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Impact of ZnO nanopriming on physiological and biochemical traits of wheat (*Triticum aestivum* L.) seedling

Priyanka Pandya[1], Sushil Kumar[1*], Ghanshyam Patil[1], Monil Mankad[1] and Zarna Shah[1]

Abstract

To ascertain the ideal dosage of ZnO NPs (Zinc Oxide Nanoparticles), we conducted an investigation on the priming effects of varying concentrations of ZnO NPs on germination and physio-biochemical parameters of wheat. In this study, ZnO NPs were synthesized and characterized for their physico-chemical properties followed by confirmation of the formation of ZnO NPs. Throughout this study, wheat seeds were subjected to ZnO NPs at various concentrations of 5, 50, 100, 250, and 500 ppm for a period of 4 h via continuous aeration. The primed seeds were sowed in plastic bags, allowed to grow for 21 days, following which comprehensive evaluations of physio-biochemical attributes were conducted. At 250 ppm, an impressive 100% of seeds successfully germinated compared to the control group. The examined physiological factors such as shoot length, root length, and fresh as well as dry weights of leaf and root tissues all exhibited notable increases with the ascending concentrations of ZnO NP up to 250 ppm. However, beyond this threshold, at 500 ppm, these parameters experienced a decline. Inductively coupled plasma atomic absorption spectrophotometer (ICP-AAS) measurements validated the progressive increase in Zinc content in the nanoprimed seedlings, further affirming the dose-dependent trend. Zinc oxide nanoparticles notably improved key biochemical features, including elevated levels of total chlorophyll, malondialdehyde (MDA), total protein, and the accumulation of osmolytes such as proline and glycine-betaine. Additionally, the presence of ZnO NPs led to increased activity of antioxidant enzymes like superoxide dismutase (SOD) and catalase in a dose-dependent mananer. Collectively, the amassed data underscores the efficacy of the 250 ppm ZnO NPs treatment, which emerged as superior in comparison to both the control group and other administered treatments. These findings underscore the potential of ZnO NPs at a concentration of 250 ppm as a valuable seed nanopriming agent, effectively enhanced germination and robust early-stage growth in young plants.

Keywords Seed priming, ZnO NPs, Wheat, Germination, Growth parameters, Oxidative stress, Anti

Introduction

Zinc, an essential micronutrient, raises pressing concerns due to its widespread deficiency, affecting over 30% of the world's arable land (Itroutwar et al. 2020). Crucial biological processes, such as protein synthesis, gene transcription, gene control, and phytohormone metabolism, hinge on the presence of zinc (Mirakhorli et al. 2021). India's agricultural landscape is marred by the grim reality that half of its farmland grapples with significant yield losses, attributed to zinc deficiency, with one instance revealing up to 34% deficiency in soil samples from Gujarat (Patel 2019). The deficiency of zinc is a significant factor reducing agricultural harvest, especially in alkaline calcareous soils. where its deficiency casts a shadow on

*Correspondence:
Sushil Kumar
sushil254386@yahoo.com; sushil_k@aau.in
[1] Department of Agricultural Biotechnology, Anand Agricultural University, Anand 388110, India

both crop production and human health (Pejam et al. 2021). Nearly 2 billion individuals globally confront malnutrition due to inadequate intake of critical micronutrients including Zn (Itroutwar et al. 2020). In light of the Zn scarcity and the need to meet agricultural demands, dedicated efforts are underway to devise innovative, safe, and profoundly effective zinc fertilizers.

Within the realm of agriculture, nanotechnology has emerged as a transformative force, yielding substantial benefits (Prasad et al. 2012). Numerous nanomaterials have been tailored for agriculture use with twin goals of elevating crop yields and reducing pesticide application (do Espirito et al. 2021). Some of the most critical parameters regulating the observed positive and negative impacts of nanomaterials on plant growth are the kind of nanomaterial, particle size, specific surface area, and the plant species at issue. (Zhu et al. 2012;). Zinc oxide nanoparticles (ZnO-NPs) are gaining prominence due to their superior efficiency compared to conventional ZnO (Foroutan et al. 2018). Comparative studies have shown that ZnO NPs are much less toxic for plants than either Zn^{2+} or ZnO bulk particles.

The concept of "seed nano-priming" introduces a novel approach involving the treatment of seeds with nanoparticles (Mahakham et al. 2017). Nanotechnology for seed priming is an emerging area of research, but preliminary findings are promising (Rai-Kalal and Jajoo 2021; Sharma et al. 2021a, b; Pandya et al. 2023). ZnO nanopriming is considered sustainable and cost efficient due to its small particle size and higher surface area, which promotes improved nutrient absorption by plants, enhancing the overall yield with minimum environmental impact. The controlled release and enhanced absorption of zinc from nanoparticles may optimize nutrient application, potentially reducing the overall cost of fertilizers. If scalable and affordable synthesis methods for zinc oxide nanoparticles are developed, this technology could be accessible to small-scale farmers, contributing to broader adoption and potential economic benefits. Furthermore, the ZnO nanopriming has been reported to modulate the expression of genes associated with plant defence mechanisms. These modulations can lead to the production of antimicrobial compounds and protein, potentially reducing the need for chemical pesticides (Malik et al. 2020). Recent studies have substantiated that nano-primed seeds elicit the activation of numerous genes during germination, notably those implicated in stress responses (Mahakham et al. 2017). However, ZnO NPs offer promising benefits for sustainable agriculture but several concerns and uncertainties remain which including the fact that zinc oxide nanoparticles (ZnO NPs) pose toxicity concerns due to their size and surface characteristics, potentially impacting human health and the environment. Their long-term environmental impact in agriculture, including soil health and biodiversity, requires thorough investigation. The use of these nanoparticles poses regulatory challenges, necessitating guidelines for responsible application and risk mitigation. The research on nanoparticles in agriculture is constantly evolving, offering insights into the benefits and risks of using zinc oxide nanoparticles in farming practices. Therefore, responsible research, development, and implementation are crucial for maximizing the potential of this technology while addressing potential risk.

