

THE REPAIR EFFECTS OF CERIUM OXIDE NANOPARTICLES ON ENHANCED UV-B RADIATION IN MUNG BEAN (*VIGNA RADIATA*) SEEDLING ROOTS

CHENG SUN^{1,3}, CHEN ZHAO^{2,3} AND RONG HAN^{2,3*}

¹College of Chemistry and Materials Science, Shanxi Normal University, Taiyuan 030000, China

²College of Life Sciences, Shanxi Normal University, Taiyuan 030000, China

³Shanxi Provincial Key Laboratory of Plant Macromolecular Adversity Response, Shanxi Normal University, Taiyuan 030000, China

*Corresponding author's e-mail: hhwrsl@163.com

Abstract

Recently, cerium oxide nanoparticles (CeO₂-NPs) have attracted much attention for their ability to enhance the resistance of plants to withstand multiple abiotic stress such as high salinity and drought. However, few studies have shown whether CeO₂-NPs can enhance plant resistance under UV-B radiation. In this study, mung bean (*Vigna radiata*) seedlings were selected as the research objects to investigate the effects of CeO₂-NPs application on their growth and development, as well as physiological and biochemical indicators. Furthermore, the repair effects of CeO₂-NPs against UV-B radiation damage of mung bean seedlings had been elaborated in terms of morphology, physiology, biochemistry, cell and molecule. The findings of the study indicated that CeO₂-NPs could reduce membrane damage and oxidative damage of taproots under enhanced UV-B radiation, regulate auxin and cytokinin-associated genes, decrease amylase activity and increase starch content. Enhanced UV-B radiation-induced curvature of mung bean taproot was repaired to some extent and the response of taproot to gravity was restored. Thus, CeO₂-NPs could enhance the stress resistance for taproots of mung bean seedlings. These results provide a new way to reduce UV-B stress.

Key words: CeO₂-NPs, UV-B radiation, Oxidative stress, Auxin, Cytokinin, Gene regulation.

Introduction

The continuous depletion of the ozone layer leads to the growing UV-B radiation, posing a serious threat to life on Earth (Yang *et al.*, 2007). As producers of ecosystems, plants are more susceptible to various environmental stress factors in nature, such as enhanced UV-B radiation, heavy metals, drought and temperature, due to their unique sessiness growth mode (Tripathi *et al.*, 2017). Enhanced effects of UV-B radiation on plants involve multiple links such as growth and development, physiology and biochemistry, molecular processes, and even genetics and evolution (Nassour & Ayash, 2021). For example, enhanced UV-B radiation will lead to a decrease in leaf area and damage photosynthesis, disrupt antioxidant defense system, induce changes in cell cycle regulation-related genes, induce different types of damage to nuclear gene DNA, and affect auxin synthesis and distribution (Jiang *et al.*, 2011; Rai & Agrawal, 2017; Alemu & Gebre, 2020; Matthew *et al.*, 2020). This ultimately inhibits plant growth and slows down plant development, resulting in short plants and root length, and abnormal "warping" phenomenon, thereby limiting the increase of plant biomass (Lv *et al.*, 2013). Enhanced UV-B radiation causes plants to produce excess reactive oxygen species (ROS), inducing oxidative damage. When the production of ROS far exceeds the clearance capacity of the antioxidant system, several cellular components (e.g., proteins, carbohydrates, lipids, and DNA) are damaged, inhibiting plant growth and ultimately leads to death (Das & Roychoudhury, 2014).

In order to alleviate the damage of UV-B stress to plants, researchers suggest using nanomaterials to improve their UV-B stress (Soni *et al.*, 2022). Azadi *et al.* found that silver nanoparticles (AgNPs) can alleviate

UV-B stress on garden thyme (*Thymus vulgaris* L.) by improving the growth and increasing yield of plant under UV radiation (Azadi *et al.*, 2021). Nanomaterials have been extensively applied to catalysis, light, electricity, magnetism and other fields since they have various advantages, including spatial dimension (1-100 nm), large specific surface area, high reactivity, outstanding physical, chemical and biological properties, and significantly superior physical and chemical properties compared with conventional materials (Kaneko *et al.*, 2007). Recent studies have confirmed that nanomaterials can improve plant growth, enhance plant resistance under unfavorable conditions, and increase crop yield. This brings new ideas and hopes for the revolution of agricultural technology and sustainable development of agriculture (Zhao *et al.*, 2020).

Among many nanomaterials, CeO₂-NPs can alternate between Ce³⁺ and Ce⁴⁺. Therefore, they have the antioxidant enzyme simulation activity and are considered as powerful scavengers of ROS (Dutta *et al.*, 2006; Walkey *et al.*, 2015). Numerous studies indicate that CeO₂-NPs not only promote plant growth but also simulate antioxidant enzymes to reduce oxidative stress, increase plant resistance and decrease yield loss under abiotic stress (Khan & Upadhyaya, 2019). For example, research has confirmed that CeO₂-NPs exhibit superoxide dismutase (SOD), catalase (CAT) antioxidant activities, which can alleviate *Arabidopsis thaliana* photosynthesis damages caused by high salt (Wu *et al.*, 2018). CeO₂-NPs entered the chloroplast of *Arabidopsis thaliana* and removed ROS caused by abiotic stress, thereby improved PSII quantum yield (Wu *et al.*, 2017). Djanaguiraman *et al.* showed that leaf spraying of CeO₂-NPs could alleviate oxidative damage by lowering the levels of superoxide free radicals, hydrogen peroxide and lipid peroxidation of cell

membrane in sorghum under drought conditions, while increase pollen germination and seed yield per plant (Djanaguiraman *et al.*, 2018).

Despite of numerous studies about effects of UV-B stress on plants and the enhancement of CeO₂-NPs resistance in plants under abiotic stress, it is still unclear whether enhanced UV-B radiation and CeO₂-NPs interact. It is hypothesized that CeO₂-NPs has a potential value in alleviating plant oxidative damage under enhanced UV-B radiation.

Mung beans (*Vigna radiata*) belong to the leguminous plant family. Rich in nutrients such as protein, dietary fiber, minerals and vitamins, mung beans play a vital role in human nutrition and are a good functional alternative food. In addition to nutrition, mung beans also contain a large number of various bioactive compounds, and it is easy to transport and store, which has important economic value. The root system of plants plays an important role in plant growth and development, such as absorbing water and nutrients, transporting them to aboveground organs, secreting certain hormones and organic compounds, and serving as a bridge between plants and their growth environment. Previously, this may have been due to limited availability of root observation, resulting in little understanding of how UV-B affects plant root structure and development at the molecular level (Kul *et al.*, 2020). In summary, the roots of mung bean seedlings were selected as the research object to explore the effect of CeO₂-NPs on their growth, and the possible mechanism by which CeO₂-NPs alleviate enhanced UV-B radiation damage to plants was elucidated.

Material and Method

Characterization of nanomaterials and preparation of suspensions: Nanomaterial CeO₂-NPs have been purchased from Beijing Dekedao Gold Technology Co., LTD. CeO₂-NPs morphological characterization was achieved by transmission electron microscopy (TEM) (FEI Talos F200X, USA). CeO₂-NPs suspensions were prepared in ultra-pure water at the concentrations of 0, 20, 60, 100, 120 and 140 mg/L, and ultrasonic treatment in a 25°C water bath for 1 h.

Cultivation and handling of plant materials: Mung bean (*Vigna radiata* (Linn.) Wilczek) variety Maolu 1, bred by Shanxi Academy of Agricultural Sciences, was adopted in the experiments.

Plant material culture: After disinfecting the healthy seeds with a 5% sodium hypochlorite solution, washed for 10 minutes (with sterile water), later wrapped by wet gauze and leted them germinate in a dark incubator at 25°C for 24 h. Germinated seeds were placed in a gauze-covered petri dish with at least 30 seeds per bowl. The seedlings were planted in a photoperiod (16 h/8 h) environment at 25°C/18°C in a light incubator.

