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RESEARCH ARTICLE

Application of zinc oxide nanoparticles immobilizes the chromium uptake in rice plants by regulating the physiological, biochemical and cellular attributes

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Abstract Zinc oxide nano particles (ZnO NPs) have been employed as a novel strategy to regulate plant tolerance and alleviate heavy metal stress, but our scanty knowledge regarding the systematic role of ZnO NPs to ameliorate chromium (Cr) stress especially in rice necessitates an in-depth investigation. An experiment was performed to evaluate the effect of different concentrations of ZnO NPs (e.g., 0, 25, 50, 100 mg/L) in ameliorating the Cr toxicity and accumulation in rice seedlings in hydroponic system. Our results demonstrated that Cr (100 µM) severely inhibited the rice seedling growth, whereas exogenous treatment of ZnO NPs significantly alleviated Cr toxicity stress and promoted the plant growth. Moreover, application of ZnO NPs significantly augmented the germination energy, germination percentage, germination index, and vigor index. In addition, biomass accumulation, antioxidants (SOD, CAT, POD), nutrient acquisition (Zn, Fe) was also improved in ZnO NPs-treated plants, while the lipid peroxidation (MDA, H_2O_2), electrolyte leakage as well as Cr uptake and in-planta accumulation was significantly decreased. The burgeoning effects were more apparent at ZnO NPs (100 mg/L) suggesting the optimum treatment to ameliorate Cr induced oxidative stress in rice plants. Furthermore, the treatment of ZnO NPs (100 mg/L) reduced the level of endogenous abscisic acid (ABA) and stimulated the growth regulator hormones such as brassinosteroids (BRs) possibly linked with enhanced phytochelatins (PCs) levels. The ultrastructure analysis at cellular level of rice revealed that the application of 100 mg/L ZnO NPs protected the chloroplast integrity and other cell organells via improvement in plant ionomics, antioxidant activities and down regulating Cr induced oxidative stress in rice plants. Conclusively, observations of the current study will be helpful in developing stratigies to decrease Cr contamination in food chain by employing ZnO NPs and to mitigate the drastic effects of Cr in plants for the sustainable crop growth.

Keywords Stress tolerance · Plant growth · Phytohormones · Seed germination · Physio-biochemical attributes

Introduction

Rice (*Oryza Sativa L.*) is being utilized as a vital food component worldwide due to its higher nutritional and economical value. The 90% global rice production is only limited to Asian countries and China is leading the world with 30.7% share in the rice market (Lin et al. 2007)). Rice plants, being hydrophytic, accumulate higher concentrations of heavy metals with ultimate storage in seed which can be a major health hazard to humans (Mao et al. 2019). These heavy metals may cause damaging effects when bioaccumulated in soft tissues of plant's body and thereby alters metabolism (Faizan et al. 2021). Soil pollution due to continuous

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addition of toxic heavy metals through various natural and anthropogenic sources threatens the life of plants as well as human beings (Kasassi et al. 2008; Su, 2014; Tanwir et al. 2021). Chromium (Cr) is ranked as the seventh highly abundant as well as second most toxic element on the earth (Oh et al. 2007). Higher levels of Cr in soil was found lethal for plants due to its persistent nature, variable oxidation states and rapid accumulation in plants (Singh and Prasad 2019; Javed et al. 2021). Major anthropogenic sources of Cr include textile, leather, petrochemicals, paints, steel, dying, and electroplating industries along with sewage water and burning of fossil fuels in agricultural soil (Kasassi et al. 2008; Sharma et al. 2020; Javed et al. 2021). Higher discharge of Cr can alter the physio-biochemical properties of soil (Habiba et al. 2018). Chromium has 0-VI oxidation states; however, Cr (III) and Cr (VI) are the highly stable forms present in the ecosystem (Khatun et al. 2019). The Cr (VI) is known as the most hazardous oxidation state because of its carcinogenic and harmful impacts on plant's growth and yield (Hayes 1982; Basit et al. 2021, 2022a). Rice plants may accumulate Cr easily by transformation of trivalent oxidative state of Cr into hexavalent state through forming a complex with plant nutrients or by using symplast pathway (Bilal et al. 2018). Chromium competes with essential nutrients for accumulation, which, as a result, cause physiological, cellular, molecular, and biochemical changes inside the plants (Samrana et al. 2020; Basit et al. 2022b). Chromium bioaccumulation at excess levels in cultivated land negatively effects the physio-biochemical and cellular process in plants and triggers oxidative damage by increasing the biosynthesis of reactive oxygen species (ROS) (Mei et al. 2002; Sinh et al. 2020). Furthermore, Cr is the major concern of researchers to find possible techniques for the mitigation of Cr stress. Plants show defense against Cr stress through enhanced production of antioxidant enzymes, phytohormones and decreased ROS generation to avoid or minimize the physiological damage (Gupta et al. 2020; Javed et al. 2021; Basit et al. 2022c).

Engineered nanoparticles (NPs), due to their distinctive physio-chemical characters have gained attention in recent years as a novel strategy in agriculture and for remediation of heavy metal contaminated soils (Salem and Fouda 2021; Azhar et al. 2019). Among various engineered nanomaterials, nanoparticles have different application especially in agriculture as plant stress mitigation agents, nutrient facilitators due to their small size, superior surface feature, tailored shape and magnetic properties (Mahakham et al. 2017; Salem and Fouda 2021). In metal contaminated soils, nanoparticles (NPs) could be used to immobilize the heavy metal uptake due to increased surface area to volume ratio of NPs along with their unique surface chemistry (Tripathi et al. 2015; Lowry et al. 2019). There are various ways to produce NPs such as chemical, physical as well as biological to attain the purpose of reclamation of heavy metal soil contamination (Parakh et al. 2019; Fouda et al. 2021). Amongst the variety of method of NPs synthesis, green synthesis has been focused and prioritized recently due to the emerging global environmental concerns.

ZnO NPs production seem to be a valuable approach for plant science, with promising aspects for improved plant growth and yield, which is one of the most significant solutions for the world's rapidly growing population (Chanu and Upadhyaya 2019). Nevertheless, at large doses and extended periods of application, ZnO NPs may induce a variety of negative effects on plant growth and crop yield and may also become a threat to human, and animal health after entering the food chain. Different plants show distinctive responses toward ZnO NPs application; some plants show beneficial effects while others exhibit toxic, but the mechanism involved is still unclear. Although, several investigations have declared that the NPs size and dose are the foremost factors that determine their upshots (Thounaojam et al. 2021). Substantial research is being conducted to generate environmentally safe ZnO NPs, with functionalization being one of the most successful strategies for bringing the NPs into a stable, less toxic, and more efficacious state. Extensive research and more effective solutions are still required to overcome the increasing exploitation and risk of ZnO NPs (Chanu and Upadhyaya 2019; Thounaojam et al. 2021).

It has been found that the phytotoxicity induced by Cr has been mitigated by the application of green copper NPs in wheat (Noman et al. 2020). Silver NPs have also been employed to diminish Cr toxicity in pea (Azhar et al. 2019), and rice. Reportedly, silica NPs augmented the growth and development of plants under cadmium stress (Cui et al. 2017), and foliar use of selenium and silicon NPs reduced the harmful effects of cadmium and lead in rice plant (Hussain et al. 2020). Similarly, zinc oxide nanoparticles (ZnO-NPs) could serve the purpose of metal toxicity alleviation in plants with a subsequent increase in growth due to the micronutrient nature of Zn (Salam et al. 2022). So, utilization of ZnO NPs in Cr polluted soils is now gradually becoming a research area among the scientific community.

