control plants. Foliar application of nano Zn-Se @ 20 mg L^{-1}under drought stress increased plant height by 6.61 % as compared to drought control plants. The stem girth was significantly decreased by 25.76 % in drought control plants as compared to irrigated control plants. Foliar application of nano Zn-Se @ 20 mg L^{-1}under drought stress at peak vegetative stage increased stem girth by 32.65 % as compared to drought control plants.

Effect of nano Zn-Se foliar spray on yield parameters

The interaction effect of drought stress and foliar spray of nano Zn-Se on pearl millet at peak vegetative stage was found non-significant ($P < 0.05$) for ear-head length (in cm) and number of seeds per ear-head but was found significant for 100 grain weight (in g) (Table 1). The drought stress significantly reduced the 100-grain weight by 47.3 % in drought control plants as compared to irrigated control. The foliar application of nano Zn -Se @ 20 mg L^{-1} under drought conditions increased the 100 grain seed weight by 31.96 % as compared to drought control.

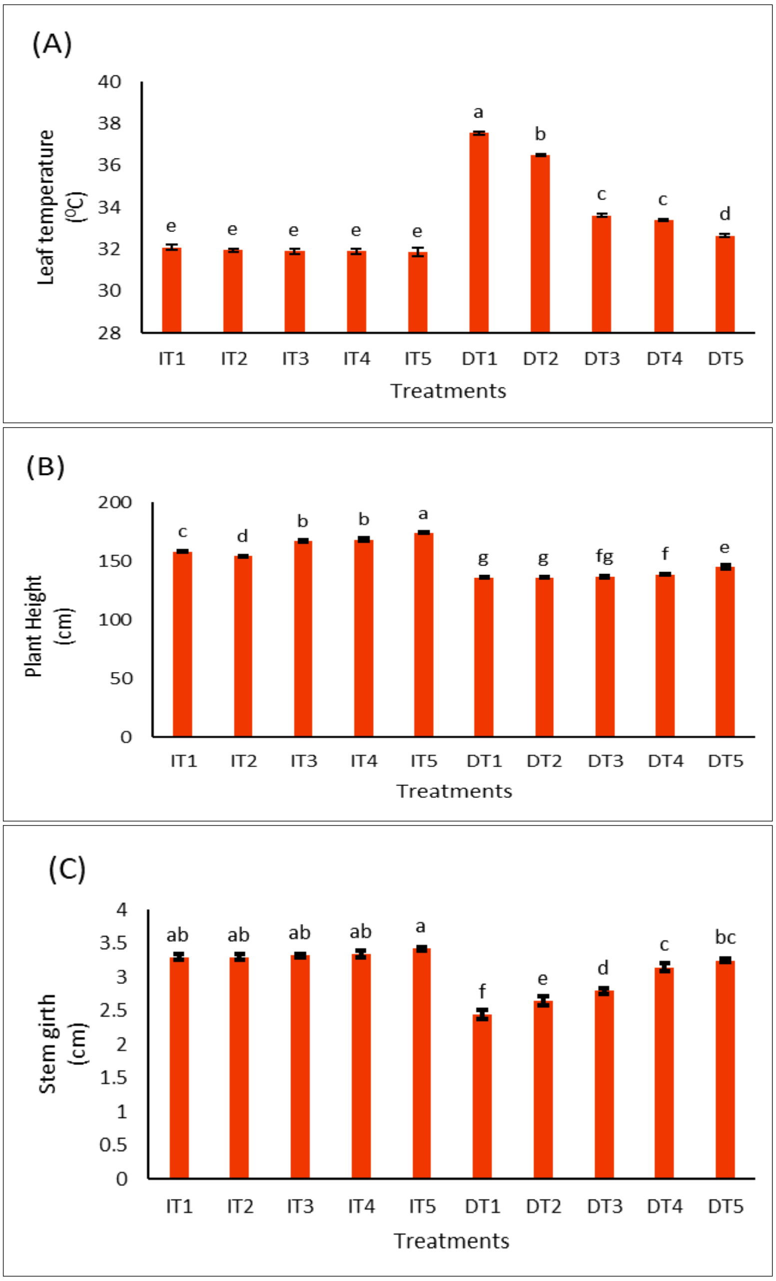

Fig. 3. Interaction effects of the moisture levels [Irrigated control (**I**): watered every evening to maintain field capacity; Drought stress (**D**): by withholding irrigation for 15 days] and foliar spray of nano Zn-Se concentrations [$\mathbf{T_1}$: water (control), $\mathbf{T_2}$: 5 mg L^{-1}nano Zn-Se, $\mathbf{T_3}$: 10 mg L^{-1}nano Zn-Se, $\mathbf{T_4}$: 15 mg L^{-1}nano Zn-Se, $\mathbf{T_5}$: 20 mg L^{-1}nano Zn-Se] on (**A**) Leaf temperature (°C), (**B**) plant height (cm) and (**C**) stem girth (cm). Each datum was the mean ± standard error of the mean (n=4). Means with different letters are significantly different at $P < 0.05$.

Table 1. Interaction effects of the moisture levels.

Treatment	Ear head length (cm)	No. of grains per earhead	100 grain weight (g)
IT1	18.25	1622.25	1.205[a]
IT2	20.13	1633.25	1.21[a]
IT3	19.00	1642.25	1.178[a]
IT4	20.88	1720.50	1.218[a]
IT5	21.75	1769.50	1.215[a]
DT1	17.28	905.75	0.635[c]
DT2	16.75	812.50	0.6[c]
DT3	17.25	920.00	0.637[c]
DT4	18.50	932.50	0.782[b]
DT5	19.25	1173.50	0.838[b]
SE(d)	1.429	53.80	0.03
CD	NS	NS	0.061*

Significant difference are indicated at 0.05 level; NS - Nonsignificant; Mean with same letter are not significantly different. [Irrigated control (**I**): watered every evening to maintain field capacity; Drought stress (**D**): by withholding irrigation for 15 days] and foliar spray of nano Zn-Se concentrations [**T_1**: water (control), **T_2**: 5 mg L^{-1} nano Zn-Se, **T_3**: 10 mg L^{-1} nano Zn-Se, **T_4**: 15 mg L^{-1} nano Zn-Se, **T_5**: 20 mg L^{-1} nano Zn-Se] on ear-head length, number of seeds per ear-head and 100 grain weight.