Treatments of CeO₂-NPs and enhanced UV-B: Seedlings exposed to neither CeO₂-NPs nor enhanced

UV-B radiation were considered controls (CK). In this paper, different concentrations of CeO₂-NPs (CK: 0 mg/L, C1:20 mg/L, C2:60 mg/L, C3:100 mg/L, C4:120 mg/L and C5:140 mg/L) were applied to mung bean seedlings to observe their effects. Mung bean seedlings treated with the optimal concentration of CeO₂-NPs solution (120 mg/L) as CeO₂-NPs treatment group (CeO₂-NPs). After the seedlings were grown to 3 days, UV-B radiation (6 KJ/m²) was added by using UV lamp (FLB30T8E/5C, Nanjing Huaqiang Electronics Co., LTD, China). Seedlings in the above groups were halved for 3 consecutive days of enhanced UV-B irradiation treatment group (UV-B) and CeO₂-NPs combined with enhanced UV-B treatment group (CeO₂-NPs+UV-B).

Determination of plant growth index: More than 30 mung bean seedlings were randomly selected from the control group and treatment group for phenotypic index determination. Plant height and root length were measured using a steel ruler, while fresh and dry weight were measured using an electronic balance.

Determination of Ce by inductively coupled plasma mass spectrometry (ICP-MS): Roots of mung bean seedlings were dried in a 70°C oven for 48 h. After completes drying, they were digested with HNO₃ and H₂O₂ (4:1) at 120°C for 1 h, and then the Ce content in the digestive solution was analyzed by ICP-MS (NexION 300D, Perkin Elmer, USA).

Determination of MDA and antioxidant: Mung bean seedlings taproots cuttings from different treatment groups were mixed and weighed 0.1 g for the determination of physiological and biochemical indices. MDA, enzyme antioxidant (SOD, peroxidase (POD), CAT, ascorbateperoxidase (APX)) and non-enzymatic antioxidants (proline, ascorbic acid, total flavonoids and hydroxyl radical) were measured using biochemical kit (Jiangsu Jingmei Biotechnology Co., Ltd., China) through microplate method.

Transparent staining of plant roots: We selected approximately 1cm of taproots tip of mung bean seedlings, and the dehydration, transparency and magnification were adjusted according to the method used by Shi in the cell tissue of *Arabidopsis thaliana* root system (Shi *et al.*, 2005) to make mung bean root tip transparent and observe the cell structure of it. First, a 4% glutaraldehyde fixing solution was used to fix mung bean seedlings roots overnight at room temperature. After aspirating the fixative, dehydration was performed on different concentration gradients (50%, 65%, 75%, 85%, 95%, and 100%) of alcohol. The dehydrated root tips were kept transparent in a mixture of benzyl benzoate and benzyl alcohol (benzyl benzoate: benzyl carbinol 2:1) for 6-8 h. A confocal laser microscope (FV1000, Olympus, Japan) with excitation wavelength of 488 nm was used to observe the structure of the apical cells, and the focal length was adjusted to the level of the apical middle column for image acquisition.

Determination of auxin and cytokinin: Under a type mirror (Chongqing Aote Optical Instrument Co., LTD, China), mung beans of each treatment were cut along the central axis about 1 cm from the root tip and divided into curved inner side (irradiated surface i) and outer side (non-irradiated surface ni). Auxin and cytokinin contents were determined by high performance liquid chromatograph (LC-20A, Shimadzu, China) according to HPLC method.

Detection of RT-qPCR: Extracted total RNA from fresh 6-day-old root tips (1cm) of each experimental group via the Trizol method, and reversed transcribe them using a reverse transcription kit to obtain cDNA strands. Detection of changes in gene expression using QuantStudio 3 Real-Time PCR System (Applied Biosystems, USA), and further calculation of relative gene expression using Livak *et al.*'s method (Livak and Schmittgen 2001). *Actin* was an internal reference gene. Supplementary Table S1 contains all primer sequences for this experiment.

Table S1. Primer of qRT-PCR.

Gene name	Sequence
<i>ACTIN</i>	F: GGCGGTGTTCCCTAGCATTG
	R: AGCGGTGCCTCGGTAAGAAG
<i>YUC2</i>	F: GGGAGTGACATGTTTCAGTGAGAAAGATG
	R: TCATGCAAGTGGACAAGGAAACTCC
<i>YUC8</i>	F: GGCCAAGATTAGATCCGGTG
	R: CTCACCTGAAGCCAATAAGGC
<i>IAA</i>	F: GTGACAGTGTTCCTCTCTATCC
	R: GTTCTCGTTCCTCAAGACACC
<i>CYCB</i>	F: CTTCTCTCAGTCTCATCCGTTAC
	R: ACACAAACGGCATCAAGATAG
<i>LOG</i>	F: GCAGGTGGGTCTGTTGAATGTG
	R: CCACACCAACTTGGACACAAC

I₂-KI staining: I₂-KI staining was used to observe the changes of starch granule content in plant tissues. Added 3 g of potassium iodide (KI) to 100 mL of distilled water and dissolved. Continue to add 1 g iodine (I₂) to the solution and after I₂ dissolved, dilute the solution 3-5 times to prepare I₂-KI solution. The plant samples in I₂-KI dyeing solution were rinsed with ddH₂O and washed with distilled water. Finally, decolorized the samples with 50% chloral hydrate for 40s. The images were obtained and collected under V16 Motorized Materials Zoom Microscope (Zeiss, Germany). The staining intensity was quantified by Image J software.

Determination of starch content and amylase activity

Starch content determination: Slightly modified from previous studies (Li *et al.*, 2016), the absorbance was measured using an ultraviolet spectrophotometer (UV-29100 UV-vis, Varian Inc., CA, USA) to calculate the starch content of mung bean taproot.

Amylase activity determination: According to the principle that amylase can catalyze the hydrolysis of starch to produce reducing sugar, we weighed 0.5g of mung bean taproot and grinded them thoroughly in a mortar. Centrifuged them at a speed of 3000r/min for 10 min and aspirated the supernatant. Afterwards, the increase rate of OD value of 540 nm was measured with the help of UV spectrophotometer. The relative maltose content, and the activity of α -amylase and β -amylase were calculated.

Statistical analysis: All experiments were repeated three times. Based on Newman-Keuls multiple comparison test, one-way variance analysis ($p \leq 0.05$) was used to analyze the experimental data (GraphPad Prism 5).

Results and Discussion

Characterization of CeO₂-NPs: The TEM image clearly shows that CeO₂-NPs were mostly spherical with a particle size of about 20nm (Fig. 1).

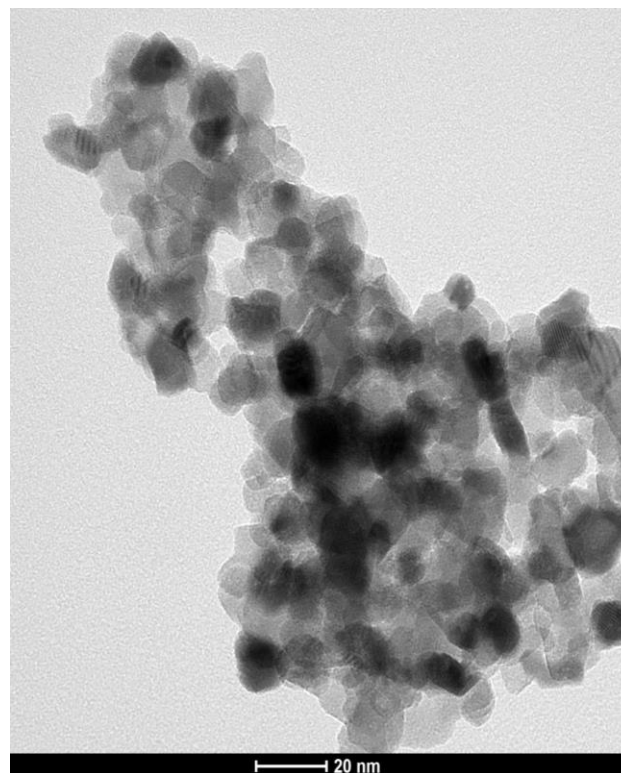


Fig. 1. TEM image of CeO₂-NPs (20nm).