The recent study hypothesized that the ZnO NPs ameliorate Cr stress by reducing oxidative stress through improved antioxidant concentration and diminishing Cr accretion in rice plants. Thus, the key objective of the recent study was (a) to study the role of ZnO NP in improving rice growth, photosynthetic attributes, nutrient acquisition and decreasing Cr uptake in rice plants (b) to determine the role ZnO NPs affect lipid peroxidation and antioxidant activities at cellular level in rice seedlings under Cr stress (c) to assess the potential role of ZnO NPs in modulating phytohormone production under Cr stress.

Materials and methods

Hydroponic experiment

ZnO NPs with an average diameter 15–30 nm, surface area of 50 m^2 g^{-1}, spherical in shape and distinct crystallite structures were obtained from Alfa Aesar (Massachusetts, USA). Rice genotype named Chunyou-927 (CY927) was used in this experiment, which was conducted in the growth chamber of Seed Science Center, Zhejiang University, Hangzhou, China. Surface sterilization of seeds was done with 1% H_2O_2 for 15 min, carefully washed with (dd H_2O) and a seed germination analysis was conducted. Afterwards, 50 sterilized rice seeds were cultivated in plastic germination boxes (12 cm × 18 cm). The temperature of the growth chamber was set at 25/22 °C with an interchanging 16 h/8 h light/dark period for 14 days (Zheng et al. 2006). Incubated seeds were then transferred into 1L plastic boxes, in which 100 µM Cr was present with nutrient media solution. The nutrient solution was composed of 0.5 µM calcium nitrate ($Ca(NO_3)_2$), 0.5 µM potassium nitrate (KNO_3), 2.5 µM monopotassium phosphate (KH_2PO_4), 100 µM ferric EDTA (Fe–K–EDTA), 0.5 µM magnesium sulfate $MgSO_4$, 30 µM boric acid (H_3BO_3), 2.5 µM ammonium chloride (NH_4Cl), 1 µM copper sulfate ($CuSO_4$), 5 µM manganese monosulfate ($MnSO_4$), 5 µM manganese sulfate ($MnSO_4$), 1 µM copper sulfate ($CuSO_4$), 1 µM zinc sulfate ($ZnSO_4$), 1 µM ammonium heptamolybdate ($(NH_4)_6Mo_7O_{24}$) and 1 µM zinc sulfate ($ZnSO_4$) (Basit et al. 2021). Nutrient medium pH was maintained at 5.0 with hydrochloric acid (HCl) and sodium hydroxide (NaOH) (Crouch et al. 1990). Then, four different concentrations of ZnO NPs (0, 25, 50, and 100 mg/L) in powdered form were mixed in distilled water, sonicated for proper suspension, and applied to the hydroponic nutrient media. The solution was renewed after two days and plants treated with no exogenous Cr stress and ZnO NPs were considered as control (CK). After 21 days, plants were collected, roots and shoots were freezed at -80 °C after instantaneously dipping into liquid nitrogen for further biochemical analysis.

Germination parameters

Total germinated seeds were tallied after germination on 5th days and were considered as germination energy. Furthermore, on day 14th germination percentage % (GP) was measured. Later, Germination Index, Mean Germination Time, as well as Vigor Index was calculated by using following formulas (Hu et al. 2005).

$$GI = \Sigma(Gt/Tt) \tag{1}$$

$$MGT = \Sigma(Gt \times Tt)/\Sigma Gt \tag{2}$$

$$VI = Ger\min ation\,(\%) \times \left[Shoot\ length\,(Clouse\ et\ al.) + Root\ length\,(Clouse\ et\ al.)\right] \tag{3}$$

whereas Gt is the total calculated number of germinated seeds on day *t*, and Tt is the time conforming to Gt in days.

Plant growth attributes

Plants were harvested after 21 days of germination to check the growth parameters such as length of roots and shoots. Moreover, roots and shoots weights were measured on the electronic balance after washing it with ddH_2O_2 and dried on Whatman filter paper grade 1. The plants were oven dried for 48 h at 80 °C to estimate dry weight.

Determination of photosynthetic pigments

Fresh leaf sample (0.2 g) were standardized with 3 mL 95% (v/v) ethanol and the homogenized mixture was centrifuged at 5000 × *g* for 10 min for pigment extraction. Afterwards, 9 mL ethanol was poured in 1 mL aliquot in test tubes and left for overnight. Chlorophyll content was assessed at 645 and 663 nm a UV-VIS spectrophotometer (Ultrospecific 3000 Biochrom Ltd. Cambridge, England) (Lichtenthaler 1987). The measurement of total chlorophyll was done by using formula:

$$Total\ chlorophyll\ content\,(a+b) = \left[20.2\,(OD645 - 8.02\,(OD663)\,x\,V/1000\,x\,W)\right] \tag{4}$$

The quantities of chlorophyll content was presented as mg g^{-1} of plant extract.

Malondialdehyde and H_2O_2 content

To estimate the hydrogen peroxide (H_2O_2) (0.5 g) plant samples were ground in 5.0 mL of TCA (0.1%) and centrifuged at 12,000 g for 15 min and absorbance was tested at 390 nm (Junglee et al. 2014). MDA content was examined as 2-thiobarbituric acid (TBA) volatile metabolites 20. Almost, 1.5 mL aliquot was extracted in 2.5 mL of 5% TBA poured in 5% trichloroacetic acid (TCA). Subsequently, centrifugation for 10 min at 5000 g was conceded; optical density of the extract was determined at 532 nm. Amendment of non-specific turbidness was by subtracting the absorbance value assessed at 600 nm (Heath and Packer 1968). The content of MDA was estimated in terms of nM g^{-1} FW.

Electrolyte leakage

To determination the electrolyte leakage (dSm^{-1}), A piece of fresh leaf (1.0 cm) was cut down and used to determine the water potential (Ψp) with a vapor pressure osmometer (Wescor Inc., Logan, UT, USA). Then, the relative electrolyte leakage (dSm^{-1}) of seedling was measured according to the reported method (Ista et al. 2004).

Antioxidant activities

Catalase activity was measured by taking the 0.1 g grinded leaves with 5 mL phosphate buffer (25 Mm, pH 7.0) and centrifugated at 10,000 g for 10 min. The sample was homogenized with H_2O_2 (0.75 mM) and the reduction in absorbance was investigated at 240 nm (Aebi 1984). Superoxide dismutase (SOD) content was analyzed for its capability to reduce the photochemical activity of superoxide nitroblue tetrazolium (Giannopolitis and Ries 1977). One unit of SOD was referred to as quantity of enzyme needed to cause half of the amount of inhibition of NBT at 560 nm wavelength. Determination of Peroxidase (POD) activity was performed by using the protocol described by Klessig (1995). The optical density of the supernatant was measured at 470 nm. The modification in absorbance through 0.01 units/min was corresponding to one-unit peroxidase activity.

