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RESEARCH ARTICLE

Effect of gibberellic acid on growth, biomass, and antioxidant defense system of wheat (*Triticum aestivum* L.) under cerium oxide nanoparticle stress

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Abstract

Recently nanoparticles (NPs) are ubiquitous in the environment because they have unique characteristics which are the reason of their wide use in various fields. The release of NPs into various environmental compartments mainly ends up in the soil through water bodies which is a serious threat to living things especially plants. When present in soil, NPs may cause toxicity in plants which increase significance to minimize NPs stress in plants. Although gibberellic acid (GA) is one of the phytohormones that has the potential to alleviate abiotic/biotic stresses in crops plant, GA-mediated alleviation of cerium oxide (CeO_2) NPs in plants is still unknown, despite the large-scale application of CeO_2-NPs in various fields. The present study was performed to highlight the ability of foliar-applied GA in reducing CeO_2-NPs toxicity in wheat under soil exposure of CeO_2-NPs. We observed that CeO_2-NPs alone adversely affected the dry weights, chlorophyll contents, and nutrients and caused oxidative stress in plants, thereby reducing plant yield. GA coupled with CeO_2-NPs reversed the changes caused by CeO_2-NPs alone as indicated by the increase in plant growth, chlorophylls, nutrients, and yield. Furthermore, GA alleviated the oxidative stress in plants by enhancing antioxidant enzyme activities under CeO_2-NPs exposure than the NPs alone which further provided the evidence of reduction in oxidative damage in plants by GA. Overall, evaluating the potential of GA in reducing CeO_2-NPs toxicity in wheat could provide important information for improving food safety under CeO_2-NPs exposure.

Keywords Wheat · Nanoparticles · Gibberellic acid · Crop yield · Nutrients

Introduction

Nanotechnology is a branch of technology in which the particles being dealt have at least one dimension less than 100 nm and also have different properties as compared with the products in bulk. In the recent years, nanotechnology contributes in a variety of industries, providing a great possibility of novel applications (Piccinno et al. 2012; Rafique et al. 2018; Irshad et al. 2020). Thus, the production and use of nanoparticles (NPs) on such a large scale have become a matter of concern because these particles end up in the soil and cause toxicity in the food chain. Cerium oxide (CeO_2) NPs (CeO_2-NPs) are those NPs that are being investigated with regard to ecosystem and human health as well as the toxicity induced by them in the plants. CeO_2-NPs are the metal oxide NPs that can shift their oxidation states depending on the available oxygen (Reshma and Ashwini 2017; Reed et al. 2014). Due to this property, CeO_2-NPs have been extensively used in UV coatings, chemical and mechanical planarization and polishing,

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fuel catalysis, catalytic conversion, and others (Younis et al. 2016). CeO_2-NPs are now used in skin care products at large scale because of their photoprotection properties and also extensively exploited as a photocatalyst. Most of the research have reported that CeO_2-NPs present in fuel additives and sewage effluents can easily interact with the environment and develop pathways towards biological receptors.

Earlier studies showed the varying effects of NPs on the plants that includes negative, positive, or neutral effect of NPs (Rizwan et al. 2017, 2019; Hussain et al. 2018, 2019). Nonetheless, in most of the studies, the parameters addressed are easily observable, like growth- and germination-related features. Plant response to the application of the NPs did not only depend upon plant species but also on the properties and doses of NPs. For instance, a dose-dependent experiment on tomato (*Solanum lycopersicum* L.) showed higher Ce accumulation when treated with CeO_2-NPs than CeO_2 (Singh et al. 2019). The lower concentrations of CeO_2-NPs had no significant effect on lettuce, while at higher concentration NPs suppressed the biomass content and growth of the plant (Gui et al. 2015). Moreover, CeO_2-NPs modified the macromolecule composition and activities of antioxidant stress enzymes in rice plants (Rico et al. 2014), but in corn plants, NPs did not affect the ion leakage and oxidative stress (Zhao et al. 2012).

The interaction of the NPs with the plant cellular structure varies with the shapes, size, atomic arrangement, and composition and yet is difficult to understand. It is mandatory to explore the interaction among CeO_2-NPs and plants to highlight their effects in plants and transfer to other environmental compartments (Miralles et al. 2012; Rizwan et al. 2017). It was supposed that CeO_2-NPs entered in plant tissues through the stomatal pathways on leaves and pores in the cell wall and then reach to other plant compartments (Wang et al. 2013; Rizwan et al. 2017). The release of Ce^{3+} ions from CeO_2-NPs may be the cause of NPs toxicity in plants (Zhang et al. 2015). CeO_2-NPs have large surface area and smaller size due to which they interact directly with the macromolecule and damage the DNA or membranes. Most importantly, CeO_2-NPs showed a dose-dependent increase in the reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2) and superoxide radical (O_2^-). It has been proposed that the unnecessary production of ROS might be the cause of lipid peroxidation and causes membrane leakage and impairment by the destruction of lipid components; that is one of the primary reasons of plants' nanotoxicity. To scavenge excessive level of ROS, plants have a well-equipped system consisting of antioxidants that are both enzymatic and nonenzymatic in nature. However, plants face oxidative stress under excessive NPs accumulation that may deteriorate the defense system of the plants (Rizwan et al. 2017).

Cereals are one of the most important crops in the world those are used as main food source (Koehler and Wieser 2013; Abbas et al. 2019). Major cereal crops are wheat, rice, and maize, and these are consumed worldwide. To better understand the interaction of the NPs with the cereals and the other agriculture crops, similarities in farming/cultivation practices (i.e., annual yield of plants with one planting harvest) and botanical classification (i.e., they belong to monocot family of the grasses) play an important role. Wheat is the main portion of human food and hence may be considered a major pathway for the uptake of the NPs. Previous report has revealed significant effects of NPs on the quality of the seeds, grain physiology, and nutrient profile in wheat (Wang et al. 2019). The extensive use of CeO_2-NPs and deliberate entrance to the environment may enhance possible health-related problems to people of the NPs-polluted areas via food chain movement. Therefore, for the security and safety of the food chain, elucidation of strategies that can reduce NPs uptake and improve plant tolerance to NPs toxicity is critical. Previous studies on CeO_2-NPs deepened our knowledge on phytotoxicity, but till date, no reports related to the toxicity of CeO_2-NPs are available.