Effect of CeO₂-NPs on mung bean seedlings growth:

The phenotype diagram of 6 days old mung bean seedlings indicated that CeO₂-NPs treatment promotes mung bean seedlings growth to different degrees compared to the CK group (Fig. 2a). Further statistical analysis of the root length and plant height of mung beans in each group (Fig. 2b) showed that the root length of C1, C2, C3, C4 and C5 treatment groups was increased significantly by 9%, 12%, 13.4%, 17% and 12.4%, respectively, compared to the CK group. In addition, the plant height of C1, C2, C3 and C4 mung bean seedlings were increased by 1.7%, 6.6%, 7.5% and 14%, respectively, while there was no significant change in C5 (Fig. 2c). Rico *et al.* indicated that CeO₂-NPs treatment promoted the growth of wheat plants and increased aboveground biomass and yield (Rico *et al.*, 2014). Other studies have also confirmed that CeO₂-NPs significantly promotes root growth in cucumber and maize (Lopez-Moreno *et al.*, 2010). In summary, it can be concluded that CeO₂-NPs can promote the root growth of mung bean seedlings, and there is a concentration response relationship among different concentrations of CeO₂-NPs. This stems from the fact that CeO₂-NPs decrease the oxidative stress of plants during the growth period,

allowing for increased root elongation. It is also possible that the nanomaterials entering plants change the content of plant hormone analogues, thus promoting plant growth (Lopez-Moreno *et al.*, 2010; Li *et al.*, 2019). Finally, CeO₂-NPs with the most significant concentration (120 mg/L) in promoting root length were selected for the follow-up studies.

Effect of enhanced UV-B radiation on mung bean seedlings growth: In this experiment, enhanced UV-B was used to treat mung beans with radiation. Compared with the CK group, mung bean seedlings in the UV-B group showed serious damage, and their root growth was significantly inhibited, and root development was stunted and shortened and severely browned (Fig. 3). The statistical results of root length and plant height in the UV-B group showed a reduction of 29.3% and 24%, respectively, compared to the CK group. The fresh weight and dry weight of the above-ground part were reduced by 16.1% and 12.9%, and those of the underground part were decreased by 26.1% and 25.4%, respectively (Fig. 4).

This is consistent with the phenomenon observed by Zhang *et al.* in soybeans, where root related growth parameters such as root length, root dry weight, and root surface area were reduced under UV-B stress (Zhang *et al.*, 2019). Numerous studies have demonstrated that UV-B radiation inhibits plant growth and reduces biomass accumulation (Kumari *et al.*, 2009; Chen *et al.*, 2014; Nassour & Ayash, 2021). In addition, the taproot of mung bean seedlings lost its geotropism and bent towards the side of UV-B radiation source, resulting in an abnormal "warped root" phenomenon.

Under enhanced UV-B radiation, CeO₂-NPs treatment resulted in a decrease of 0.9% and 20% in root length and plant height of mung beans compared to the control group, a decrease of 10.2% and 7% in fresh and dry weight of aboveground parts, and a decrease of 14.5% and 11.7% in fresh and dry weight of underground parts, respectively (Fig. 4). It is easy to notice that compared to the UV-B group, the addition of CeO₂-NPs increase the root length, fresh and dry weight of mung beans and reduce their "warped root" phenomenon, while do not significantly alleviate the inhibition of plant height. CeO₂-NPs can effectively alleviate the damage on mung bean seedlings caused by UV-B radiation.

Absorption of CeO₂-NPs in taproot of mung bean seedlings: In order to verify whether the added CeO₂-NPs can be absorbed by mung bean, the Ce content in the taproot of mung bean in different treatment groups was determined. The Ce content taproot of mung bean was significantly increased compared to the CK group and UV-B groups, whether in the CeO₂-NPs treated alone or in the CeO₂-NPs+UV-B group (Fig. 5). Whether CeO₂-NPs can be absorbed by plants and transported within their bodies are species dependent. Previous root division methods by Ma *et al.* showed that CeO₂-NPs could be transported from root to aboveground through plant xylem, then transferred from aboveground to root through phloem, and may be discharged into the environment through root tips (Ma *et al.*, 2017). Schwabe

et al., confirmed that CeO₂-NPs could be transferred from the stem of pumpkin to the bud, while in wheat, CeO₂-NPs firmly adhered to the surface of its roots (Schwabe *et al.*, 2013). This study also confirmed that CeO₂-NPs can be absorbed by the roots of plants.

Determination of MDA in the taproot of mung bean seedlings: Malondialdehyde (MDA) is often regarded as a marker of ROS in plants (Lidon & Ramalho, 2011). The higher the content, the more serious the damage of plant membrane lipid. According to Fig. 6, there is no obvious difference between the MDA content of seedlings treated with CeO₂-NPs alone and the control group. The MDA content of mung bean seedlings was significantly increased in the UV-B group, indicating that the cell membrane of mung bean was seriously damaged. This is consistent with the research findings of Dai *et al.* that UV-B radiation causes damage to rice cells, accompanied by an increase in MDA and hydrogen peroxide (H₂O₂) concentrations (Dai *et al.*, 1997). Compared with the UV-B group, the MDA content in mung bean seedlings significantly decreased in the CeO₂-NPs+UV-B group, indicating a reduction in damage to mung bean root cells.

Changes in antioxidants in the taproot of mung bean seedlings: As active oxidants in plant cells, the ROS family mainly members mainly include H₂O₂, superoxide anions (O₂^{•-}), and hydroxide anion radicals (•OH). Excessive ROS will cause oxidative stress to plants, so a balance between ROS production and removal is crucial (Huang *et al.*, 2019). Enhanced UV-B radiation induces excessive ROS production in plants (Mahdavian *et al.*, 2008). Plants have developed multiple antioxidants to deal with ROS induced by UV radiation stress. These antioxidants include antioxidant enzymes represented by SOD, CAT, POD, APX, glutathione reductase (GR), and non-enzymatic systems (including flavonoids, ascorbic acid (AA), ascorbate peroxidase (AsA), reduced glutathione (GSH), and proline (Pro)) (Frohmeyer & Staiger, 2003; Jansen *et al.*, 2008; Kurdziel *et al.*, 2018).

Various antioxidants in the taproots of mung bean seedlings were determined. As can be seen from the results of antioxidant enzymes detection in Fig. 7, there are not significant differences in SOD, POD and CAT activities of mung bean root between the CeO₂-NPs and the CK group. UV-B treatment increased the activities of SOD, POD, CAT, and APX in mung bean seedling taproots by 31.8%, 30%, 58%, and 37%, respectively, compared to the CK group. Compared with the UV-B group, the antioxidant activity of mung bean roots in the CeO₂-NPs+UV-B group was significantly decreased. The determination results of non-enzymatic antioxidants in Fig. 8 indicated that UV-B radiation treatment significantly increased Pro and total flavonoid content by 71.3% and 42.4%, respectively, compared to the CK group, while AsA content was decreased by about 34.2%. CeO₂-NPs alone did not significantly affect non-enzymatic antioxidants. The Pro and total flavonoid content in the CeO₂-NPs+UV-B group was decreased by 16.3% and 15.6%, respectively, while the AsA content significantly increased by 36% compared with the UV-B group.

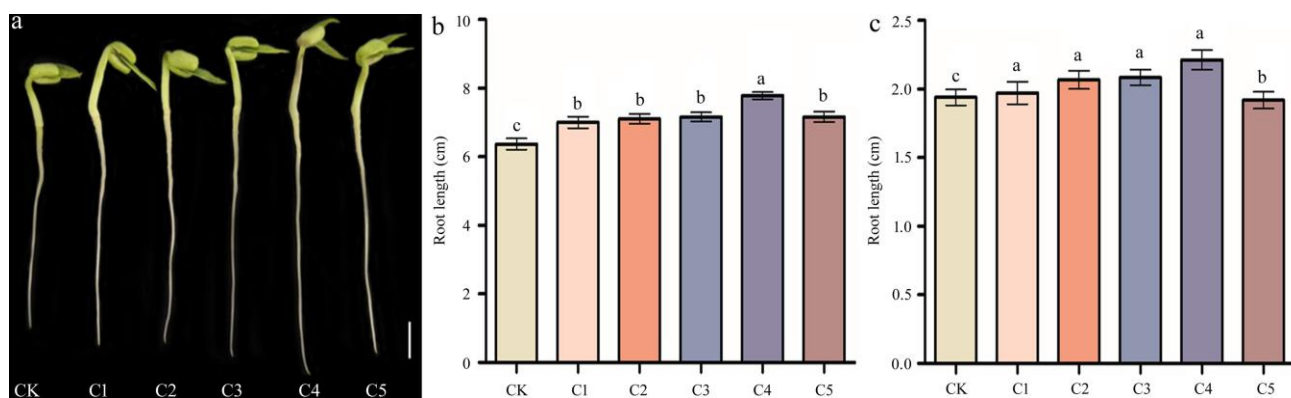


Fig. 2. Effect of CeO₂-NPs on mung bean seedlings growth: a, Phenotypic map; b, root length; c, plant height; Different letters represent significant differences in mung bean roots between groups ($p < 0.05$). Scale bar: 1 cm.