Estimation of Cr and mineral content in rice roots and shoots

The estimation of Cr content was done by taking dried samples of roots and shoots. Dry samples of both roots and shoots each (0.2 g) were integrated by utilizing 5 mL concentrated HNO_3 and $HClO_4$ (5:1, v/v) in digestion flask on a hot plate at 70 °C for almost 5 h. The digestion material was diluted by using 2% HNO_3 upto volume of 10 mL and examined. The digested samples were used to measure the Cr, Fe, and Zn content using an atomic absorption spectrometer (iCAT-6000–6300, Thermo Scientific, USA) (Khan et al. 2013).

Endogenous BR content

Plant sample (0.8 g) after homogenization with 4 mL acetonitrile was shifted into a centrifuge tube (10 mL) and placed at 20 °C overnight. The extraction, dehydration as well as double-layered solid phase extraction (DL/SPE) were carried out (Chen et al 2009). The HPLC mass spectrometry was used for quantification of BR content by applying a calibration curve with established quantities of standards and depending on the ratios of the summed area of the multiple reaction monitoring (MRM) transitions for BRs. The Data acquisition, as well as evaluation, were accomplished by Xcalibur Data System (Thermo Fisher Scientific, Waltham, MA, USA). The level of BRs was exposed as pM g^{-1} dry weight (DW) (Ding et al. 2013).

Phytochelatin (PC) content

The HPLC quantification was used to determine the PC content in rice plants. The dry weight of almost 500 mg tissues of seedlings was standardized in 5 mL of 0.1 M HCl. Afterward, the homogenate was centrifuged for 10 min at 6000 g. The aliquot were further filtered by using 0.4-µm millipore filters and utilized for the HPLC (Waters 2489 UV/Visible Detector) analysis of PCs according to the technique of (Döring et al. 2000).

Endogenous ABA content

The endogenous titers of ABA were investigated according to the previously reported method of Wang and Xiao (2009).

Ultrastructure changes

Leaf tissues without veins were cut from indiscriminately selected seedlings after 14 days of treatment and fixed in 2.5% glutaraldehyde (v/v) made in 0.1 M PBS sodium phosphate buffer, pH (7.4) overnight and splashed three times with similar PBS. Earlier, the leaves were post fixed in 1% OsO_4 [osmium (VIII) oxide] for 1 h. Moreover, samples were eroded three times in 0.1 M PBS by 10 min gap between each wash. Subsequently, after 15–20 min gap, the leaves were dried in the classified classification of ethanol (50–100%) and lately splashed through absolute acetone for 20 min. The leaves were at that moment intimated further embedded in Spurr's resin the whole night. Consequently, the samples were stewed at 70 °C for 9 h and ultra-thin sects. (80 nm) were cut. The sections were attached on copper nets for ultra-structure studies through a transmission electron microscope (JEOLTEM- 1230EX) at a hastening voltage of 60.0 kV.

Statistical analysis

All experimental data were analyzed by applying one-way analysis of variance through the least significant difference (LSD) at $p \leq 0.05$ and 0.01 level as a posthoc test at 95% assurance interlude amongst frequent data set between mean values using Statistix (8.1) software. The standard deviation (StD) were represented with the data.

Results

Effect of ZnO NPs on seed vigor and germination traits

Results observed for germination attributes viz., germination energy (GE), germination percentage (GP), germination index (GI) and mean germination time (MGT) as well as vigor index (VI) have been presented in (Table 1). As expected, exposure to Cr stress resulted in significant reduction of GE, GP, GI, MGT and VI compared to control. Our results showed that ZnO NPs (100 mg/L) application showed marked increase in GE, GP, GI, and VI as compared to control. Moreover, MGT value recorded higher under Cr stress in contrast to control (CK) plants. The results displayed that ZnO NPs 25, and 50 mg/L significantly reduced the MGT by 9.52%, and 18.7%, respectively, but the reduction was more prominent (34.9%) in rice plants applied with ZnO NPs (100 mg/L) under Cr toxicity in contrast to the Cr alone exposed rice plants (Table 1).

Effect of ZnO NPs on rice growth

Exposure to Cr stress caused significant changes in plant's phenotypes such as reduction in shoot length (SL), and root length (RL) as compared to control plants (Fig. 1). Results also revealed significant decline in fresh weight (FW), as well as dry weight (DW) of rice seedling treated with Cr alone in contrast to control (Table 2). However, significant increase in SL, RL and plant biomass (fresh and dry weight) was observed under all applied levels of ZnO

Table 1 Effect of different concentrations of ZnO NPs on germination energy (GE), germination percentage (GP), germination index (G.I), mean germination time (MGT), and vigor index (VI) under 100 µM Cr stress

Treatments	GE (%)	GP (%)	GI (%)	MGT (days)	VI
CY-927 (CK)	94.67 ± 3.06^{a}	100.00 ± 0.00^{a}	31.65 ± 1.14^{a}	2.28 ± 0.07^{c}	2.07 ± 0.01^{a}
CY-927 + Cr	40.67 ± 1.15^{c}	52.00 ± 2.00^{d}	8.44 ± 0.43^{e}	3.78 ± 0.13^{a}	0.23 ± 0.01^{e}
ZnO 25 mg/L + Cr	67.33 ± 2.31^{b}	72.67 ± 3.06^{c}	13.51 ± 0.37^{d}	3.42 ± 0.24^{b}	0.63 ± 0.01^{d}
ZnO 50 mg/L + Cr	70.00 ± 3.46^{b}	76.00 ± 2.00^{c}	16.13 ± 0.74^{c}	3.07 ± 0.29^{b}	0.87 ± 0.03^{c}
ZnO 100 mg/L + Cr	90.67 ± 1.15^{b}	91.33 ± 1.15^{b}	23.15 ± 0.26^{b}	2.46 ± 0.14^{c}	1.82 ± 0.03^{b}

Each treatment value represents the mean of three replicates ± standard deviation. Same letters are representing no significant differentiation at 95% probability level ($p < 0.05$)

Fig. 1 The physiological impact of Cr (100 µM) toxicity on rice seedlings and its mitigation through different concentrations of ZnO NPs (25 mg/L ZnONPs, 50 mg/L ZnONPs, and 100 mg/L ZnONPs)

Table 2 Effect of various concentrations of ZnO NPs on fresh weight (F/W), dry weight (D/W), shoot length (S/L), and root length (R/L) under 100 µM Cr stress

Treatments	F/W (g)	D/W (g)	S/L (cm)	R/L (cm)
CY-927 (CK)	0.92 ± 0.01^a	0.09 ± 0.00^a	16.63 ± 0.15^a	13.47 ± 0.15^a
CY-927 + Cr	0.24 ± 0.01^e	0.03 ± 0.00^d	7.33 ± 0.06^e	6.57 ± 0.06^e
ZnO 25 mg/L + Cr	0.45 ± 0.01^d	0.05 ± 0.00^c	11.20 ± 0.17^d	9.63 ± 0.06^d
ZnO 50 mg/L + Cr	0.50 ± 0.01^c	0.05 ± 0.00^c	12.00 ± 0.10^c	10.36 ± 0.04^c
ZnO 100 mg/L + Cr	0.82 ± 0.01^b	0.08 ± 0.00^b	15.50 ± 0.10^b	12.37 ± 0.15^b

Each treatment value represents the mean of three replicates ($n = 3$) ± standard deviation. Same letters are representing no significant differentiation at 95% probability level ($p < 0.05$)

NPs i.e., 25, 50, 100 mg/L in plants exposed with Cr as compared to control. Moreover, addition of 100 mg/L ZnO NPs displayed more prominent increase in morphological attributes of rice seedlings such as SL, RL, FW, and DW as compared to other applied concentrations of ZnO NPs under Cr toxicity (Table 2).