Plant growth regulators (PGR) are the compounds that regulate plant growth and enhance the tolerance of plants under stressed environment and play a crucial part in survival of plants under stressed environment (Santner et al. 2009; Zhang et al. 2020; Ahammed et al. 2014). It has been well established that PGR such as jasmonic acid, gibberellic acid (GA), and indole acetic acid can alleviate abiotic stresses in plants (Tuna et al. 2008; Saleem et al. 2015; Dai et al. 2020; Zhang et al. 2020). Among PGR, GA, a plant growth stimulating hormone, regulates numerous physiological and biochemical processes in plants (Vishal and Kumar 2018). GA also plays a vital role in the enhancement of the plants against various abiotic stresses such as salinity, drought, and chilling and toxic trace element stress (Upreti and Sharma 2016). Gibberellic acid may protect the plants against environmental stresses by regulating ROS and antioxidant enzyme activities (Shu et al. 2018). For example, Ali et al. (2015) have shown that foliar use of GA effectively reduced Ni uptake in mungbean plants and enhanced the yield and growth. Amri et al. (2016) assessed that GA alleviated the Cd toxic effects in barley. Although the importance of GA under abiotic stresses is well established, the role of GA in reduction of NPs stress in plants is not well-established. In our previous study, it has been reported that GA improved the tolerance in wheat against zinc oxide NPs stress (Iftikhar et al. 2019). However, the impact of GA on wheat plants in the presence of CeO_2-NPs is not known and still remains elusive. It was hypothesized that foliar application of GA could significantly affect wheat growth, photosynthesis, nutrient contents, oxidative stress, and antioxidant system under CeO_2-NPs stress. The present study explored the changes

of wheat plants by the exogenous GA with respect to growth, nutritional value, and antioxidant system in response to the systematic addition of CeO_2-NPs in the soil. The current experiment is expected to give a better insight regarding the efficiency of GA in CeO_2-NPs-mediated oxidative stress which might be helpful for food safety in NPs-polluted areas.

Materials and methods

Soil collection and characterization

A field used for research purposes was finalized for the collection of soil for the further use in the current study. A soil depth of 0 to 20 cm from surface of the field was selected for the collection of soil, and unwanted materials were removed from the soil such as roots and other debris if present and then dried without direct sunlight. The samples of the soil were analyzed for soil sand silt and clay percentage (Bouyoucos 1962). Soil pH and EC were determined by mixing the soil in water at 1:2.5 ratio. Organic matter contents in the soil were measured with standard procedure (Walkley and Black 1934). The soil was also analyzed for selected cations/anions by the methods reported earlier (US Salinity Laboratory Staff 1954). The soil characteristics have been demonstrated in a previous study (Iftikhar et al. 2019). Briefly, soil texture was sandy clay loam, with pH, EC, and OM of 7.82, 1.42 dS/m, and 0.73%. Total zinc, manganese, and iron contents in the soil were 34.57, 41.29, and 125.05 mg/kg.

NPs spiking in the soil and wheat growth

Cerium(IV) oxide nanopowder (CeO_2-NPs) were of Alfa Aesar with size, purity, and surface area of 15–30 nm, 99.5%, and 30–50 m^2/g. Different treatments of CeO_2-NPs were selected such as 100, 200, 300, 400, and 600 mg/kg of soil including control without NPs. The selected treatments were added in the soil by preparing the solution of NPs, and then the solutions were ultrasonicated for about 30 min and mixed in the soil to reach final levels. After 1 week, 5 kg of soil was added in each pot, and seeds of the selected wheat variety were sown in the pots. Five healthy seedlings were maintained in each pot after 2 weeks of seed sowing. The three levels of GA were selected, i.e., 0, 100, and 200 mg/l, and GA was applied by foliar spray method at different intervals during the growth. The GA was applied at 2nd, 4th, 6th, and 8th week of seed sowing. The replicates of each treatment were placed together during foliar application of GA. Total 1.5 l of GA per treatment were used in the whole experiment. The control plants were simultaneously sprayed with distilled water. There were four replicates of each treatment. The plants were fertilized with primary macronutrients (NPK) by using a rate of 120/50/25 kg per hectare by using the commonly available salts including $(NH_2)_2CO$, $(NH_4)_2HPO_4$, and K_2SO_4. The plants were irrigated with tap water by maintaining approximately 70% of soil water holding capacity. The experiment was done under natural conditions of the environment, i.e., 64 ± 5% RH and 32/21 °C day/night at sowing time.

Harvesting and sampling

The plants were grown in the soil for 124 days from the seed sowing, then harvested and separated into various parts, and further processed. Just before harvesting, a meter scale was used to measure the height of plants and length of spikes. The various tissues were carefully washed with tap water and d-H_2O to ensure that there was no dust or other undesirable materials on the surface of the samples. The seeds were separated from spikes and roots were carefully taken from the pots. All the tissues were dried using oven at 70 ± 5 °C for 4 days to ensure constant dry biomass of each sample, and weight was measured, and samples were crushed into powder for nutrient analyses.

Estimation of chlorophyll contents

The photosynthetic pigments from the leaves were measured after 75 days of sowing the seeds in the pots when there was an apparent difference among treatments. The 2nd fully developed leaves were used for this purpose, and the known quantity of the samples was extracted in an acetone (85% v/v) without direct light and 4 °C till the whole color was extracted from the samples. A spectrophotometer was used for recording the readings at various wavelengths, and finally chlorophyll contents were calculated by using equations described by Lichtenthaler (1987).

Estimation of EL, H_2O_2, MDA, and antioxidant enzyme activities

The above parameters were measured from the leaves, and sampling for this purpose was done at the time of chlorophyll content measurements. The electrolyte leakage (EL) from the leaves was measured by cutting the leaves into small pieces and placing them vertically in the tubes with already 8.0 ml of d-H_2O, and then heating was done for 2 h at 32 °C followed by cooling and measuring EC of the solution named as EC_1. The same samples were again heated for 20 min at 121 °C followed by cooling and measuring the EC named as EC_2 (Dionisio-Sese and Tobita 1998). The EL was measured by putting these EC values in an equation as $EL = (EC_1/EC_2) \times 100$ (1).