Fig. 3. Growth morphology of mung bean seedlings under different treatments.

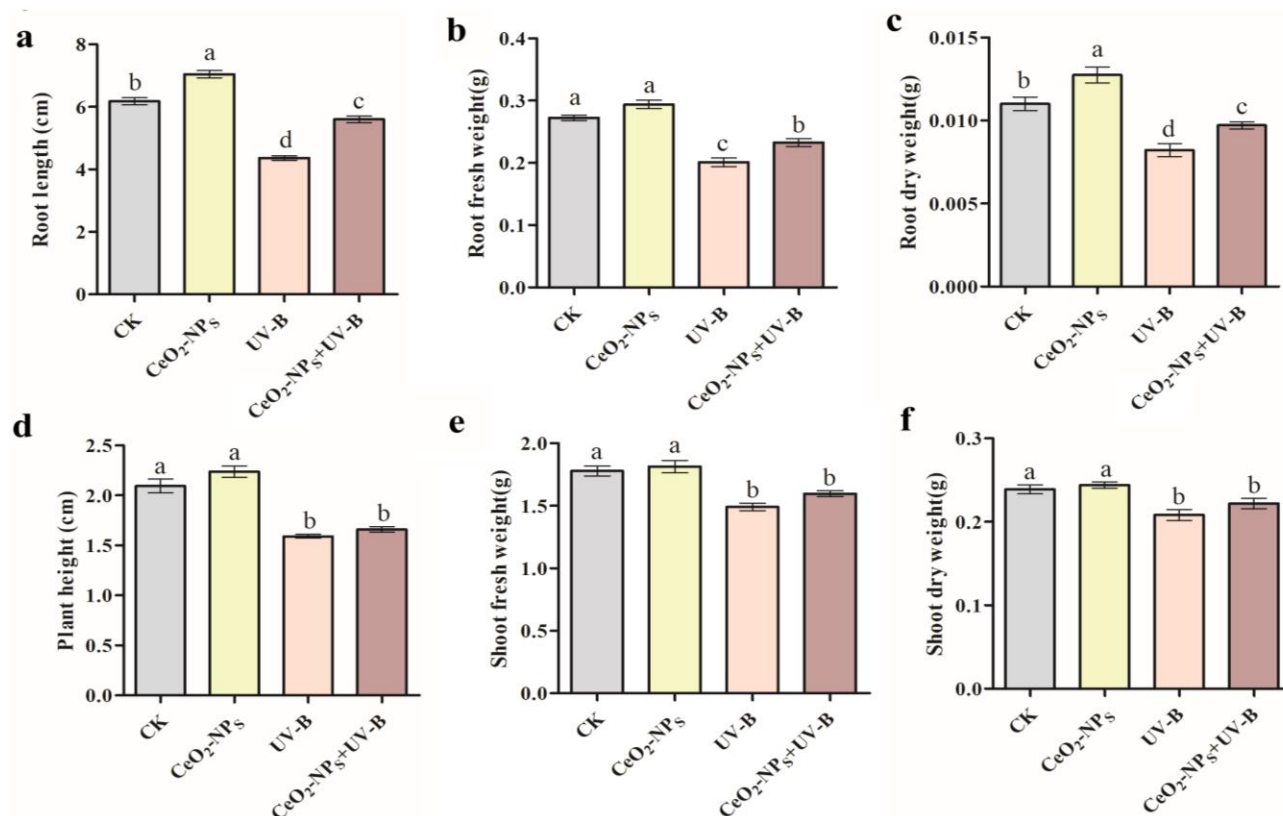


Fig. 4. Physiological indicators of mung beans: a, root length; b, fresh weight of the underground portion; c, dry weight of the underground portion; d, plant height; e, fresh weight of the above-ground portion; f, dry weight of the above-ground portion. Different letters represent significant differences in mung bean roots between treatments ($p < 0.05$). Scale bar: 1 cm.

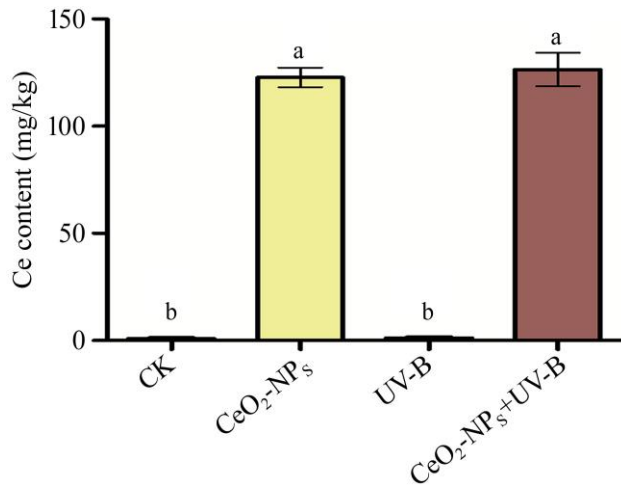


Fig. 5. The content of Ce in mung beans. Different letters represent significant differences in mung bean roots between treatments ($p < 0.05$).

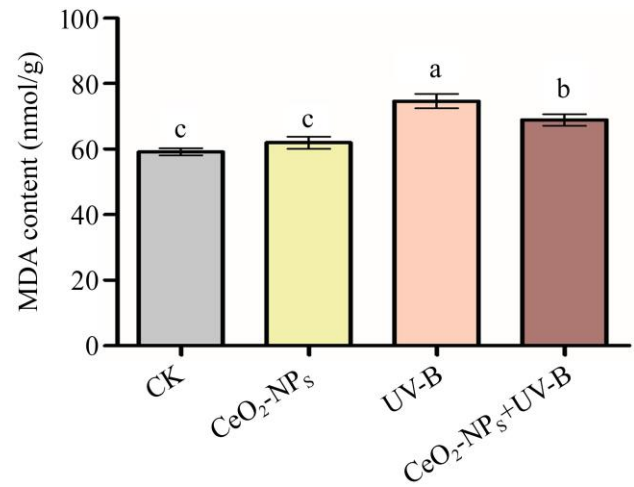


Fig. 6. The MDA content of mung beans. Different letters represent significant differences in mung bean roots between treatments ($p < 0.05$).

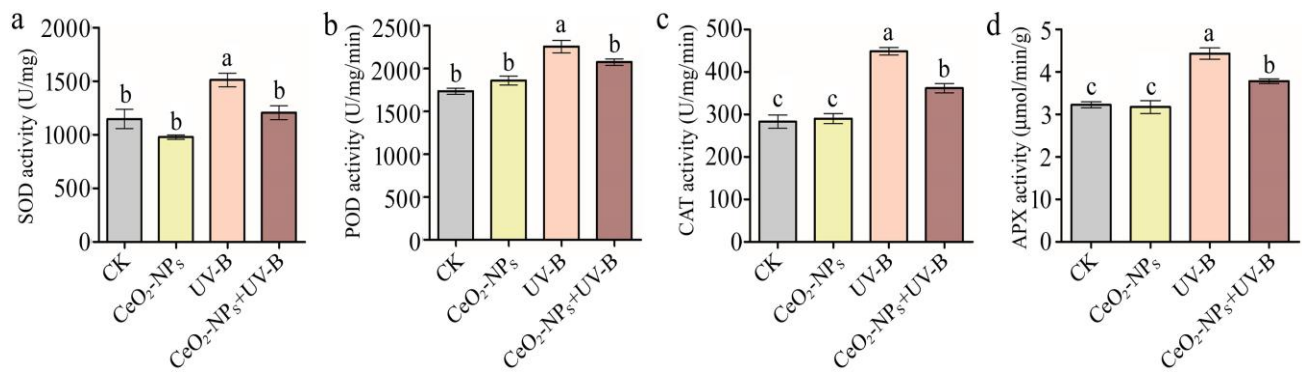


Fig. 7. Changes in antioxidant enzyme activities in mung bean seedlings: a, SOD enzyme; b, POD enzyme; c, CAT enzyme; d, APX enzyme. Different letters represent significant differences in mung bean roots between treatments ($p < 0.05$).