Wheat (*Triticum aestivum* L.) is cultivated all over the world because it is highly nutritious and can thrive in a variety of climates (Abou-Zeid et al. 2021). Wheat supplies are expected to fall as global consumption is anticipated to exceed production (FAO 2018). A research gap exists in the realm of understanding how ZnO NPs impact the complicated physiological and biochemical processes of wheat plants during a crucial 21-day growth phase (a critical developmental stage). The facile metabolization of Zn ions by plants provides a fine line between the detrimental and beneficial effects of ZnO NPs; hence, we have selected a wide range of Zn concentration (5–500 ppm) to explore possible dose-dependent effects (Stałanowska et al. 2023). Focusing on practical applications for Gujarat farmers, we investigated the effects of ZnO nanopriming on the *Triticum aestivum* variety GW 451, known for its high yield potential. We hypothesize that: (1) nanopriming will enhance seed germination and seedling growth, and (2) different ZnO NPs concentrations will trigger dose-dependent changes in plant growth parameters and potentially improve the physiological status through biochemical alterations. This study uniquely investigates the impact of a wider range of ZnO NP concentrations (5–500 ppm) on previously unexplored biochemical parameters in wheat seedlings, potentially unraveling novel insights into its dose-dependent effects, paving the way for safer and more efficient sustainable agricultural practices.

Materials and methods

Synthesis and characterization of ZnO nanoparticles (ZnO NPs)

The zinc oxide nanoparticles employed in this research were synthesized using a modified chemical synthesis method developed within our laboratory. In brief, to synthesize ZnO nanoparticles, a 500 mL of deionized water was combined with 0.1 M zinc sulfate hydrate ($ZnSO_4.7H_2O$) at ambient temperature (25–30 °C) utilizing a magnetic stirrer. 1N NaOH solution was gradually added into the former solution until pH of the reactant becomes 11 (Singh and Haque 2016; Ribut et al. 2018). The solution's pH was meticulously regulated through

the gradual addition of freshly prepared 1N NaOH. Within a span of 3 h, ZnO NPs precipitated, manifesting as discernible white deposits. Subsequently, the solution underwent centrifugation at 6000 rpm for 10 min, leading to the formation of a pellet that was then resuspended in Milli-Q water to eliminate any potential impurities. This purification process was reiterated twice employing Milli-Q water. The resultant pellets were mixed with a 500 mL solution of the dispersing agent, namely STPP (sodium tripolyphosphate). This mixture was subjected to ultrasonic treatment using sonicator (Qsonica, USA) at 20 kHz for a duration of 10–15 min (Farroh et al. 2020). Particles size, polydispersity index (PDI) and zeta potential were measured using Malvern Zeta Sizer, USA (Model: ZS-90). Functional group of ZnO NPs were analyzed using Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer, Spectrum II) in the mid-IR region of 400–4000 cm^{-1} and it were generated using Perkin Elmer Spectrum-II instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets. XRD pattern was utilized to analyze the phase composition and crystal structure of synthesized ZnO NPs in the scanning angle range of $2\theta = 20–80$.

Experimental details

The wheat seeds of variety GW 451 procured from Agricultural Research Station, Dhandhuka, AAU were utilized in a pot/bag experiment at Anand Agricultural University, India, during 2021–2022. The plants were grown in a net house with natural light, a temperature of 28/18 °C day/night, and a relative humidity of 60–70%. The seeds were surface sterilized with 4% sodium hypochlorite for 5 min, and then rinsed three times with distilled water (Saber et al. 2021). ZnO NPs solution with concentrations of 5, 50, 100, 250, and 500 ppm were freshly prepared by diluting a solution of ZnO NPs in Milli-Q water to the appropriate concentration. Healthy and uniform sized wheat seeds were primed (30 seeds/treatments) in 100 ml of pre-determined five concentration of ZnO NPs suspension for 4 h and kept on shaker at low speed. Then, the solution was discarded and seeds were dried on blotting paper for one hour. The control group involved seed immersion in sterile distilled water, establishing a positive control. Conversely, seeds in the negative control group were directly planted without any prior soaking. Plants for both the control and primed groups were frequently watered after germination into plastic bags (12×12 cm) with a mixture of 7:2:1 soil, cocopeat, and vermicompost. The soil pH, EC, and zinc content were determined prior to filling the bags. The pH and EC were 7.5 and 1.6 dS/m, respectively, with an initial Zn content of soil was 0.11 ppm. The seedlings were collected at the age of twenty-one days for biochemical and physiological investigation. Using a randomized approach, each treatment was conducted three times (CRD). The whole experiment was conducted twice, one for destructive physiological parameters such as shoot and root length, shoot fresh and dry weight, root fresh and dry weight and another for the remaining physio-biochemical parameters.

Physio-biochemical analysis of different parameters

Pre-decided physio-biochemical parameters were studied from the leaf and root tissues of both control and ZnO NPs-treated plants.

Germination and growth parameters

ZnO nanoparticles impact on germination was determined by placing both untreated and treated seeds (20 seeds per replication) in Petri dishes and incubating them for 3–4 days according to the International Seed Testing Association (ISTA) germination test guidelines (Itroutwar et al. 2020). After 24, 48, and 72 h, germination was recorded (Hajra and Mondal 2017). This germination rate was determined as:

$$\text{Germination}(\%) = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Three randomly selected plants per treatment were measured using a millimeter ruler for their shoot lengths (SL) and root lengths (RL). Three seedlings for each treatment were weighed for fresh weight (FW) immediately after harvesting, and dry weights (DW) were weighed and recorded in milligrams after drying for 72 h at 65 °C.

Relative water content (RWC) and membrane stability index (MSI)

The relative water content (RWC) of leaf disc from 10 fully expanded leaves (200 mg) was determined as per Weatherley (1950). These leaf discs were placed in distilled water for 24 h at 4 °C under dark condition. The blotted leaf discs were weighed immediately to obtain the turgid weight (TW). The turgid leaf disc was then oven-dried at 65 °C for 48 h and dry weight (DW) was recorded. The RWC (%) was determined using following equation:

$$\text{RWC}\ (\%) = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

The membrane stability index (MSI) was determined as per Premachandra et al. (1990), based on the electrical conductivity (C1 and C2) of two leaf samples without midribs heated at two different temperatures, 40 °C and 100 °C for 30 and 10 min, respectively and the MSI (%)

was calculated using the following equation (Hajra and Mondal 2017).

$$MSI = 1 - \frac{EC1}{EC2} \times 100$$

Zinc content

Zn content was estimated by oven dried the tissues (leaf and root) at 70 °C for 48 h. Microwave-assisted digestion was performed on 0.1 g of dried plant material by combining it with 8 ml of HNO_3 and heating the mixture to 150 °C for 2 h in Microwave Digestion System (Anton Paar, Austria) and then diluting the samples with 50 ml with deionized water. Inductively Coupled Plasma-Atomic Absorption Spectrophotometer (ICP-AAS) was then used to determine the amount of Zn present in the plant tissues.