The leaf tissue lipid peroxidation (LPO) levels were recorded by measuring the contents of malondialdehyde (MDA) which is the product of LPO. In this regard, thiobarbituric acid was considered a reaction mixture (Heath and Packer 1968) with minor changes by Dhindsa et al. (1981) and then by Zhang and Kirkham (1994). Hydrogen peroxide (H_2O_2) contents in leaf tissues were determined by extracting the samples in 3.0 ml of 50 mM phosphate buffer at pH 6.5 followed by homogenization and centrifugation at 6000 rpm for specific time (25 min). 3.0 ml of this extractant were added in 1.0 ml titanium sulfate (0.1%) in 20% v/v sulfuric acid followed by homogenization and centrifugation at 6000 rpm for 15 min. The final color of the solution was yellow, and the intensity of this color was recorded at a specific wavelength (410 nm). The H_2O_2 concentration was recorded with 0.28 $mmol^{-1}$ cm^{-1} extinction coefficient.

For the estimation of antioxidant enzyme activities, the leaf tissues were crushed for standardization in a 0.05 M solution of phosphate buffer maintaining at a pH of 7.8. The superoxide dismutase (SOD) and peroxidase (POD) activities in leaf tissues were determined spectrophotometrically after homogenization and centrifugation at 12000 rpm for 10 min under controlled temperature (4 °C) accordingly mentioned in the literature (Zhang 1992). Catalase (CAT) activity in leaf tissues was estimated by using the mixture of 3.0 ml, 100 μl enzyme extract, 100 μl H_2O_2 (300 mM), 2.8 ml phosphate buffer (50 mM), and citric acid (CA) of 2 mM at 7 pH (Aebi 1984). The disappearance of H_2O_2 ($\varepsilon = 39.4$ mM^{-1} cm^{-1}) was recorded at 240 nm spectrometrically. For the estimation of ascorbate peroxidase (APX), the reaction mixture includes 100 μl of each enzyme extract, ascorbate (7.5 mM), H_2O_2 (300 mM), phosphate buffer (25 mM), and 2.7 ml of CA (2.0 mM at 7 pH). Finally, oxidation activity of ascorbate ($\varepsilon = 2.8$ mM^{-1} cm^{-1}) was recorded by measuring the variation in wavelength at 290 nm.

Measurement of nutrients in tissues

The selected macronutrients (P and K) and micronutrients such as Fe and Mn in different tissues were determined after digestion of the samples in concentrated HNO_3 and $HClO_4$ at a ratio of 3:1 by heating the solution with samples at about 350 °C. The K, Fe, and Mn concentrations were measured by atomic absorption spectrophotometer. Phosphorus concentration was measured by developing the green color and recording the measurement at 630 nm through spectrophotometer (Ohno and Zibilske 1991).

Statistical analyses

All the data were analyzed statistically by the software (SPSS 21.0 software for Windows). A two-way analysis of variance (ANOVA) was used for analysis. The comparison of the mean values was performed by the Tukey test at $p \leq 0.05$.

Results

Growth and biomass

The effects of foliar GA on growth and chlorophyll concentrations under CeO_2-NPs toxicity were evident and showed that plant growth increased with increasing concentration of GA (Fig. 1). There appeared inverse relationship between plant growth and CeO_2-NPs alone. According to the results, growth of wheat plant was considerably reduced by increasing the concentrations of CeO_2-NPs, especially under higher treatments (600 mg/kg) CeO_2-NPs. Likewise, the height and length of plants and spikes decreased by 42 and 38%, respectively, following the treatment of seedlings to 600 mg/kg CeO_2-NPs as compared with untreated plant (Fig. 1a, b). For the investigation of the potential role of GA in wheat in CeO_2-NPs tolerance, different concentrations of GA were selected. The combined use of GA and CeO_2-NPs significantly affected the growth and chlorophyll concentrations in tissues positively compared with those of CeO_2-NPs (both 100 and 600 mg/kg) treatments without GA application. As shown in Fig. 1, all selected concentrations of GA caused significant enhancement in biomass accumulation when compared with CeO_2-NPs treatments alone (Fig. 1). The grain yield was the highest in 200 mg/l GA without NPs, whereas lowest grain yield was detected in 600 mg/kg CeO_2-NPs treatment alone (Fig. 1e). The growth attributes were positively correlated with the mineral nutrients in tissues (Table 1).

Chlorophyll pigments are important physiological attributes to determine the plant health and plant growth development status. Therefore, the response of chlorophyll contents was measured in plants under all treatments applied (Fig. 2). Amount of chlorophyll contents declined CeO_2-NPs treatments without GA, and this effect was further increased with the increasing CeO_2-NPs treatments in the soil. Foliar sprayed GA linearly enhanced the parameters concentrations over the CeO_2-NPs with no GA treatments. The photosynthetic pigment concentrations were higher in higher treatment of GA as compared with lower treatments of GA.

EL, H_2O_2, and MDA concentrations in leaves

Malondialdehyde, H_2O_2, and EL are oxidative markers in the plants subjected to abiotic/biotic stresses. To understand the toxicity induced by CeO_2-NPs and alleviative effect of GA, we examined values of these parameters for all the treatments in leaves of wheat plant. CeO_2-NPs treatments stimulated the MDA, EL, and H_2O_2 concentrations in leaf tissues. With the higher dose of NPs, values of these parameters were

