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Cerium oxide nanoparticles alleviate drought stress in apple seedlings by regulating ion homeostasis, antioxidant defense, gene expression, and phytohormone balance

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Drought stress is one of the most important environmental constraints that negatively affect the growth and production of crops worldwide. Recently, nanotechnology has been increasingly used to improve the tolerance of plants exposed to abiotic stresses such as drought. The present study was designed to investigate the moderating effect of cerium oxide nanoparticles (CeO_2 NPs) on alleviating drought stress for the apple cv. 'Red Delicious' on M9 rootstock. Drought stress caused a significant increase in CAT, GPX, APX, and SOD enzyme activities compared to control plants. Drought decreased the content of macro and microelements, and the application of CeO_2 NPs led to significant changes in the content of these elements in plants under drought stress. CeO_2 NPs significantly reduced chlorophyll damage under high drought levels. In addition, they alleviated the damage caused by drought, which was shown by lower levels of MDA and EL. When these nanoparticles were used during drought stress, they greatly increased the production of abscisic acid and indole-3-acetic acid hormone. In response to drought stress, the expression of *DREB1A* and *DREB1E* genes increased. The use of CeO_2 NPs in stressful and non-stressful conditions had a positive effect on improving the studied traits of the apple plants and enhancing nutrient levels. Taken together, the findings suggest that CeO_2 NPs can be used as promising drought stress-reducing agents in apples. Therefore, understanding the mechanisms of abiotic stress in global horticulture and the role of nanoparticles is essential for developing improved, drought-tolerant crops and the adoption of measures to deal with changing climatic conditions.

Keywords Abiotic stress, Apple, Elements, Gene, Nanoparticle, Oxidative stress

Abbreviations

CAT	Catalase
APX	Ascorbate peroxidase
GPX	Guaiacol peroxidase
Y (II)	Effective photochemical quantum yield of photosystem II
SOD	Superoxide dismutase
IWC	Initial water content
RWP	Relative water protective
ELWR	Excised leaf water retention
WSD	Water saturation deficit
EL	Electrolyte leakage
F0	Minimal fluorescence

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MDA	Malondialdehyde
Y (NO)	Quantum yield of non-regulated non-photochemical
RWL	Relative water loss
LWC	Leaf water content
ETR	Electron transport rate
Protein	Total soluble protein
RWC	Relative water content
Fv/Fm	The ratio of variable fluorescence to maximal fluorescence
Fv	Variable fluorescence
Chlb	Chlorophyll b
Total chl	Total chlorophyll
Chla	Chlorophyll a
Fm	Maximal fluorescence
LWL	Leaf water loss
ELWL	Excised leaf water loss
ABA	Abscisic acid
DREB1A and 1E	Dehydration response element binding 1A and 1E

Plants are typically exposed to various environmental stresses during growth. Drought is an important abiotic stress that severely limits plant growth, productivity, and survival worldwide[1,2]. Understanding the response of plants to drought conditions is important and paves the way to improve drought tolerance. In the long period of evolution, different varieties of plant species have responded to, adapted to, and survived drought stress with different strategies, including drought avoidance, escape, and tolerance, all of which include several physiological and molecular adaptation mechanisms[3,4].

Photosynthesis is a physiological process that is sensitive to drought stress and plays an important role in the physiological processes of plants during drought adaptation[5]. It is also one of the key processes that regulate carbon fixation and metabolism. Drought stress reduces the rate of photosynthesis and changes the distribution and metabolism of carbon in the plant, thereby reducing energy and yield[6]. Stomatal closure is a primary response of plants to drought stress, which reduces transpiration and the rate of photosynthesis. By limiting transpiration, stomatal closure can also improve water use efficiency and thus indirectly affect plant productivity under drought stress[3,4]. The accumulation of reactive oxygen species (ROS) is another primary effect of drought stress[7]. At high concentrations, ROS production can cause oxidative damage to the photosynthetic system and other essential functions of cells by destroying oxidative lipids, proteins, and nucleic acids[8]. To deal with the harmful effects of ROS accumulation, plants rely on antioxidant defense systems through enzymatic or non-enzymatic pathways. ROS-inhibiting enzymes include superoxide dismutase (SOD), catalase (CAT), and peroxidases and non-enzymatic antioxidants such as proline, carotenoids, phenolic acids, and flavonoids that play a role in ROS detoxification. Under normal conditions, homeostasis between ROS and antioxidant enzyme activity is maintained. However, in drought conditions, this balance is disturbed due to high levels of ROS, which leads to an increase in oxidative stress and a decrease in antioxidants[9]. On the other hand, molecular responses to abiotic stresses include stress reception, signal transmission to cellular components, gene expression, and finally, metabolic changes that create stress tolerance[10].

Nanotechnology is an advanced tool with high potential in agricultural science to improve crop productivity. It has been reported that metal nanoparticles at different sizes, concentrations, and surface charges affect the growth and development of different plant species[9]. Cerium is a rare earth metal that exists as a free metal or oxide and can act as an oxygen buffer between the oxidation state of cerous (Ce^{3+}) and ceric (Ce^{4+})[11]. Relevant biochemical research has shown that such particles significantly change photosynthetic processes, oxidative stress, gene expression, and antioxidant enzyme activities in plants. It has been reported that nanoparticles such as CeO_2 NPs improve plant tolerance to abiotic stress to a large extent by increasing the capacity of the antioxidant system[12,13]. Under drought stress conditions, nanoceria catalyzes ROS produced in chloroplasts, leading to improved photosynthesis and chlorophyll content index. After application of ceria nanoparticles under stress conditions, an increase in compatible solutes is generated to counteract cellular ion imbalance, thereby reducing osmotic stress and maintaining membrane function and integrity[9,13].

Depending on stress type and intensity, abscisic acid can stimulate the expression of genes related to stress or metabolic changes in the plant. By stomatal closing, it reduces water loss[14]. The apple M9 rootstocks were more resistant to short-term drought stress than the MM111 rootstocks, which grew strong roots with a higher ABA content. ABA also induces the expression of several ORGs that increase plant tolerance to osmotic stress. These genes are mainly involved in the synthesis of plant hormones, signal transduction pathways, osmolyte accumulation, and antioxidant activity[15]. Increased ABA in response to stress activates the transcription of stress-related genes. Dehydration response element binding (DREB) proteins play an important role in plant tolerance to several abiotic stresses[16]. Indeed, 68 MdDREB members have been identified in apples, most of which are induced in response to drought, salt, cold, and heat stress[17].

Molecular responses to abiotic stresses include stress perception, signal transduction to cellular components, gene expression, and finally, metabolic changes that create stress tolerance. Therefore, the genes induced by stress not only protect cells from stress by producing important metabolic proteins but also regulate downstream genes for signal transmission. Transcription factors interact with cis-elements in the promoter regions of various stress-related genes to regulate the expression of many downstream genes, thus conferring stress tolerance[18,19]. DREB proteins constitute a large family of transcription factors that induce the expression of a large number of functional genes and confer stress tolerance in plants. Dehydration responsive element (DRE) was found as a cis-acting element in the promoter regions of many genes induced by drought and low temperature[20]. This review

specifically focuses on DREB proteins and their role in regulating abiotic stress responses in plants, with an emphasis on Arabidopsis, grasses, and other crop plants, as well as their application in crop breeding programs through genetic engineering and marker-assisted selection[18].

Apple is one of the economically important fruits with a large extent of consumption in the world. Drought stress inhibits its growth and causes premature leaf drop, which leads to a decrease in fruit yield and quality[21]. The ability of apple trees to deal with drought stress and achieve high yields in these conditions will be of great economic importance. The correct selection of drought-resistant cultivars and rootstocks is a way to reduce the impact of this stress and contribute to the sustainability of apple production[22]. Tworkoski et al.[23] studied drought stress on M9 and MM111 apple rootstocks. They found that MM111 was highly drought tolerant and attributed it to a more extensive root system. The present study was conducted to investigate the effect of drought stress on the apple 'Red Delicious' on M9 rootstock using physiological and biochemical traits. It also investigated the modulating effect of CeO_2 NPs as an efficient and rapid method at different levels of drought stress (low stress and severe stress) by improving ionic homeostasis, antioxidant system defense, and increasing the activity of hormones involved in the response to drought stress by increasing the expression of transcription factors.

Materials and methods

Materials

To carry out the research, apple seedlings of cv. 'Red Delicious' on M9 rootstock were grown in pots containing agricultural soil in a greenhouse (Table 1). The homogeneous apple seedlings of cv. 'Red Delicious' on M9 rootstock were provided by a local nursery in Maragheh, Iran by the relevant institutional and national guidelines and legislation. The two-year-old seedlings were kept in a greenhouse at a day/night temperature regime of 25–28/18–20 °C and a diurnal cycle of 16 h light/8 h darkness. During the growth period, necessary care was taken regularly, such as irrigation and other operations. After the full growth of the leaves of the seedlings, the treatments were applied. Two-year-old apple seedlings were treated with drought stress and foliar spraying with Nano Ceria 50 days after the start of the growing season in greenhouse conditions when complete vegetative growth and leaf maturity were achieved. The test factors included three irrigation levels (FC 100%, FC 50%, and FC 25%) and three levels of CeO_2 NPs (0, 50, and 100 mg/L of CeO_2 NPs). Foliar spraying on the leaves of the seedlings was done with a sprayer using a hand-held sprayer with a powder nozzle (3 cycles with an interval of 2 weeks). After applying the treatment, the parameters were measured (Fig. 1). All leaf samples were collected two days after treatment for molecular, biochemical, and physiological analyses, immediately frozen in liquid nitrogen, and then stored at − 80 °C until further use. The third and fourth leaves from the apical meristem were used to measure photosynthesis-related parameters. The fourth and fifth leaves were used for ABA quantification and molecular analyses.

Synthesis of CeO_2 NPs

Nanoparticles of CeO_2 with a mean basic particle size of 30 nm were bought from US-NANO Company, California, USA. The purity of CeO_2 nanopowder is 99.97%, the specific surface area is 30–50 square meters/gram, it has a yellow color, and the true density of the nanoparticle is 7.132 g/cm^3. They were synthesized via a facile sonochemical procedure. The precursor materials used in this research were $Ce(NO_3)_3$ hexahydrate and urea. In this regard, 0.05 M of $(Ce(NO_3)3.6H_2O)$ was dissolved in 17 ml of water and kept for ultra-sonication for 20 min. After that, 20 g of urea was added to the solution and allowed to sonicate for 2 h. Subsequently, the solution was centrifuged at 8000 rpm for 15 min and washed with water and ethanol to remove the unreacted materials. Finally, the precipitate material was dried at 50 °C overnight. Later, the dried powder was calcined at 850 °C for 5 h.

Ceria is of interest as a material for solid oxide fuel cells (SOFC) due to its relatively high oxygen ionic conductivity (i.e., oxygen atoms move easily through it at moderate temperatures of 500–650 °C) and lower enthalpy. Cerium (IV) oxide cycle, Cerium (III) oxide or CeO_2/Ce_2O_3 cycle is a two-step thermochemical water-splitting process based on cerium (IV) oxide and cerium (III) oxide to produce hydrogen. Nanoceria has been considered as a biological antioxidant. CeO_2 NPs also have potential applications for environmental remediation. The photocatalytic activities of CeO_2 nanotubes and nanoparticles have commercial applications.

Measurement of chlorophyll fluorescence

After applying drought stress, chlorophyll fluorescence parameters were measured in the last developed leaf using a fluorometer (PAM 2500-Walz, Germany) according to the method of Genty et al.[24]. Using the parameters determined in the leaves adapted to light, variable fluorescence = Fv, [Maximum photochemical quantum yield of photosystem II = Fv/Fm, Effective photochemical quantum yield of photosystem II = Y(II)], (Quantum yield of non-regulated non-photochemical (Y(NO)), and electron transport rate (ETR) were calculated based on Formulas (1) to (5).

$$\mathrm{Fv} = \mathrm{Fm} - \mathrm{F0} \tag{1}$$

$$\mathrm{Fv/Fm} = (\mathrm{Fm} - \mathrm{F0})\,/\mathrm{Fm} \tag{2}$$

Clay (%)	Silt (%)	Sand (%)	K (ava) ppm	P (ava) ppm	Total N %	Organic carbon %	T.N.V. %	pH	EC × 10^3
12	34	54	610	17.65	0.068	0.72	5.25	7.79	1.56

Table 1. Physicochemical properties of the soil sample utilized in the present experiment.

Fig. 1. Apple seedlings of cv. 'Red Delicious' on M9 rootstock under different treatments of levels of irrigation (Field capacity) and CeO_2 NPs.

$$Y\,(II) = \left(Fm' - Ft\right)/Fm' \tag{3}$$

$$Y\,(NO) = Ft/Fm \tag{4}$$

$$ETR = Y\,(II) \times PFDa \times (0.5) \tag{5}$$

Measurement of chlorophyll a and b and carotenoids

For chlorophyll and carotenoid measurement, 0.5 g of the sample was digested with 5 ml of 80% acetone, centrifuged at 6000 rpm for 10 min, and read at 663, 645, and 470 nm[25].

Measurement of leaf relative water relations

Five leaves were randomly separated from the seedlings and their average weight was recorded as FW. Then, three leaves were selected and placed in an incubator for two hours at 25 °C and their average weight was recorded immediately (W1), after 4 h (W2), and after 6 h (W3). Then, these samples were oven-dried at 80 °C for 24 h to obtain the dry weight (DW). The two remaining samples were placed in distilled water at room temperature for 18 h, and after dewatering, they were weighed[26].

Measurement of the concentration of macro and micro elements

The wet digestion method was used to measure the concentration of macro and microelements[27] for which 500 mg of dry and ground leaf samples were digested in a mixture of sulfuric acid (H_2SO_4) and nitric acid (HNO_3) at a ratio of 1:5 (v/v) at 60 °C for 24 h. In the next step, the homogenous mixture was exposed to nitric and perchloric acid ($HNO_3/HClO_4$) with a ratio of 1:5 (v/v). The zinc, manganese, and iron content was measured by atomic absorption spectrophotometer (model AA-6300, Kyoto, Japan). A flame photometer (Sherwood, model 410, England) was used to determine the K content.

Electrolyte leakage measurement

Ten identical pieces of leaves were separated from the selected leaves and placed in 20 ml of distilled water. The samples were shaken for 24 h at laboratory temperature and electrical conductivity 1 (EC1) was determined with an electrical conductivity (EC) meter. Then, the samples were autoclaved for 2 h at 120 °C, and electrical conductivity 2 (EC2) was obtained. Finally, electrolyte leakage was obtained using the following equation[28].

$$\mathrm{EC} = \mathrm{EC1}/\mathrm{EC2} \times 100$$

Measurement of proline concentration

Using Bates's[29] method, 0.5 g of plant sample was first digested with 10 ml of 3% sulfosalicylic acid. After centrifugation, 2 ml of the extract, 2 ml of ninhydrin acid (0.31 g ninhydrin + 7.5 ml acetic acid + 5 ml phosphoric acid), and 2 ml of glacial acetic acid were mixed and placed in a bain-marie. Then, 4 ml of toluene was added, and read at 520 nm.

Measurement of hydrogen peroxide concentration

To measure the amount of hydrogen peroxide, 0.2 g of leaf sample was homogenized in 2 ml of 0.1% chloroacetic acid solution (weight-volume) and centrifuged at 12,000 rpm for 15 min. Then, the reaction complex was obtained by combining 0.5 ml of supernatant, 0.5 ml of 10 mM phosphate buffer with pH = 7, and 1 ml of 1 M potassium iodide. The absorbance of the samples was measured at 390 nm using spectrophotometry[30].

Measurement of malondialdehyde concentration

The method of Heath and Packer[31] was used to measure the amount of malondialdehyde. At first, 0.2 g of fresh plant leaf sample was homogenized with 1.5 ml of 0.1% trichloroacetic acid (TCA). Then, the samples were centrifuged at 4 °C for 10 min at 10,000 rpm. 0.5 ml was removed from the supernatant solution, and then 1 ml of thiobarbituric acid (TBA) solution containing 20% trichloroacetic acid was added. The resulting mixture was heated in a hot water bath at a temperature of 95 °C for 30 min. The heated mixture was rapidly placed in an ice bath for 30 min to stop the reaction. After cooling the mixture, centrifugation was performed at 10,000 rpm for 10 min. Finally, the absorbance of the mixture was read by a spectrophotometer at two wavelengths of 532 nm and 600 nm. In calculating the MDA content, the extinction coefficient of 155 cm^{-1} mM^{-1} was also considered. Finally, MDA content was calculated for nm g^{-1} FW^{-1} using the following formula.

$$\mathrm{MDA} = [(532\mathrm{nm} - 600\mathrm{nm}) \times 20] / 155 \times 100$$

Measurement of phenol

For the measurement of phenol, 1 g of the plant sample was digested with 2 ml of acidic methanol and centrifuged at 12,000 rpm for 10 min. Then, 1.59 ml of distilled water, 100 µl of Folin 10%, and 20 µl of the extract were mixed and kept for 10 min. Finally, 300 µl of 7.5% sodium carbonate was added to the mixture, kept in darkness for 2 h, and read at 765 nm[32].

Measurement of flavonoid

The plant extract was prepared by homogenizing 1 g of leaf sample with 80% methanol. The samples were centrifuged at 14,000 rpm for 15 min at 4 °C. The reaction complex was prepared with a mixture of 200 µL of extract, 600 µL of 95% methanol, 40 µL of 10% aluminum chloride, 40 µL of 1 M potassium acetate, and 1120 µL of distilled water. Using a spectrophotometer, the amount of light absorbed by the reaction complex was read at a wavelength of 415 nm[33]. Lastly, the amount of flavonoid was calculated according to the standard curve of quercetin.

Measurement of total soluble protein concentration

To measure total soluble protein concentration, 5 ml of Bradford's reagent was added to 0.1 ml of protein extract from each sample, and then it was vortexed for 20 min. Then, the absorbance was recorded at 595 nm. In this method, the standard curve obtained from certain concentrations of the standard protein is used to determine protein amounts[34].

Antioxidant enzymes

To measure total dissolved protein, catalase, and guaiacol peroxidase, 0.5 g of the plant sample (leaf) was homogenized in liquid nitrogen, and then, 5 ml of cold phosphate buffer (pH = 7.5) containing 0.5 mM EDTA was added to it. The homogenized samples were centrifuged at 15,000 rpm at 4 °C for 15 min and separated for measurement[35].

Assay of catalase enzyme activity

The catalase (CAT) enzyme activity was investigated, given the reduction of hydrogen peroxide at 240 nm. The reaction mixture consisted of 50 mM of phosphate buffer (pH = 7) and 15 mM of hydrogen peroxide. The reaction was started by adding 100 ml of the enzyme extract to the final volume, which was 3 ml. Adsorption changes were recorded at 240 nm for 3 min. The enzyme activity was then expressed as changes in adsorption per minute per milligram of protein[36].

Assay of ascorbate peroxidase enzyme activity

To measure the activity of the ascorbate peroxidase enzyme, the reaction mixture consisted of 250 mM of phosphate buffer (pH = 7), 1.2 mM of hydrogen peroxide, 0.5 mM of ascorbic acid, and 1.0 mM of EDTA.

The enzymatic activity was initiated by adding hydrogen peroxide to the mixture. The light absorption, which decreased due to ascorbic acid peroxidation, was read for 2 min by a spectrophotometer at 290 nm. The changes in absorption per minute per milligram of protein were used to calculate the enzyme activity[37].

Assay of guaiacol peroxidase enzyme activity

To measure the activity of the guaiacol peroxidase (GPX) enzyme, the reaction medium consisted of 25 mM of potassium phosphate buffer (pH = 6.8), 40 mM of hydrogen peroxide, and 20 mM of guaiacol. The reaction was started by adding 100 µl of enzyme extract to the final volume; i.e., 3. The increased adsorption was recorded by tetraguaiacol formation at 470 nm for 3 min. The enzyme activity was then expressed as the change in absorption per minute per milligram of protein per minute[37].

Assay of superoxide dismutase enzyme activity

To measure the activity of superoxide dismutase enzyme, 1500 µl of 100 mM phosphate buffer, 200 µl of 0.2 mM methionine, 100 µl of EDTA (3 mM), 900 µl of distilled water and 100 µl of sodium carbonate ($NaCO_3$) 1.5 Molar, and 100 µl of riboflavin were mixed, and at the end, 50 µl of the enzyme sample were added to each test tube. Then the test tubes were placed at a distance of 30 cm from the light source for 15 min. After that, they were kept in complete darkness for 15 min, and at the end of the work, the absorption changes of the samples were read using a spectrophotometer at 560 nm[38].

Measurement of abscisic acid (ABA), Indole-3-acetic acid (IAA), and Gibberellic acid (GA3)

For measurement hormones, 2 to 5 g of leaf samples were selected, immediately frozen in liquid nitrogen, and then powdered. The milled samples were dissolved in 20 ml of a solution containing methanol, ethyl acetate, and acetic acid in the ratio of 1:50:50, respectively, to which 20 mg of BHT was added as an antioxidant, and a homogeneous solution was created. The resulting solution was filtered through Whatman No. 1 paper and then the final volume of the solution was adjusted to 100 ml. To remove the solvent, the final solution was placed inside the rotary balloon under vacuum conditions and at a temperature of 35 °C. Then, 10 ml of ethyl acetate was added to the final solution. For separation, ethyl acetate containing hormones (ABA, IAA, and GA3), and potassium hydrogen phosphate solution was used from a decanter funnel, and sodium sulfate was used to extract water from the ethyl acetate containing hormones solution. After evaporating the ethyl acetate, 5 ml of methyl chloride was added to the remaining dry extract, and the samples were kept for 24 h. They were kept under the hood for the evaporation of methyl chloride. After the evaporation of methyl chloride, 300 µl of the methanol solution containing 1% acetic acid was added to the remaining dry extract, and the resulting solution was filtered again by a Millipore filter and the final solution was used to determine the hormone's concentration. The standard solution of the individual acid was prepared in the mobile phase and chromatographed separately to determine the retention time for each acid. The signal of the compounds was monitored at 208, 265, and 280 nm for GA3, ABA, and IAA, respectively. The sample was passed through a 0.45 polytetrafluoroethylene filter and then injected into the HPLC column. HPLC separated the solution's components with a C18 column, a flow rate of 0.7 ml/min, and a solvent of 0.2% acetic acid and 100% methanol in a ratio of 50:50 at 40 °C[39].

RNA extraction and DNA synthesis

Total RNAs were extracted and purified from the leaves following the method described by Gasic et al.[40]. Only the extractions having an A260/A280 ratio of 1.8–2.0 and an A260/A230 ratio > 2.0 were chosen for further analysis. The integrity of the extracted RNAs was verified using 2% agarose gel electrophoresis followed by ethidium bromide staining. Oligo-dT was used for the first strand cDNA synthesis. The reaction mixture (Table 2) was prepared in a microtube on ice and was made up to 20 µl using RNase-free water.

RT-qPCR analysis

The RNA sequences of *DREB1A* and *DREB1E* genes were taken from NCBI (www.ncbi.nlm.nih.gov), and the forward and reverse primers were designed by Oligo 7 (Table 3). RT-qPCR analysis applied by an ABI StepOne Detection System (Applied Biosystems, USA), using the SYBR Green PCR Master Mix (TaKaRa, Toyota, Japan). The reaction mixture (Table 4) was made up to 20 µl total volume per sample. An initial denaturation step was performed at 95 °C for 10 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 60 s. A melting curve analysis was performed following amplification to guarantee the absence of primer dimers and other nonspecific products.

Reactive	Volume
Vivantis RT enzyme mix	0.5 µl
Buffer RT enzyme	2 µl
Oligo RT primer (50 µM)	0.5 µl
Random 6 mers (100 µM)	0.5 µl
dNTP	1 µl
DDW	11.5 µl
Total RNA (500 ng)	5 µl
Total	20 µl

Table 2. Reaction mixture for cDNA synthesis.

Primer	Sequence (5′→3′)
DREB1A-F	GGATAGTATGGCGGAAGGGC
DREB1A-R	GGTCAACGAACGAAAGCGTC
DREB1E-F	GGGTTTTCGAAGGCAGCATTACT
DREB1E-R	CGCATAGGCAAGTTCCGAAGT
EF-1α-F	CAAGCGTGTCATCGAGAGAT
EF-1α -R	ATACCACGTTCACGTTCAGC

Table 3. Primer sequence of the genes *VvCBF4*, *VvNAC1* and *MDH* used for expression analysis in the present experiment.

Reactive	Volume
RT reaction solution (cDNA)	2 µl
Primer F	0.4 µl
Primer R	0.4 µl
Power SYBR green PCR master mix	10 µl
DDW	7.2 µl
Total	20 µl

Table 4. The composition of reaction mixture for RT-PCR.

Relative quantification was executed by the comparative CT ($2^{-\Delta\Delta Ct}$). To quantify the transcript level, a standard curve (copy number as a function of Ct) was created by a 10 × mass dilution series of each cDNA fragment. The exact copy number was presented by extrapolating the Ct value for each cDNA on the standard curve and determining it as the copy number ng^{-1} of cDNA.

Statistical analysis
The research was analyzed based on a factorial experiment in the form of a completely random design and using the statistical software MSTATC (Ver. 2.10). The means of the data were compared using Duncan's multi-range test at the probability level of 5%.

Results and Discussion
Chlorophyll fluorescence
Based on the results, the highest amount of Fm was observed in the control and the treatment of 100 mg/L CeO_2 NPs at the no stress level, and the lowest amount was observed in CeO_2 NPs treatment at the stress level of 25% FC. Drought stress reduced the Fv content but the application of CeO_2 NPs significantly increased it so that the highest Fv content was obtained at the CeO_2 NPs level of 100 mg/L without stress and the lowest at 25% FC with no CeO_2 NPs application. The results showed that the application of 50 and 100 mg/L CeO_2 NPs increased Fv/Fm at the stress level of 25% FC by 15% and 22%, respectively. Also, the highest Fv/Fm was observed in no stress level with the highest level of CeO_2 NPs and the lowest in the drought stress level of 25% FC with no CeO_2 NPs treatment. Drought stress led to a decrease in Y (II). According to the results, the lowest amount was observed at the highest level of stress and the highest was observed in without stress with treatment CeO_2 NPs. The highest content of Y (NO) was obtained at 25% FC and the lowest in control. The use of CeO_2 NPs improved the electron transfer rate (ETR) under drought stress, so the largest decrease was 2.1 folds observed in 25% FC with no CeO_2 NPs application. The highest ETR was observed at the highest CeO_2 NPs level at without stress (Table 5). Drought is one of the factors that limit plant growth and production by affecting a series of morphological, physiological, biochemical, and metabolic processes. Today, the use of nanoparticles to reduce damage caused by stress has been noticed, and significant results have been obtained from their use. CeO_2 NPs have shown positive effects in plants[41–43], but research has been limited on the role of CeO_2 NPs in inducing drought tolerance in plants. However, there have been studies about salinity stress and the role of nanoceria, which attributed some mechanisms of creating tolerance to salinity stress and drought stress including (1) scavenging excessively accumulated ROS to maintain ROS homeostasis[44,45], (2) maintaining mesophyll K^+ capacity[46], (3) improving the production of NO (nitric oxide)[47], (4) modulation of alpha-amylase activities[48], and (5) reduction of lipoxygenase activity to decrease the oxidative damage on the membrane[49].

Chlorophyll fluorescence parameters are known as ideal predictors of photosynthetic ability[50] Fv/Fm, known as the maximum quantum efficiency for primary photochemistry, can provide a simple and rapid way to assess when plants are exposed to a stressful environment[43,51]. Environmental stresses increase F0 and decrease Fv/Fm, indicating the discontinuity of photochromic pigments from PSII as F0 is related to quinone oxidation capacity (Qa). Drought significantly decreased Fm, Fv, Fv/Fm, and ETR and increased Y(II), Y(NO), and F0, which is consistent with the results of[52,53]. The plant's response to drought depends on the species. One of the main causes for the decrease in the photosynthesis rate in plants is the decline in the movement of CO_2 from the atmosphere to the carbon assimilation site in chloroplasts due to stomatal closure, which increases the

Drought	Ce-NPs	Fm	Fv	Fv/Fm	F0	Y(II)	Y(NO)	ETR
100	0	3.624 ± 0.031a	3.042 ± 0.014c	0.839 ± 0.015c	0.582 ± 0.010d	0.655 ± 0.010d	0.315 ± 0.003d	32.34 ± 0.454c
	50	3.328 ± 0.047a	2.821 ± 0.016b	0.847 ± 0.015b	0.507 ± 0.008f	0.686 ± 0.010c	0.292 ± 0.006e	45.64 ± 3.154b
	100	3.654 ± 0.048ab	3.101 ± 0.055a	0.848 ± 0.022a	0.553 ± 0.011g	0.781 ± 0.009a	0.214 ± 0.007f	60.63 ± 3.013a
50	0	3.326 ± 0.034b	2.034 ± 0.028f	0.611 ± 0.012h	1.292 ± 0.008b	0.579 ± 0.024e	0.394 ± 0.008b	27.33 ± 1.310d
	50	3.181 ± 0.034c	2.347 ± 0.035e	0.737 ± 0.016f	0.834 ± 0.011d	0.646 ± 0.011d	0.336 ± 0.008c	35.74 ± 1.093c
	100	3.129 ± 0.051d	2.574 ± 0.067d	0.822 ± 0.017d	0.555 ± 0.018e	0.746 ± 0.008b	0.281 ± 0.013e	49.04 ± 3.219b
25	0	3.121 ± 0.022c	1.733 ± 0.044g	0.555 ± 0.011i	1.388 ± 0.015a	0.488 ± 0.027f	0.446 ± 0.004a	19.10 ± 1.711e
	50	2.984 ± 0.017d	1.958 ± 0.026f	0.656 ± 0.008g	1.026 ± 0.021c	0.594 ± 0.020e	0.386 ± 0.013b	26.86 ± 1.865d
	100	2.984 ± 0.014d	2.142 ± 0.063e	0.717 ± 0.019e	0.842 ± 0.008d	0.686 ± 0.014c	0.323 ± 0.010cd	34.75 ± 0.895c
S.O.V								
Drought		0.336**	4.371**	0.221**	0.070**	0.038**	0.028**	854.597**
Ce-NPs		0.071**	0.426**	0.051**	0.073**	0.075**	0.029**	1107.791**
Drought × Ce-NPs		0.12**	0.015*	0.004**	0.002**	0.001*	0.000*	34.164**
Erorr		0.002	0.004	0.0001	0.0001	0.0001	0.0001	5.726
CV (%)		1.59	2.33	3.09	1.21	3.62	3.17	7

Table 5. Mean comparisons for the effects of CeO_2 NPs under drought stress on the fluorescence chlorophyll of apple cv. 'Red Delicious' on M9 rootstock. ns, * and ** indicated no significant difference, significant at 5% probability level and significant at 1% probability level, respectively. Means with the same letter are not significantly different by Duncan grouping at ($p \leq 0.05$) in each column. S.O.V. and C.V. refers to the source of variation and coefficient of variation.

production of ROS[9,54]. The results showed that the foliar application of CeO_2 NPs improved the fluorescence parameters compared to its non-application, which was consistent with the findings of Djanaguiraman et al.[9]. The results showed that the application of nanocerium oxide led to the improvement of fluorescence parameters under stress conditions. Researchers showed that CeO_2 NPs can enhance gas exchange and photosynthetic efficiency by increasing the photosynthetic light reactions, NADPH synthesis and RuBP regeneration, as well as activating the Rubisco enzyme. Nanoceria increased the chlorophyll a content in leaves and simultaneously decreased chlorophyll b biosynthesis. This effect was observed at concentrations of 100 and 500 mg/kg CeO_2 NPs. Inhibition of chlorophyll production is a common response of plants under stress conditions. As a result, plants produce more chlorophyll b to compensate for the amount of light absorbed[55]. The beneficial role of CeO_2 NPs in photosynthesis was also identified in chickpea (*Lathyrus oleraceus* Lam.)[56,57]. Hydroponic cultivation of plants supplemented with nanoceria (100 mg/L) increased leaf net photosynthesis (40%), stomatal conductance (36%), and water use efficiency (30%) compared to the control[55]. This effect was probably initiated by the catalytic properties of CeO_2 NPs, which accelerate the photochemical phase of photosynthesis. Furthermore, the authors demonstrated that nanoceria modulated the toxicity of zinc oxide nanoparticles by protecting the photosynthetic apparatus in chickpea leaves from oxidative stress induced by excess zinc[56].

Similarly, Arabidopsis plants treatment with CeO_2 NPs exposed to abiotic stress showed up to 19% increase in photosystem II quantum yield[45]. Fm, which shows the maximum quantum efficiency of photosystem-II for converting absorbed light into chemical energy, has been widely used as a valid indicator to show stress-induced disturbances in photochemical centers and photoinhibition. The reduction of Fv/Fm indicates the reduction of the maximum quantum efficiency of photosystem II[58]. The results of this experiment also indicated a decrease in Fv and Fv/Fm (Table 5). In the research conducted by Arif et al.[59] in strawberry plants, a decrease in Fv/Fm was also observed under drought stress. According to studies by other researchers, the amount of ETR decreased under cold stress conditions.. Reducing the rate of photosynthetic activity and carbon dioxide fixation reduces the amount of energy required, thereby reducing the rate of electron transfer[58,60]. Therefore, the plants that have a lower reduction in the electron transfer rate show a higher photosynthetic rate and, as a result, more resistance to drought.

In the conditions of stress, the activity of Rubisco enzyme, followed by the functioning of the Calvin cycle and CO_2 stabilization, and as a result, the consumption of NADPH, H^+ as one of the products of the light stage of photosynthesis, decreases. Reducing the consumption of NADPH, H^+ causes its accumulation and reducing the ratio of $NADP^+$ to NADPH, H^+. In this case, the electron is transferred from ferredoxin to oxygen and oxygen free radical is produced, which will eventually lead to damage to the components of the electron transfer chain and create optical inhibition. The occurrence of photoinhibition increases Y(NO) and decreases Y(II)[58,59].

Chlorophylls and carotenoid

The results showed that drought stress decreased photosynthetic pigments and the application of CeO_2 NPs improved them so that the greatest reduction in chlorophyll a, b, and total was observed at the highest level of stress with no CeO_2 NPs treatment. The results for carotenoid content were similar to those for chlorophyll (Fig. 2a–d). The content of chlorophyll a, b, total, and carotenoids decreased by 19.84%, 19.97%, 19.9% and 59.78% under severe drought stress (FC 25%), respectively. The highest content of chlorophyll a, b, and total was observed in the control with 100 mg/L CeO_2 NPs and the lowest in 25% FC without the treatment of CeO_2 NPs. The highest and lowest levels of carotenoid were obtained from the control with 100 mg/L CeO_2 NPs and at 25% FC without

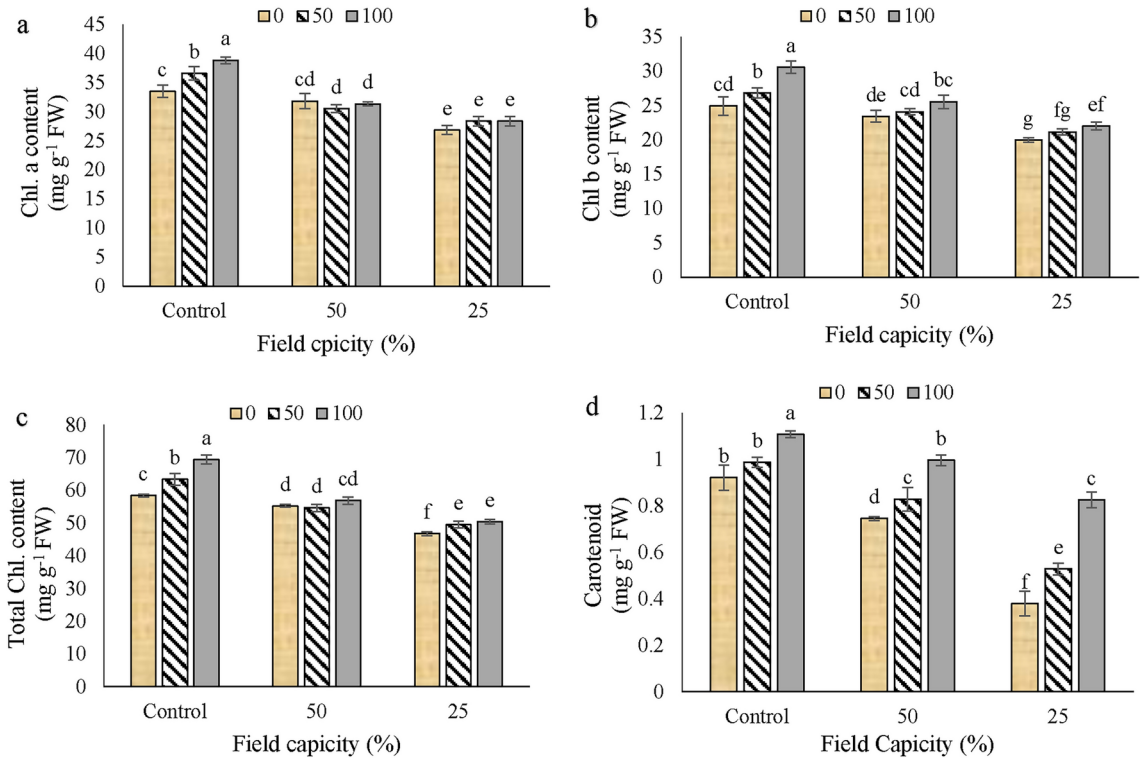

Fig. 2. Effect of CeO_2 NPs on Chlorophyll a (**a**), b (**b**) and total (**c**) and carotenoid (**d**) of apple cv. 'Red Delicious' on M9 rootstock under drought stress. Means followed by the same letter on columns are not significantly different at 0.05 level, according to Duncan's multiple range test. Data are mean ± SD (n = 3 replicates).

the treatment of CeO_2 NPs, respectively. The use of CeO_2 NPs in severe drought stress conditions (Fc 25%) and the control improved total chlorophyll by 7.14% and 15.88% and carotenoid content by 54.87% and 16.87%, respectively (Fig. 2a–d). Chlorophyll is the main photosynthetic pigment in plants, which is directly involved in the absorption, transmission, and conversion of light energy[61]. Chlorophyll is sensitive to environmental stresses[62]. It has been reported that the reduction of photosynthesis is caused by the loss of chlorophyll, and this is a general parameter to measure the degradation of the photosynthetic apparatus[63]. Franzoni et al.[64] also found a decrease in chlorophyll content in lettuce grown under drought stress. Our results showed a high content of chlorophyll in plants exposed to CeO_2 NPs in both stressed and non-stressed conditions. Similarly, Gui et al.[65] reported an increase in chlorophyll content at low concentrations of CeO_2 NPs. Drought stress in plants increases H_2O_2 production, which activates the abscisic acid signaling pathway, leading to stomatal closure. However, in plants sprayed with nanoceria, H_2O_2-mediated stomatal closure of chloroplasts can be reduced by reducing H_2O_2. ROS is involved in the destruction of chlorophyll and the damage of proteins and chloroplast membranes, and as a result, the rate of photosynthesis decreases. Nanoceria prevents ROS production in chloroplasts by the oxygen vacancies in the CeO_2 network structure, which improves photosynthesis and chlorophyll content index[9,11]. Carotenoids are also responsible for scavenging singlet oxygen, and carotenoid depletion under drought stress may also contribute to increasing ROS that further oxidizes photosynthetic pigments[66,67]. Plants sprayed with nanoceria had epicuticular wax intact. There were no changes in leaf mesophyll and vascular cell anatomy by drought treatments. Intact epicuticular wax in plants with nanoceria foliar application may be due to increased transpiration and leaf cooling along with higher stomatal conductance[9].

Relative water relations of leaves

The results showed that the application of CeO_2 NPs improved relative water relations at different stress levels. The highest value of relative water content (RWC) was observed in the control and the lowest in the highest stress level without the treatment of CeO_2 NPs. The results showed that the drought stress reduced RWC and the foliar application of CeO_2 NPs improved it. Based on the results of foliar application at the highest stress level, CeO_2 NPs at the rates of 50 and 100 mg/L increased RWC by 51% and 31%, respectively (Table 6). The application of CeO_2 NPs at different levels of drought stress improved water saturation deficit (WSD) so that the highest amount was obtained from 25% FC without the treatment of CeO_2 NPs and the lowest from CeO_2 NPs at the rate of 100 mg/L under no stress conditions. The greatest decrease in RWL was two folds obtained from 25% FC compared to the control. The highest content of IWC was obtained from the highest level of drought stress and treatment of CeO_2 NPs at the rate of 100 mg/L and the lowest from the control (Table 6). Based on the results, the application of CeO_2 NPs improved the effect of drought stress on LWC. The results showed that the highest ELWL content was obtained from the control and the lowest from the highest level of drought

Drought	Ce-NPs	RWC (%)	WSD (%)	RWL (%)	IWC (%)	LWC (%)	ELWL (%)	ELWR (%)	LWL (%)	RWP (%)
100	0	81.30 ± 0.49a	31.85 ± 0.85e	34.65 ± 0.69c	95.80 ± 2.36f	57.00 ± 0.73bc	69.65 ± 0.36a	54.65 ± 1.26e	26 ± 0.24a	29.50 ± 0.98f
	50	75.28 ± 2.79b	28.10 ± 0.57f	45.30 ± 1.30b	121.8 ± 2.62e	58.30 ± 1.30b	47.77 ± 0.97c	75.10 ± 1.22bc	20.45 ± 0.69b	79.30 ± 2.44a
	100	80.24 ± 0.64a	20.80 ± 1.55g	54.53 ± 1.17a	128.4 ± 1.13d	61.95 ± 0.93a	41.80 ± 2.25de	71.70 ± 1.30c	12.95 ± 0.69c	50.15 ± 1.18d
50	0	55.50 ± 0.57e	45.80 ± 0.81b	28.10 ± 1.14d	121.2 ± 2.30e	53.38 ± 1.12d	63.50 ± 2.61b	65.05 ± 2.24d	10.95 ± 0.69d	35.55 ± 1.91e
	50	63.60 ± 0.73d	34.80 ± 0.49d	36.00 ± 2.19c	130.7 ± 0.63d	55.65 ± 0.20bc	44.08 ± 1.31d	75.35 ± 0.77b	8.100 ± 0.16e	52.60 ± 2.36d
	100	69.52 ± 1.65c	29.45 ± 0.61f	43.40 ± 0.98b	136.5 ± 1.72c	58.50 ± 0.98b	40.75 ± 0.97e	72.00 ± 0.89bc	7.050 ± 0.20f	53.00 ± 1.38d
25	0	40.50 ± 0.65f	57.75 ± 0.69a	18.09 ± 1.32e	137.3 ± 1.45c	45.10 ± 0.99f	43.85 ± 1.26de	73.35 ± 2.49bc	7 ± 0.08f	57.10 ± 1.87c
	50	54.20 ± 1.06e	43.00 ± 1.143c	25.75 ± 4.17d	143.9 ± 1.82b	50.03 ± 1.45c	40.90 ± 0.32de	83.55 ± 1.02a	5.500 ± 0.16g	63.15 ± 2b
	100	61.38 ± 2.82d	35.11 ± 0.74d	28.57 ± 1.19d	150.9 ± 1.10a	55.60 ± 1.31cd	35.10 ± 0.65f	72.70 ± 1.14bc	4 ± 0.65h	53.15 ± 1.10d
S.O.V										
Drought		1650.316**	760.293**	968.805**	1858.473**	179.965**	413.247**	201.361**	506.910**	260.951**
Ce-NPs		286.544**	632.690**	524.977**	988.901**	106.807	951.416**	421.981**	99.502**	1330.366**
Drought × Ce-NPs		115.223**	33.351**	17.910*	110.059**	7.729*	102.964**	66.076**	24.071**	460.750**
Erorr		3.603	1.182	5.214	4.603	1.701	2.971	3.301	0.339	4.720
CV (%)		2.94	2.99	6.54	1.66	2.37	3.63	2.54	5.14	4.13

Table 6. Mean comparisons for the effects of CeO_2 NPs under drought stress on the Relative water relations of apple cv. 'Red Delicious' on M9 rootstock. ns, * and ** indicated no significant difference, significant at 5% probability level and significant at 1% probability level, respectively. Means with the same letter are not significantly different by Duncan grouping at ($p \leq 0.05$) in each column. S.O.V. and C.V. refers to the source of variation and coefficient of variation.

stress and CeO_2 NPs at the rate of 100 mg/L. Also, the ELWL content showed the greatest decrease of 98% compared to the control. The highest amount of ELWR was observed at 50 mg/L with 25% FC and the lowest amount was observed in the control. Under drought stress, LWL decreased and the results showed that foliar application led to a decrease of 27% and 75% with the treatment of CeO_2 NPs at the rates of 50 and 100 mg/L at the highest stress level, respectively. The highest content of RWP was obtained from the CeO_2 NPs level of 50 mg/L without stress and the lowest from 50% FC with no CeO_2 NPs application (Table 2). It has been reported that leaf RWC is a better indicator of drought stress than other plant growth or biochemical parameters. Leaf RWC is highly responsive to drought stress and has been shown to correlate with drought tolerance[68]. It seems that the decrease in leaf water and increase in WSD is due to high transpiration and the inability to replace leaves due to the unavailability of water to roots under drought stress[69]. Ghassemi et al.[67] reported that the reduction of LWC under drought stress may depend on the reduction of plant vigor. The present results showed that the application of CeO_2 NPs increased LWC under drought stress. RWP is also one of the main physiological parameters affecting plant-water relations. The results revealed that significant changes in RWP were observed under normal and drought stress conditions and RWP content increased under stress. Our results in RWL and ELWR traits agree with the findings of Lonbani and Arzani[70]. The strong water-holding capacity of plants allows them to limit the rate of decline in relative water content and to increase water saturation deficits[50,53].

Elemental content

The findings revealed that the treatment with 100 mg/L CeO_2 NPs without stress resulted in the maximum potassium content in the roots and shoots, whereas the lowest was achieved with no CeO_2 NPs application at a stress level of 25% FC. The application of CeO_2 NPs increased potassium concentration, with the biggest drop being 1.1-fold in shoots and 2.4-fold in roots at the lowest stress level with no CeO_2 NPs. The foliar spray of CeO_2 NPs boosted magnesium concentrations under drought conditions. The plants treated with 100 mg/L CeO_2 NPs and subjected to a stress level of 50% FC had the greatest magnesium content in the shoot, whereas those treated with 100 mg/L CeO_2 NPs under no stress circumstances had the highest magnesium content in the roots. The lowest magnesium concentration in both shoots and roots was associated with the most severe drought stress when no CeO_2 NPs were applied. Furthermore, the findings show that applying 50 and 100 mg/L CeO_2 NPs at the greatest degree of drought stress raised root magnesium concentrations by 24% and 76%, respectively, compared to the control group. Manganese was strongly impacted by drought stress. Manganese levels were greatest in shoots and roots after treatment with 100 mg/L CeO_2 NPs without stress, and lowest in both shoots and roots after 25% FC without CeO_2 NPs (Table 7). Zinc levels were greatest in the shoots and roots of plants treated with 50 and 100 mg/L CeO_2 NPs under no-stress circumstances. The lowest concentration was seen in the shoots and roots of plants treated with no CeO_2 NPs at 25% FC. According to the results for the foliar application at the highest stress level, the treatments of 50 and 100 mg/L CeO_2 NPs increased the iron concentration by 56% and 72%, respectively. The highest amount of iron under no-stress conditions was observed with the treatment of 100 mg/L CeO_2 NPs and the lowest was observed at the highest level of stress without CeO_2 NPs, respectively (Table 7). With the increase in drought stress, the concentration of potassium, magnesium, manganese, zinc, and iron decreased and the application of CeO_2 NPs improved them. Minerals play an inevitable role in the growth, quality characteristics, and productivity of plants[71]. Potassium is a cofactor that is required for the activation of more than 50 enzymes[72] and also plays an important role in cytosolic pH homeostasis, protein synthesis, and cellular activities of stomatal opening and closing[73,74]. CeO_2 NPs modulate

Drought	Ce-NPs	Shoot K (mg g^{-1} DW)	Root K (mg g^{-1} DW)	Shoot Mg (mg g^{-1} DW)	Root Mg (mg g^{-1} DW)	Shoot Mn (mg g^{-1} DW)	Root Mn (mg g^{-1} DW)	Shoot Zn (mg g^{-1} DW)	Root Zn (mg g^{-1} DW)	Shoot Fe (mg g^{-1} DW)	Root Fe è (mg g^{-1} DW)
100	0	1.330 ± 0.047f	0.745 ± 0.022d	0.575 ± 0.050cd	0.366 ± 0.028c	1.418 ± 0.007c	0.774 ± 0.036c	1.519 ± 0.072b	0.623 ± 0.025c	1.543 ± 0.042b	0.681 ± 0.028cd
	50	1.797 ± 0.041c	1.009 ± 0.029b	0.652 ± 0.040bc	0.495 ± 0.044b	1.508 ± 0.012b	0.934 ± 0.048ab	1.694 ± 0.060a	0.743 ± 0.034b	1.599 ± 0.037b	0.765 ± 0.044bc
	100	2.382 ± 0.024a	1.232 ± 0.022a	0.724 ± 0.016ab	0.696 ± 0.074a	1.644 ± 0.041a	1.027 ± 0.037a	1.692 ± 0.0.47a	0.846 ± 0.026a	1.822 ± 0.062a	0.950 ± 0.074a
50	0	1.209 ± 0.002g	0.558 ± 0.034e	0.443 ± 0.018ef	0.235 ± 0.042d	1.096 ± 0.041e	0.494 ± 0.059d	1.261 ± 0.016d	0.338 ± 0.040e	1.092 ± 0.060d	0.447 ± 0.042e
	50	1.563 ± 0.024e	0.771 ± 0.032d	0.615 ± 0.053c	0.347 ± 0.053c	1.246 ± 0.039d	0.724 ± 0.028c	1.416 ± 0.034c	0.447 ± 0.044d	1.261 ± 0.038c	0.607 ± 0.053d
	100	1.997 ± 0.044b	1.053 ± 0.024b	0.763 ± 0.045a	0.413 ± 0.006bc	1.424 ± 0.053c	0.902 ± 0.065b	1.584 ± 0.035b	0.646 ± 0.040c	1.353 ± 0.040c	0.817 ± 0.006b
25	0	1.084 ± 0.004h	0.361 ± 0.031f	0.180 ± 0.037g	0.131 ± 0.035e	0.789 ± 0.027g	0.222 ± 0.070e	1.007 ± 0.017f	0.136 ± 0.031f	0.496 ± 0.020f	0.180 ± 0.035f
	50	1.353 ± 0.030f	0.604 ± 0.016e	0.370 ± 0.044f	0.163 ± 0.043de	0.992 ± 0.006f	0.444 ± 0.035d	1.162 ± 0.060e	0.359 ± 0.026e	0.777 ± 0.001e	0.465 ± 0.043e
	100	1.703 ± 0.067d	0.951 ± 0.042c	0.503 ± 0.009de	0.231 ± 0.043d	1.174 ± 0.023d	0.726 ± 0.056c	1.408 ± 0.046c	0.484 ± 0.011d	0.854 ± 0.049e	0.615 ± 0.043d
S.O.V											
Drought		0.470**	0.287**	0.235**	0.267**	0.652**	0.452**	0.441**	0.389**	2.019**	0.323**
Ce-NPs		1.517**	0.619**	0.158**	0.093**	0.221**	0.339**	0.202**	0.193**	0.203**	0.288**
Drought × Ce-NPs		0.036**	0.004*	0.008*	0.011*	0.005*	0.013*	0.015*	0.005*	0.011*	0.009*
Erorr		0.002	0.001	0.002	0.003	0.002	0.004	0.003	0.002	0.003	0.003
CV (%)		2.84	4.39	8.71	15.89	3.10	8.85	4.05	7.66	4.35	9.01

Table 7. Mean comparisons for the effects of CeO_2 NPs under drought stress on the elements content of apple cv. 'Red Delicious' on M9 rootstock. ns, * and ** indicated no significant difference, significant at 5% probability level and significant at 1% probability level, respectively. Means with the same letter are not significantly different by Duncan grouping at ($p \leq 0.05$) in each column. S.O.V. and C.V. refers to the source of variation and coefficient of variation.

the activity of ROS-activated NSCC (non-selective cation channels) channels to provide better mesophyll K^+ retention and thus plant tolerance to environmental stresses[45]. The expression of *KOR*, a gene responsible for salt-induced K^+ leakage, was significantly decreased in nanoceria-treated plants compared to the control under salt stress[74]. Tadayyon et al.[75] reported that increased drought stress resulted in decreased potassium, magnesium, and iron. Hassanpouraghdam et al.[71] reported that the application of CeO_2 NPs improved the concentration of elements. By increasing the photosynthetic performance of the plant and reducing the apoplastic barrier of the root, nanoceria improved the salt tolerance of canola to enable more Na^+ transfer to the shoot. CeO_2 NPs play a role in maintaining the cytosolic K^+/Na^+ ratio by sequestering vacuolar Na^+ and removing Na^+ from aerial parts, thereby improving plant salt tolerance[43,74]. CeO_2 NPs reduced nitrogen uptake and increased its accumulation in the green parts of plants. At the same time, nanoparticles promoted potassium utilization by increasing the potassium level in the roots and shoots. The effect of CeO_2 NPs on phosphorus accumulation depends on the concentration of nanoparticles in the nutrient solution. The effect of CeO_2 NPs on metal homeostasis in plants was evaluated by various researchers and colleagues. They concluded that nano cerium oxide can change the nutritional value of plants. Plant growth in soil enriched with nanoparticles not only changed the standard pathways of element uptake and accumulation by plants. This can be proven by the much higher accumulation of calcium, iron, phosphorus, and S at relatively low concentrations of CeO_2 NPs, as well as copper, potassium, magnesium, manganese, and zinc at moderate doses[76,77].

Osmolytes and electrolyte leakage

Drought stress and foliar treatment of CeO_2 nanoparticles dramatically influenced proline content. It was augmented by increasing drought stress. The maximum content was recorded at a stress level of 25% FC with 100 mg/L CeO_2 nanoparticles. Drought stress (25% FC) with CeO_2 nanoparticles increased proline content by 72% relative to the control (Fig. 3a). The stress resulted in a notable reduction in total soluble protein concentration, whereas the administration of CeO_2 nanoparticles enhanced this characteristic under stress conditions. The maximum total soluble protein content was recorded in the control group with CeO_2 nanoparticles (100 mg/L), whereas the lowest level was noted under drought stress (25% field capacity) without foliar treatment (Fig. 3b). As drought stress intensified, electrolyte leakage rose markedly. CeO_2 nanoparticles enhanced cell membrane stability by diminishing electrolyte leakage under drought stress. CeO_2 nanoparticles decreased electrolyte leakage by 53% under drought stress at 25% field capacity (Fig. 3c). The hydrogen peroxidase levels were affected by the interplay between drought stress and CeO_2 NP treatments. During drought stress, hydrogen peroxidase levels increased, and the use of CeO_2 nanoparticles enhanced this effect, with the peak value recorded at the maximum stress level without CeO_2 nanoparticles, while the lowest value occurred at no stress and 100 mg/L CeO_2 nanoparticles (Fig. 3d). The maximum concentration of malondialdehyde was recorded at a stress level of 25% FC without CeO_2 NPs treatment, whereas the minimum was noted at 100 mg/L CeO_2 NPs without stress. The foliar application of CeO_2 nanoparticles at concentrations of 50 and 100 mg/L reduced malondialdehyde levels by 9% and 21%, respectively, at 25% field capacity (Fig. 3e). To maintain intracellular osmotic balance, plants usually increase osmotic regulating substances such as soluble sugars and soluble proteins to regulate osmotic balance. The accumulation of soluble sugars and soluble proteins represents a critical mechanism for suppressing oxidative stress in plants[78]. In general, proline is produced and accumulated in plants during stress[64]. Proline plays an important role in regulating the osmotic pressure of plants, and its concentration increases significantly with increasing stress, including drought stress[79,80]. Our results are consistent with the results of Sahitya et al.[81] under drought stress. The increase in proline accumulation in plants treated with CeO_2 NPs may help stabilize subcellular structures and osmotic balance in the cytosol[82]. To know the physiological state of the plant, the protein content may be a significant indicator[83]. Increasing the dry level to 25% FC resulted in a significant decrease in the total protein content of the plants. Based on our results, the application of CeO_2 NPs improved the significant reduction of leaf soluble protein caused by different levels of drought stress. Faraji and Sepehri[84] reported that foliar application increased total protein content under different levels of drought stress. Plants with low electrolyte leakage index are stress tolerant, which is seen in several plant species[85–87]. The current findings showed that dryness increased electrolyte leakage in the plasma membrane, but the leakage decreased with the CeO_2 NPs treatment at low concentrations, which is consistent with the findings of Mohammadi et al.[13]. ROS such as H_2O_2 are produced during metabolic processes such as photosynthesis or respiration, and their production is induced under dry salinity, high light intensity, and high temperature. The rate of H_2O_2 synthesis is strongly related to the strength and duration of stress. In addition, the amount of H_2O_2 varies in different parts of the cell and these levels can be strongly related to stress type[88]. Nanomaterials such as nanoceria with the ability to inhibit ROS, especially hydroxyl radicals, may have the potential to maintain ROS homeostasis in plants under stress and thus improve plant stress tolerance[74]. The results of the plant studies showed that adaptation processes are initiated in plants to protect cells from oxidative stress. The initial increase in H_2O_2 content and APOX activity in tissues is proportional to the final concentration of CeO_2 NPs. Plant exposure to CeO_2 NPs increased the level of HSP70 proteins in plants, and this effect remained throughout the treatment period. The excessive production of ROS in plant tissues can be confirmed as a response to plant exposure to nanoceria[77].

MDA, one of the products of lipid peroxidation, has been widely studied as a measure of lipid oxidative stress[88]. In this study, the effect of drought stress on MDA production was investigated. As a response to drought stress, a significant increase in MDA levels was observed in apple leaves. In the present study, the increased activity of antioxidant enzymes can explain the decreased levels of H_2O_2, MDA, and electrolyte leakage. These findings are consistent with the results of Mohammadi et al.[13] about MDA improvement with the application of CeO_2 NPs.

Recent studies have revealed the multifaceted role of nanoparticles in modulating plant responses to stress. Specifically, this study highlights advancements in nanoparticle-assisted regulation of ROS, including their influence on osmotic adjustments and metabolic pathways. Furthermore, the study emphasizes the role of

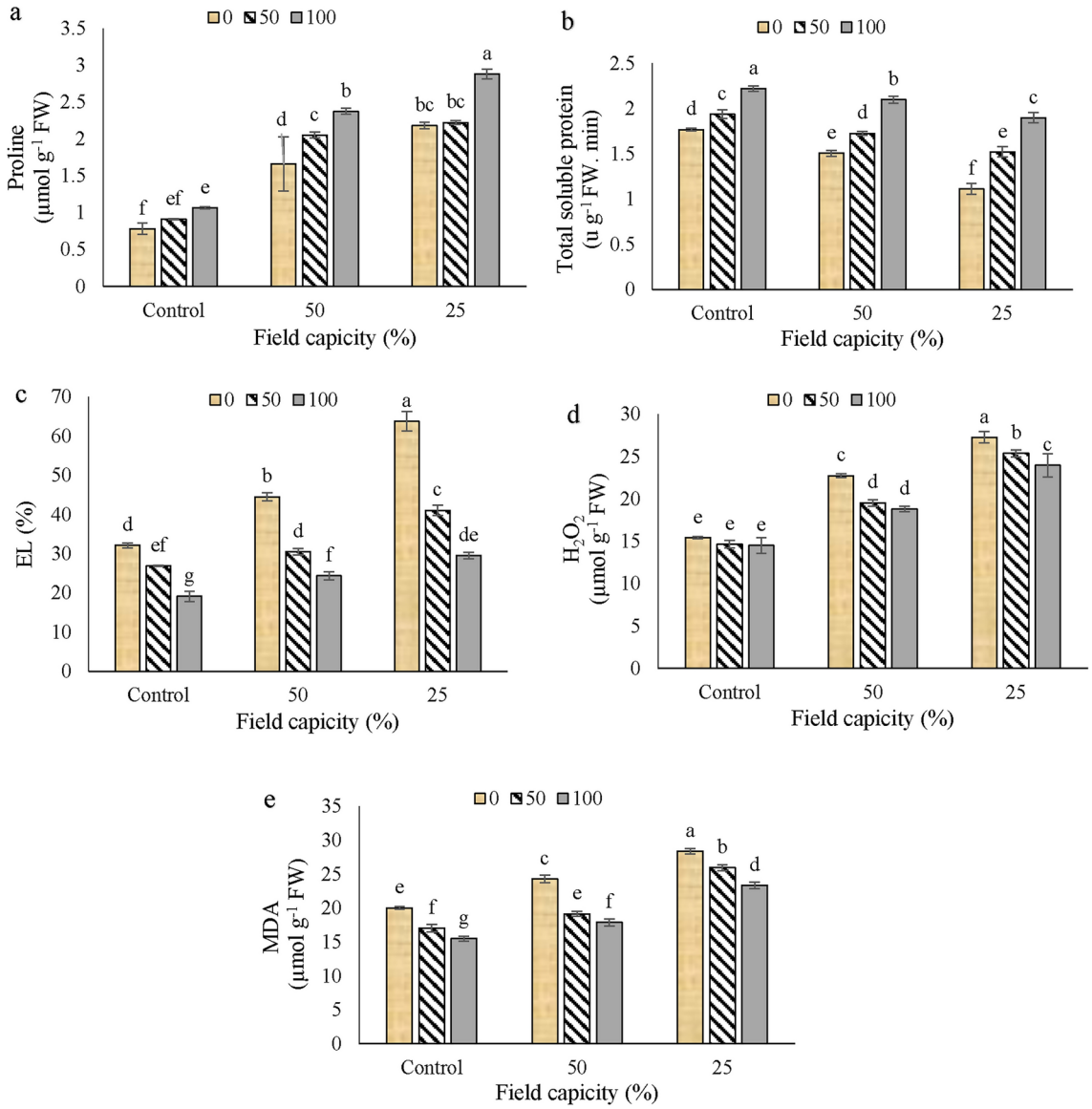

Fig. 3. Effect of CeO_2 NPs on proline (**a**), total soluble protein (**b**), Electrolyte leakage (EL) (**c**), H_2O_2 (**d**) and Malondialdehyde (MDA) (**e**) of apple cv. 'Red Delicious' on M9 rootstock under drought stress. Means followed by the same letter on columns are not significantly different at 0.05 level, according to Duncan's multiple range test. Data are mean ± SD (n = 3 replicates).

nanoparticles in shifting carbohydrate metabolism as a critical determinant of plant resilience under stress, suggesting overlaps with our findings on CeO_2 NPs[89]. A broader comparison with additional recent nanoparticle studies could further validate our observations. For example, nanoparticles such as ZnO and TiO_2 have shown similar effects, including enhanced stress tolerance via ROS detoxification and regulation of osmotic parameters[90]. This is particularly significant in the context of carbohydrate-mediated osmotic balance, as it safeguards photosynthetic mechanisms to a greater extent than other nanoparticles.

The role of carbohydrate dynamics in regulating osmotic balance across different plant tissues, particularly under stress conditions, is a critical aspect of plant physiology that warrants detailed discussion. Carbohydrates, including soluble sugars such as glucose, fructose, and sucrose, play a dual role as both metabolic substrates and osmolytes, contributing significantly to osmotic adjustment and stress tolerance. Under stress conditions, such as drought, salinity, or extreme temperatures, plants often accumulate carbohydrates in various tissues to maintain cellular turgor, stabilize membranes, and protect cellular structures. This osmotic adjustment is essential for sustaining physiological processes, such as photosynthesis, respiration, and nutrient transport, under adverse environmental conditions[91].

In roots, for instance, carbohydrate accumulation helps maintain water uptake by lowering osmotic potential, thereby facilitating continued hydration even under water-deficit conditions. In leaves, sugars act as signaling molecules that modulate stress-responsive pathways, including the synthesis of protective compounds like proline and antioxidants. Additionally, carbohydrates stored in stems and other vegetative tissues serve as reserves

that can be mobilized to support growth and recovery once the stress is alleviated. The dynamic redistribution of carbohydrates across tissues highlights their central role in plant stress adaptation and underscores the importance of understanding these processes in the context of osmotic regulation[92].

Recent studies have shown that the use of nanoparticles has been investigated to modulate carbohydrate metabolism and increase stress tolerance in plants. For example, nanoparticles have been shown to influence sugar metabolism by altering the activity of key enzymes involved in carbohydrate synthesis and degradation. These findings suggest that nanotechnology-based approaches could be leveraged to optimize carbohydrate dynamics and improve osmotic balance under stress conditions. Comparing the current findings with such studies would provide valuable insights into the potential applications of nanoparticles in enhancing plant resilience[89,93,94].

Phenol and flavonoid

The highest phenol content was observed in 50% FC with 100 mg/L CeO_2 NPs and the lowest in untreated control and 25% FC without the treatment (Fig. 3a). The highest flavonoid content was obtained from the stress level of 25% FC with 100 mg/L CeO_2 NPs and the lowest content from the control (Fig. 3b). The application of CeO_2 NPs under drought stress compared to the control increased total phenols and flavonoids by %28.96 and %34.26, respectively (Fig. 4a and b). Phenolics and flavonoids are secondary metabolites that play an essential role in the reaction between cells and their environment and even in the enzymatic activities of plants[88,95]. Total phenol content was also affected by drought stress. Several previous reports on lettuce[96], wheat[97], pepper[98], and kale[99] showed that plants subjected to drought stress conditions had higher and lower TPC depending on the plant, indicating that drought stress has a different effect on the plant genotype. Kalisz et al.[100] found that the foliar application of cerium oxide increased phenols and flavonoids.

Antioxidant system

Drought stress increased the activity of antioxidant enzymes (CAT, APX, GPX, and SOD). The CAT activity was markedly influenced by both drought stress and CeO2 nanoparticle treatment. The peak CAT activity was seen at a stress level of 25% FC and a concentration of 100 mg/L CeO_2 NPs. The use of CeO_2 nanoparticles at concentrations of 50 and 100 mg/L enhanced its efficacy under maximum drought stress by 0.4 and 1.9 times, respectively (Fig. 5a). The APX activity elevated in response to drought stress. The use of CeO_2 nanoparticles enhanced its efficacy under both stress and control settings. The enzyme exhibited peak activity at 25% FC with 100 mg of CeO_2 nanoparticles, demonstrating an 82% increase in activity relative to the control (Fig. 5b). The activity of guaiacol peroxidase increased during drought stress. The incorporation of CeO_2 nanoparticles enhanced its activity. The peak activity was recorded at 25% FC with 100 mg of CeO_2 nanoparticles, demonstrating a 68% increase in activity relative to the control (Fig. 5c). The SOD activity elevated in response to drought stress. The peak activity occurred at a CeO_2 NPs concentration of 100 mg/L with stress levels of 50% and 25% field capacity (FC), and at a concentration of 50 mg/L with a 50% FC level, while the untreated control exhibited the lowest activity. The maximum SOD activity rose by 26% under stress with CeO_2 NPs foliar application compared to the control (Fig. 5d). Water deficit can increase ROS in many plants and lead to oxidative stress. Plants can protect themselves against drought damage by increasing the activity of antioxidant enzymes, such as catalase, ascorbate peroxidase, and peroxidase. There is an internal ROS scavenging system in plants that reduces oxidative damage, thus ensuring normal cellular function[89,101]. Several studies have shown that CeO_2 NPs induce ROS release and interfere with plant antioxidant defense mechanisms. In addition, nanoceria has enzyme-like activity and induces antioxidant and oxidant effects in plants, therefore it is considered a nanoenzyme for plant abiotic stress tolerance[102].

When plants are exposed to water deficiency, it is necessary to activate the defense system to resist ROS damage. The activity of antioxidant enzymes, such as SOD, CAT, ascorbate peroxidase, and peroxidase, also increases under different environmental stresses[67]. Drought stress increases the production and accumulation

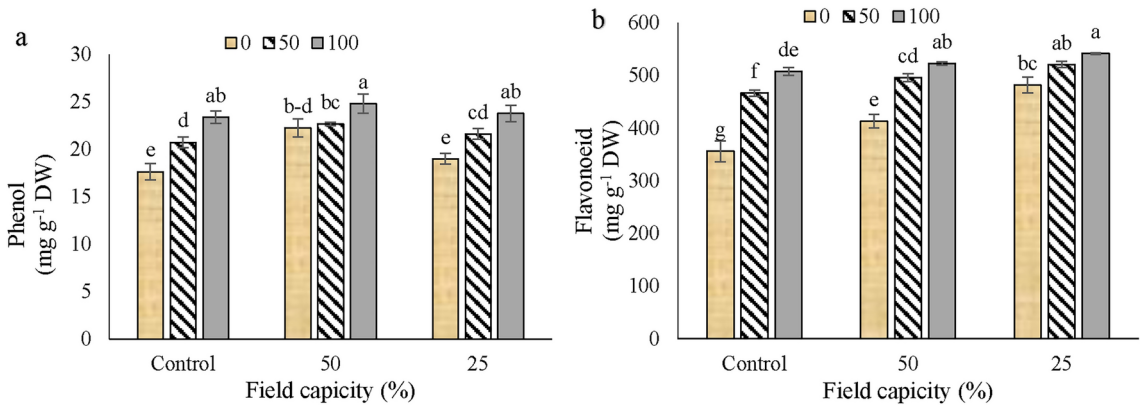

Fig. 4. Effect of CeO_2 NPs on phenol (**a**) and flavonoid (**b**) of apple cv. 'Red Delicious' on M9 rootstock under drought stress. Means followed by the same letter on columns are not significantly different at 0.05 level, according to Duncan's multiple range test. Data are mean ± SD (n = 3 replicates).

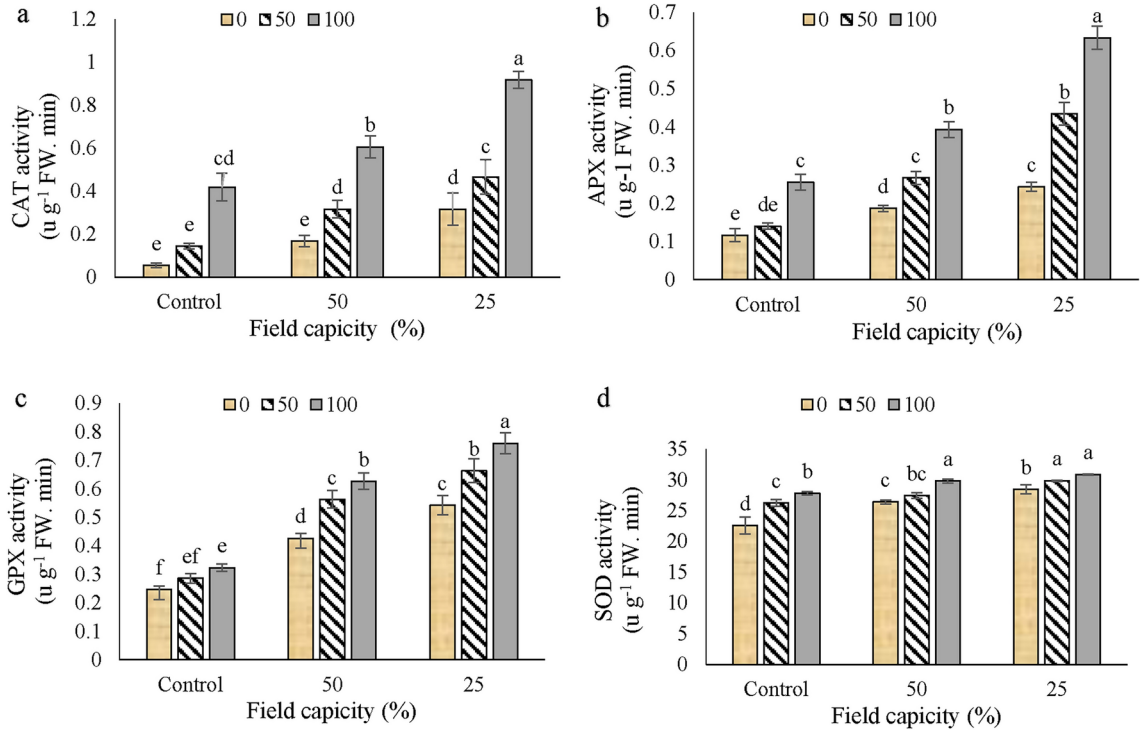

Fig. 5. Effect of CeO_2 NPs on CAT activity (**a**), APX activity (**b**), GPX activity (**c**) and SOD activity (**d**) of apple cv. 'Red Delicious' on M9 rootstock under drought stress. Means followed by the same letter on columns are not significantly different at 0.05 level, according to Duncan's multiple range test. Data are mean ± SD (n = 3 replicates).

of O^{2-} and H_2O_2 and reduces the activity of antioxidant enzymes. The effect of foliar application of FeO NPs under salt stress was studied on some plants. The results showed higher activity of four antioxidant enzymes (CAT, GPX, GR, and APX) in the shoots and roots of plants induced by FeO NPs, thus helping to neutralize the negative effects of stress[102].

Nanoceria converts O^{2-} to H_2O_2 with higher efficiency than SOD. The surface area of nanoceria in relation to its volume alternates between Ce^{4+} and Ce^{3+} oxidation states with high redox capacity, which can scavenge ROS produced under drought conditions[9,103]. The application of nanoceria increased the regulation of *SOD*, *POD*, *CAT*, *GSH*, and *ASA* genes[104]. In cotton, RNAseq data showed that nanoseria mainly regulated *POD* (peroxidases) and *GST* (glutathione S-transferases) genes to improve tolerance to salt stress[105]. In this study, the content of CAT, ascorbate peroxidase, guaiacol peroxidase, and SOD increased with increasing the concentration of CeO_2 NPs, which indicates the antioxidant defense system in apples. The application of CeO_2 NPs, in addition to scavenging ROS, modulated the antioxidant system of apple plants and helped to maintain ROS homeostasis, which indicates that the application of CeO_2 NPs is a positive stimulation but does not pose plants to stress.

Correlation coefficient

Pearson's correlations of morphological, biochemical, and antioxidant traits are exhibited in Fig. 6. The results revealed that photosynthesis pigments, root Mg, root K, Zn, K, Fe, and Mn, shoot Mg, and Zn, Fv, Fv/Fm, Y (NO), RWC, total soluble protein content, LWC, RWL correlated with each other positively, while they showed negative correlations with H_2O_2, MDA, F0, EL, and WSD. Also, a positive significant correlation was noted among phenol, IWC, SOD, flavonoids, Y (II), GPX, proline, APX, and CAT. On the other hand, these traits had negative significant correlations with ELWL, LWL, and Fm. The results confirmed that the traits such as photosynthesis pigments, root Mg, root K, Zn, K, Fe, and Mn, shoot Mg, and Zn, Fv, Fv/Fm, Y (NO), RWC, total soluble protein content, LWC, RWL improved with CeO_2 NPs application under drought stress, while the other characteristics for example H_2O_2, MDA, F0, EL, and WSD increased under drought stress and can be a helpful marker for the water deficit evaluation. RWC showed a meaningful negative correlation with EL.

The biplot of variables classified the traits into three groups. Class I contained the root Mg, root K, Zn, K, Fe, Mg, and Mn, shoot Mg, Fe, Mn, and Zn, photosynthesis pigments, protein, ETR, Y (No), RWL, LWC, Fv/Fm, Fv, Fm, LWL, and RWC. Class II contained phenol, flavonoids, Y (II), CAT, APX, SOD, ELER, IWC, GPX, and proline. Finally, class III included H_2O_2, MDA, WSD, F0, EL, and ELWL (Fig. 7).

The Pearson correlation analysis revealed that IAA and ABA have significantly correlated with each other, while GA3 had a negative correlation with ABA, although this negative correlation was low and non-significant. Also, the genes of *DREB 1A* and *DREB 1E* had a positive significant correlation with IAA and ABA content, but these genes negatively correlated with GA3 content. Finally, a significant positive correlation has been shown between the genes of *DREB 1A* and *DREB 1E*. (Fig. 8).

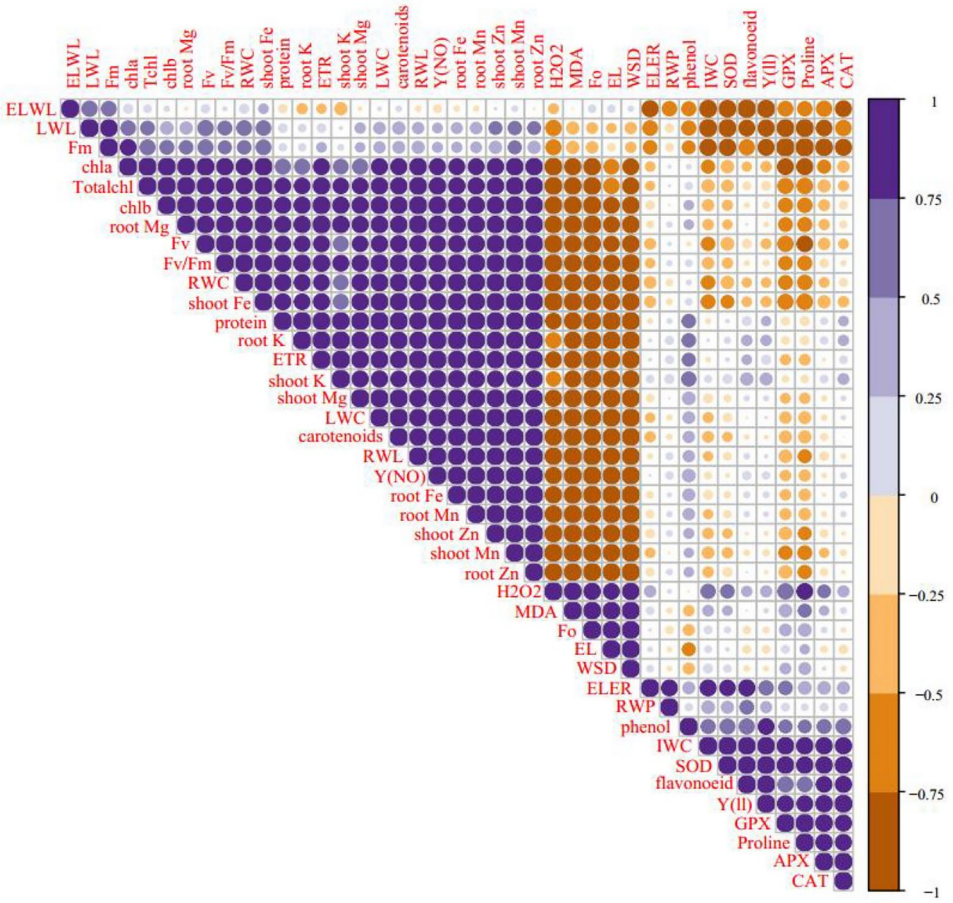

Fig. 6. Heat map Pearson correlation analysis of the studied attributes included CAT (Catalase activity), APX (Ascorbate peroxidase activity), proline, GPX (Guaiacol peroxidase activity), Y (II) (Effective photochemical quantum yield of photosystem II), flavonoeid, SOD (Superoxide dismutase activity), IWC (Initial Water Content), Phenol, RWP (Rlative Water Protective), ELWR (Excised Leaf Water Retention), WSD (Water Saturation Deficit), EL (Electrolyte leakage), F0 (Minimal fluorescence), MDA (Malondialdehyde), H_2O_2, Root Zn, Shoot Mn, Shoot Zn, Root Mn, Root Fe, Y (NO) (Quantum yield of non-regulated non-photochemical), RWL (Relative Water Loss), Carotenoids, LWC (Leaf Water Content), Shoot Mg, Shoot K, ETR (Electron transport rate), Root K, protein (Total soluble protein), Shoot Fe, RWC (Relative Water Content), Fv/Fm (The ratio of variable fluorescence to maximal fluorescence), Fv, Root Mg, Chlb (chlorophyll b), Totalchl (Total chlorophyll), Chla (Chlorophyll a), Fm (Maximal fluorescence), LWL (Leaf Water Loss), ELWL (Excised Leaf Water Loss).

Abscisic acid, Indole-3-acetic acid and gibberellic acid content

The concentration of abscisic acid rose with heightened drought stress. The maximum concentration of abscisic acid was achieved at 25% field capacity with foliar application of 100 mg/L CeO_2 nanoparticles. The little content was noted in the control group. Elevating the concentration of CeO_2 nanoparticles increased the levels of abscisic acid. The foliar spray of 100 mg/L CeO_2 nanoparticles at 25% field capacity elevated the abscisic acid content by 62% relative to the control (Fig. 9a). Intense drought stress elevated the levels of IAA. The maximum concentration of IAA was associated with 25% FC stress after foliar application of 100 mg/L CeO_2 nanoparticles. The IAA concentration in FC 25% with 100 mg/liter CeO_2 nanoparticles rose by 1.7 times relative to the control (Fig. 9b). The GA3 content decreased under drought stress, with a reduction that was 50% greater in the 50% FC treatment. The maximum concentration of GA3 was recorded in the treatment with 100 mg/L CeO_2 nanoparticles in non-drought conditions, whereas the lowest concentration was noted in the FC 50% treatment without foliar application. The GA3 concentration in the FC 50% treatment without foliar application exhibited a reduction of 29.6% relative to the control (Fig. 9c). A decrease in water potential is associated with the accumulation of leaf ABA[106]. M9 root showed a high concentration of ABA in basal and induced conditions. M9 shortening rootstock seems to have better tolerance to drought stress due to higher ABA. In a study, dwarf rootstocks showed more drought tolerance than their strong counterparts, such as MM111[107]. In a study, it was shown that the dwarf rootstock of peach had a lower tolerance capacity than the strong rootstock, which does not always correlate with tree size and plant tolerance. Tolerance to drought stress under ABA can be associated with negative effects on tree productivity, causing senescence and leaf fall[15]. The expression of SnRK, DREB, ERD, and MYC2 genes

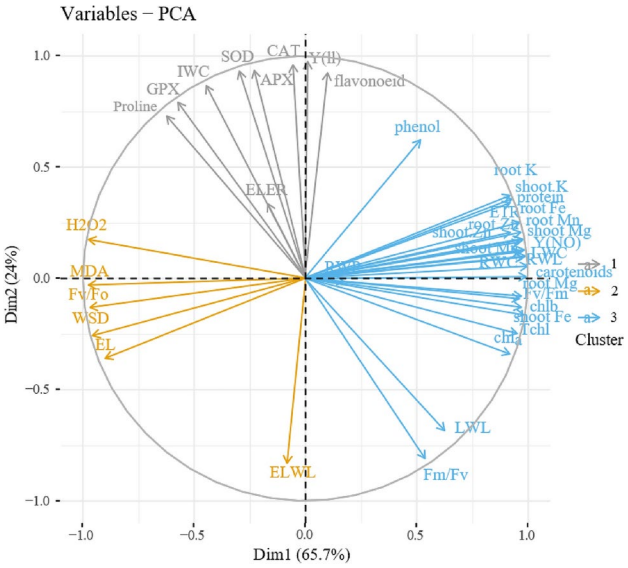

Fig. 7. The loding biplot of analysis of the studied attributes included CAT (Catalase activity), APX (Ascorbate peroxidase activity), proline, GPX (Guaiacol peroxidase activity), Y (II) (Effective photochemical quantum yield of photosystem II), flavonoeid, SOD (Superoxide dismutase activity), IWC (Initial Water Content), Phenol, RWP (Rlative Water Protective), ELWR (Excised Leaf Water Retention), WSD (Water Saturation Deficit), EL (Electrolyte leakage), F0 (Minimal fluorescence), MDA (Malondialdehyde), H_2O_2, Root Zn, Shoot Mn, Shoot Zn, Root Mn, Root Fe, Y (NO) (Quantum yield of non-regulated non-photochemical), RWL (Relative Water Loss), Carotenoids, LWC (Leaf Water Content), Shoot Mg, Shoot K, ETR (Electron transport rate), Root K, protein (Total soluble protein), Shoot Fe, RWC (Relative Water Content), Fv/Fm (The ratio of variable fluorescence to maximal fluorescence), Fv, Root Mg, Chlb (chlorophyll b), Totalchl (Total chlorophyll), Chla (Chlorophyll a), Fm (Maximal fluorescence), LWL (Leaf Water Loss), ELWL (Excised Leaf Water Loss).

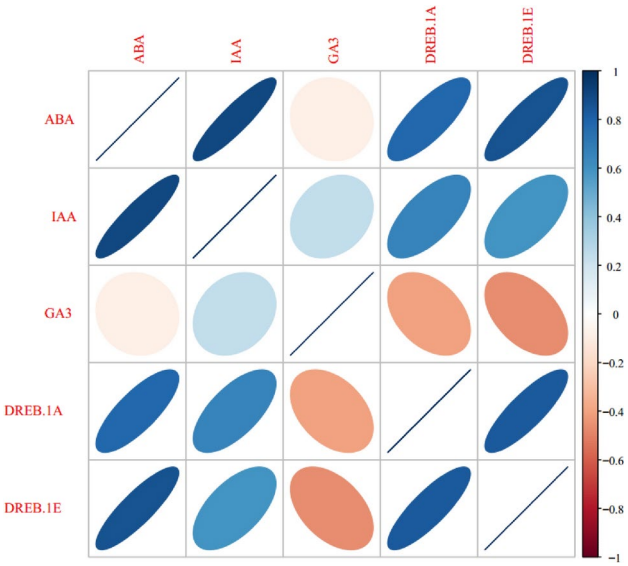

Fig. 8. The pearson correlation analysis between the genes of DREB 1A and DREB 1E with ABA, IAA and GA3 content.

in apple rootstocks with higher ABA levels showed a higher regulation. In addition to the positive role of these genes in improving the tolerance of apple rootstocks, it shows the vital role of ABA as a factor[108]. Foliar spraying with CeO_2 NPs under drought stress and control conditions increased ABA concentration in apples. In a study, an increase in ABA in strawberry plants treated with Se-NPs increased the root biomass and RWC, as well as the overall plant growth under salt stress[109]. Endogenous hormones are necessary to increase tolerance to environmental stresses in plants. IAA acts as a growth hormone and is also involved in the response to abiotic stress in plants. It plays an important role in root water transport as a regulator under drought stress, and IAA

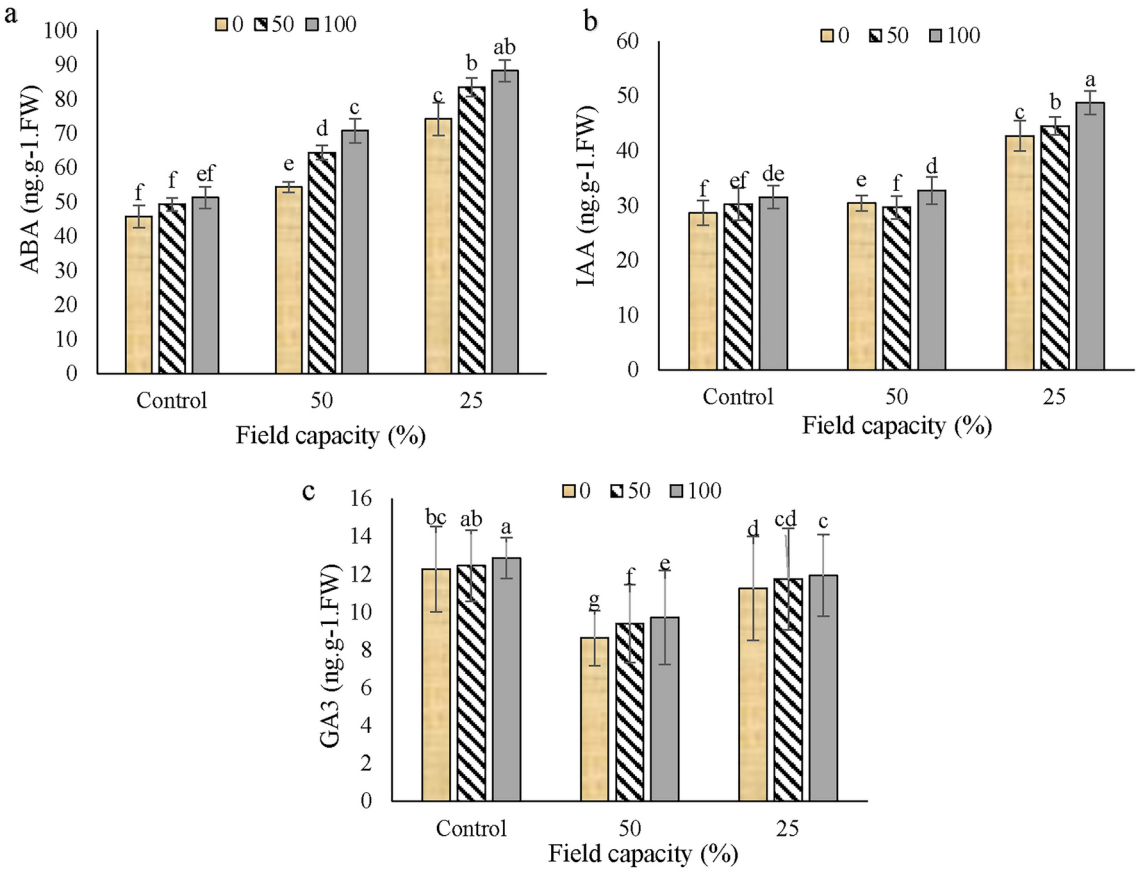

Fig. 9. Effect of CeO_2 NPs on Abscisic acid (ABA) (**a**), Indole-3-acetic acid (IAA) (**b**) and Gibberellic acid (GA3) (**c**) of apple cv. 'Red Delicious' on M9 rootstock under drought stress. Means followed by the same letter on columns are not significantly different at 0.05 level, according to Duncan's multiple range test. Data are mean ± SD (n = 3 replicates).

accumulation can improve plant drought tolerance[110]. Application of exogenous brassinosteroids can increase IAA concentration in wheat and cucumber seedlings under stress conditions[111,112]. We also found that CeO_2 NPs treatment significantly increased IAA concentration in leaves, which may contribute to growth protection. IAA regulates vascular tissue growth and cell elongation, and increases root biomass, which contributes to efficient uptake of water and nutrients under stress[113]. Melatonin was shown to positively regulate ABA and IAA contents to help maintain the growth and survival of olive seedlings under saline conditions[114]. Phytohormones can act as regulators of plant growth, development, and response to water deficit. This is an important ability to reduce water loss by inducing stomatal closure, improving antioxidant capacity, and regulating photosynthesis and stress response genes[115]. A large number of transcription factors belonging to the DREB/CBF subfamily have been reported that, when overexpressed under the control of strong constitutive stimuli, increase the durability of transgenic plants by regulating stress-responsive downstream genes. However, constitutive overexpression of stress-related regulatory genes often results in severe growth retardation and/or reduced seed yield under natural growth conditions[116].

Gene expression

DREB1A gene expression increased with increasing drought stress. The peak expression of the *DREB1A* gene occurred at 25% FC after the foliar application of 100 mg/L CeO_2 nanoparticles. The minimal expression of the *DREB1A* gene was recorded under the control condition with 50 mg/L nano CeO_2. The peak expression of the *DREB1A* gene exhibited an 84% increase relative to the control. The expression of the *DREB1E* gene escalated with heightened drought stress. The foliar application of CeO_2 nanoparticles enhanced the expression of the *DREB1E* gene at all levels. The peak expression level of the *DREB1E* gene (3.92-fold) was recorded at 25% FC with the application of 100 mg/L CeO_2 nanoparticles by foliar spraying. The minimal expression of the *DREB1E* gene was seen under the control condition with 50 mg/L CeO_2 nanoparticles. The peak expression of the *DREB1E* gene exhibited an 88% increase relative to the control (Fig. 10). One of the important ways to achieve tolerance to multiple stress conditions is the overexpression of transcription factors that control multiple genes from different pathways. The overexpression of several transcription factors of the drought response element in transgenic plants using different stimuli has led to greater tolerance of plants to drought, salt, heat, and freezing stresses. Transcription factors (TFs) play an important role in signal transduction networks that cover

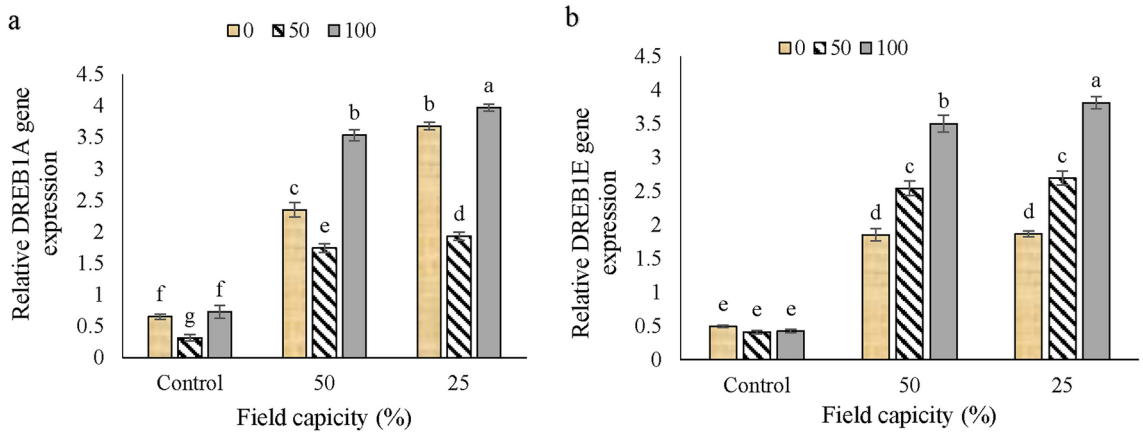

Fig. 10. Effect of CeO_2 NPs on DREB1A (**a**) and DREB1E (**b**) gene expression of apple cv. 'Red Delicious' on M9 rootstock under drought stress. Means followed by the same letter on columns are not significantly different at 0.05 level, according to Duncan's multiple range test. Data are mean ± SD (n = 3 replicates).

the perception of stress signals and the expression of relevant stress-responsive genes. TFs are multifunctional proteins that may simultaneously control multiple pathways during stress in plants. This makes them powerful tools for manipulating regulatory and stress-responsive pathways. Structure–function relationships of many plant TFs involved in drought expression have been elucidated, leading to practical strategies for engineering plants with increased stress tolerance. Extensive data are available on WRKY, MYB, and drought response element binding proteins/C-repeat binding factor (DREB/CBF), shine (SHN), and wax-like production (WXPL) TFs. Consequently, this information is useful in tailoring the design of different TFs, which will enhance our understanding of their functional states, such as post-translational modification patterns, protein–protein interactions, and their abilities to recognize downstream target DNA sequences[117,118].

Genes involved in hormone and osmolyte synthesis have been extensively cloned and studied, along with transcription factors (TFs) involved in hormone biosynthesis pathways and the activation of genes involved in modulating the effects of drought stress. Overexpression of these genes alters the accumulation of hormones and osmolytes and often increases stress tolerance[117,119].

Several reports have implicated TFs in a range of stresses, identifying TFs that regulate upstream or downstream genes. Simultaneously, more than 200 TFs belonging to 20 gene families are shown to be up- or down-regulated by ABA at a single developmental stage, although the molecular mechanisms of individual TFs are not well understood. Many drought-responsive genes are involved in ABA signaling pathways, although reports indicate that some drought-induced genes show no response to ABA signaling. Based on these observations, the pathways leading to plant adaptation to abiotic stresses are divided into ABA-dependent and ABA-independent pathways[118,120].

Transgenic Arabidopsis plants expressing DREB1B/CBF1 or DREB1A/CBF3 under the control of the Cauliflower mosaic virus promoter showed strong tolerance to freezing, drought, and high salinity stresses, suggesting that DREBs/CBFs target multiple genes. Lines overexpressing DREB1A/CBF3 under non-stress conditions accumulate osmotic protectors such as proline and various sugars[117,118]. CBF/DREB proteins are fundamental compounds in the expression of specific genes involved or responsive in abiotic stress tolerance. These proteins are target compounds of the breeding programs for environmental stressors. Otherwise, several studies have claimed that DREB/CBF genes are induced by several stressors and ABA activates a bunch of DREB/CBF genes[118,119]. Seemingly, CBF/DREB genes act as connectors or interaction points of several biosynthetic pathways and simultaneously regulate the tolerance to the stressors like drought, cold, and salinity. Transgenic Arabidopsis lines of DREB1B/CBF1 or DREB1A/CBF3 exhibited reasonable salinity tolerance. It seems that DREBs/CBFs target several genes[120].

The use of nanoceria increases economic productivity in the production of horticultural products by increasing water efficiency. The surface properties of nanoparticles can be used to recover and reuse nitrogen and phosphorus, as well as effectively destroy pathogens in water streams. Nanoceria improved the efficiency of light use in apples. Some plants, especially fruit trees such as apples, which have a more compact crown, use only a few percent of incoming light photons, nano-ceria is justified by increasing the productivity of plants and economical production of nano materials[121]. Nanomaterials such as nanoceria can reduce the efficiency of using nutrients and toxins and thus reduce environmental damage and energy loss. All crops require significant amounts of nitrogen, phosphorus, potassium, and micronutrients such as zinc, copper, and iron to maintain growth. Excessive use of fertilizers and low absorption efficiency (less than 50%) lead to significant release of nutrients and harmful effects on water and surrounding soils. Nanomaterials can improve nutrient utilization efficiency by providing new substrates and pathways for nutrients[9,71,121] In this way, in addition to reducing adverse environmental effects, reducing the use of nano-fertilizers and increasing efficiency will increase production efficiency and increase economic efficiency.

Conclusions

The results showed that photosynthetic pigments decreased in Red Delicious on M9 rootstock apple plants with increasing drought stress, but the content of osmolytes and antioxidant enzymes increased. Drought stress destroyed ion homeostasis and reduced nutrients. In addition, it increased the accumulation of abscisic acid and indole-3-acetic acid, vital stress signaling molecules, and the expression of genes that play a role in regulating various morphological, physiological, and molecular responses of plants to drought stress. The use of CeO_2 NPs in stressed and non-stressed conditions had a positive effect on improving the studied traits of the apple plants and improving nutrient levels. In conclusion, this nanomaterial represents an innovative approach that can be successfully used in apple plants to improve performance under drought stress. However, further validation is needed to determine their effectiveness in other plant species under greenhouse and field conditions.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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Declarations

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