Effect of exogenous ZnO NPs on chlorophyll pigment content

Current study revealed that Cr stress significantly diminished the chlorophyll content of rice seedling in contrast to plants without any Cr treatment (control) (Fig. 2A). However, the foliar supply of ZnO NPs showed remarkable increase in chlorophyll pigment in rice plants against Cr stress in contrast to control plants. Results indicated that the application of ZnO NPs 25, and 50 mg/L increased chlorophyll content upto 42.3%, and 52.4%, respectively, in rice leaves, under Cr toxicity compared to plant applied with Cr alone. Further, (100 mg/L) ZnO NPs substantially augmented the chlorophyll pigment by 61.9% compared to plants without ZnO NPs application under Cr stress as shown in (Fig. 2A).

Expression of MDA and H_2O_2 under ZnO NPs

After the exposure of Cr, MDA content was drastically enhanced in rice seedlings. Further, the treatment of ZnO NPs significantly reduced the lipid peroxidation linked with decreased MDA content in Cr stressed rice plants (Fig. 2B). The reduction was more prominent (75.1%) with the treatment of 100 mg /L ZnO NPs in rice plants in contrast to control plants. Moreover, the reduction was more pronounced (61.8%) with foliar application of ZnO NPs 100 mg/L (Fig. 2C) compared to control plants.

ZnO NPs supplementation reduces electrolyte leakage in planta

The adverse effect of Cr toxicity triggered elevated levels of electrolyte leakage in rice seedlings compared to their controls (Fig. 2D). However, foliar supply of ZnO NPs ameliorated the Cr initiated toxicity and decreased the EL in rice plants in contrast to control plants. Among the ZnO NP applications, 100 mg/L concentration exhibited 71.15% decrease in EL when compared with 25 and 50 mg/L ZnO NPs which showed 2.94%, 17.83% reduction in EL compared to the plants treated without any ZnO NPs supplied under Cr stress.

Activities of antioxidative system under ZnO NPs application

After the exposure of Cr, antioxidative enzyme activities such as SOD, CAT and POD were increased significantly compared to their controls (Fig. 2). However, addition of ZnO NPs resulted in significant increase in SOD content upto (3.5%), (6.1%) and (17.1%) with foliar application of 25, 50 and 100 mg/L ZnO NPs as compared to control (Fig. 2E). Moreover, the addition of ZnO NPs i.e., 25, 50, and 100 mg/L enhanced CAT activity in rice seedlings upto 10.1%, 12.7%, and 31.3%, respectively, compared to the rice plants treated without any ZnO NPs (Fig. 2F). The Cr treatment significantly enhances the POD activity in rice seedlings compared to control. The addition of ZnO NPs further enhanced the POD activity in rice plants subjected to Cr stress and the increase in POD content was observed upto 14.17%, 15.69%, and 34.59% with the foliar addition of 25, 50 and 100 mg/L ZnO NPs concentrations, respectively, as compared to Cr stressed plants without ZnO NPs treatment (Fig. 2G).

Effect of ZnO NPs on Cr contents and nutrient uptake

Treatment of rice plants with Cr stress caused high Cr levels in root and shoot, whereas the Cr accumulation in roots and shoots tissues of rice genotype was reduced considerably with the supply of ZnO NPs in order: root > shoots as shown in Fig. 3. The rice seedling applied with 25 mg/L ZnO NPs + Cr exposed plants displayed lower Cr root and shoot contents upto 17.2% and 13.9%, respectively, compared to the plants supplied with Cr alone. Whereas the rice cultivar added with 50 mg/L ZnO NPs + Cr showed