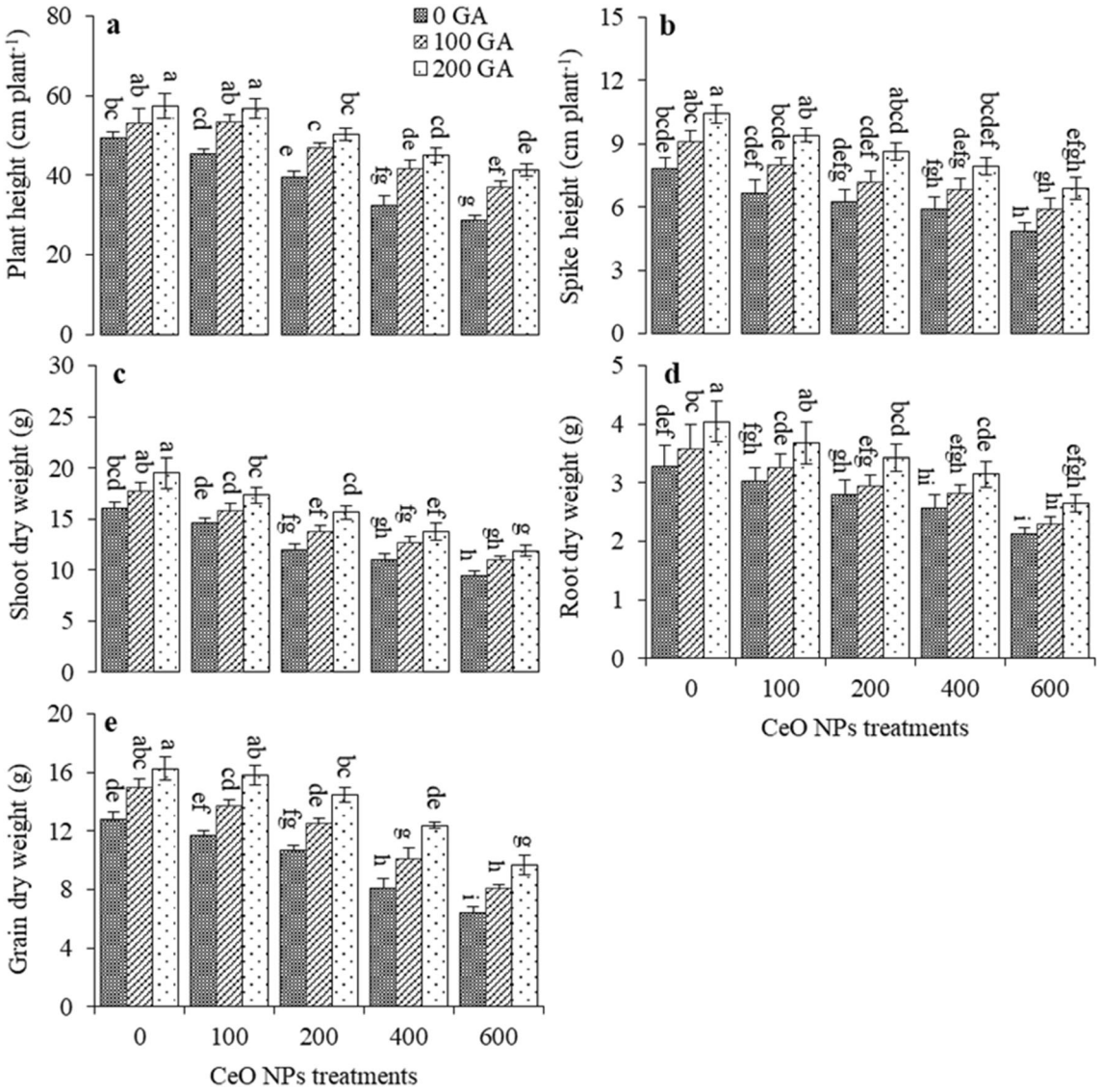

Fig. 1 Effect of various treatments of soil applied CeO_2-NPs (mg/kg) and foliar application of gibberellic acid (mg/l) on plant height and dry biomass of shoot, roots, and grains of wheat. The data presented are means of 4 replicates with standard errors. The different letters on the bars indicate significant differences at $p \leq 0.05$

significantly increased over the control. At 600 mg/kg CeO_2-NPs, EL, MDA, and H_2O_2 contents enhanced by 75, 99, and 83%, respectively, over the control. However, the combined treatment of GA and NPs substantially reduced the MDA, EL, and H_2O_2 contents over the respective CeO_2-NPs levels alone. The highest treatment of GA + CeO_2-NPs reduced MDA, H_2O_2, and EL contents by 44, 59, and 32% when compared with 600 mg/kg CeO_2-NPs treatment alone.

Antioxidant enzyme activities in leaves

It was expected that higher levels of CeO_2-NPs could affect the activities of antioxidants in the test plants. Responses of antioxidant enzyme (SOD, POD, CAT, and APX), towards CeO_2-NPs either alone or in combination with GA treatments, have been shown in Fig. 4. CeO_2-NPs decreased the activity of all the antioxidant enzymes being tested in all the treatments in comparison with the untreated control. With the increase in the value of CeO_2-NPs, there was a substantial decline in the antioxidant enzyme activities. As compared with the control, 600 mg/kg CeO_2-NPs level decreased the SOD, POD, CAT, and APX activities by 58%, 45%, 48%, and 60%. Combined treatment of GA and CeO_2-NPs predominantly elevated the activities of all studied enzymes, over the CeO_2-NPs alone. There was a dose-additive effect of GA on antioxidant enzyme activities. 200 mg/l GA demonstrated the highest activities, whereas 600 mg/kg CeO_2-NPs demonstrated the lowest enzyme activities.

Table 1 Correlation coefficient between different parameters

	Plant height	Spike length	Shoot DW	Root DW	Grain DW	Chl a	Chl b	Carotenoids	EL	H_2O_2	MDA	SOD	POD
Plant height	1												
Spike length	0.887**	1											
Shoot DW	0.901**	0.906**	1										
Root DW	0.835**	0.904**	0.907**	1									
Grain DW	0.950**	0.919**	0.934**	0.898**	1								
Chl a	0.915**	0.867**	0.945**	0.850**	0.915**	1							
Chl b	0.890**	0.896**	0.943**	0.912**	0.933**	0.905**	1						
Carotenoids	0.889**	0.893**	0.929**	0.885**	0.919**	0.928**	0.929**	1					
EL	−0.935**	−0.872**	−0.861**	−0.778**	−0.900**	−0.901**	−0.850**	−0.865**	1				
H_2O_2	−0.889**	−0.859**	−0.798**	−0.765**	−0.874**	−0.795**	−0.783**	−0.784**	0.901**	1			
MDA	−0.905**	−0.889**	−0.858**	−0.850**	−0.934**	−0.859**	−0.875**	−0.848**	0.887**	0.929**	1		
SOD	0.893**	0.884**	0.945**	0.858**	0.902**	0.921**	0.893**	0.905**	−0.856**	−0.745**	−0.811**	1	
POD	0.903**	0.933**	0.920**	0.884**	0.916**	0.886**	0.900**	0.887**	−0.874**	−0.851**	−.0866**	0.914**	1
CAT	0.904**	0.908**	0.959**	0.890**	0.947**	0.929**	0.929**	0.943**	−0.863**	−0.802**	−0.861**	0.940**	0.922**
APX	0.915**	0.896**	0.949**	0.894**	0.944**	0.934**	0.936**	0.923**	−0.859**	−0.804**	−0.868**	0.938**	0.916**
Root P	0.918**	0.910**	0.911**	0.843**	0.916**	0.919**	0.873**	0.909**	−0.895**	−0.824**	−0.855**	0.919**	0.906**
Shoot P	0.918**	0.929**	0.914**	0.880**	0.947**	0.881**	0.910**	0.876**	−0.869**	−0.870**	−0.897**	0.886**	0.908**
Root K	0.926**	0.936**	0.938**	0.883**	0.948**	0.918**	0.910**	0.911**	−0.886**	−0.878**	−0.909**	0.910**	0.945**
Shoot K	0.928**	0.908**	0.874**	0.841**	0.928**	0.855**	0.863**	0.861**	−0.895**	−0.936**	−0.915**	0.860**	0.910**
Root Fe	0.932**	0.861**	0.899**	0.823**	0.932**	0.919**	0.898**	0.883**	−0.904**	−0.816**	−0.880**	0.897**	0.864**
Shoot Fe	0.933**	0.861**	0.909**	0.851**	0.937**	0.905**	0.906**	0.866**	−0.900**	−0.862**	−0.898**	0.876**	0.879**
Grain Fe	0.931**	0.910**	0.894**	0.875**	0.953**	0.890**	0.895**	0.902**	−0.915**	−0.895**	−0.931**	0.871**	0.896**
Root Mn	0.901**	0.924**	0.883**	0.876**	0.943**	0.851**	0.901**	0.848**	−0.853**	−0.892**	−0.929**	0.858**	0.919**
Shoot Mn	0.891**	0.908**	0.834**	0.801**	0.893**	0.811**	0.814**	0.812**	−0.861**	−0.923**	−0.884**	0.804**	0.895**
Grain Mn	0.905**	0.946**	0.905**	0.879**	0.919**	0.861**	0.883**	0.878**	−0.867**	−0.903**	−0.882**	0.873**	0.941**