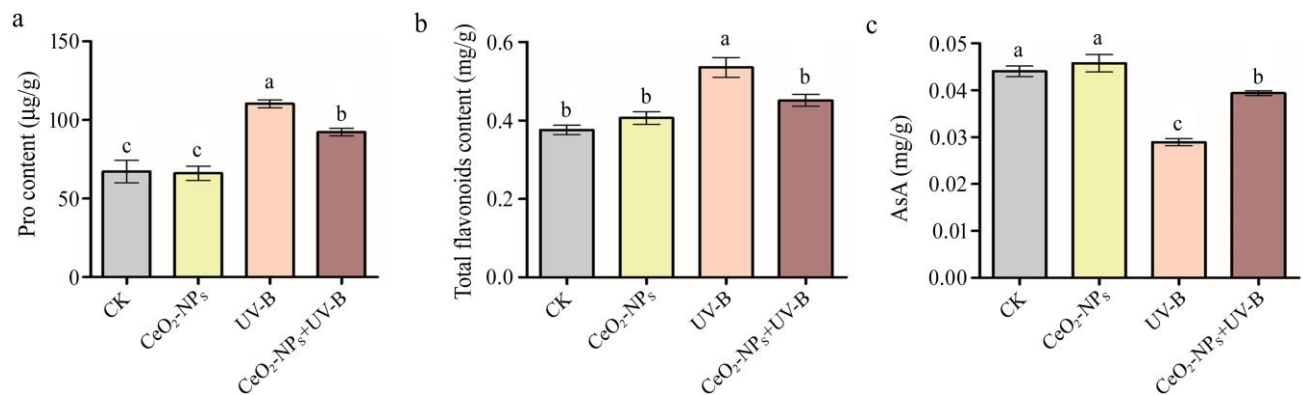


Fig. 8. Changes in non-enzymatic antioxidant system in mung bean seedlings: a, Pro content; b, Total flavonoids content; c, AsA content. Different letters represent significant differences in mung bean roots between treatments ($p < 0.05$).

Usually, the antioxidant response exhibited by exposure to UV radiation varied among plant species. Kurdalle *et al* confirmed that SOD, APX, GR activities in wheat, oat and barley seed embryos were increased under UV treatment, while SOD activity in endosperm was decreased (Kurdziel *et al.*, 2018). Olivastra Seggiane plants showed increased SOD activity, decreased GPX activity, and high flavonoid levels under UV-B treatment (Piccini *et al.*, 2021). Current work reveals that enhanced UV-B radiation can enhance various antioxidants activities

in the Mung bean seedlings taproots. This may be a stress response of plants to resist the damage of reactive oxygen species. Rikabad *et al* showed that titanium dioxide (TiO₂) nanoparticles could enhance the antioxidant activity of the stigma, increase total flavonoids and phenolic compounds contents, hereby helping soybeans improve their resistance to UV-B stress (Rikabad *et al.*, 2019). Here, the treatment with CeO₂-NPs also alleviated the oxidative stress of plants under UV-B radiation. We speculate that after CeO₂-NPs enter the plant body, they still exert enzymatic activity and

accelerate the removal of free radicals. CeO₂-NPs significantly alleviate oxidative damage in plants under UV-B radiation by reducing antioxidant substances activities and alleviating antioxidant reactions.

Morphology and structure of taproot cells in mung bean seedlings: In order to investigate the morphology changes of mung bean taproot cells under different treatments, 1cm of mung bean taproot tip was selected for transparency treatment and observed by confocal laser microscope. As shown in Fig. 9, whether in the CK group or the CeO₂-NPs group, the cell structure of mung bean taproot was complete and clear, and the cells on both sides were arranged neatly, with almost no difference in number and size. Under enhanced UV-B radiation, mung bean taproot tip curved towards UV-B radiation, and the number and size of cells on both sides of the root were significantly different. This is consistent with the phenomenon observed by Rong *et al.*, that UV-B radiation induces temporary loss of gravitropism in plants and causes the roots of plants to bend towards the radiation source (Rong *et al.*, 2007; Wan *et al.*, 2018). Compared with the UV-B group, CeO₂-NPs treatment repaired the cell structure on both sides of the root, and significantly alleviated the "warping" phenomenon of the taproot of mung bean without obvious difference compared to the control group.

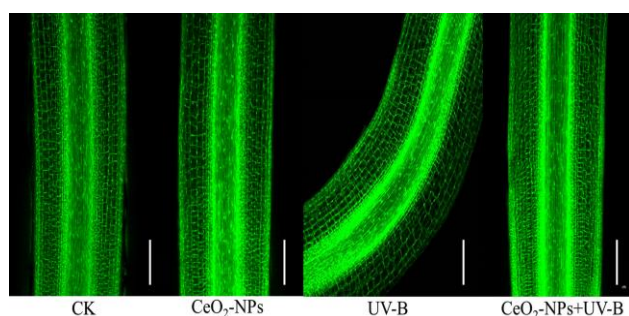


Fig. 9. Morphology of mung bean taproot cells in different treatment groups. Scale bar: 200 μm .

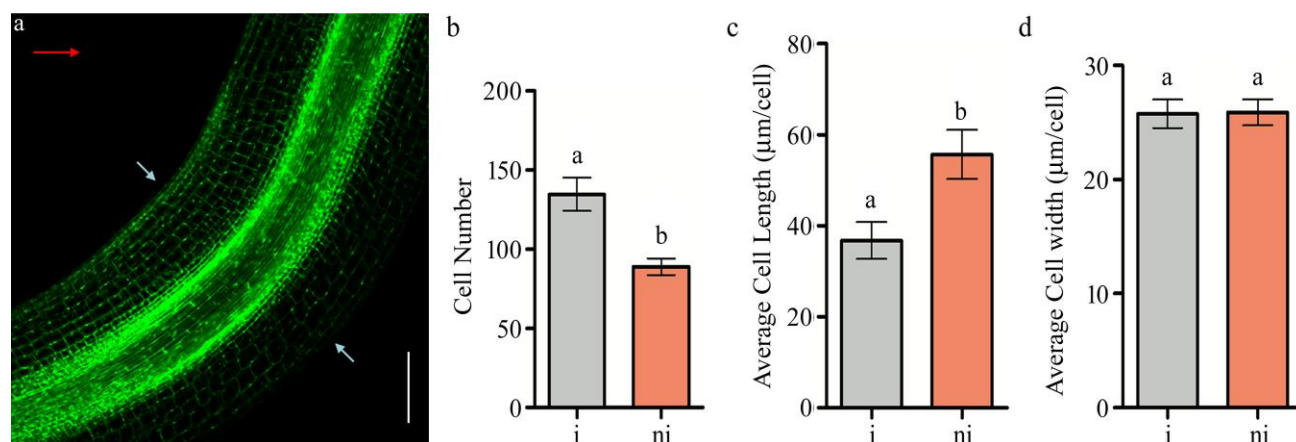


Fig. 10. Effect of UV-B radiation on the structure of mung bean taproot cells: a, Observation of cells on both sides of mung bean root tips, red arrows represent UV-B radiation direction, white arrows represent the radiating side (i) and non-radiating side (ni) of the root; b, The number of cells on both sides of the taproot of mung bean; c, Average cell length on both sides of the taproot of mung bean; d, Mean cell width on both sides of the taproot of mung bean. Different letters represent significant differences in mung bean roots between the radiating side (i) and non-radiating side (ni) ($p < 0.05$). Scale bar: 200 μm .