Discussions

Drought stress negatively affects plant growth and causes significant yield reduction in crop plants. Foliar application of nanoparticles under abiotic stress conditions can be a promising stress mitigating approach to sustain the crop growth and yield under such situations (24). In this study the drought stress at vegetative phase has significantly affected the growth and photosynthesis related traits in pearl millet. It greatly reduced the stomatal conductance of the plant. It may be the result of plant response to abiotic stress, as plants could sense the environment and respond to external stimuli of drought stress leading to closure of stomata (25). This reduction in stomatal conductance strongly controls the gas exchange happening in plants thus leading to reduced availability of CO_2 for photo-synthesis (26). But the application of nano Zn-Se showed to increase the stomatal conductance. The similar findings were seen by application of nano ZnO in tomato (27). The nanoparticles strongly regulate the stomata causing an increase in stomatal conductance. Although the mechanism behind it is not clearly known it may be because of the reduced ROS which acts as a signal for stomatal closure. Application of nano Zn-Se had shown significant decrease in ROS in maize (22). Also, the reduced stomatal conductance under drought may be responsible for reduction of transpiration rate. Transpiration is necessary to maintain a steady state in plants. It helps in trans-location of nutrients as a study in green pepper shows that how drought can contribute to reduced nutrient content in final fruits (28). Also, transpiration helps to regulate leaf temperature. As observed in this study, the pearl millet plants that have recorded low transpiration rate showed higher leaf temperature. Under drought stress the transpiration gets reduced drastically which resulted in significant reduction of growth and rise in leaf temperature. As most of the enzymes are thermo-labile the increased temperature adds up to the stress by destabilizing cellular homeostasis (29). But with application of nano Zn-Se it helped to increase the transpiration in nano Zn-Se treated plants as compared to drought control. Thus benefitting the plant growth as seen in strawberry by application of copper oxide nanoparticles and selenium nanoparticles (30). But at the same time it maintained the reduced transpiration rate as compared to irrigated control which contributes to the better water use efficiency as observed in case of maize by application of nano Zn-Se (22).