	CAT	APX	Root P	Shoot P	Root K	Shoot K	Root Fe	Shoot Fe	Grain Fe	Root Mn	Shoot Mn	Grain Mn
Plant height												
Spike length												
Shoot DW												
Root DW												
Grain DW												
Chl a												
Chl b												
Carotenoids												
EL												
H_2O_2												
MDA												
SOD												
POD												

Table 1 (continued)

CAT	1														
APX	0.958**	1													
Root P	0.930**	0.910**	1												
Shoot P	0.912**	0.912**	0.928**	1											
Root K	0.950**	0.938**	0.955**	0.952**	1										
Shoot K	0.890**	0.884**	0.911**	0.931**	0.946**	1									
Root Fe	0.891**	0.904**	0.917**	0.913**	0.897**	0.877**	1								
Shoot Fe	0.891**	0.906**	0.904**	0.913**	0.917**	0.908**	0.934**	1							
Grain Fe	0.892**	0.892**	0.906**	0.924**	0.921**	0.929**	0.915**	0.910**	1						
Root Mn	0.903**	0.911**	0.898**	0.951**	0.951**	0.945**	0.895**	0.918**	0.908**	1					
Shoot Mn	0.853**	0.837**	0.887**	0.914**	0.923**	0.948**	0.831**	0.886**	0.881**	0.930**	1				
Grain Mn	0.913**	0.911**	0.913**	0.941**	0.965**	0.956**	0.841**	0.881**	0.909**	0.944**	0.942**	1			

**Correlation is significant at the 0.01 level (2-tailed)

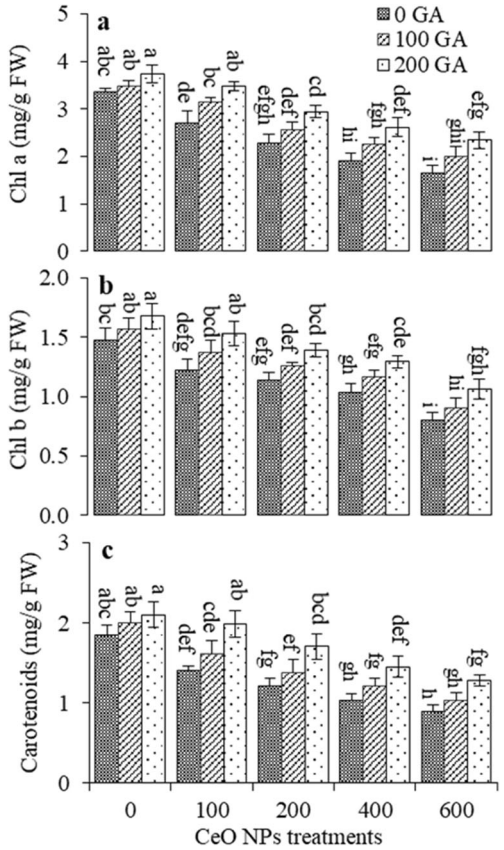

Fig. 2 Effect of various treatments of soil applied CeO_2-NPs (mg/kg) and foliar application of gibberellic acid (mg/l) on photosynthesis of wheat. The data presented are means of 4 replicates with standard errors. The different letters on the bars indicate significant differences at $p \leq 0.05$

P, K, Fe, and Mn contents in tissues

To understand whether GA affected the nutrients in plant under CeO_2-NPs stress, the concentrations of selected nutrients in various tissues were determined, and the results have been shown in Figs. 5 and 6. The results related to nutrient concentration of K and P in wheat have been reported in Fig. 5. Irrespective of the applied treatments, concentrations of P and K were high in belowground tissues than shoots but decreased with the application of CeO_2-NPs alone. The P concentration decreased in roots and shoots by 31 and 46% in 600 mg/kg CeO_2-NPs alone, than that of control. The K contents decreased in roots and shoots by 49 and 44% in 600 mg/kg CeO_2-NPs alone, over control. The foliar use of GA elevated P and K contents in plant in CeO_2-NPs stress. The P concentration increased in roots and shoots by 24 and 47% in the highest GA + CeO_2-NPs, over NPs treatment alone. The K contents increased in roots and shoots by 41 and 77% in the highest GA + CeO_2-NPs, over NPs treatment alone.

Figure 6 shows the results about Fe and Mn contents in various tissues of plants. The CeO_2-NPs alone reduced the Fe and Mn contents in wheat tissues, and the response was

dose-additive. The highest CeO_2-NPs alone treatment minimized Fe concentrations in roots, shoots, and grains by 64, 54, and 50% over the untreated control. The highest CeO_2-NPs alone decreased the Mn concentrations in roots, shoots, and grains by 53, 48, and 49% over the untreated control. The foliar-applied GA under NPs enhanced Fe and Mn contents in various tissues over CeO_2-NPs only treatments. The Fe concentrations increased in roots by 48%, in shoots by 44%, and in grains by 58% in 600 mg/kg CeO_2-NPs + 200mg/l GA than this NPs treatment alone. The Mn concentrations increased in roots by 57%, in shoots by 94%, and in grains by 80% in 600 mg/kg CeO_2-NPs + 200 mg/l GA than this NPs treatment alone.