Analyzed the differences in the number, length, and width of cells on both sides of the bend of mung bean taproot in the UV-B group to further identify the reasons for the structural changes of mung bean root cells. Cells number on the UV-B irradiated side increased compared with the non-irradiated side, while the cell length decreased, accompanied by no obvious changes (Fig. 10). Our research indicates that enhanced UV-B irradiation mainly affects cell proliferation and cell elongation on both sides of mung bean taproot, causing the root tip to bend in the direction of UV-B radiation, i.e., the phenomenon of "warping root".

The content of auxin and cytokinin in the taproot of mung bean seedlings: It is well known that hormones can affect the development of plant roots in the normal life activities of plants from cell, tissue and molecular levels, and affect the establishment of plant root morphological structure. The gravitational growth of plant roots is inseparable from the action of auxin, which can also promote cell elongation, division and differentiation. Cytokinin is also an important hormone that promotes cell growth, proliferation and differentiation, and has direct or indirect effects on plant growth under abiotic stress (Kazan, 2013). Considering the importance of auxin and cytokinin in cell growth and proliferation, combined with the above experimental results, it can be speculated that the difference in cell length and number between the irradiated (i) and non irradiated (ni) surface of mung bean taproot may be related to the asymmetric distribution of auxin and cytokinin on both sides of the root. Therefore, the contents of auxin and cytokinin in both sides of the taproot cells of mung bean in each treatment group were determined.

In the CK group, there was little difference in the content of auxin and cytokinin on both sides of the taproot cells of mung beans, and the trend was basically the same in the CeO₂-NPs group. The auxin level on the non-irradiated side (ni) of the taproot of mung bean in the UV-B group was significantly higher than that on the irradiated side (i), while the cytokinin level was significantly lower than that on the irradiated side (i). After CeO₂-NPs combined treatment, the asymmetric distribution of auxin and cytokinin on both sides of the taproot of mung beans was significantly improved (Fig. 11).

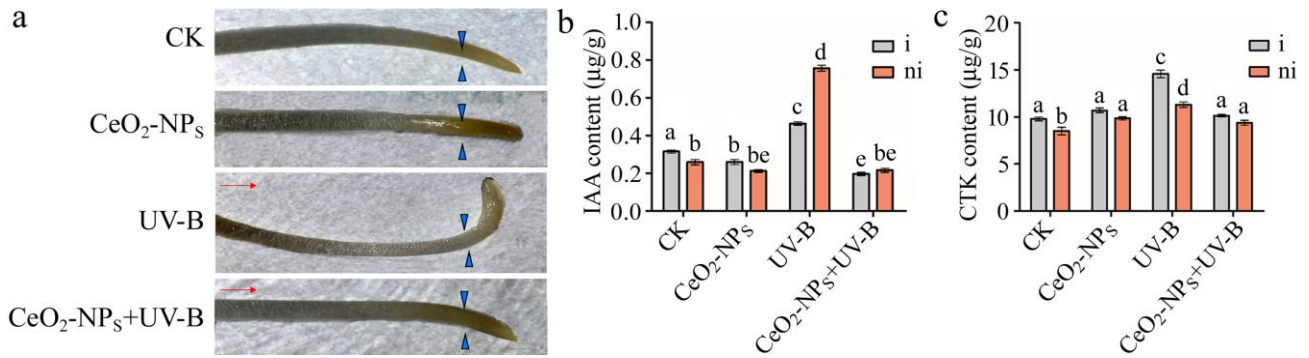


Fig. 11. The content of auxin and cytokinin in the taproot of mung bean seedlings: a, Observation of mung bean root tips under different treatments, the red arrow represents the UV-B radiation direction, and the blue arrow represents the radiating side (i) and the non-radiating side (ni) of the root; b, auxin content of cells on both sides of mung bean root tips; c, Cytokinin content of cells on both sides of mung bean root tips. Different letters represent significant differences in mung bean roots between treatments ($p < 0.05$).

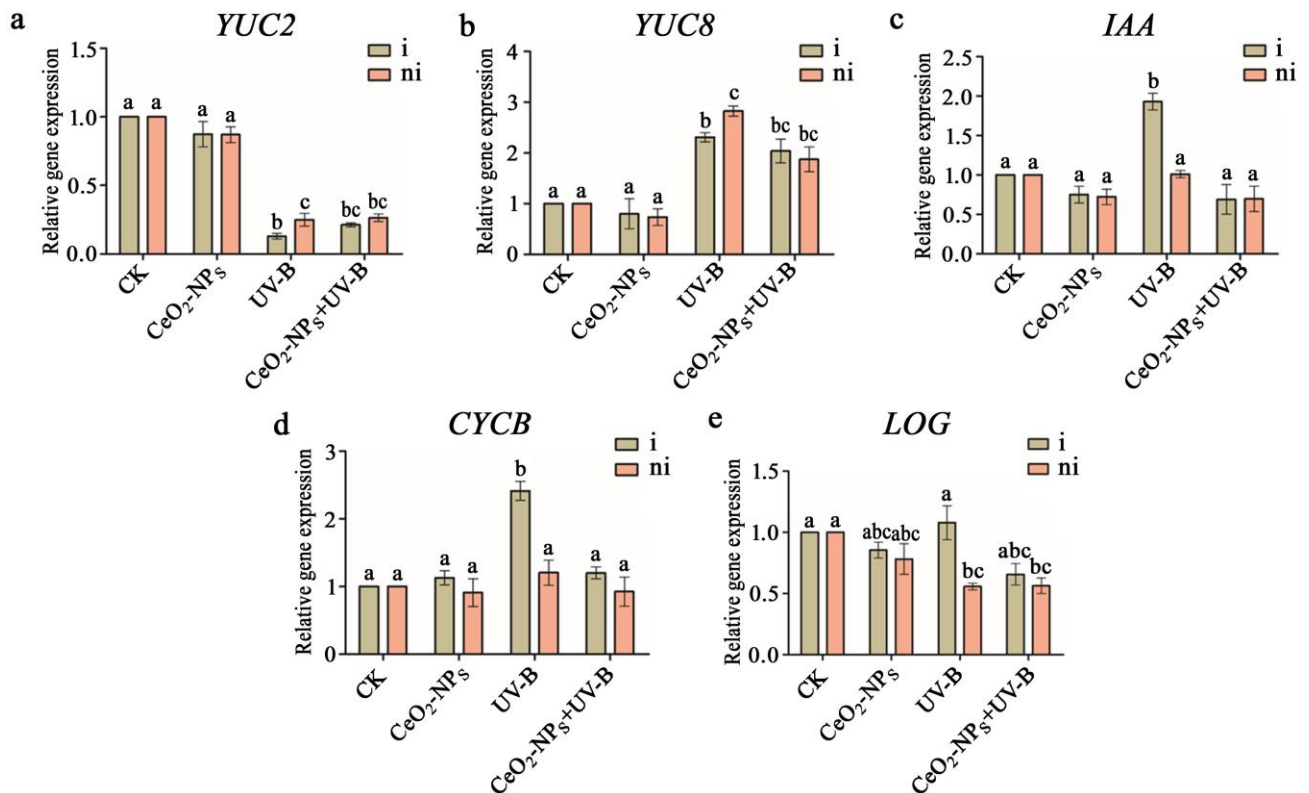


Fig. 12. Transcription levels of auxin and cytokinin-related genes in mung bean taproot. i represents the radiation side and ni represents the non-radiation side. The relative expression of: a, *YUC2*; b, *YUC8*; c, *IAA*; d, *CYCB*; e, *LOG*. Different letters represent significant differences in mung bean roots between treatments ($p < 0.05$).

To further clarify whether the upstream regulatory genes of auxin and cytokinin had undergone changes at the molecular level, we detected the expression of to auxin and cytokinin-related key genes on both sides of mung bean root tips. Among them, *YUC2* and *YUC8* are key genes for auxin synthesis, *IAA* is a gene encoding auxin suppressor protein, *CYCB* is a gene regulating cyclin, and *LOG* is a key gene for cytokinin synthesis. The expression differences of the above-mentioned genes on both sides of mung bean root tips in the CK group and CeO₂-NPs group were not significant. The expressions of *YUC2* and *YUC8* on the non-irradiated side (ni) of mung bean seedlings in the UV-B group were higher than those on the irradiated side (i), while the expressions trends of *IAA*, *CYCB* and *LOG* were opposite to them. Enhanced

UV-B radiation inhibited the synthesis of auxin on the non-irradiated (ni), promotes its cytokinin production, and leads to asymmetric distribution of auxin and cytokinin on both sides of the taproot of mung bean seedlings. CeO₂-NPs treatment equalized the difference in gene expression between the two sides of the taproot cells of mung bean seedlings in the UV-B group (Fig. 12). TiO₂-NPs altered the expression levels of biosynthesis and transport-related genes of auxin in *Arabidopsis thaliana*, thereby promoting its root growth (Wei *et al.*, 2020). This study supports a defense mechanism to help plants cope with external stress that CeO₂-NPs restore root gravitropism by regulating the redistribution of apical hormones, thus alleviating the phenomenon of taproot growth in mung beans.

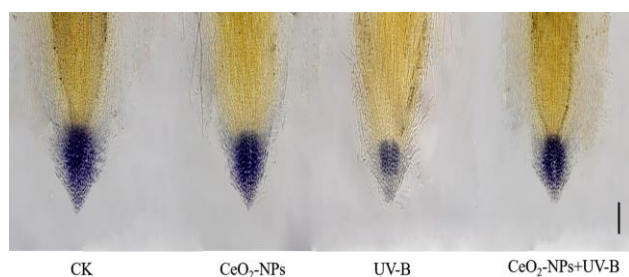


Fig. 13. Starch grain staining of mung bean taproot. Scale bar: 50 μ m.

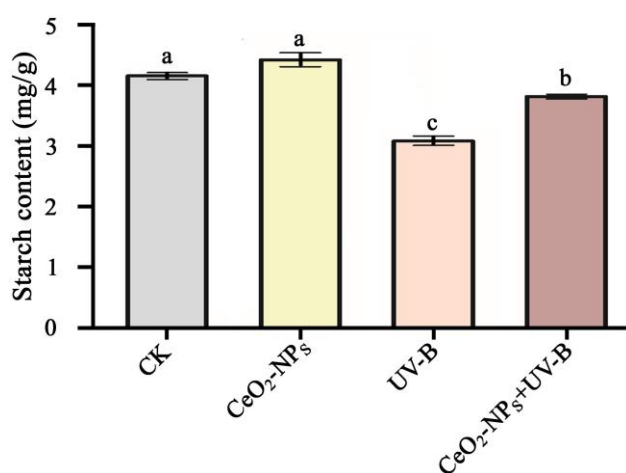


Fig. 14. Starch content in mung bean taproot. Different letters represent significant differences in mung bean roots between treatments ($p < 0.05$).

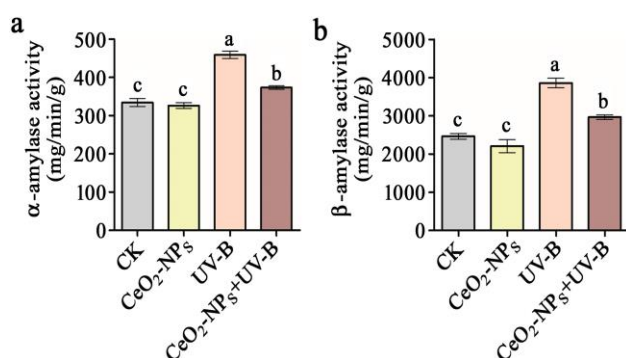


Fig. 15. Amylase activity in mung bean taproot: a, α -amylase activity; b, β -amylase activity. Different letters represent significant differences in mung bean roots between treatments ($p < 0.05$).

Changes in starch granules and starch contents in taproot of Mung bean seedlings:

According to the starch-equilibrium stone hypothesis, changes in starch granules lead to changes in the response of plant roots to gravity (Band *et al.*, 2012). Herein, it was inferred that "warps" occurred in the taproot of mung bean under UV-B stress, and that the loss of gravitation might also be related to the change of starch content. During plant growth, starch is present in plant cells in the form of starch granules. First, the taproot tips of mung bean seedlings in different treatment groups were stained with iodine-potassium iodide solution. Compared with the CK group, the staining changes of starch granules in the taproot of mung bean in the CeO₂-NPs group were not significant - they all showed darker colors, while UV-B

radiation made the staining of starch granules lighter. Compared to the UV-B group, the staining of starch granules in the CeO₂-NPs + UV-B group significantly deepened, and basically recovered to a level similar to the staining effect of the CK group. The treatment of CeO₂-NPs can significantly improve the phenomenon of reduced starch deposition at the root tips of mung bean seedlings under UV-B stress (Fig. 13).

The starch content of mung bean taproot in different treatment groups was determined. The difference in starch content of mung bean taproots between the CeO₂-NPs group and the CK group is not significant. Meanwhile, the starch content in the enhanced UV-B radiation group was significantly decreased compared to the CK group. CeO₂-NPs significantly alleviate the decrease in the starch content in the taproot of mung bean under the enhanced UV-B radiation (Fig. 14). Changes in starch content of mung bean root tips between different treatment groups are in accordance with the above trend of starch granules staining results.

The amylase in plants mainly exists in the form of α -amylase and β -amylase, which can affect the starch content by hydrolyzing starch. In order to explore the reasons for the differences in starch content in mung bean roots among different treatment groups, the amylase activity of each group was further measured. Compared to the CK group, the separate treatment of CeO₂-NPs had little effect on the α -amylase and β -amylase activities of mung bean taproots. The activities of both enzymes in the UV-B group was significantly increased, while the CeO₂-NPs + UV-B group had the opposite effect (Fig. 15).

As a result, increased activity of α -amylase and β -amylase in mung bean taproot under UV-B stress resulted in increased hydrolysis of α -amylase and β -amylase, thus leading to decreased starch content, changes of starch granules as gravity signal receptors, and ultimately curvature of the root. The applied CeO₂-NPs increased the content of starch by regulating α -amylase and β -amylase activities, restored the response of plant roots to gravity, and greatly repaired the phenomenon of "warping root" caused by UV-B radiation. Zhao *et al.*'s findings suggest that the use of CeO₂-NPs can also increase the starch content in cucumbers (Zhao *et al.*, 2014).

Conclusion

CeO₂-NPs could alleviate the inhibition of UV-B stress on mung bean seedlings taproot growth and increase their biomass. Applying CeO₂-NPs to mung beans treated with enhanced UV-B could help in balancing the production and removal of ROS, and reduce oxidative damage. CeO₂-NPs balanced the expression of auxin and cytokinin in cells on both sides of the mung bean taproots, while reducing the amylase activities of the taproots to increase starch content, thereby restoring the main root's response to gravity and improving the phenomenon of mung bean taproots "warping" caused by UV-B radiation. This provides a new way to improve plant tolerance to UV-B radiation, but the potential molecular mechanism by which CeO₂-NPs enhance plant tolerance under UV-B stress still needs further research.

Acknowledgement

This work was supported by the Graduate Innovation Project of Shanxi Normal University (NO.2021Y443).