Drought stress also leads to an increase in minimal fluorescence value which is the result of damaged photo-system leading to increased non radiative energy dissipation. But the application of nano Zn-Se led to decrease in non-radiative energy dissipation indicating the positive effect of nanoparticle on photosystem. When the excitation energy of light reaction is transferred efficiently from antenna pigments to the reaction center it leads to decrease in F_0 value leading to reduced generation of ROS and enhanced photochemistry (31). Similar results were observed in sorghum by application of nanoceria (26). The F_v/F_m ratio was also decreased under the influence of drought stress, but its value was increased by application of nano Zn-Se which could be the result of reduced photo-oxidative damage of PS II by better scavenging of ROS produced in chloroplast at the time of light reaction. Similar effects were observed in sorghum under drought stress by foliar application of nano Selenium which reduced the minimal fluorescence (F_0) by 21 % and increased F_v/F_m ratio by 13 % (12). The chlorophyll index (SPAD values) which was reduced under drought stress was increased by nano Zn-Se application. The increase in chlorophyll content may be the result of the combined effect of reduced membrane damage, decreased leaf temperature thus promoting the chloroplast stability. Similar effect was observed by foliar application of nanoceria in sorghum (14). The activity of RuBP and PEPcase is greatly influenced by temperature where the higher temperature inhibits of the activity of these enzymes thus inhibiting the photosynthesis (32). Although the application of nano Zn-Se led to significant decrease in leaf temperature and thus enhancing the enzyme's activity, increased stomatal conductance made more CO_2 available for photosynthetic reaction and reduced the PS II damage leading to increased efficiency of the photo-synthetic machinery. These altogether were responsible for the increased photosynthetic rate observed in this experiment. Similar effect was recorded by application of nano selenium in sorghum under drought situation (12).

Thus observed enhancement in plant's internal system was visible by increment in growth parameters such as increase in plant height and stem girth along with an improvement in yield Similar enhancement was observed in wheat by application of nano selenium (33) and nano ZnO (34). Among all the treatments, the foliar application of nano Zn-Se @ 20 mg L^{-1} has performed best in all the parameters studied. Thus, indicating it to be the optimal concentration for mitigating drought stress in pearl millet. With this it is evident that the mitigation of drought stress in pearl millet by nano Zn-Se is an integrated effect of enhancing the photosynthesis related traits, eventually improving the growth and development of pearl millet plants under drought conditions at vegetative stage.

Conclusion

The study shows that drought stress during the vegetative stage significantly affects growth and photosynthesis-related traits in pearl millet. It decreases the transpiration rate, stomatal conductance, photosynthetic rate, maximum quantum yield of PS II and chlorophyll index, while increasing minimum fluorescence and leaf temperature, all of which ultimately contribute to suppressed growth. Foliar application of nano Zn-Se helped to mitigate the inhibitory effects of drought stress by enhancing photo-synthesis related traits and improving the growth of pearl millet plants. This shows the bio-stimulant nature of nano Zn-Se under drought as well as normal conditions. Also, it becomes evident that out of all the concentrations of nano Zn-Se, 20 mg L^{-1} concentration was very effective in mitigating the negative effects of drought stress in pearl millet.

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Authors' contributions

HRK, VBRP and MD conceptualized the study. HRK and VBRP conducted the study and prepared the manuscript. All authors reviewed the final version and approved the manuscript for submission.

Compliance with ethical standards

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

Ethical issues: None

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