Estimation of total chlorophyll and total protein content

The chlorophyll content per unit area of a fully expanded leaf sample of 21 days old seedlings were measured using SPAD 502 M (Minolta Co., Japan) and total chlorophyll content was expressed in SPAD value (Haider et al. 2018).

The total protein content was estimated using the folin reaction of Lowry et al. (1951) method. The protein extract (0.1 mL) was mixed with 5 mL of an alkaline copper solution, then 0.5 mL of Folin and Ciocalteu's reagent was added. After 30 min of incubation at room temperature in the dark, the absorbance was measured at 660 nm against a blank (without protein solution) on a UV-Spectrophotometer (Hajra and Mondal 2017). The protein quantity was calculated using the standard curve of the standard protein solution (Bovine serum albumin, BSA).

Quantification of proline and glycine-betaine content

Proline content of root and leaf tissues was estimated using the method described by Bates et al. (1973). Proline content was determined from a standard curve and calculated on a fresh weight basis using the following formula by:

$$\text{Proline}(\mu g/g, FW) = [(\mu g \text{ proline/ml} \times \text{ml toluene})/115.5\,\mu g/\mu\text{mole}] / [(\text{g sample})/5]$$

where, total proline in micrograms were divided by the molecular weight of proline (115.5 µg/µmol) to express it in micromoles.

The technique of Grieve and Gratten (1983) was used to determine the amount of glycine betaine (GB) content, which was calculated using a standard curve derived from betaine solutions with known values ranging from 100 to 1000 ppm.

Malondialdehyde (MDA) content

Level of lipid peroxidation was measured by estimating malondialdehyde (MDA) content using thiobarbituric acid (TBA) reaction as described by Heath and packer, (1968). Based on MDA's molar extinction value (€) of 155 mM^{-1} cm^{-1}, the MDA amount was determined as per following formula (Rai-Kalal and Jajoo 2021):

$$\text{MDA}\,(\mu\text{mol/g, FW}) = 1000 \times \frac{A532 - A600 \times V}{n} \times W$$

where, € is molar extinction value (155 mM^{-1} cm^{-1}), V is the volume of crushing medium, W is the fresh weight of leaf and root tissues, A532 represented the absorbance of the TBA-MDA complex; A600 represented the correction for non-specific turbidity.

Determination of antioxidant enzymes activity

Fresh leaf and root tissues from the control and treated plants were homogenized in a pre-chilled mortar and pestle with liquid nitrogen until a fine powder was produced for the enzyme assay. After that, the powder was suspended in 5 mL of sodium phosphate buffer (0.2 M, pH 7.2), and it was centrifuged at 8000 × g for 15 min at 4 °C. The supernatant was used to calculate the activity of the superoxide dismutase (SOD) and catalase (CAT) using the following methods. Inhibition of the photochemical nitroblue tetrazolium (NBT) technique was used to quantify superoxide dismutase (SOD) activity (Beauchamp and Fridovich 1971). The SOD enzyme unit was determined using the formula (Roy et al. 2021):

Percent inhibition (%) = Control OD- treatment OD/ Control OD x 100

Where, Control OD represent the optical density of blank and treatment OD represent the optical density (OD) of the samples

$$\text{SOD activity}(\mu g/mg) = \frac{\text{Percent inhibition} \times \text{Dilution factor}}{50\% \times \text{enzyme extract taken} \times \text{FW of sample in mg}}$$

H_2O_2 (10 mL) and phosphate buffer (50 mM; 1.9 mL) were added to 100 µL of fresh enzyme extract to measure CAT activity. To determine the degree of H_2O_2-dependent oxidation, absorbance at 240 nm was measured for 180 s (Aebi 1984). The CAT enzyme unit was determined as per following formula:

$$\text{Enzyme activity} = \frac{\text{Change in OD/min}}{\text{Fresh weight of sample present in enzyme extract}}$$

Statistical analysis

The experiment was carried out in a completely randomized block design (CRD), with three replicates of each treatment repeated twice. Results were analyzed using a one-way ANOVA followed by the Duncan's multiple range test (DMRT). Analyses were made using R statistical software (4.0.5) and Microsoft Excel. Data were represented using the mean ± standard error (SE). A p value ≤ 0.05 was considered statistically significant in a DMRT.

Result and discussion

Synthesis and characterization of ZnO NPs

The results of dynamic light scattering (DLS) studies showed that the hydrodynamic diameter of ZnO NPs was 71.13 nm with PDI of 0.29 (Fig. 1A). The results showed that ZnO nanoparticles were on the nanoscale, and PDI showed that the nanoparticles were distributed monodispersely in solution, without any aggregation (Tarafdar et al. 2014). The significant repulsion between the nanoparticles was confirmed by the average zeta potential value of -33 mV for the synthesized ZnO NPs, which further improved the stability of the formulation by preventing any aggregation (Fig. 1B). Similar to current study, previous reports of Darezereshki et al. (2011) and Alves et al. (2018) aslo showed same zeta potential. This similarity among various reports concludes that ZnO NPs are more stable and aggregate less when their zeta potential is in the aforementioned range.

Absorption peaks was detected in the FTIR spectra of synthesized ZnO NPs (Fig. 1C) which was ranged from 500 cm^{-1} to 4000 cm^{-1}. Bands at 1635 cm^{-1} indicated C=C stretching vibration of alkene, 1422 cm^{-1} indicated O–H bending of carboxylic acid, 1035 cm^{-1} indicated S=O stretching vibration of sulfoxide group; and 3852, 3745, and 3420 cm^{-1} were assigned to the OH group stretching mode of alcohols, indicated the presence of a small amount of water adsorbing capacity of the zinc oxide nanoparticles surfaces (Ghasemzadeh et al. 2015). The peak between 400 and 600 cm^{-1} was attributed to the bond stretching vibrations of the zinc oxide molecule. The FTIR spectrum revealed that every functional group present in ZnO NPs contributes to their encapsulation and stability. According to Kasivelu et al. (2020), the hexagonal phase of Zn–O was shown by the band at 487 cm^{-1} in the FTIR spectrum of ZnO nanoparticles, while the bands at 1624 and 1104 cm^{-1} were found to be responsible for interacting and capping the surface of ZnO nanoparticles, respectively. This suggested that the nanoparticles were small and stable enough for use in nano-fertigation, also known as seed priming.

Effect of ZnO NPs on physio-biochemical traits

Seed germination (%)

Figure 2A shows the effect of ZnO nanoparticles on wheat seed germination; compared to the control group, the germination rate increases dramatically as ZnO nanoparticle concentration is raised from 0 to 250 ppm. The germination rate of the ZnO NPs-treated seeds were much greater than those of the hydroprimed and unprimed seeds. After 72 h, 100% of the ZnO-treated seeds had germinated, whereas the germination rate was lower in the first 48 h. Seeds treated with ZnO NPs at 250 ppm germinated 100% after 72 h, which is 15.38% and 20% greater than hydro- and unprimed seeds, respectively. Zn plays a key role in a cascade of biochemical changes in the seed that are needed to initiate the germination process, including dormancy breaking, hydrolysis or metabolization of inhibitors, optimal reactive oxygen species (ROS) production, imbibition, and enzyme activation, all of which contribute to the higher germination percentage observed in ZnO NP-treated seeds (Harris et al. 2007; Samad et al. 2014). This is validated by research of Sharma et al. (2021), which found that rice seeds primed with nanopriming solutions (ZnO at20 ppm and ZnO at 40 ppm) experienced faster germination and earlier protrusion than their hydroprimed counterparts. Priming rice seedlings with seaweed-based biogenic ZnO nanoparticles at 10 ppm resulted in improved germination rates (100%) as reported by Itroutwar et al. (2020). Wheat and maize seedlings treated with ZnO nanoparticles exhibited improved germination and growth due to increased amylase activity (Srivastav et al. 2021).

Effect of ZnO NPs on root and shoot attributes

Data on wheat root and shoot length and biomass are presented in Table 1. The data showed that the morphological characteristics varied significantly with NPs concentration. Wheat seedlings shoot and root lengths, as

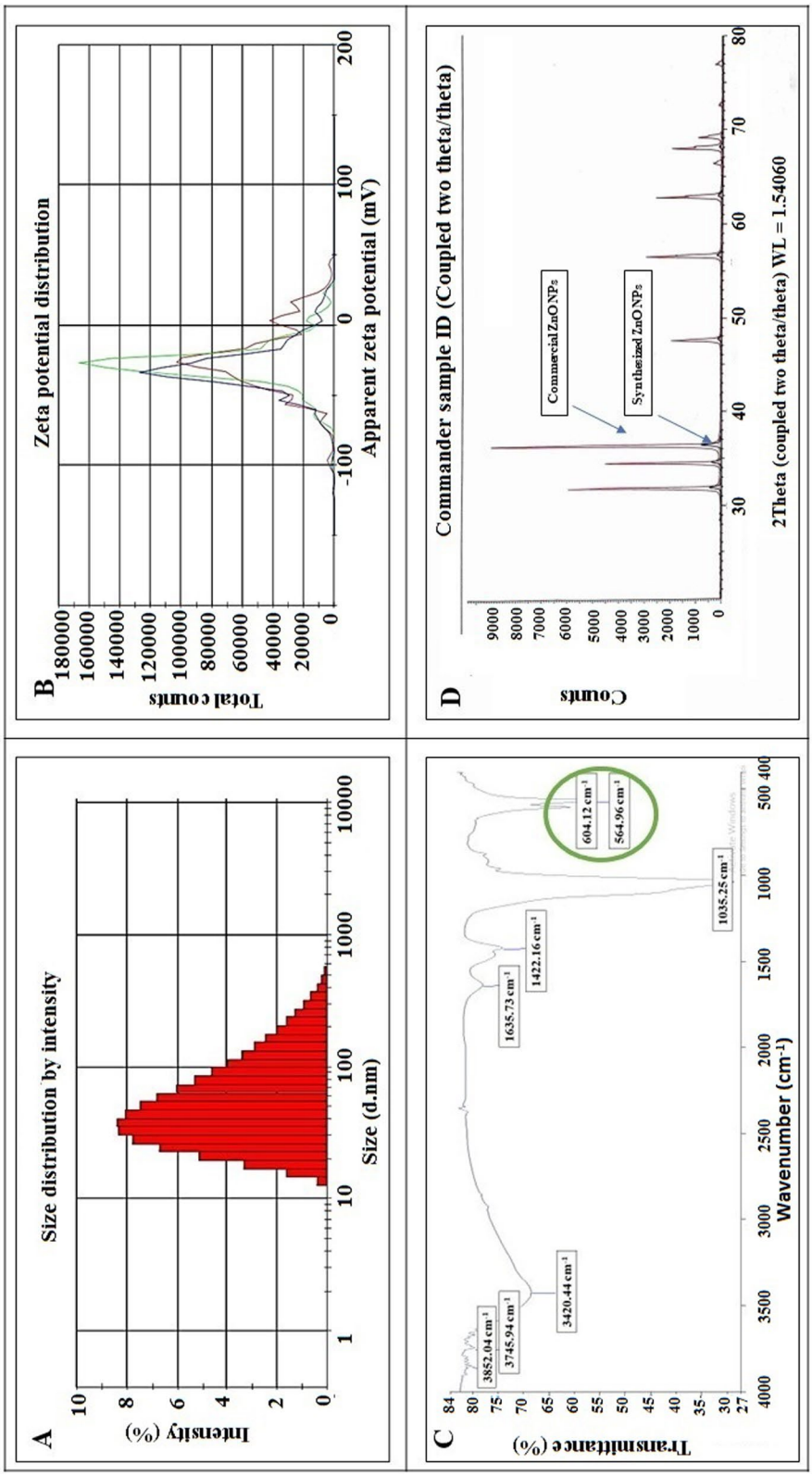

Fig. 1 **A** The hydrodynamic size diameter of ZnO NPs **B** The zeta potential distribution of ZnO NPs **C** FTIR spectrum of ZnO NPs **D** XRD patterns of ZnO NPs

Table 1 Morphological variation of wheat (*Triticum aestivum* L.) plant under ZnO nanoparticles treatment

Parameters	Control		T_1 500 ppm	T_2 250 ppm	T_3 100 ppm	T_4 50 ppm	T_5 5 ppm	SEm±	CD @ 0.05	CV (%)
	Water control	Negative control								
Root length (cm)	14.33 ± 0.88^{cd}	13.67 ± 0.67^{d}	22.00 ± 1.52^{a}	23.00 ± 1.15^{a}	16.33 ± 0.88^{b}	16.00 ± 0.57^{bc}	14.33 ± 0.33^{cd}	0.934	2.83	9.46
Shoot length (cm)	31.66 ± 0.88^{cd}	29.83 ± 1.09^{d}	34.00 ± 1.00^{bc}	38.00 ± 0.57^{a}	35.33 ± 0.88^{ab}	34.00 ± 0.58^{bc}	33.33 ± 0.66^{bc}	0.833	2.53	4.27
Shoot Fresh weight (mg)	2550 ± 50.33^{de}	2436 ± 59.25^{e}	2916 ± 161.69^{bc}	3866 ± 61.73^{a}	3126 ± 63.59^{b}	2883 ± 44.09^{bc}	2783 ± 72.64^{cd}	82.17	249.8	4.845
Shoot Dry weight (mg)	819 ± 12.17^{e}	880 ± 16.95^{de}	944 ± 23.79^{cd}	1385 ± 10.83^{a}	1117 ± 69.58^{b}	988 ± 5.00^{cd}	902 ± 14.31^{cde}	30.79	90.01	5.122
Root Fresh weight (mg)	156 ± 3.33^{cd}	140 ± 5.77^{d}	140 ± 11.54^{d}	233 ± 12.01^{a}	186 ± 6.66^{b}	180 ± 5.77^{bc}	160 ± 5.77^{cd}	7.868	23.86	7.972
Root Dry weight (mg)	91 ± 2.02^{ef}	86 ± 4.40^{f}	112 ± 3.46^{cd}	163 ± 6.00^{a}	137 ± 4.16^{b}	118 ± 4.58^{c}	103 ± 2.72^{de}	4.096	12.42	6.116

Each value is the mean ± SE of three replicates (n = 3); significant at p less than 0.05 ($p \leq 0.05$)); SEm ±: Standard Error of the Mean; CD: Critical Difference at α = 0.05; indicates statistically significant difference between treatment groups at 5% level

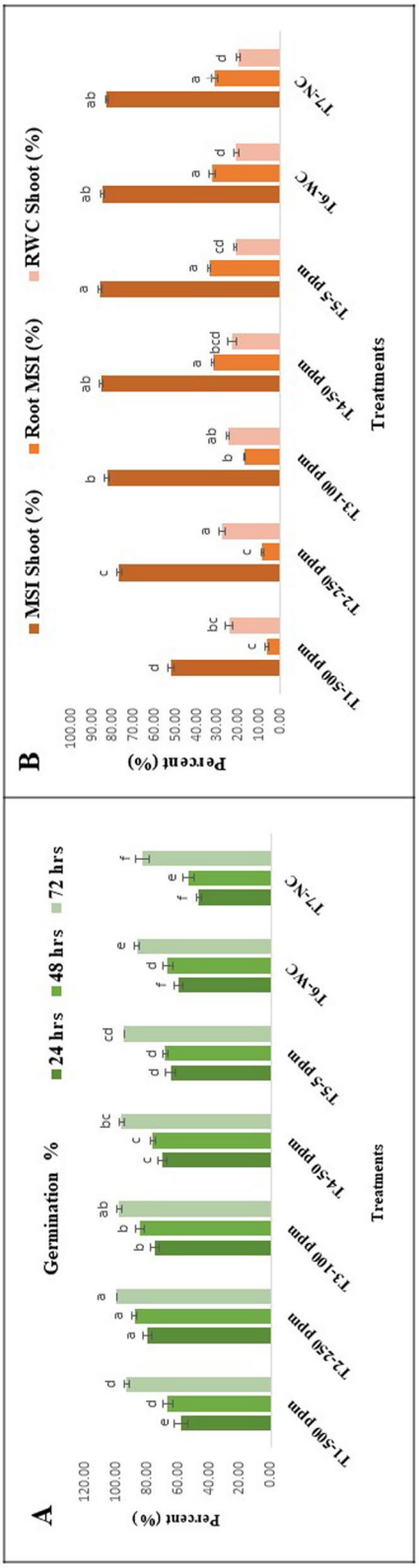

Fig. 2 Effect of different concentration of ZnO NPs on **A** seed germination at various time interval **B** root and shoot MSI and root RWC of 21 days old wheat seedlings; Error bar: standard error of mean (±SE). Different alphabets: significant difference among treatment over control ($p \leq 0.05$; Duncan's multiple range test)

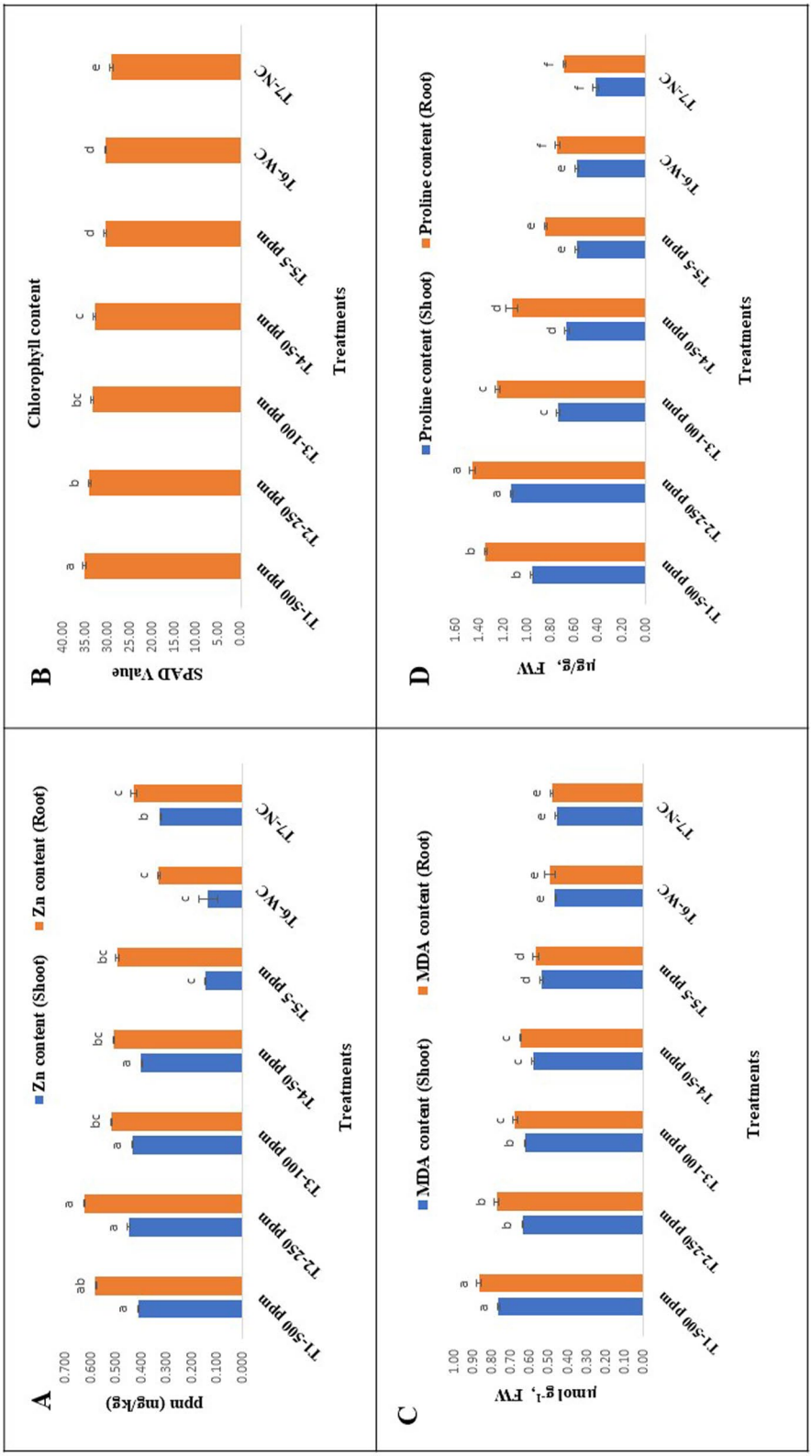

Fig. 3 Effect of different concentration ZnO NPs on **A** Zn content **B** Chlorophyll content **C** MDA content **D** Proline content of 21 days old wheat seedlings; Error bars indicates standard error of mean (± SE), Different alphabets indicate significant difference among treatments over control ($p \leq 0.05$; Duncan's Multiple Range test)

well as their biomass, increased gradually with increasing doses of ZnO nanoparticles. Furthermore, 250 ppm ZnO nanoparticles treatment resulted in the highest SL (38 cm), RL (23 cm), SFW (3866 mg), RFW (233 mg), SDW (1385 mg), and RDW (163 mg), which significantly differed from the water control by 60%, 64%, 52%, 49%, 69%, and 79%, and from the negative control by 59%, 68%, 58%, 66%, 57%, and 89%. The priming of seeds was significantly impacted by the concentration of NPs. The priming treatment applied prior to wheat seed germination increased shoot and root proliferation in all doses. Increased cell division and cell elongation under the impact of increased IAA activity may account for the notable increases in plant length as seen at varying nanoparticle concentration. Higher quantities of ZnO nanoparticles were associated with a decrease in groundnut plant height, as reported by Prasad et al. (2012). Similar to previous research, this study found that increasing the concentration of ZnO nanoparticles had a negative effect on plant height, possibly due to nanoparticle toxicity. Low dosages of ZnO nanoparticles were sufficient to elicit a good response in a separate investigation on chickpea seedlings, while higher doses were associated with a delay in early development metrics (Mahajan et al. 2011; Burman et al. 2013). Cyriac et al. (2020) also discovered that when peas and rice were cultivated with ZnO NPs, their root growth was significantly enhanced compared to control. Better plant development was also observed by Avinash et al. (2010) who reported that NPs enhances the level of IAA in the roots (sprouts). They used HPLC analysis to confirm an elevated amount of IAA. ZnO nanoparticles had a considerable effect on the plant samples biomass up to 250 ppm, and then a decreasing effect at 500 ppm. The improved photosynthetic activity of the wheat plant was attributed to the higher Zn content in the plant's root and leaf tissues, suggesting a beneficial effect of ZnO NPs on plant biomass. The fresh weight of wheat shoots and roots from 21 days old seedlings was found to increase by 57% and 88%, respectively, when treated with 100 ppm ZnO NPs, compared to the control group. However, when treated with 800 ppm ZnO NPs, the fresh biomass of shoots and roots decreased significantly in earlier report (Saber et al. 2021). The fresh weight (by 55%) and dry weight (by 27%) of seedlings in nano-primed rice were found to be the highest compared to hydroprimed and unprimed seeds by Rai-kalal and Jajoo (2021). Similar increases in dry biomass of shoot were observed by Srivastav et al. (2021) for maize plants treated with 100 ppm and wheat plants treated with 150 ppm ZnO NPs. The root biomass was significantly affected by the ZnO NPs concentration used in the foliar treatment. Zn treatments enhanced biomass production which attributed to the role of Zn in photosynthetic activities and cell division (Torabian et al. 2016).

Membrane stability index and relative water content

Increasing the ZnO nanoparticle concentration dramatically reduced the MSI of the shoot and root. Compared to water and the control, ZnO NPs at 5 ppm greatly increased the membrane stability of both the shoot and the root (Fig. 2B). These data demonstrate that priming treatments induced oxidative stress, which in turn disrupted membranes. In the case of chickpea, identical findings have been reported by Hajra and Mondal (2017). According to their findings, MSI increased at higher doses of ZnO NPs but then reduced, while MSI decreased at lower doses of TiO_2 nanoparticles but then increased with higher concentrations. Root and shoot membrane injury is likely to occur at greater ZnO nanoparticle concentrations. The membrane integrity of plant cells is compromised by the oxidative stress and electrolytic leakage caused by nanoparticles (Hatami and Ghorbanpour 2014). According to the results, RWC variations between treatments was significant (Fig. 2B). Increased dose caused a dramatic increase in RWC of the leaf tissues up to 250 ppm, but after that the RWC began to decrease. In comparison to hydropriming and the negative control, the RWC was 32% and 38% higher, respectively, with treatment of ZnO NPs at 250 ppm. Ghani et al. (2022) found that the RWC of cucumber seedlings exposed to ZnO NPs (100 ppm) was 7.66% greater than the control. The RWCs of rice roots significantly increased by 10.8% in rice seedlings treated with 25 ppm ZnO NPs compared to control (Yang et al. 2021). The increased RWC could be the result of increased water uptake by wheat seeds treated with nano-priming.

Zn content (ppm)

ZnO NPs treatments consistently resulted in a greater increase in Zn content in leaf and root tissues compared to the control (Fig. 3A, E). Compared to other treatments, the Zn content of nanoprimed (with 250 ppm) leaf and root tissues was 39% and 37% higher, respectively, and 89% and 45% higher, respectively, than that of hydro- and unprimed seedlings. However, this difference was not statistically significant. This suggested that root tissue collected more Zn than leaf tissue as ZnO NPs dissolved in the root rhizosphere and improved uptake of Zn^{+2} ions. Though overall Zn content of wheat tissues increased in comparison to control. Wheat root cortex cells took up ZnO NPs, suggesting that they could be taken up systemically by the plant and used elsewhere (Chen et al. 2018). Priming seeds with zinc improve their nutrient content, germination rate, and subsequent plant growth. Priester et al. (2012) also found that the root collects more Zn at

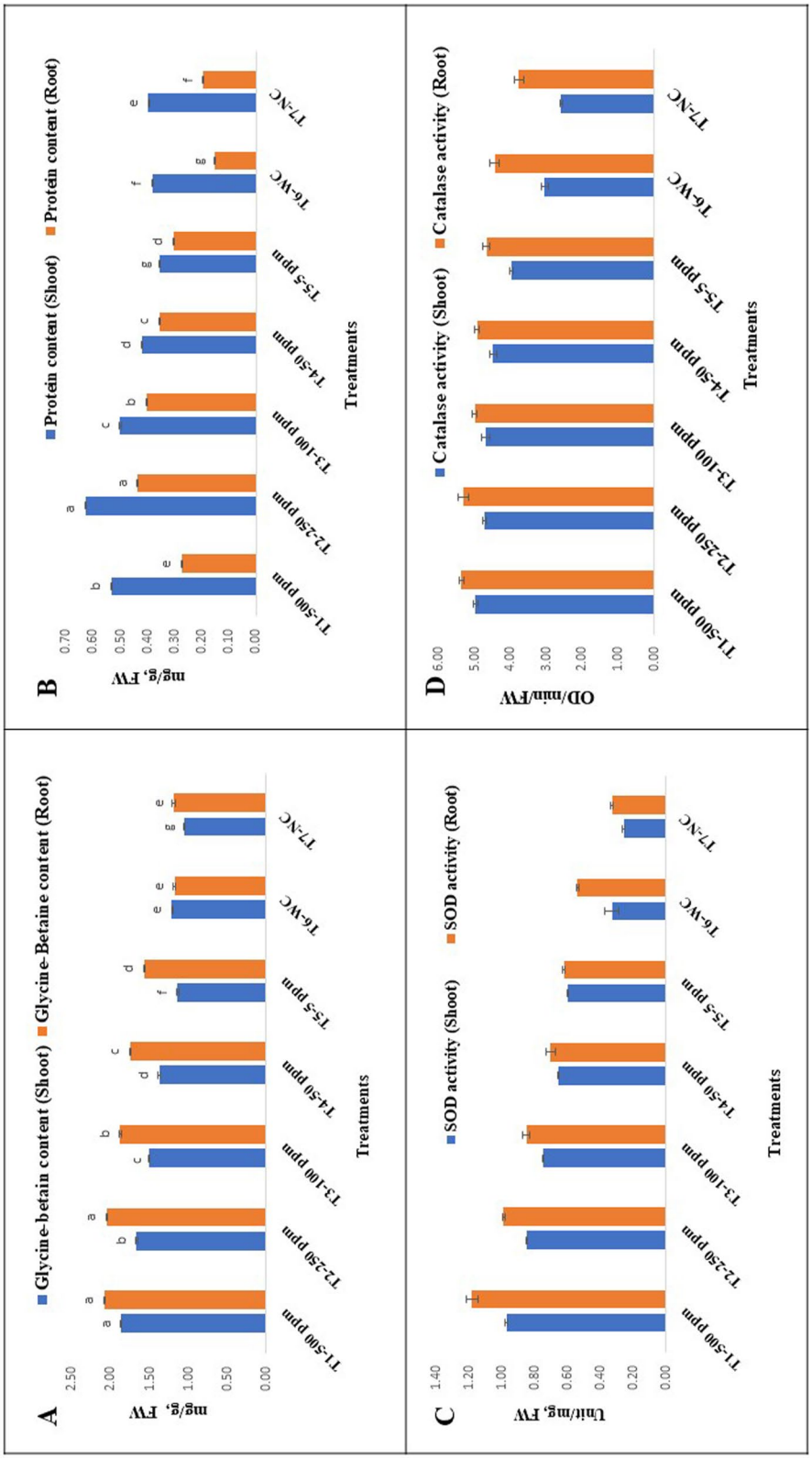

Fig. 4 Effect of different concentration ZnO NPs on **A** Glycine betaine content **B** Total Protein content **C** SOD activity **D** Catalase activity of 21-d old wheat seedlings; Error bars indicates standard error of mean (± SE), Different alphabets indicate significant difference among treatments over control ($p \leq 0.05$; Duncan's Multiple Range test)

greater doses of ZnO nanoparticles. According to Chen et al. (2018), rice plants have a higher Zn content in their roots than their shoots. Zinc concentration in the control group rice seedlings was 0.150 ppm, and it was shown to increase in a dose-dependent way in the nanoprimed ZnO nanoparticles by Itroutwar et al. (2020). Zn is more concentrated in the roots of wheat and maize plants than in their shoots, according to research by Srivastav et al. (2021). The enhanced zinc content of nanoprimed rice seeds compared to the hydroprimed control suggests that zinc acquisition may have improved the nutritional value of the seed (Sharma et al. 2021).

Chlorophyll content (SPAD)

Chlorophyll levels reflect the efficiency of photosynthesis and the rate of plant development. Figure 3B shows that the concentration of chlorophyll changed in response to ZnO nanoparticles. Chlorophyll content significantly varied between experimental ZnO nanoparticle treatments. The chlorophyll content of wheat seedlings was 15.20% and 20.12% greater, respectively, than it was in hydroprimed and unprimed seedlings. This indicated that ZnO NPs might have a role in chlorophyll synthesis, which in turn might boost wheat photosynthetic efficiency. Srivastav et al. (2021) found a similar pattern in maize and wheat and reported an increasing concentrations of ZnO NPs resulted in higher levels of Chl a, Chl b, and total chlorophyll content. Wheat plants exposed to salt stress showed a significant increase in total pigments content after being primed with varying amounts of ZnO NPs (Abou-Zeid et al. 2021). In addition to elevating the amount of the photosynthetic pigments, priming with ZnO NPs did so even in the absence of salt stress. Leaf chlorophyll content was shown to change over time after treatments, as reported by Bala et al. (2019). The synergistic effect of other intrinsic nutrients including magnesium, iron, and sulphur was proposed by Prasad et al. (2012) as a possible explanation for increased leaf chlorophyll content.

MDA content (µmol/g, FW)

All wheat seedlings treated with ZnO NPs showed a dramatic increase in MDA content. Figure 3C demonstrates that as the concentration of ZnO NPs in root and leaf tissues was increased, the MDA content also increased. ZnO NPs treatment with 500 ppm increased the MDA concentration in both the leaf and root tissues by 67% and 77%, respectively, compared to hydro- and un-primed seedlings. While the lowest MDA content was found in 5 ppm. Lipid peroxidation, oxidative stress, and damage to the cell membrane result after ZnO NPs treatment, as indicated by the increased MDA level (Hajra and Mondal 2017). The quantities of hydrogen peroxide and malondialdehyde (MDA) in the shoots and roots of a soybean plant treated with ZnO NPs were considerably greater than in a control samples (Saber et al. 2021). Plants treated with ZnO NPs also displayed elevated MDA levels, peaking at 500 ppm and then 1000 ppm. It is likely that ROS production causes the higher buildup of MDA during the presence of nanoparticle (Hajra and Mondal 2017).

Proline and glycine-betaine content (µg/g, FW)

ZnO NPs considerably altered the proline and glycine-betaine concentrations in both the shoot and root tissues (Figs. 3D, 4A). Plants exposed to 250 ppm of ZnO NPs showed the greatest increase in proline content, by 162% in leaf tissue and 91% in root tissue compared to the negative and water control, respectively. However, when treated with larger concentrations of ZnO NPs, glycine betaine content increased dramatically. According to the results, 500 ppm ZnO NPs treatment resulted in the greatest increase in glycine-betaine concentration in both leaf and root tissues (79% and 50%, respectively) compared to the negative and water controls. The increased accumulation of proline in ZnO NPs treatments may serve as a defense mechanism or higher tolerance to metals despite the growth inhibition by shielding the plant from singlet oxygen and free radical-induced damages caused by excess ROS. According to a study conducted by Salehi et al. (2021), when ZnO NPs were sprayed onto the leaves of a bean plant, the endogenous levels of proline increased in a dose-dependent way, with a 131% rise at 2000 ppm ZnO NPs treatment compared to the control. A similar 45.1% and 23.6% rise in proline concentrations was observed between the control and the ZnO NP foliar spray and ZnO NP -soil treatments (Mirakhorli et al. 2021). Glycine-betaine, on the other hand, functions as osmolytes to keep cellular turgor pressure stable under stressful conditions. This suggests that glycine-betaine buildup enhances ROS scavenging, helps keep redox equilibrium and enzyme activities stable (Kaya et al. 2013). Plants subjected to 10 and 20 µM arsenic (As) stress produced significantly more glycine betaine compared to plants non-As-fed plants. Plants exposed to 10 and 20 µM As had increased glycine betaine by 11.08% and 28.66%, respectively, when supplemented with 50 ppm and 100 ppm ZnO NP. Therefore, ZnO NPs are useful for reducing heavy metal toxicity in plants (Ahmed et al. 2021).

Total protein (mg/g, FW)

The total protein content of plant tissues exposed to any concentration of ZnO NPs was shown to increase

significantly (Fig. 4B). Total protein content in leaf tissues treated with 250 ppm ZnO NPs was increased by 58.97% and in root tissues by 63% compared to hydro- and unprimed seedlings. Zinc is a micronutrient that plays a role in many physiological processes in plants due to its roles as a cofactor for many enzymes and as a key structural element of regulatory and structural proteins. It is also involved in chlorophyll biosynthesis and increased antioxidant enzyme activity. This finding suggests that the overall protein content of wheat plants rose after being treated with ZnO nanoparticles. These findings are consistent with those of Faizan et al. (2021), who discovered that treating tomato leaves with 8 ppm ZnO NPs boosted protein content by 45.0% compared to control and other treatments.

Antioxidant enzymes

Plants rely heavily on antioxidant enzymes as a first line of defense against ROS (Willekens et al. 1997). Wheat seedlings oxidative status was studied by examining the impact of different priming treatments on antioxidant enzyme activities such superoxide dismutase (SOD) and catalase (CAT). Antioxidant activity in both root and leaf tissues was significantly boosted by ZnO NPs (Fig. 4C, D). However, SOD and CAT activity were both greater in root tissues than in leaf tissues. Wheat seedlings treated with 500 ppm ZnO NPs showed a 2.90- and 3.69-fold increase in SOD in leaf tissues and a 2.18- and 3.57-fold rise in SOD in root tissues, respectively, compared to hydro- and unprimed seedlings. Similarly, CAT activities were 1.63- and 1.91-fold greater in leaf tissues and 1.21- and 1.4- folds higher in root tissues in comparison to water and the control, respectively, when exposed to 500 ppm ZnO-NPs. The SOD and CAT activities in the root and leaf tissues of wheat seedlings were found to be significantly elevated after receiving ZnO nano-priming treatments. Zinc is an antioxidant and helps plants quench ROS because of its well-known activity as a cofactor of superoxide dismutase (SOD). The antioxidant enzyme composition and the defense mechanism of wheat and maize plants were found to be altered after ZnO NPs were introduced in experiment, as reported by Srivastav et al. (2021). The sustained development of wheat was also observed by Amooaghaie et al. (2017), who found that nano-Zn and nano-ZnO boosted SOD activity in wheat. Compared to the unprimed control, NP and bulk $ZnSO_4$ primed wheat plants shown a 47% and 55% increase in CAT enzyme activity, respectively (Raikalal and Jajoo 2021). Weisany et al. (2012) revealed that zinc is indirectly required for high activity of the enzyme involved in H_2O_2 detoxification, such as CAT, which is similar with current findings. According to Azarin et al. (2022), CAT activity was boosted in plant roots and leaves treated with either nano or bulk ZnO. The increment was noticed when *Hordeum vulgare* was exposed to nano-ZnO.

Conclusion

The current research showed that wheat seeds primed with 250 ppm of zinc oxide nanoparticles (ZnO NPs) improved wheat growth in the GW 451 cultivar. ZnO NPs significantly improved growth-related physiological and morphological characteristics, and they also increased Zn content, which enhances the nutritional value of wheat plants. ZnO NPs considerably improved biochemical characteristics after being applied to the plants. These characteristics include chlorophyll and total protein concentrations as well as proline and glycine betaine levels. In addition, ZnO NPs treatment was found to improve plants resistance to ROS via changing the antioxidant enzyme composition in both root and leaf tissues. However, it was discovered that the reaction of plants to ZnO NPs was dose-dependent. Hence, future research should be carried out to examine the impact of various doses of ZnO NPs in field conditions and stressed environment. ZnO NPs are a Zn nutrient for sustainable agricultural development and an effective seed nano-priming agent for boosting plant growth.

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Author contributions
SK and GBP conceptualized and supervised the experiment, interpret the data and annotate the original draft; PP performed the seed priming, physio-biochemical evaluation and wrote original draft, MM synthesised and characterized nanoparticles, ZS helped in physio-biochemical evaluation.

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Declarations

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Not applicable.

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