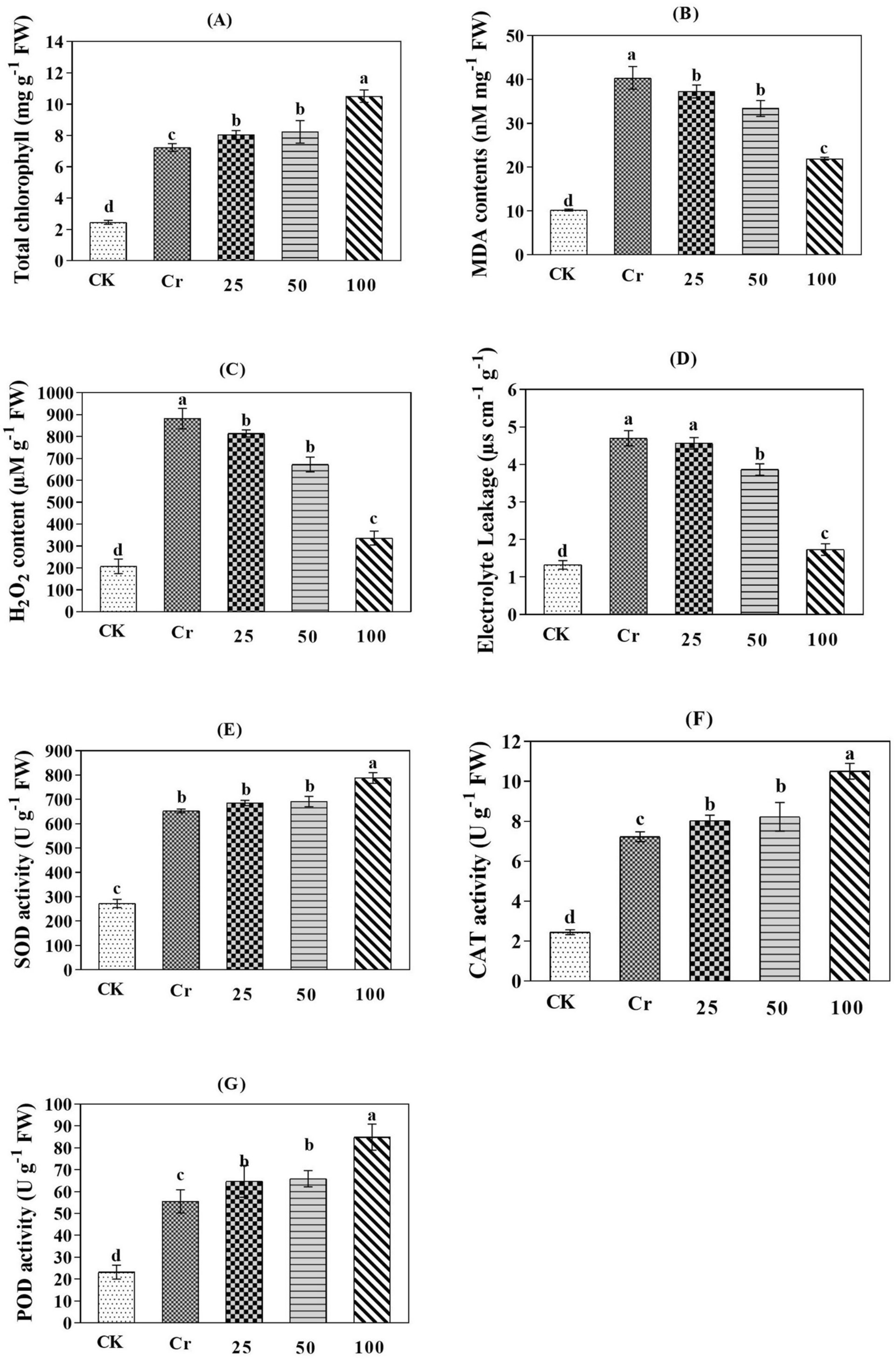

Fig. 2 Effect of various concentrations of ZnO NPs CK (⬚), Cr (▩), 25 mg/L ZnO NPs (▦), 50 mg/L ZnONPs (▤), and 100 mg/L ZnO NPs (▧) on total chlorophyll **A**, malondialdehyde MDA **B**, hydrogen peroxide H_20_2 **C**, Electrolyte leakage EL **D**, Superoxide dismutase SOD **E**, Catalase CAT **F** and Peroxidase POD **G** in rice plants under Cr (100 µM) stress. Each treatment value represents the mean of three replicates (n = 3) ± standard deviation. Same letters are representing no significant differentiation at 95% probability level ($p < 0.05$)

decreased root and shoot Cr contents upto 29.7% and 29.1%, respectively. Among ZnO NPs treated rice plants 100 mg/L ZnO NPs + Cr showed much lower Cr content in roots and shoots tissues upto 52.5% and 60.3%, respectively, compared to control plants. While, the highest decline in the translocation of Cr was observed (48.75%) in plants treated with ZnO NPs 100 mg/L under 100 µM Cr stress as compared to the plants added with Cr alone (Fig. 3A, B). Moreover, the ZnO NPs' 100 mg/L application showed maximum increase in nutrient uptake in Cr treated plants compared to other ZnO NPs' applied concentration (Fig. 3). Different treatment levels of ZnO NPs such as 25, 50, and 100 mg/L increased the uptake of Zinc

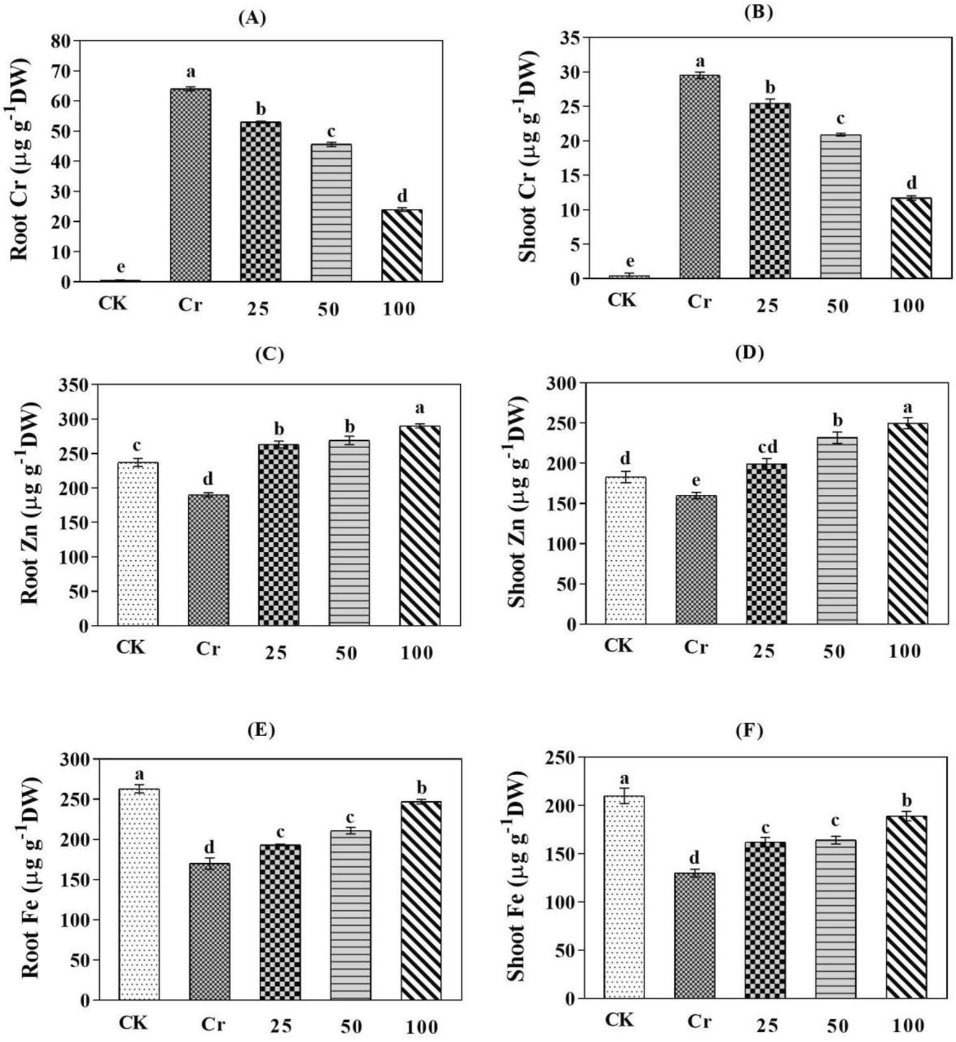

Fig. 3 Effect of various concentrations of ZnO NPs CK (⬚), Cr (▩), 25 mg/L ZnO NPs (▦), 50 mg/L ZnONPs (▤), and 100 mg/L ZnO NPs (▧) root Cr content **A**, shoot Cr content **B**, root Zn content **C**, shoot Zn content **D**, root Fe content **E**, and shoot Fe content in rice seedlings under Cr (100 μM) stress conditions. Each treatment value represents the mean of three replicates (n = 3) ± standard deviation. Same letters are representing no significant differentiation at 95% probability level ($p < 0.05$)

(Zn) 8.29%, 26.83%, and 37.29%, respectively, as well as upregulated the uptake of iron (Fe) 11.92%, 19.43%, and 31.17%, respectively, compared to the plants treated with 0 mg/L ZnO NPs under Cr toxicity (Fig. 3 C,D,E,F).

The ZnO NPs treatments stimulated the level of BRs under Cr stress

The different treatments of ZnO NPs were applied under Cr stress in rice seedlings to observe the level of endogenous BRs. The results have shown that exogenous addition of ZnO NPs stimulated the level of growth hormones i.e. BRs in rice seedlings exposed to Cr stress. Our observations indicated that treatments of 25 and 50 mg/L concentration of ZnO NPs showed no significant impact on BR content compared to plants subjected to Cr alone treatment in rice seedlings. However, the 100 mg/L concentration of ZnO NPs showed a significant rise (2.08-fold) in the endogenous contents of BRs as compared to control plants under Cr stress in rice seedlings (Fig. 4A).

Phytochelatin (PC) content in plants

In current study, the impact of ZnO NPs on PC content level and its ability for metal detoxification were examined (Fig. 4B). Results showed that PC contents were enhanced under Cr stress condition compared to control. Application of ZnO NPs (25, and 50 mg/L) showed no significant rise in the level of PC contents as compared to 0 mg/L ZnO NPs under similar stress conditions (Fig. 4B). However, the addition of ZnO NPs (100 mg/L) showed a significant increase in PC contents under Cr toxicity (Fig. 4B). Whereas the concentrations of ZnO NPs (25, and 50 mg/L) showed no significant rise in the level of PC content compared to control (Fig. 4B).

ZnO NPs modulated the level of ABA under Cr stress

The ABA is stress responsive phytohormones; related to stress management. In present study, the level of ABA was observed under various applied treatments of ZnO NPs

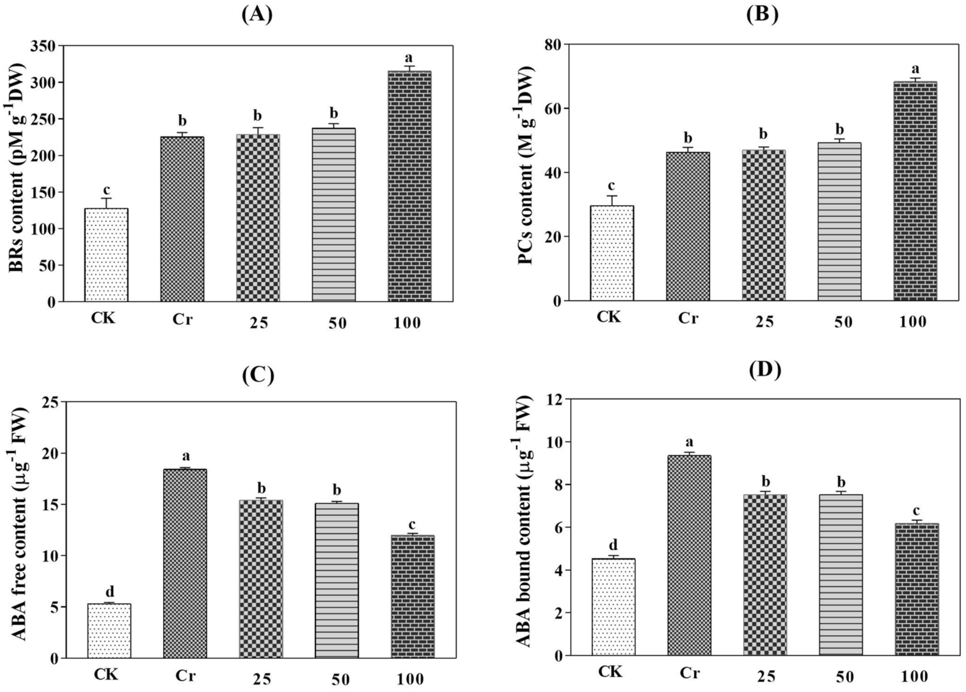

Fig. 4 Effect of different concentration of ZnO NPs CK (░), Cr (▩), 25 mg/L ZnO NPs (▦), 50 mg/L ZnO NPs (▤) and 100 mg/L ZnO NPs (▧) on brassinosteroid content BRs **A**, phytochelatins content PCs **B**, level of endogenous free ABA content **C**, and level of endogenous bound ABA content in rice seedlings under Cr (100 µM) stress conditions. Each treatment value represents the mean of three replicates (n = 3) ± standard deviation. Same letters are representing no significant differentiation at 95% probability level ($p < 0.05$)

under the Cr stress and its association in Cr detoxification. Rice seedling under Cr stress indicated a significant enhancement in free (3.83-fold) and bound (3.12-fold) levels of ABA, respectively, compared to control (Fig. 4C, D). The exogenous application of various ZnO NPs under Cr toxicity significantly decreased the ABA level. Nevertheless, the 100 mg/L concentration of ZnO NPs caused noticeable reduction in the level of ABA both free (2.43-fold) and bound (1.89-fold), correspondingly (Fig. 4C, D).

Ultrastructural analysis

In the current investigation, ultrastructural analysis of leaf mesophyll cells in control and ZnO NPs applied conditions have been demonstrated m in Fig. 5. The electron micrographs represented normal structure contained clean and thin cell walls, normal organelles, healthy chloroplast, and granule thylakoids (Fig. 5A) in cells exposed to ZnO NPs. However, leaf mesophyll cells under Cr toxicity presented a damaged structure of nucleolus with double-layered nuclear membrane enlargement and it also has revealed the impairment in chloroplast development and granule thylakoids (Fig. 5A, B). The exogenic treatments of ZnO NPs such as 25 and 50 mg/L showed a slightly damaged structure of nucleolus (double nucleolus) and injury in the chloroplast as well as granule thylakoid impairments (Fig. 5C, D) as compared to the control (Fig. 5B). Interestingly, the 100 mg/L concentration of ZnO NPs exposed better development of chloroplast with less damage of granule thylakoid as well as the membrane was developed more precisely than 0, 25, and 50 mg/L of ZnO NPs (Fig. 5B, C, D, and E), and the development of nucleolus was observed normal (Fig. 5E).

Discussion

Rice researchers are striving to solve the predicament of Cr contamination of food chain owing to serious health hazards that Cr presents to the health of millions worldwide who consumes rice (Hussain et al. 2018). It is therefore pertinent to find viable ways of decreasing the Cr contamination from contaminated soils into food crops thereby regulating the plant growth through wide array of cellular and metabolic developments important for plant development without curtailing the crop yield (Javed et al. 2021). In past years, the use of nanotechnology-based approaches in agriculture are being focused to enhance metal tolerance in plants as well as decrease its mobility and accumulation frequency in different plant parts (Faizan et al. 2021; Yang et al. 2021). For this purpose, nanomaterials are being investigated for nutrient and gene delivery agents and surface adsorbents to reduce the metal accumulation in plants. The main objective of current work was to illuminate the role of ZnO NPs in the amelioration of Cr stress in rice plants. During plants growth, seed germination is an important early stage that stimulates the crop growth and yield. Our results depicted that plant's physiological parameters such as germination energy, germination percentage exhibited decline following the application of Cr stress. Moreover, germination index and vigor index were also substantially

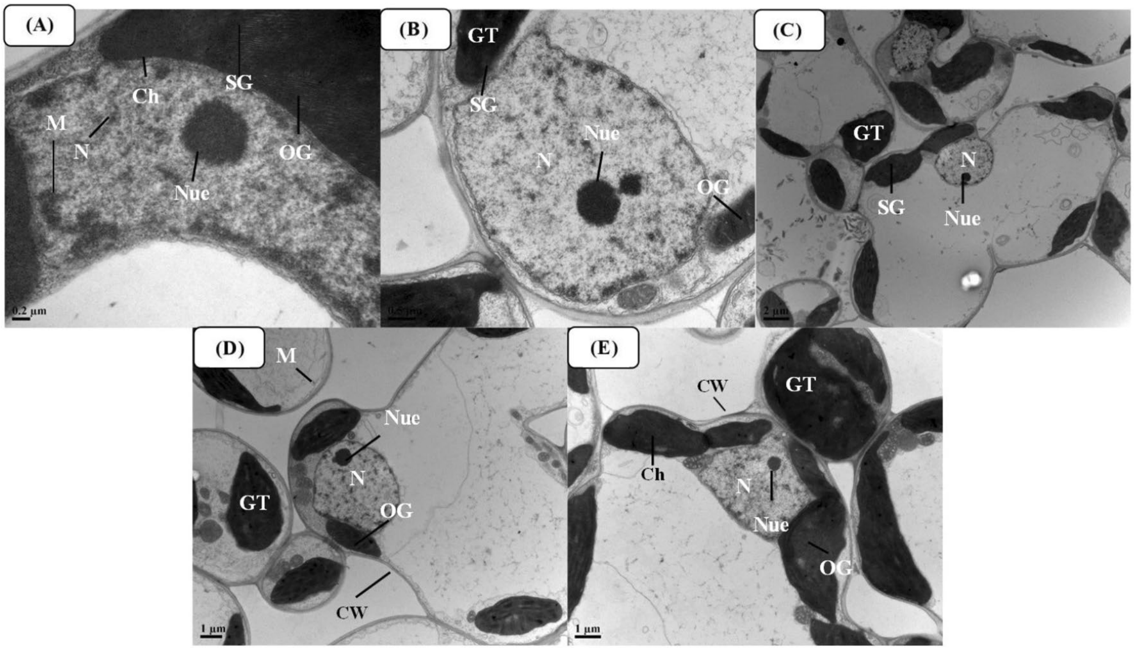

Fig. 5 Visual analysis of ZnO NPs treatment on ultrastructural changes caused by Cr (100 µM) stress in rice leaf mesophyll cells using electron micrographs. **A** Leaf mesophyll cell at control level. **B** Leaf mesophyll cell under alone treatment of Cr toxicity. **C** Leaf mesophyll cell treated with ZnO NPs (25 mg/L) under Cr stress. **D** Leaf mesophyll cell treated with ZnO NPs (50 mg/L) under Cr stress. **E** Leaf mesophyll cell treated with ZnO NPs (100 mg/L) under Cr stress. N (nucleus); CW (cell wall); Ch (chloroplast); GT (granule thylakoids); M (mitochondria); Nue (nucleolus); NM (nuclear membrane)

decreased in rice plants subjected to Cr stress compared to their respective controls (Table 1). This vigorous decrease in seed germinations efficiency might be due to harmful effects of Cr on the utilization of preserved food including starch, protein and phytate for developing embryos which lead to inhibition of germination traits (Arshad et al. 2017; Khan et al. 2020). Previous studies have confirmed that deterioration in germination traits results due to Cr accumulation in seed coat and seed radicle which might suppress the α and β amylase activity linked with decreased sugar availability during seed germination under Cr toxicity in the medium (Bewley and Black 2012; Sethy and Ghosh 2013). However, our findings revealed that addition of ZnO NPs ameliorated the Cr induced adverse effects on seed germination in relation to the Cr-alone treated rice plants (Table 1). Besides, supplementation of ZnO NPs (100 mg/L) showed optimum germination characters under Cr toxicity environment which may possibly be inferred due to ZnO NPs induced upregulation of ABA catabolism and GA synthesis which promotes seed germination in rice plants. Few studies have indicated that nanoparticles could enter into seed coat pores, triggering water molecule penetration and mediate the activity of ROS generating/starch-degrading enzymes which increased germination traits (Mahakham et al. 2018; Itroutwar et al. 2020).

Our results showed that Cr contamination drastically decreased the plant weight and height in contrast to control plants (Table 2). Similar results were underpinned by several studies in plants (Gill et al. 2015; Sehrish et al. 2019) which corroborate our results. The lower biomass of rice plants might result from Cr induced cellular damages in numerous plant parts which disrupt the regular function of the plants (Gill et al. 2016; Li et al. 2018). Furthermore, the Cr stress ruptures the root cell surface that inhibit root cell division and elongation which ultimately disturbs water balance as well as plant nutrient uptake (Panda 2007; Arshad et al. 2017). However, in this experiment, addition of ZnO NPs to the Cr stressed plants considerably increased the plant height as well as biomass of rice plants in contrast to Cr alone (Table 2). It was envisaged that ZnO NPs neutralized the Cr toxic effects via enhanced uptake of available nutrients such as Zn and diminished the Cr bioavailability which in turn increased the plant growth. Moreover, enhanced levels of Zn in the plants regulates the expression of genes associated with metal homeostasis which is likely to be linked with improved nutrient status, antioxidant system of plants under toxic metal stress (Mustafa and Komatsu 2016; Faizan et al. 2021).

Our results displayed that chlorophyll content were substantially decreased in rice plants treated with Cr stress in contrast to their controls without any ZnO NPs application

(Fig. 2). Lower photosynthetic traits might originate from displacement of Mg essential for biosynthesis of chlorophyll and preeminent levels of chlorophyllase under heavy metal toxicity (Habiba et al. 2015; Farid et al. 2018; Abbas et al. 2020a, b). Besides, decrease in photosynthetic apparatus may possibly be correlated with acropetal translocation of Cr which may disrupt laminar membrane of chloroplast (Danish et al. 2019; Salem et al. 2021). The outcomes of current study highlighted that ZnO NPs significantly augmented the gaseous exchange traits of rice plants by ameliorating the Cr stress (Fig. 2). The decreased photosynthetic rate dismantled ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) efficiency and causes inhibition of electron transport chain that induced decrease in overall plant biomass (Salam et al. 2022). Our findings corroborate with earlier reports signifying that exogenous supply of ZnO NPs enhanced chlorophyll content by alleviating heavy metal stress yielding higher photosynthesis and thereby plant performance (Latef et al. 2017; Rizwan et al. 2019). Furthermore, enhanced photosynthetic traits assessed after ZnO NPs application might be associated with maintained nutrient acquisition and reduced ROS induced oxidative damage in the toxic environment (Rizwan et al. 2019; Faizan et al. 2021; Abdelaziz et al. 2022; Al Jabri et al. 2022).

Our results also showed higher levels of lipid peroxidation as MDA content, initiation of H_2O_2 and electrolyte leakage in rice plants treated with Cr stress compared to their control (Fig. 2). Higher levels of MDA, H_2O_2 and EL promotes ROS accumulation which impairs the composition and activity of cellular membrane owing to interference and uptake of Cr with essential nutrients in rice under metal toxicity (Javed et al. 2021). Chromium holds the affinity to change the K^+ efflux together with electron transport chain which in turn enhances levels of OH^- and O^- consequently cause EL increment in rice (Yu et al. 2019; Farid et al. 2019). We analyzed that plants given with ZnO NPs maintained lower levels of MDA, H_2O_2 and EL in ascending order of ZnO NPs (25, 50, 100 mg/L) applications under Cr toxicity which in turn safeguards the cellular membrane integrity of Cr stressed plants (Fig. 2). Our results are in agreement with earlier studies that NPs instigate lower MDA, H_2O_2 and EL in seedlings under adverse abiotic stress conditions (Rizwan et al. 2019; Sheikhalipour et al 2021). It was perceived that exogenous application of ZnO NPs in particular may be linked with increased antioxidant machinery which enhanced ROS scavenging, diminished membrane damage through decreased subcellular distribution and Cr translocation in plants (Khan et al. 2020).

Different reports stated that Cr application in rice plants stemmed in enhanced Cr retention in the root apoplast that triggered higher Cr levels in metal treated rice plants (Zeng et al. 2010; Basit et al. 2021, 2022b). In present study, higher levels of Cr toxicity in medium triggered significant rise in Cr uptake/bioaccumulation in various plant parts (root and shoot) (Fig. 2). Higher accretion of Cr in plant roots possibly linked with Cr deposition in vacuoles as a defense mechanism. Hence, we analyzed that ZnO NPs diminishes the Cr bioaccumulation in root and shoot tissues of rice plants (Fig. 2). It was perceived that NPs decreased the Cr mobility in the root- shoot direction thereby impacts the absorption and translocation of Cr in aerial plant parts as depicted by different studies under Cr stressed environment (Ahmad et al. 2015; Faizan et al. 2021).

In current research results, we found enhanced antioxidant levels (SOD, CAT, POD) in Cr stressed plants compared to their respective controls which might be attributed to balance in ROS formation to avoid oxidative injury under stressful environment (Fig. 2). Our observations are similar to previous studies stating that antioxidants are known to escalate under oxidative stress that protect plants by regulating ROS assembly in plants (Andre et al. 2010)). Further, outcome of present research highlighted that exogenous supply of ZnO NPs augmented the efficiency of antioxidant system including SOD, CAT, POD in the presence of Cr stress (Fig. 2). Previous research validates our findings which reported that ZnO NPs mediated increased levels of antioxidant defense system might results from expression of genes or enhanced efficacy of various metabolic processes under Cd stressed conditions in *Lycopersicon esulentum* plants (Faizan and Hayat 2019; Shah et al. 2021).

Undeniably, Cr interferes with uptake of mineral nutrients and significantly diminished ionic content of Fe, Zn in both roots and shoots of plant after Cr stress application (Fig. 3). A burgeoning effect of enhanced Cr uptake and decreased mineral nutrient uptake has been associated with inhibition of plasma membrane H+—ATPase activity in Cr treated rice plants (Kharbech et al. 2020). Similar findings by (Zaheer et al. 2020) documented that Cr stress encounter decreased Fe, Zn content in *Spinacia oleracea* and *Brassica napus* under metal toxicity regimes. The application of ZnO NPs showed substantial increase in Fe, Zn levels in Cr treated rice plants and the prominent uptake of mineral nutrients in ZnO NPs (100 mg/L) can be related to optimal supply of these nutrients (Fig. 2). Increased influx of nutrients as a result of ZnO NPs application had a strong effect in alleviating the negative impacts induced by Cr stress. Similar results indicated increased bioavailability of mineral nutrients with the application of NPs in other crops was earlier represented in other crops (Azimi et al. 2021; Faizan et al. 2021).

Brassinosteroids are well-established plant growth regulators that confers tolerance in crop plants against adverse climatic conditions (Bukhari et al. 2016). Our observations displayed that ZnO NPs significantly maintained the higher levels of BRs and played a chief role in diminution of Cr stress via improved photosynthesis and reduction in lipid peroxidation in Cr treated plants (Fig. 4). Our results were further validated by previous studies which indicated that BR application reinstated the growth, photosynthesis and stomatal regulation in Tomato plants under different Cr stress application (Jan et al. 2020). Phytochelatins are family of peptide that are produced enzymically from glutathione by enzyme PC synthase. Phytochelatins are rapidly generated in many plants in response to different heavy metals and act as metal detoxifier (Diwan et al. 2010). Phytochelatins (PCs) are considered as metal detoxification markers and increased level of PC indicates the metal toxicity management inside the plants. In current study, an enhanced level of PC was found in plants supplemented with different ZnO NPs under Cr stress conditions (Fig. 4). Our results corroborate with previous finding which showed that inductions of PCs together with improved antioxidant system in response to Cr contamination indicates the cumulative function of PCs in conferring Cr tolerance in *B. Juncea* plants (Diwan et al. 2010). The outcomes of our study indicated that Cr increases both free and bound ABA contents in rice seedlings without any ZnO NPs application (Fig. 4). Abscisic acid has critical roles in various developmental processes of plants viz., seed development, vegetative growth and leaf abscission (Hadiarto and Tran 2011; Vishwakarma et al. 2017). Higher quantities of ABA act antagonistic on plant growth causing stomatal closure thereby negatively affecting the photosynthetic efficiency of plants under hostile conditions (Nishiyama et al. 2011; Shah et al. 2021).We observed that upregulated levels of ABA might be linked with decreased photosynthetic apparatus as well as plant biomass in Cr toxicity conditions. Furthermore, our results demonstrated that significant decrease in ABA levels were found in ZnO NPs in ascending order (20, 50, 100 mg/L) in Cr stressed rice plants as compared to Cr alone treatment (Fig. 4C). The lower ABA levels in plants supplemented with NPs might originate from improved Cr tolerance capacity of rice plants which ultimately enhanced the photosynthetic attributes as well as antioxidative activities under Cr stress (Vishwakarma et al. 2017).

In our experiment, the ultrastrucure of chloroplast was visibly altered under Cr stress which might be linked with over production of ROS in cellular organelles (Fig. 5) (Daud et al. 2013). Furthermore, we found significant reduction of gas exchange attributes under Cr stress which is directly associated with disorganized cells. Conversly, we have observed that ZnO NPs application decreased the cellular distortion by lowering the excessive ROS production and ameliorated the damage in the ultrastrcutre development of rice plants under Cr stress (Fig. 5). Improvement in ultrastrucutre under Cr stress treatment might involve upregulation of defense related genes and stablization of photosynthetic membrane due to optimum supply of mineral elements in ZnO NPs treated plants (Salam et al., 2022).We assumued that the exogenous supply of ZnO NPs might decreased the damage to thylakoids in chloroplast and assisted in the recovery of photosynthetic machinery in rice plants under Cr induced stress. Further, we observed that ZnO NPs' addition evidently recovered the shape of chloroplast and enhanceed the size of starch grains in contrast to Cr stress. Our results are in conformity with previous studies (Mapodzeke et al. 2021) who reported the zinc supply could improve chloroplast ultrastructure via decreased Cd uptake, improvement in planta ionomics and lipid peroxidation in rice genotypes.

Conclusions

The application of ZnO NPs significantly reduced in-planta Cr accumulation in rice plants and attenuated ROS induced oxidative damage, while improved seed germination, growth traits and plant biomass. The results demonstrated that ZnO NPs at suitable levels can prevent the Cr bioaccumulation and counteract Cr toxicity via increased growth, nutrient acquisition as well as modulating the phytohormone levels of ABA and BRs resulting in enhanced PC contents that could enhance plant growth under Cr stress. It was concluded that ZnO NPs foliar application confer higher Cr tolerance potential in terms of enhanced seed germination, plant height, fresh dry biomass, photosynthetic pigments, antioxidative activities (SOD, POD, CAT), nutrient accumulation (Zn, Fe) while diminishing lipid peroxidation (MDA, H_2O_2, EL) and Cr retention. More importantly, the ZnO NPs substantially protected and restored the cell organelles from ROS induced oxidative stress by immobilizing the Cr translocation in rice plants. This study provided an insight into important mechanisms responsible for ZnO NPs mediated Cr tolerance in rice plants and their application to increase crop yield and productivity in heavy metal polluted soils. However, more investigations are required in more heterogeneous soil media with different crops for potential application of ZnO NPs in in soil contaminated with Cr for food safety and increasing crop productivity. Furthermore, integration of "omics" techniques are needed to explore the mechanisms involved in ZnO NPs contributed Cr tolerance in rice plants at gene and protein levels. Understandings of such mechanisms will be of great utility to assimilate strategies to reduce metal contamination of agricultural crops.

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Data availability The data of this research can be obtained upon request to the corresponding author.

Declarations

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