Discussion

It is evident from the current research (Figs. 1 and 2) and previous findings that the use of NPs on large scale might be a serious threat to food safety and crop cultivation (Chichiricco and Poma 2015; Van Aken 2015). For the sustainable production of the crops in NPs-polluted marginal lands, advance approaches that can alleviate NPs-induced phytotoxicity are important. In this current study, we showed that foliar spray of GA could be used as an amendment for the CeO_2-NPs-induced phytotoxicity. GA efficiently alleviated the CeO_2-NPs-induced oxidative stress by stimulating the activities of antioxidant enzymes (Fig. 4). In addition, GA also positively affected the morphology and nutrients of the NPs stressed wheat plant (Figs. 5 and 6). This study delivered the data that put forward the beneficial role of the GA in alleviating the tolerance of CeO_2-NPs toxicity while ensuring food safety.

The NPs-mediated toxic impacts on plants are not only due to large surface area, small size, and reactivity of NPs that allow the NPs to interact with biological macromolecules (nucleic acid and protein) but also related with the metal constituents. According to the prior investigation, NPs rapidly dissolve releasing metal ions which promote generation of ROS including OH and H_2O_2 that are capable of damaging biomolecule such as protein, DNA, and lipids (Van Aken 2015). After dissolution, the NPs penetrate into the plant producing deleterious effect. In this study, CeO_2-NPs caused inhibition in the wheat growth and biomass accumulation. Similar to our study results, high concentrations of CeO_2-NPs often lead towards substantial toxicity in plants. For example, Gui et al. (2015) observed that low concentration (100 mg/kg) of CeO_2-NPs has positive effect on the growth, but at the higher concentration (1000 mg/kg), dry mass of root and shoot was decreased significantly. CeO_2-NPs visibly damaged the trifoliate leaves in soybean plant (Priester et al. 2017). In another study, high concentration of CeO_2-NPs reduced the chlorophyll content in

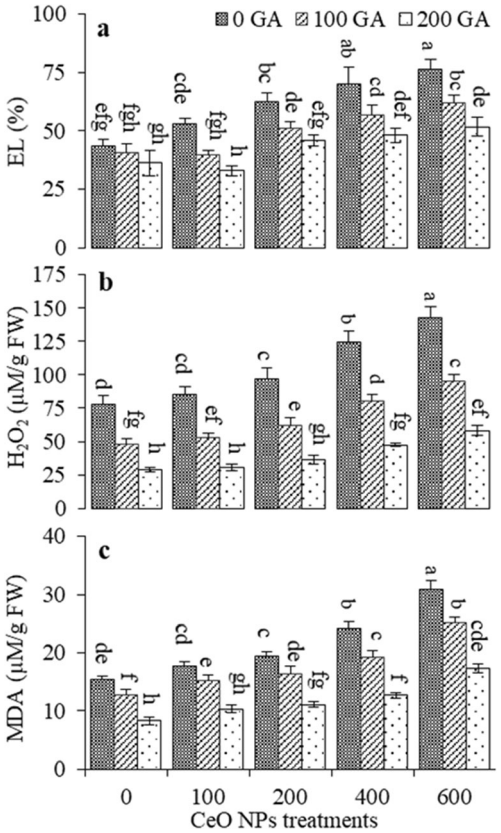

Fig. 3 Effect of various treatments of soil applied CeO_2-NPs (mg/kg) and foliar application of gibberellic acid (mg/l) on electrolyte leakage (EL), hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) of wheat leaves. The data presented are means of 4 replicates with standard errors. The different letters on the bars indicate significant differences at $p \leq 0.05$

wheat (Du et al. 2015). Conversely, CeO_2-NPs at 500 mg/kg in soil increased the plant growth, grain yield, and shoot biomass in wheat plant (Rico et al. 2014). According to a comparative study between CeO_2-NPs and cerium oxide (CeO_2), CeO_2-NPs caused more inhibitory effect on growth of the tomato than the CeO_2 (Singh et al. 2019). CeO_2-NPs stimulate photosynthetic rate and plant growth at 100 mg/kg, but at higher concentration (500 mg/kg), photosynthesis rate was reduced by 36% in soybean plant (Cao et al. 2017). Gui et al. (2015) reported that lettuce cultivated in soil treated with 100 mg/kg CeO_2-NPs produced higher growth compared with control.

Beside regulating the different developmental processes in plant (such as stem elongation and flowering), GA also plays a critical role in controlling certain biological processes against stress (Hamayun et al. 2017; Urano et al. 2017; Wang et al. 2017). In our previous study results, GA minimized the ZnO-NPs stress by activating antioxidant potential and reducing H_2O_2 accumulation in wheat. In conformity with previous data, it was observed that foliar-sprayed GA enhanced the yield and growth of wheat plant under various levels of CeO_2-NPs (Figs. 1 and 2). Function of the GA

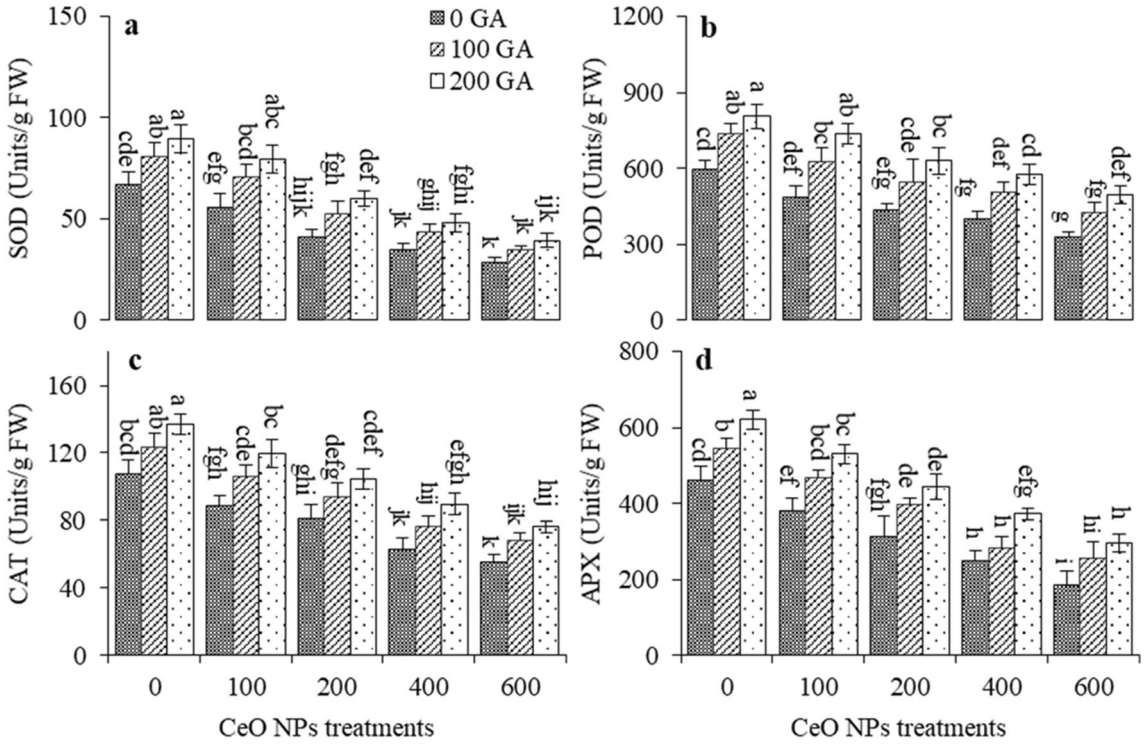

Fig. 4 Effect of various treatments of soil applied CeO_2-NPs (mg/kg) and foliar application of gibberellic acid (mg/l) on SOD, POD, CAT, and APX of wheat leaves. The data presented are means of 4 replicates with standard errors. The different letters on the bars indicate significant differences at $p \leq 0.05$

was dose-dependent as we perceived that increasing the GA dose increased the stress resistance towards plant. However, results have also showed that despite the dose-dependent response under stressful environment, plant species, application method, and growth environment also matter.

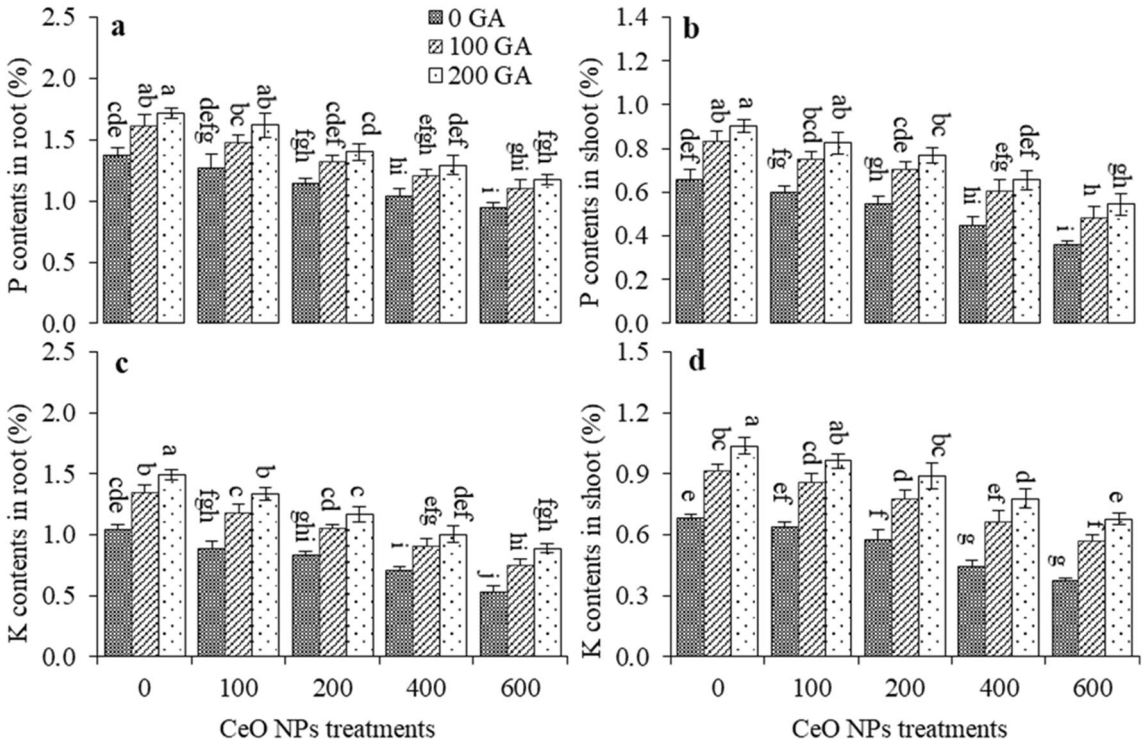

Fig. 5 Effect of various treatments of soil applied CeO_2-NPs (mg/kg) and foliar application of gibberellic acid (mg/l) on P and K contents of wheat. The data presented are means of 4 replicates with standard errors. The different letters on the bars indicate significant differences at $p \leq 0.05$