References

- Alemu, S.T. and H. Gebre. 2020. Impact of ultraviolet-b radiation based on altitude on photosynthetic efficiency, growth performance and crop yield: A review. *J. Hort. Posthar. Res.*, 3(2): 285-296. www.doi.org/10.22077/jhpr.2020.2885.1102.
- Azadi, M., S.S. Moghaddam, A. Rahimi, L. Pourakbar and J. Popović-Djordjević. 2021. Biosynthesized silver nanoparticles ameliorate yield, leaf photosynthetic pigments, and essential oil composition of garden thyme (*Thymus vulgaris* L.) exposed to uv-b stress. *J. Environ. Chem. Eng.*, 9(5): 105919. www.doi.org/10.1016/j.jece.2021.105919.
- Band, L.R., D.M. Wells, A. Larrieu, J. Sun, A.M. Middleton, A.P. French, G. Brunoud, E.M. Sato, M.H. Wilson, B. Péret, M. Oliva, R. Swarup, I. Sairanen, G. Parry, K. Ljung, T. Beeckman, J.M. Garibaldi, M. Estelle, M.R. Owen, K. Vissenberg, T.C. Hodgman, T.P. Pridmore, J.R. King, T. Vernoux and M.J. Bennett. 2012. Root gravitropism is regulated by a transient lateral auxin gradient controlled by a tipping-point mechanism. *Proc. Natl. Acad. Sci. USA*, 109(12): 4668-4673.
- Chen, H., Y. Gong and R. Han. 2014. Cadmium telluride quantum dots (CDTE-QDS) and enhanced ultraviolet-b (UV-B) radiation trigger antioxidant enzyme metabolism and programmed cell death in wheat seedlings. *Plos One*, 9(10): e110400.
- Dai, Q., B. Yan, S. Huang, X. Liu, S. Peng, M.L.L. Miranda, A.Q. Chavez, B.S. Vergara and D.M. Olszyk. 1997. Response of oxidative stress defense systems in rice (*Oryza sativa*) leaves with supplemental uv-b radiation. *Physiol. Plant.*, 101(2): 301-308.
- Das, K. and A. Roychoudhury. 2014. Reactive oxygen species (ros) and response of antioxidants as ros-scavengers during environmental stress in plants. *Front. Environ. Sci.*, 2: 53. www.doi.org/10.3389/fenvs.2014.00053.
- Djanaguiraman, M., R. Nair, J.P. Giraldo and P.V.V. Prasad. 2018. Cerium oxide nanoparticles decrease drought-induced oxidative damage in sorghum leading to higher photosynthesis and grain yield. *ACS Omega*, 3(10): 14406-14416.
- Dutta, P., S. Pal, M.S. Sehra, Y. Shi and R.D. Ernst. 2006. Concentration of ce³⁺ and oxygen vacancies in cerium oxide nanoparticles. *Chem. Materials*, 18(21): 5144-5146. www.doi.org/10.1021/cm061580n.
- Frohnemeyer, H. and D. Staiger. 2003. Ultraviolet-b radiation-mediated responses in plants. Balancing damage and protection. *Plant Physiol.*, 133(4): 1420-1428.
- Huang, H., F. Ullah and Y. Zhao. 2019. Mechanisms of ros regulation of plant development and stress responses. *Front. Plant Sci.*, 10: 440478. www.doi.org/10.3389/fpls.2019.00800.
- Jansen, M.A., K. Hectors, N.M. O'Brien, Y. Guisez and G. Potters. 2008. Plant stress and human health: Do human consumers benefit from uv-b acclimated crops? *Plant Sci.*, 175(4): 449-458.
- Jiang, L., Y. Wang, L.O. Björn and S. Li. 2011. Uv-b-induced DNA damage mediates expression changes of cell cycle regulatory genes in *Arabidopsis* root tips. *Planta*, 233(4): 831-841.
- Kaneko, K., K. Inoke, B. Freitag, A.B. Hungria, P.A. Midgley, T.W. Hansen, J. Zhang, S. Ohara and T. Adschiri. 2007. Structural and morphological characterization of cerium oxide nanocrystals prepared by hydrothermal synthesis. *Nano Lett.*, 7(2): 421-425.
- Kazan, K. 2013. Auxin and the integration of environmental signals into plant root development. *Ann. Bot.*, 112(9): 1655-1665.
- Khan, Z. and H. Upadhyaya. 2019. Impact of nanoparticles on abiotic stress responses in plants: An overview. In: (Eds.): Tripathi, D.K., P. Ahmad, D.K. Chauhan and N.K. Dubey. *Nanomaterials in plants, algae and micro-organism: Concepts and controversies*. Publisher: Elsevier. 2: pp. 305-322. www.doi.org/10.1016/B978-0-12-811488-9.00015-9
- Kul, R., M. Ekinici, M. Turan, S. Ors and E. Yildirim. 2020. How abiotic stress conditions affects plant roots. In: (Ed.): Yildirim, E. *Plant roots*. IntechOpen Limited, England and Wales., pp. 1-24. www.doi.org/10.5772/intechopen.95286
- Kumari, R., S. Singh and S.B. Agrawal. 2009. Effects of supplemental ultraviolet-b radiation on growth and physiology of *Acorus calamus* L. (sweet flag). *Acta Biol. Cracov.*, 51(2): 19-27.
- Kurdzziel, M., M. Filek and M. Łabanowska. 2018. The impact of short-term uv irradiation on grains of sensitive and tolerant cereal genotypes studied by epr. *J. Sci. Food Agri.*, 98(7): 2607-2616.
- Li, H., J. Huang, Y. Liu, F. Lu, J. Zhong, Y. Wang, S. Li, Y. Lifshitz, S.T. Lee and Z. Kang. 2019. Enhanced rubisco activity and promoted dicotyledons growth with degradable carbon dots. *Nano Res.*, 12(7): 1585-1593.
- Li, S., L. Su, S. Ma, Z. Shi and Z. Sun. 2016. The impacts of exogenous h₂o₂ on primary root horizontal bending of pea (*Pisum sativum* L.). *Plant Growth Regul.*, 78(3): 287-296.
- Lidon, F. and J. Ramalho. 2011. Impact of uv-b irradiation on photosynthetic performance and chloroplast membrane components in *Oryza sativa* L. *J. Photochem. Photobiol.*, 104(3): 457-466.
- Livak, K.J. and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative pcr and the 2^{-ΔΔct} method. *Method.*, 25(4): 402-408.
- López-Moreno, M.L., G. de la Rosa, J.A. Hernández-Viezcas, J.R. Peralta-Videa and J.L. Gardea-Torresdey. 2010. X-ray absorption spectroscopy (xas) corroboration of the uptake and storage of ceo (2) nanoparticles and assessment of their differential toxicity in four edible plant species. *J. Agric. Food Chem.*, 58(6): 3689-3693.
- Lv, Z., X. Zhang, L. Liu, Y. Guo, Y. Fan, X. Yang, Y. Li and W. Zhang. 2013. Comparing intraspecific responses of 12 winter wheat cultivars to different doses of ultraviolet-b radiation. *J. Photochem. Photobiol.*, 119: 1-8.
- Ma, Y., X. He, P. Zhang, Z. Zhang, Y. Ding, J. Zhang, G. Wang, C. Xie, W. Luo, J. Zhang, L. Zheng, Z. Chai and K. Yang. 2017. Xylem and phloem based transport of ceo2 nanoparticles in hydroponic cucumber plants. *Environ. Sci. Technol.*, 51(9): 5215-5221.
- Mahdavian, K., M. Ghorbanli and K. Kalantari. 2008. The effects of ultraviolet radiation on the contents of chlorophyll, flavonoid, anthocyanin and proline in *Capsicum annuum* L. *Turk. J. Bot.*, 32(1): 25-33.
- Matthew, R.T., P.J. Aphalo, A.K. Banaś, P.W. Barnes, C.C. Brelsford, G.I. Jenkins, T.K. Kotilainen, J. Łabuz, J. Martínez-Abaigar, L.O. Morales, S. Neugart, M. Pieristè, N. Rai, F. Vandenbussche and M.A.K. Jansen. 2020. A perspective on ecologically relevant plant-uv research and its practical application. *Photochem. Photobiol. Sci.*, 18(5): 970-988.

- Nassour, R. and A. Ayash. 2021. Effects of ultraviolet-b radiation in plant physiology. *Agriculture*, 67(1): 1-15. www.doi.org/10.2478/agri-2021-0001.
- Piccini, C., G. Cai, M.C. Dias, M. Araújo, S. Parri, M. Romi, C. Faleri and C. Cantini. 2021. Olive varieties under uv-b stress show distinct responses in terms of antioxidant machinery and isoform/activity of rubisco. *Int. J. Mol. Sci.*, 22(20): 11214.
- Rai, K. and S. Agrawal. 2017. Effects of uv-b radiation on morphological, physiological and biochemical aspects of plants: An overview. *J. Sci. Res.*, 61: 87-113.
- Rico, C.M., S.C. Lee, R. Rubenecia, A. Mukherjee, J. Hong, J.R. Peralta-Videa and J.L. Gardea-Torresdey. 2014. Cerium oxide nanoparticles impact yield and modify nutritional parameters in wheat (*Triticum aestivum* L.). *J. Agri. