Influence of zinc nanoparticles on maize productivity under heat stress caused by climate variability

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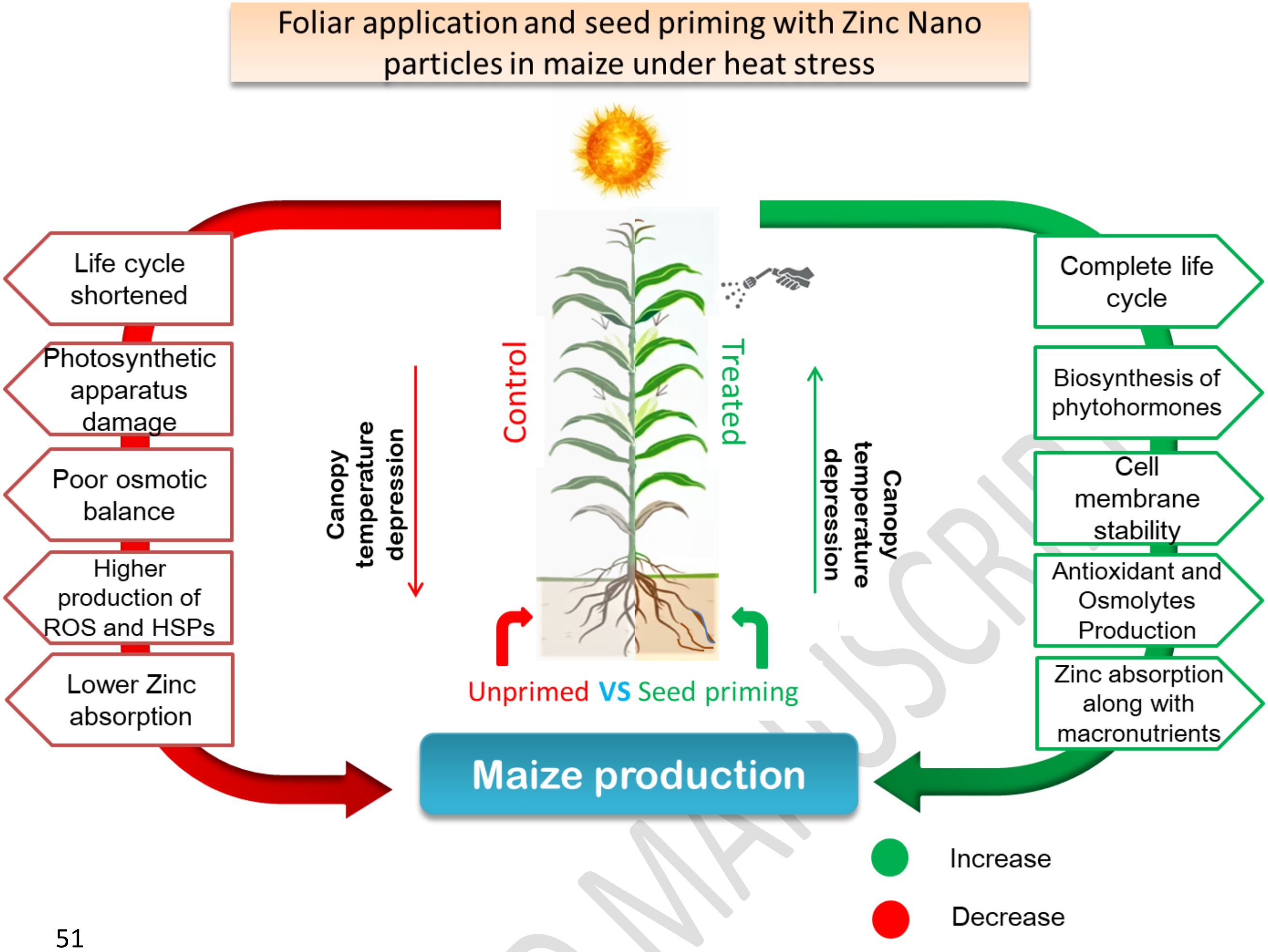
Abstract

Heat stress is among the most devastating abiotic stresses responsible for the reduction of maize yield. Therefore, the application of zinc nanoparticles (ZnNPs) is considered one of the precise and sustainable practices to meet the nutritional and food security demands of the global population. In this context, a field trial was conducted at Agronomy Research Farm, The University of Agriculture Peshawar, Pakistan, to assess the effect of ZnNPs, applied as foliar and seed priming, on the growth, biochemical and productivity of maize. The treatments consisted of foliar application of ZnNPs (100 and 150 mg L^{-1}) and seed priming with ZnNPs (100 and 150 mg L^{-1}) along with control. Heat stress indeed hindered the production of maize. However, the application of ZnNPs via foliar spray and seed priming showed a positive impact as compared to the control. Foliar spray of ZnNPs at the rate of 150 mg L^{-1} increased leaf area, plant height, thousand-grain weight, biological yield, grain yield, grain zinc content, and total zinc uptake by 15.25, 9.03, 5.88, 7.54, 7.73, 53.03 and 34.65% as compared to control. Similarly, seed priming with 150 mg L^{-1} of ZnNPs improved leaf area, plant height, grains ear^{-1}, thousand-grain weight, biological yield, grain yield, grain zinc content, and total zinc uptake by 12.21, 18.47, 6.26, 15.30, 16.71, 9.40, 26.16 and 59.77% as compared to control. In addition, ZnNPs improved chlorophyll pigments (a, b, and carotenoids) and net photosynthetic rate as well as stabilized transpiration rate, electrolyte leakage, increased canopy temperature depression (CTD), and reduced heat shock protein (HTPs). Therefore, it is concluded that applying ZnNPs at a rate of 150 mg L^{-1} via foliar spray and seed priming is an effective strategy to enhance maize resilience against heat stress, leading to improved growth and yield components, thereby contributing to food security and agricultural sustainability.

Keywords: Nanoparticle, Maize (*Zea mays*), Photosynthesis, Plant homeothermy, Heat stress



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52 **Graphical abstract**

Introduction

Maize (*Zea mays* L.) holds a significant agricultural importance, which grows best in tropical and subtropical climatic conditions (Bayar et al., 2024). Maize is primarily used as human food, animal feed and feedstock to produce starches, corn oil, baby corn, corn syrup, flakes, and biofuel industries (Revilla et al., 2022), which makes it a crucial food and feed in many countries (Li et al., 2021; Maitra et al., 2021). However, the prevailing global climate change and the depletion of natural resources has risen the day and night temperature that is threatening modern agriculture (Lowery et al., 2019; Tamez et al., 2020). High-temperature stress has a huge impact on the growth cycle, affecting metabolic and morphological traits that cause irreversible losses to maize yield (Shim et al., 2017).

Heat stress is the most damaging abiotic factor affecting seasonal growth and spatial variations of various crops (Sallam et al., 2018; Magaña Ugarte et al., 2019). The rising global temperatures, driven by population growth and industrial development, have become a significant concern (Baus, 2017). The impact of high temperatures varies among different plants and cultivars, also across various developmental stages within the same species. Among agricultural crops, field crops are particularly sensitive to high temperatures during their reproductive stage (Ravikiran et al., 2020). Temperatures exceeding 32°C disrupts several metabolic processes of maize crop, inhibiting photosynthesis and increasing surface transpiration rate (Sharma et al., 2020), pollen sterility (Gourdji et al., 2013) and shrinking grains (Rezaei et al., 2015), all these leading to a significant yield loss. Heat stress disrupts chlorophyll, electron flow, photosystem II, and carbon fixation that collectively impede photosynthetic function (Hussain et al., 2021). Several techniques are being adapted to combat heat stress, such as assisting Quantitative Trait Locus (QTL) markers, plant breeding and genetic engineering as well as agrochemicals application, which require time and have different ethical, environmental and economic concern.

Nanotechnology have transformed agriculture by increasing crop yield and provide significant potential for elevating plant tolerance against abiotic stresses (Arif et al., 2020). One innovative method involves the use of zinc nanoparticles (ZnNPs) that has recently attracted significant research attention due to its unique properties and diverse applications across various fields (Sturikova et al., 2018). Zinc NPs are considerably more effective in enhancing crop productivity and Zn absorption to its high surface area to volume ratio (Khan et al., 2021). The optimal dosage and application methods of ZnNPs are being reported for enhancing growth, yield and nutritional status of different crops while minimizing potential environmental risks (Jalal et al., 2022; 2023a, b, c).

Zinc NPs are applied either to soil, foliar, or seeds (Abbasi et al., 2020), however, foliar and seed treatments are considered the most effective in terms of leaf or plant nutritional status (Tabesh et al., 2020). Foliar spray of Zn can mitigate oxidative stress caused by factors like extreme temperature (Khan et al., 2023). Zinc NPs enhance antioxidant defense system to scavenge free radicals by altering microRNA expression and regulating various morphological, physiological, and metabolic processes of the plant (Kambe et al., 2021). Seed Zn nano-priming has the capability to activate a germination process and genes expression related to plant stress tolerance (An et al., 2020). Alfalfa seed priming with ZnNPs altered the ultrastructure of chloroplasts, mitochondria, and cell walls, thereby preventing heat-induced damage and promoting plant growth (Kareem et al., 2022a).

Based on the comprehensive overview, where the existing studies have highlighted the potential of ZnNPs in enhancing plant tolerance mechanisms under abiotic stress. However, there is still lack of focused research on the performance of maize in response to application methods of ZnNPs under heat stress conditions. There is a need for systematic investigation into the comparative effectiveness of these application methods under heat stress scenarios in maize. Therefore, this research assessed the growth rate, chlorophyll content, photosynthetic efficiency, and yield performance to determine the efficacy of ZnNPs via foliar spray and seed priming in enhancing maize resilience.

2. Materials and methods

2.1 Description of experimental site and design

A field experiment was performed at Agronomy Research Farm, The University of Agriculture, Peshawar, during the kharif season of 2022, which is located at 34.01° N, 71.35° E, at an altitude of 350 m above sea level in the Peshawar valley. The weather data of the experimental site is presented in Fig. 1.

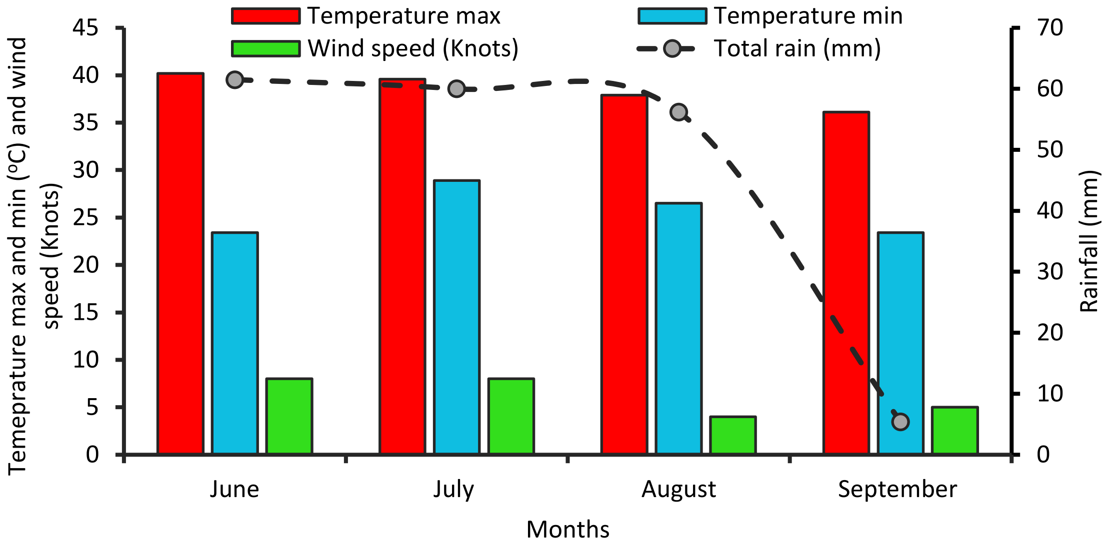


Fig. 1. Mean monthly averaged meteorological data during the summer growing season of 2022.

The experiment aimed to examine how maize crops reacted to zinc nanoparticles (ZnNPs) applied as foliar application and seed priming in response to the growth and yield of maize crops under high temperatures. The experiment was conducted in a randomized complete block design having four replications. Each plot exhibited dimensions of 3.75 by 3 meters, comprising five rows with R-R and P-P distances set at 75 and 20 centimeters, respectively. The maize variety 'Azam 1984' obtained from Cereal Crop Research Institute Pirsabak was sown at a rate of 30 kilograms per hectare. For seed priming, maize seeds were treated with varying concentrations of ZnNPs (P1= Control, P2=100 and P3=150 mg L^{-1}) while a foliar spray of ZnNPs was applied at V8 stage at various levels (F1= Control, F2= 100 mg L^{-1} and F3= 150 mg L^{-1}). For Zn priming and foliar at 0 mg L^{-1}, distilled water was used. The recommended dose of NPK were applied as a basal dose of 150, 90 and 60 kg ha^{-1} from urea, diammonium phosphate and muriate of potash. Three irrigations were applied during crop growing season at V4, V8 and milking stage. Hand weeding was done after application of second irrigation. All other cultural practices i.e. thinning, herbicide and insecticide application were kept uniform for all experimental units.

2.2. Soil Analysis

A composite of soil sample was made by homogeneously mixing thirty random samples that were taken from the field site before the experiment started. Following air drying, powdering, and sieving through a 2 mm sieve, the composite sample was subjected to the physical and chemical properties as listed in Table 1. Wet digestion method by Jackson (1968) used to determine the total amount of organic matter in the soil. The pipette method, as outlined by Avery and Bascomb (1974), was used to determine the texture of the soil. According to McLean (1982), soil pH and electrical conductivity were measured in each sample. Following the Soltanpour and Schwab (1977) methodology, the available P, K, and Zn were estimated. The Kjeldahl method was used to calculate the nitrogen content, according to the Bremner and Mulvaney (1982) methodology.

Table 1 Pre-harvest soil physiochemical properties.

Soil Analysis	**Concentration**
Texture	Silt loam
Sand	3 %
Silt	71 %
Clay	26 %
pH	7.1
Electric conductivity	0.33 dSm^{-1}
Organic matter	0.93%
Nitrogen	0.18%
Phosphorus	0.06%
Potassium	0.24%
Zinc	1.09 mg kg^{-1}

2.3. Preparation of zinc nanoparticles from clove bud extract

Plant materials (clove buds) were collected and ground into a powder using an electric grinder. In a beaker, 20 g of the clove bud powder was combined with 100 ml of distilled water. The solution was microwaved for 130 to 150 seconds at 1000 watts. The extract was then cooled to 21 ^{o}C and filtered (Bangar et al., 2023). For the preparation of the reagent, 0.65 g of zinc was dissolved in 100 ml of water. Ten milliliters of the plant extract were mixed with ten milliliters of a pre-prepared zinc sulfate solution. After four hours, the mixture was examined. One milliliter of the solution was then transferred to an Eppendorf tube and centrifuged for 10 minutes at 10,000 rpm. The resulting pellets were dissolved in 1 ml of pure water using a vortex mixer. To purify the nanoparticles, the centrifugation procedure was repeated 3-4 times with distilled water. The pellets were then stored for future use (Panwar et al., 2023).

2.4. Parameters related to the growth and yield of maize

2.4.1. Crop phenology

The number of days to tasseling was determined by counting the days from seed emergence to the point when 80% of the plants in each subplot had produced tassels. Similarly, the days to silking were recorded from the time of sowing until 80% of the plants in each subplot had produced silks. In last, the duration to reach physiological maturity was measured from the sowing date, when 80% of the plants in each subplot developed a black scar at the base of their grain.

2.4.2. Growth Parameters

To determine the leaf area within each plot, five plants were randomly selected at the tillering stage. Leaf length and width were measured using a measuring tape, and these measurements were then multiplied by a correction factor (0.75), as prescribed by

Mutisya and Geadelmann (1988). Moreover, in each subplot, the heights of eight randomly chosen plants were measured starting from the plant's top and going down to the ground.

$$LA = \text{Leaf length} \times \text{leaf width} \times 0.75$$

2.4.3. Yield characteristics

The average length of ten randomly selected ears from each plot was recorded to determine ear length. Additionally, ten ears were randomly chosen in each subplot, and the average number of grains per ear was determined and recorded. Each plot seed lot was sampled three times for 1000 grains, which were weighed and averaged**.** To find out biological yield four rows were selected and harvested with sickle from every experimental plot and sundried at harvest maturity. The dry material, after weighting, was then converted into kg ha^{-1} using equation 1. The plants harvested for biological yield were threshed, and the grains were separated from the cobs. The grains were then weighed by digital balance, and the sample data were converted to kg ha^{-1} using equation 1 to determine grain yield.

$$\text{Biological and grain yield (kg ha}^{-1}\text{)} = \frac{\text{BY/GY of four central rows}}{\text{R-R distance} \times \text{No. of rows} \times \text{row length}} \times 10000 \quad \text{(equation 1)}$$

2.4.4. Zinc nutrition and biochemical attributes

To determine grain zinc content, grain samples from each seed lot were washed with water and dried in an oven at 70°C for 48 hours. They were then ground in a Wiley mill and digested in a di-acid mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) as described by Jackson (1973). The zinc concentration in the aqueous extracts of the digested plant material was then measured using an atomic absorption spectrophotometer. To measure zinc uptake by the plant, the following equation was used.

$$\text{Zinc uptake by the plant (g ha}^{-1}\text{)} = \frac{\text{Plant zinc concentration} \times \text{Dry weight of the plant}}{1000}$$

To determine zinc use efficiency in plants the following equation was employed.

$$\text{Zinc use efficiency (kg kg}^{-1}\text{)} = \frac{\text{Dry weight of plants}}{\text{(Total zinc uptake} \times \text{Total zinc supplied)}}$$

2.5. Parameters for evaluating heat stress in maize

2.5.1 Photosynthetic pigments

The photosynthetic pigment in maize leaves at the onset of the flowering stage (30 DAS) was measured using a spectrophotometer (Lichtenthaler, 1987). The Spector-photometric method was used to determine the chlorophyll (Chl a and b) and carotenoid content. A 200 mg sample of freshly harvested leaf material was submerged in an 8 ml solution composed of 80% acetone (volume/volume). This mixture was kept at a frigid temperature of -4 °C in complete darkness for a duration of 48 hours. The concentration of carotenoid, total chlorophyll, and chlorophyll (Chl a and Chl b) were measured at absorbance (A) of wavelength 663.2, 646.8, and 470 nm. The concentrations of carotenoids, Chl a, and Chl b, were calculated using protocols of Lichtenthaler 1987, and expressed in mg g^{-1} of fresh leaf weight (FW).

$$\text{Chl a (mg g}^{-1}\text{)} = 12.25A_{663.2} - 2.79A_{646.8}$$

$$\text{Chl b (mg g}^{-1}\text{)} = 21.50A_{646.8} - 5.10A_{663.2}$$

Total Carotenoids (mg g^{-1}) = $1000A_{470}$ – 1.82Chl a – 85.02Chl b

2.5.2. Net photosynthetic and transpiration rate

Net photosynthesis rate (CO_2 umol m^{-2} s^{-1}) and transpiration rate (H_2O mmol m^{-2} s^{-1}) were measured using a portable infrared gas exchange analyzer, model Li-6400 (Li-Cor, USA).

2.5.3. Electrolyte leakage and heat shock protein analysis

The topmost expanded leaves of maize during the anthesis stage in every plot were washed in deionized water, and the leaf discs, 5 mm in diameter, were then punched out. The leaf discs were cut into slices and submerged in 30 ml of deionized water for 2 hours at 25 °C. Following the incubation period, the electrical conductivity was measured using a conductometer at 25 and 100 °C. Then electrolyte leakage was calculated using the formula: electrolyte leakage = (C1/C2) × 100, where C1 is the conductivity at 25 °C and C2 is the conductivity at 100 °C (Hniličková et al., 2019). The heat shock protein (HSP) levels in leaves were measured using the Heat Shock Protein Assay Kit (ab284527) from BioVision. The absorbance for HSP was recorded at OD 450 nm and expressed in ng g^{-1} of fresh weight (Hussain et al., 2019).

2.5.4. Canopy temperature depression

Canopy temperature depression (CTD) was measured by recording the canopy temperature of plants under study using an infrared thermometer during midday (11:00 AM to 1:00 PM) on cloud-free days. The ambient air temperature was simultaneously recorded at the same height as the canopy during anthesis stage. CTD was calculated as the difference between ambient air temperature and canopy temperature (Awika et al., 2017).

2.6. Statistical analysis

Data on all parameters of maize were sample by Randomized Complete Block Design procedures and outlined by (Jan et al., 2009) was submitted to analysis of variance (ANOVA), and average between treatments was compared using LSD (least significant difference).

3. Results

3.1. Maize phenology and growth parameters

Plant phenology and growth of maize were positively influenced by foliar spray and seed priming with zinc nanoparticles (ZnNPs), while the interaction was significant only for leaf area and plant height (Supply file 1). The number of days to tasseling, silking and maturity were delayed by 1, 3 and 5 days, respectively, with foliar-applied ZnNPs at 150 mg L^{-1} as compared to the control. Seed priming with 150 mg L^{-1} also delayed tasseling, silking and maturity by 1, 2 and 3 days, respectively as compared to the control.

Leaf area of maize was increased with application of ZnNPs via both foliar spray and seed priming. Application of ZnNPs at the rate of 150 mg L^{-1} via foliar and seed priming increased leaf area of maize by 15.25% and 12.21% respectively as compared to control. Seed priming with ZnNPs at the rate of 150 mg L^{-1} was observed with greater leaf area, which was statistically not different from the interactive effect of foliar spray at 150 mg L^{-1} of ZnNPs together with seed priming at the rate of 150 mg L^{-1} as compared to the other treatments (Figure 2a). The interaction of water priming and foliar spray at the rate of 100 mg L^{-1} was observed with small leaf area. In terms of plant height, 100 and 150 mg L^{-1} foliar ZnNPs led to tallest plants compared to the control while among priming treatments, 150 mg L^{-1} ZnNPs resulted in taller plants as compared to control. The interactive effect showed that seeds primed at 150 mg L^{-1} ZnNPs without foliar treatment produced tallest plant that was also comparable to seeds primed with 100 and 150 mg L^{-1} ZnNPs together with 150 mg L^{-1} of foliar applied ZnNPs (Figure 2b).

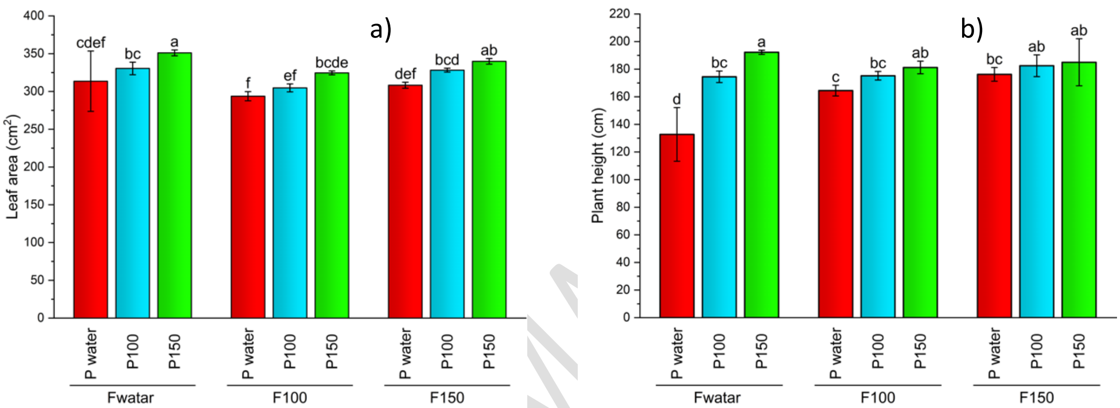


Fig. 2. Interactive effect of foliar application and seed priming with ZnNPs on leaf area and plant height of maize. Fwater, F100 and F150 is foliar ZnNPs at the rate of 0, 100 and 150 mg L^{-1} whereas, Pwater, P100 and P150 is seed priming done with 000, 100 and 150 mg L^{-1} ZnNPs, respectively. The bars represent standard deviation and different letters on the bars showed significant variation between means of same group. (n= 4 replications).

3.2. Yield and yield component of maize

The application of Zens via foliar and seed priming cause significant disparity ($P \leq 0.05$) in yield and related components of maize (Supply file 1). The combined effect of these treatments was also significant for the yield and yield-related traits. However, foliar applied ZnNPs has no significant effect on grains ear^{-1} of maize. On the other hand, seeds primed with 100 and 150 mg L^{-1} ZnNPs produced 6.26% and 4.47% maximum grains ear^{-1}, respectively, as compared to the control. The combined effect of foliar applied and seed primed with ZnNPs showed that foliar applied ZnNPs at 150 mg L^{-1} together with seed priming of 100 and 150 mg L^{-1} resulted in the highest grains ear^{-1} that was also statistically similar to foliar application at 100 mg L^{-1} when applied in combination with primed seeds (Figure 3a).

Heavier thousand grains (5.88%) were produced as compared to the control when 100 mg L^{-1} ZnNPs was applied, which were statistically similar to foliar application at 150 mg L^{-1}. In terms of priming, seeds primed with 150 mg L^{-1} ZnNPs produced 15.31% highest thousand grain weight compared to control. Interactive effect showed that thousand grains weight was improved when foliar 100 mg L^{-1} ZnNPs was applied together with primed seeds at 150 mg L^{-1} ZnNPs that was at statistical parity with 150 mg L^{-1} ZnNPs applied via foliar along with primed seeds at 150 mg L^{-1} (Figure 3b). Grain and biological yield were substantially improved with application of ZnNPs both as foliar and seed priming. Foliar application of ZnNPs at 100 and 150 mg L^{-1} increased biological yield by 7.53% and 7.54% respectively, as compared to control, while seed priming with ZnNPs at 100 and 150 mg L^{-1} significantly increased biological yield by 6.96% and 16.72% respectively, as compared to control. Interactive effect showed that seeds primed at 150 mg L^{-1} ZnNPs without any foliar treatment recorded highest biological yield that was similar to foliar application of 150 mg L^{-1} ZnNPs together with 150 mg L^{-1} ZnNPs primed seeds (Figure 3c). Similarly, foliar application of ZnNPs at 100 and 150 mg L^{-1} produced 10.83% and 7.74% more grain yield as compared to control. Similarly, seed priming with ZnNPs at 100 and 150 mg L^{-1} increased grain yield by 7.84% and 9.41% respectively, compared to control. Moreover, grain yield improved the most with 100 and 150 mg L^{-1} ZnNPs without any foliar treatment that was also comparable to foliar application of 150 mg L^{-1} ZnNPs together with 150 mg L^{-1} ZnNPs primed seeds (Figure 3d).

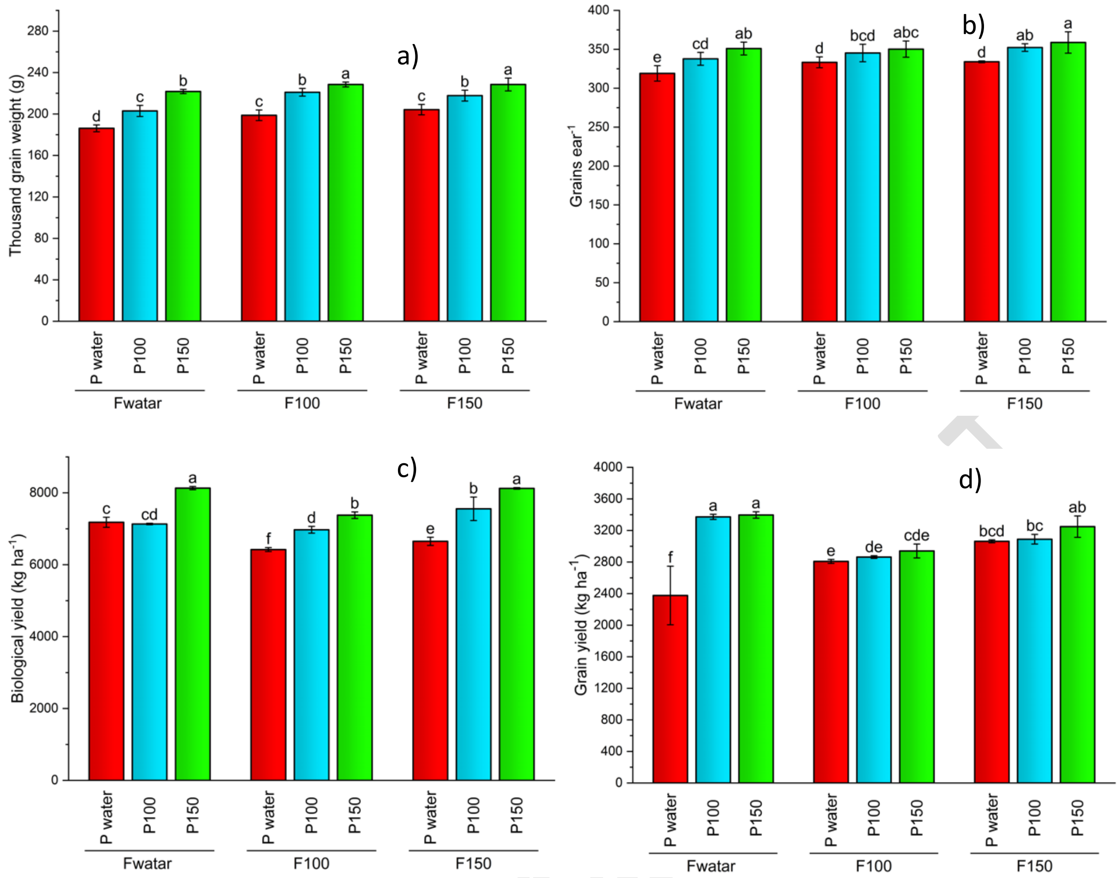
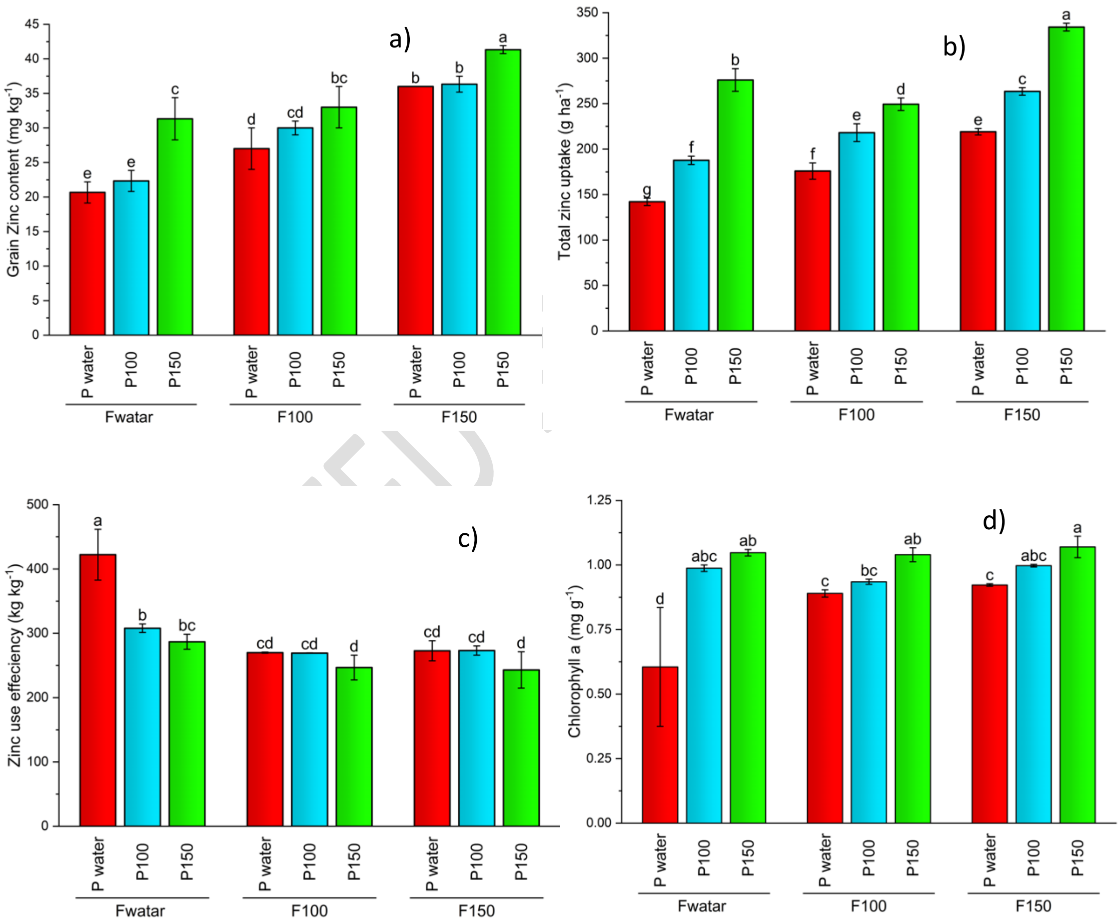


Fig. 3. Interactive effect of foliar application and seed priming with ZnNPs on yield and yield components of maize. Fwater, F100 and F150 is foliar ZnNPs at the rate of 0, 100 and 150 mg L^{-1} whereas, Pwater, P100 and P150 is seed priming done with 0, 100 and 150 mg L^{-1} ZnNPs, respectively. The bars represent standard deviation and different letters on the bars showed significant variation between means of same group. (n= 4 replications).

3.3. Zinc nutrition and biochemical attributes

Foliar application of ZnNPs and seed priming with ZnNPs resulted significant variation ($P \leq 0.05$) in terms of zinc absorption by maize with significant interaction among different treatments (Supply file 1). Application of 150 mg L^{-1} ZnNPs as a foliar increased 53.03% zinc content in the grain as compared to the control. Similarly, seed priming with 150 mg L^{-1} ZnNPs, produced 26.16% maximum zinc content in the grain as compare to the control. According to interactive effect, grain zinc content was recorded highest with 150 mg L^{-1} foliar combined with seed priming done with 150 mg L^{-1} ZnNPs compared to control plots (Figure 4a). In case of zinc uptake, application of

150 mg L^{-1} ZnNPs both as a foliar and seed priming resulted in 34.65 and 59.77% respectively, higher uptake as compared to the control as also clear from their interaction (Supply file 1; Fig 4b). Zinc use efficiency was higher in control plots over foliar applied and seed priming treatments (Supply file 1; Fig 4c). Chlorophyll a, b and carotenoid contents were recorded highest with application of 150 mg L^{-1} ZnNPs both as a foliar and seed priming and the same results were also reflected in interactive effect (Supply file 1; Fig 4d, e).



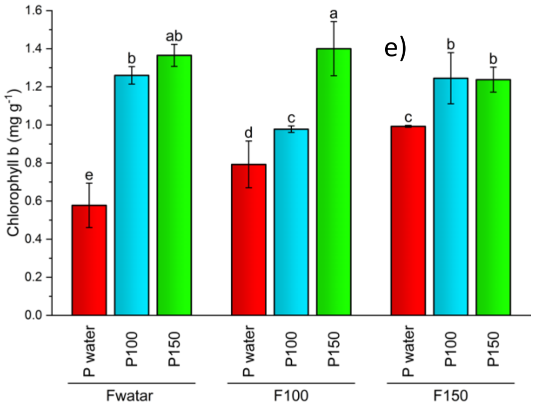


Fig. 4. Interactive effect of foliar application and seed priming with ZnNPs on Zinc nutrition and biochemical attributes. Fwater, F100 and F150 is foliar ZnNPs at the rate of 0, 100 and 150 mg L^{-1} whereas, Pwater, P100 and P150 is seed priming done with 000, 100 and 150 mg L^{-1} ZnNPs, respectively. The bars represent standard deviation and different letters on the bars showed significant variation between means of same group. (n= 4 replications).

3.4. Heat stress indicators

ZnNP application via foliar and seed priming has a significant effect on heat stress indicators (Supply file 1). Net photosynthetic rate was higher in foliar treated plots with 100 and 150 mg L^{-1} ZnNPs along with seeds primed with 150 mg L^{-1} as compared to control (Figure 5a). The application of 150 mg L^{-1} ZnNPs via foliar and seed primed significantly reduced transpiration rate as compared to 100 mg L^{-1} ZnNPs and control (Figure 5b). Electrolyte leakage was higher in control plot that was significantly lowered with the application of 100 and 150 mg L^{-1} ZnNPs both as a foliar and seed priming (Figure 5c). The value of heat shock protein was highest in control plot that was significantly lowered with 100 and 150 mg L^{-1} ZnNPs foliar application and seed priming with the respective levels (Figure 5d). Canopy temperature depression (CTD) that represents the difference between canopy and ambient temperature differed significantly. Higher CTD was noted in plots that were treated with 150 mg L^{-1} ZnNPs as a foliar and seed primed with 100 and 150 mg L^{-1} ZnNPs (Figure 5e).

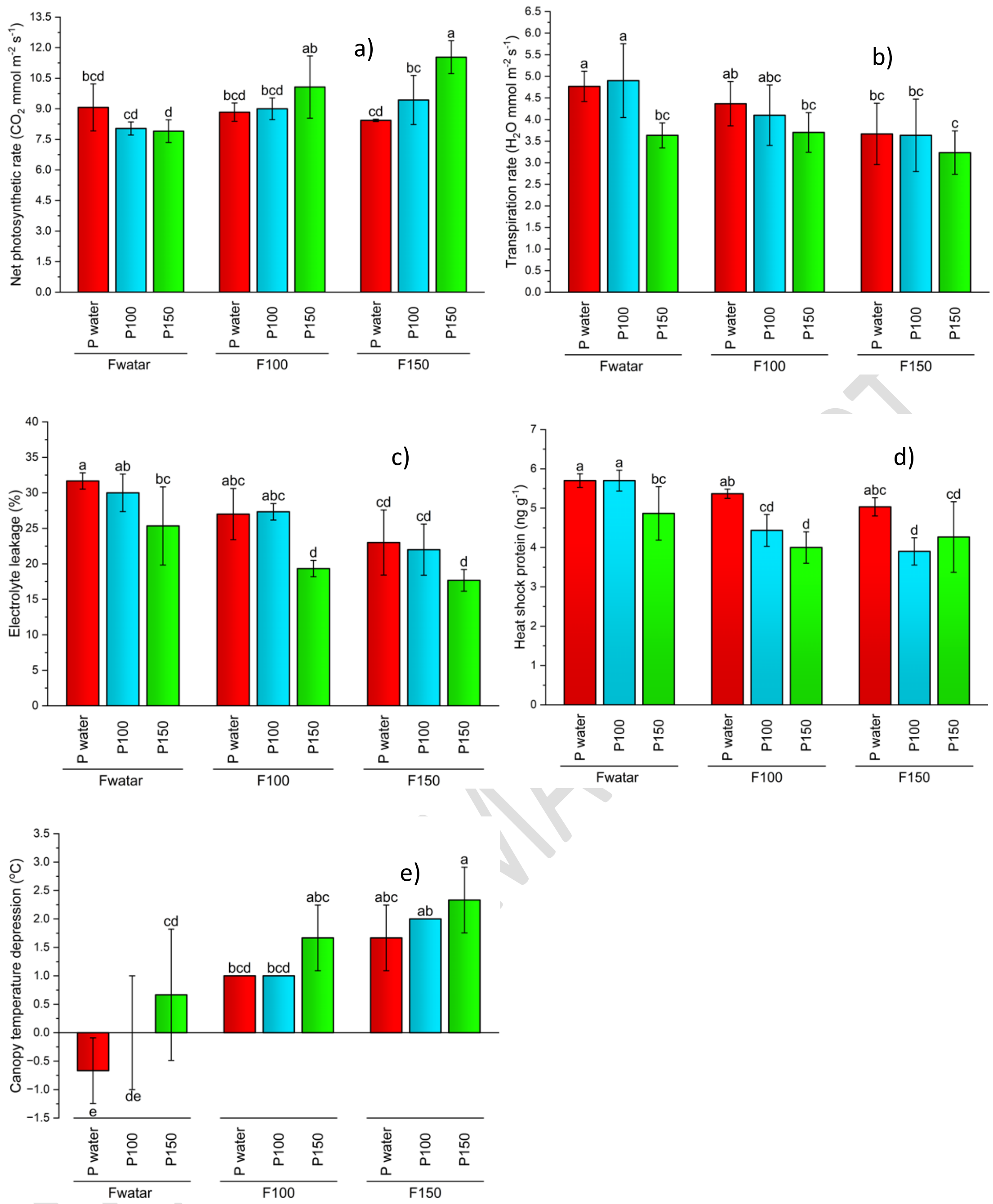


Fig. 5. Interactive effect of foliar application and seed priming with ZnNPs on heat stress indicators. Fwater, F100 and F150 is foliar ZnNPs at the rate of 0, 100 and 150 mg L^{-1} whereas, Pwater, P100 and P150 is seed priming done with 0, 100 and 150 mg L^{-1} ZnNPs, respectively. The bars represent standard deviation and different letters on the bars showed significant variation between means of same group. (n= 4 replications).

3.5. Interaction of different treatments

Correlation analysis was performed between all the evaluated traits that included phenological traits, yield and yield components, zinc nutrition and biochemical attributes and heat stress indicators when treated with ZnNPs as a foliar and seed priming under the influence of heat stress. It was revealed that maize phenology (Days to tasseling, silking and maturity) was positively correlated with maize growth, its yield and related components i.e. delayed phenology improved growth, yield and related components. Moderate to weak correlation was observed between grain zinc content and yield along with related components however, zinc uptake was strongly correlated with it. Similarly, plant photopigments also revealed positive correlation with yield. On the other hand, net photosynthetic rate, transpiration rate and electrolyte leakage was negatively correlated with yield i.e. increase it that parameters caused decrease in yield and its components. Moreover, heat shock protein had a negative effect on all the studied traits except transpiration rate and electrolyte leakage while, CTD had a moderate to weak correlation with evaluated parameters (Figure 6).

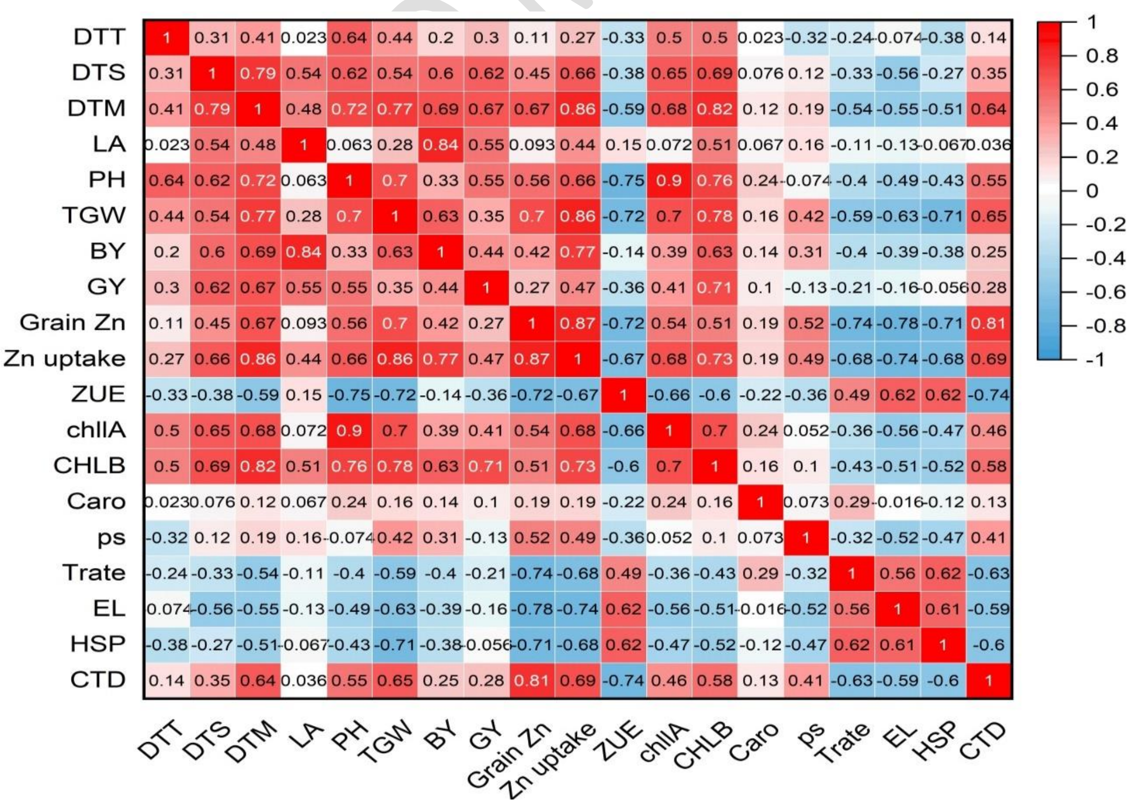


Fig. 6. Pearson correlation analysis between yield and yield component, zinc absorption and stress indicating indices of maize under foliar application and seed priming with ZnNPs.

4. Discussion

Rising temperatures and extreme heat stress are causing a worldwide decrease in maize production (El-Sappah et al., 2022) and this effect can be weakened by adaptation of modern approches such as nanotechnology (Mahakham et al., 2017). Our research revealed significant results when maize was treated with ZnNPs under elevated temperatures. In our research, the phenology of crop was delayed in terms of days to tasseling, silking and maturity by supplementation of ZnNPs via foliar and seed priming (Supply file 1). However, growth stages were shortened in control plots and that reduction in the life cycle is usual response of plants towards heat stress (Cohen et al., 2021). Prolong growth stages in ZnNPs treatments (foliar or seed priming) could be due to enhanced zinc availability and uptake (Elshayb et al., 2021), which is important for enzyme activation, protein synthesis, and growth regulation (Saleem et al., 2022). Broader leaves and taller plants was observed under the influence of combine treatments of foliar and primed seeds with ZnNPs especially at the rate of 150 mg L^{-1} over control plots (Supply file 1; Fig. 2a,b). This enhancement can be attributed to ZnNPs role in influencing the biosynthesis of phytohormones such as cytokinins and gibberellins that increases internodes per plant leading to taller plants (Tondey et al. 2021 ; Zakirov et al. 2018). Moreover, enhanced cell elongation, increased plant height and leaf area of maize (Sturikova et al., 2018). While no supplementation of ZnNPs in control plots would have reduced leaf area and plant height due to the production of reactive oxidation species in maize above 35 ^{o}C (Ashkiani et al., 2020 ; Djalovic et al., 2024; Poudela et al., 2024).

Heat stress reduces photosynthetic efficiency by damaging the photosynthetic apparatus, which in turn limits carbohydrate production, an essential process for growth and nutrient transport (Fahad et al., 2017). Moreover, heat stress can cause nutrient competition and imbalances, as plants may prioritize the uptake of certain nutrients like potassium to maintain osmotic balance, thereby reducing the uptake of other essential macronutrients such as nitrogen, phosphorus and magnesium (Bisht et al., 2023). Zinc is a vital element for cell development, multiplication, and pollen fertility, which are crucial for proper plant establishment, growth, and reproduction. Zinc, being a cofactor for nitrate reductase and glutamine synthetase ensure these enzymes function optimally, enhancing nitrogen assimilation and utilization (Maret, 2013). ZnNPs can facilitate the availability of growth promoting nutrients and improve plant resilience and efficiency in nutrient uptake and utilization enabling roots to absorb more nutrients from the soil (Wang et al., 2018). ZnNPs boost the plants antioxidant defense system by activating enzymes like superoxide dismutase (SOD) and catalase (CAT), reducing oxidative damage and maintaining cellular function for efficient nitrogen uptake (Li et al., 2019). Therefore, the current results verified that yield and its components were improved especially with foliar application of 150 mg L^{-1} ZnNPs and seed priming done with 100 and 150 mg L^{-1} ZnNPs whereas control produced lowest results possibility due to more vulnerability to elevated temperatures (Supply file 1; Fig. 3a,b,c and d). Our results are consistent with the findings of Afzal et al. (2013) and Raza et al. (2023) who reported an improvement in maize yield and yield components as a result of nutrient seed priming with $ZnSO_4$. The increase in 1000-grain weight with Zn application to seeds may be attributed to the enhanced bioavailability of this element and its direct translocation to young seedlings (Choukri et al., 2022). Similarly, a study by Rameshraddy et al. (2018) showed an increase in grain yield with the application of zinc nanoparticles compared to $ZnSO_4$ treatment. The application of ZnO nanoparticles increased grain yield by enhancing the yield related characteristics of the treated plants (Tondey et al., 2021). The yield increase can be attributed to a rise in chlorophyll content, which leads to improved photosynthetic efficiency. This enhancement can be specifically linked to increases in soluble protein content, starch content, and dry mass (Kolenčík et al., 2019).

Plant zinc concentrations and uptake were notably enhanced by the application of ZnNPs especially by interactive effects of foliar and seed priming, as reported in similar study on wheat (Ahmed et al., 2023). However, the main effect showed that more improvement was done by foliar application (Supply file 1; Fig. 4a, b). Mechanistically, ZnNPs foliar spray is more effective because it provides a direct and immediate route for zinc uptake through the leaf cuticle and stomata (Mohapatra et al., 2023). In contrast, seed priming with zinc nanoparticles involves initial absorption by seeds and subsequent translocation during germination and early growth (Yu et al. 2022), which can be less efficient due to soil interactions and environmental factors. ZnNPs also enhance photosynthetic efficiency, and our current experiment observed their positive effect on plant photopigments (Supply file 1; Fig. 4d, e). Similar, improvements in chlorophyll and photosynthesis were observed by different researcher under heat and other type of abiotic stresses (Rai-Kalal and Jajoo, 2021; Azmat et al., 2022; Khan et al., 2023; Seleiman et al., 2023). Higher transpiration rate was notable in untreated plots (Supply file 1; Fig. 5b) that were likely due to high vapour pressure deficit created due to high atmospheric temperatures that created physiological drought (Seetharam et al., 2021). While on the other hand, transpiration was stabilized by the application of ZnNPs (foliar or seed priming) as exogenous application of ZnNPs increased the production of antioxidants and osmolytes (Kareem et al., 2022). As per

Disante et al. (2011), the reduction of reactive oxygen species by ZnNPs decreased oxidative damage and lipid peroxidation by facilitating Cu/Zn-SOD (an anti-oxidative defense mechanism), thus preserving the integrity of cell membranes, including those in the stomata.

Electrolyte leakage, revealing membrane damage and mechanisms of cell death, was observed to be higher in control plots (Table 5; Fig. 5c). However, the percentage of damage was reduced by ZnNPs, particularly under the highest levels of ZnNPs applied through foliar and seed priming. ZnNPs create existing antioxidant defense system stronger that likely reduce electrolyte leakage as also noted in other crops like wheat (Azmat et al., 2022), rice (Yan et al., 2021), cucumber (Ghani et al., 2022) and soybean (Ahmad et al., 2020). Moreover, elevated values for heat shock protein were observed in untreated plots in response to heat stress at grain filling stage (Table 5; figure 5d). Perras and Sarhan (1989) observed that at 40°C, normal cellular metabolism declines, leading to disruptions in regular protein production and structure (Monjardino et al., 2005). This stress response triggers the increased heat shock proteins, which help plants tolerate high temperatures. However, less heat shock proteins observed with ZnNPs treated plots, indicated that plants can better cope with high-temperature stress because ZnNPs enhance antioxidants and osmolytes, reducing reactive oxygen species and lipid peroxidation, which ultimately decreases the need for heat shock proteins in plants under heat stress conditions (Kareem et al., 2022; Vinay et al., 2023). The recorded temperature difference between atmosphere and canopy showed variation under ZnNPs treatments (foliar and seed priming) compared to control (Supply file 1; Fig. 5e). Maize plants have poor homeotherm behavior (Sobejano-Paz et al., 2023) that led to higher canopy temperatures and reduce cooling efficiency causing lower canopy temperature depression. Plots treated with 150 mg L^{-1} foliar applied and seed primed

ZnNPs and this could be due to improvement in chlorophyll content and photosynthetic pigments as discussed above. This increased CO_2 assimilation and subsequent carbohydrate production, which can cool the plant canopy through increased transpiration and metabolic activity. However, the direct impact of ZnNPs on canopy temperature depression has not been examined. In this context, the current research indicated a positive correlation between maize phenology and its growth, yield, and components, with delayed phenology enhancing these factors. These findings highlight the potential of nanotechnology in modern agriculture, particularly in regions prone to extreme heat stress, and contribute to the broader goal of achieving global food security.

5. Conclusions

The research demonstrated that the application of zinc nanoparticles (ZnNPs) via foliar spray and seed priming significantly enhances maize resilience against heat stress, leading to improved growth, yield, and biochemical properties. Specifically, foliar application and seed priming at 150 mg L^{-1} increased growth parameters, grain yield, zinc content, and overall zinc uptake compared to the control. Additionally, ZnNPs also mitigate heat shock protein expression, contributing to improved plant health and productivity under elevated temperatures, making this approach a viable and sustainable strategy to improve maize productivity under heat-stress conditions, thereby supporting global food security efforts. However, a significant gap remains between laboratory research and field applications, which often hinders the widespread adoption of nanotechnology in agriculture. Bridging this gap requires collaboration among scientists and with business and political stakeholders to unlock the transformative potential of nanoparticles, fostering agricultural and economic prosperity while maintaining the commitment to global food production.

Conflicts of interest/Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Availability of data and material

All the data will be made available on the request.

Authors' contributions

Naseer Ahmad: Writing – original draft; **Shahen Shah:** Writing – review & editing; **Jalal Bayar:** Software; **Shereen Magdy Korany:** Software, Formal analysis; **Uzair Ahmad:** Conceptualization; **Atia Gul**: Investigation; **Waleed Khan**: Visualization; **Arshad Jalal**: Writing – review & editing, Resources, Project administration; **Emad A Alsherif**: Investigation, Data curation; **Nawab Ali:** Writing – review & editing, Conceptualization. **Danyah A. Aldailami**: Writing – review & editing, Validation;

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