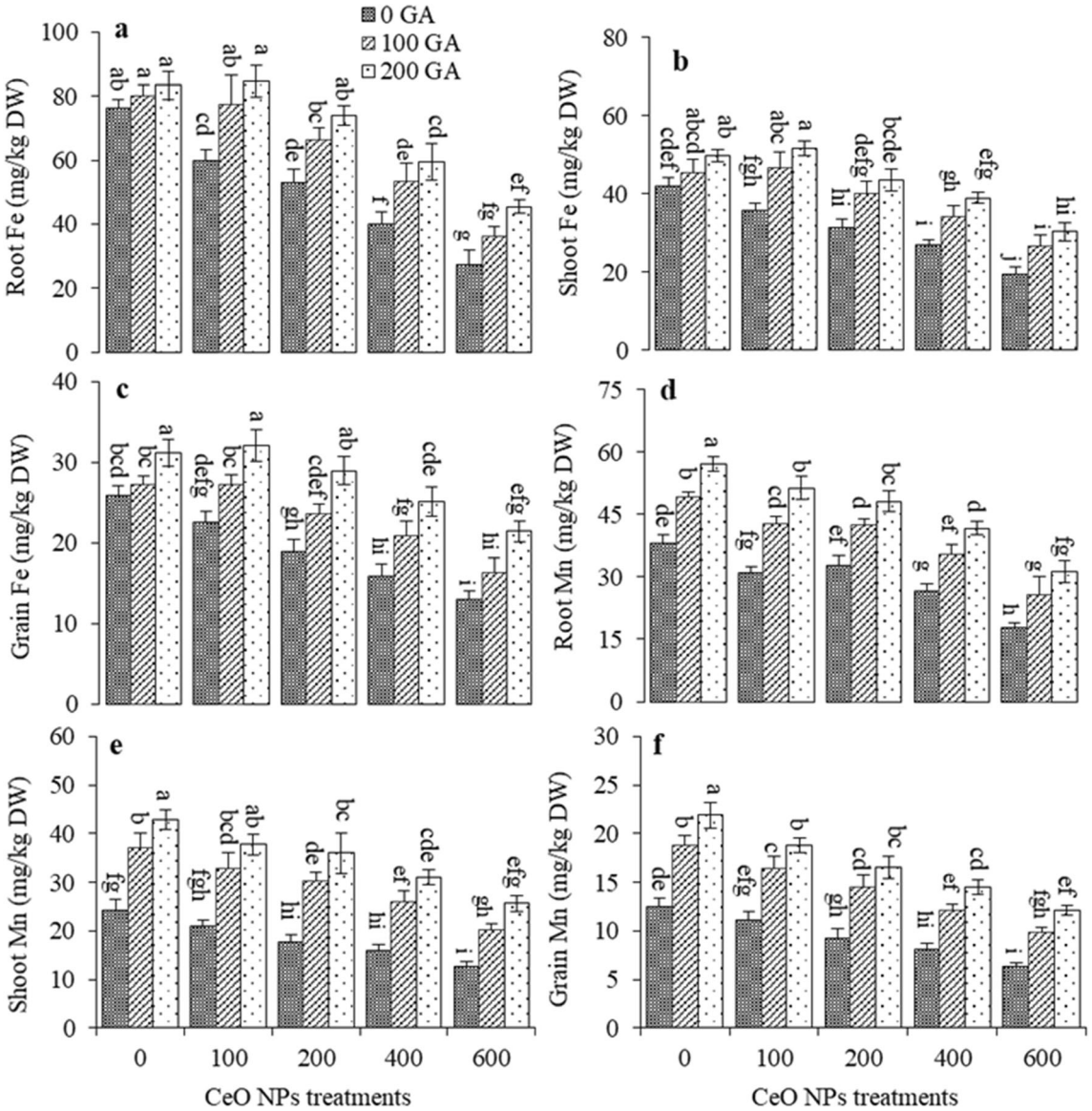

Fig. 6 Effect of various treatments of soil applied CeO_2-NPs (mg/Kg) and foliar application of gibberellic acid (mg/l) on Fe and Mn concentrations in plants. The data presented are means of 4 replicates with standard errors. The different letters on the bars indicate significant differences at $p \leq 0.05$

It has been reported that NPs can cause stress in plants via ROS-mediated cellular damage (Hernandez-Viezcas et al. 2011). Accumulation of the CeO_2-NPs in the roots could become the cause of oxidative stress. CeO_2-NPs increased/decreased the antioxidant enzyme activities which varied with doses, types of antioxidants, and plant tissues (Rico et al. 2013). The CeO_2-NPs at high concentration increased the CAT and SOD activities in tomato plant compared with control (Singh et al. 2019). Similar results were also observed in CeO_2-NPs-treated wheat leaves in this study (Figs. 3, and 4). Excessive production of ROS can induce LPO in cell membrane and/or promote the internalization and accumulation of the NPs into cells and finally lead towards cell death (Ma et al. 2013). CeO_2-NPs upregulated the antioxidant enzyme activities in tomato plant depending upon the applied levels of NPs. Tomato plant exposure with the low levels of CeO_2-NPs (20 and 100 mg/l) were beneficial and exhibited improved antioxidant enzyme activities, whereas at higher levels (500 and 1000 mg/l), CeO_2-NPs produced lethal effects to plant (Singh et al. 2019).

GA effectively scavenged ROS production and minimized MDA level in wheat plant under CeO_2-NPs treatments (Figs. 3 and 4). These results indicated that the foliar-sprayed GA reduced the oxidative damage and exhibited positive effect on wheat plant. According to our prior study, a typical ROS, H_2O_2, was intricately involved in GA-mediated stress response (Iftikhar et al. 2019). It is reported that GA application to the NaCl-stressed okra plant impacted the enzymes activity

depending upon the concentration of GA (Zhu et al. 2019). Treatment of the seeds at low concentration (150 μM) had best effect on antioxidant enzyme activity and growth-related parameters rather than the higher concentration (300 μM). However, our results illustrated that GA promoted the enzymes activity in CeO_2-NPs-stressed wheat leaves (Fig. 4).

Given that wheat is a vital component of daily food, its interaction with NPs is necessary to understand for the safety of public and environment health. Concerning the relation between GA and food security, the published researches have depicted that GA could decrease abiotic stress and heavy metal uptake in plant. The normal growth of the plants is mainly dependent on the nutritional status of the plants. NPs could cause nutrient deficiencies in plants (Rizwan et al. 2017). Our results highlighted that CeO_2-NPs reduced the concentrations of P, K, Fe, and Mn in various tissues of the plant (Figs. 5 and 6). Foliar GA subcutaneously improved the nutritional status of the wheat under CeO_2-NPs stress (Figs. 5 and 6). The GA improved the nutritional status of wheat under ZnO-NPs stress (Iftikhar et al. 2019). These results might be used to highlight that GA improved wheat growth under CeO_2-NPs and or other NPs stress via increase in nutrients in plants and decrease in oxidative stress as depicted in this study.

Conclusion

Wheat represents an important cereal crop being widely consumed worldwide. However, NPs may cause toxicity in wheat if present in excessive amount in the soil posing health risks when consumed. Therefore, the safe production of agricultural crops is important including wheat. The present experiment has explored that CeO_2-NPs negatively affected the growth, photosynthesis, and nutrient accumulation by plants. Excessive amounts of CeO_2-NPs in the soil could severely alter the balance between ROS production and antioxidant enzymes in plants. Foliar-applied GA improved the growth and nutritional quality of the wheat under CeO_2-NPs exposure and increased overall level of antioxidant enzymes in the leaves, thereby suppressing the ROS production in plants. The findings of the current study showed the effectiveness of GA in alleviating the toxicity of NPs in wheat which may help to enhance food safety.

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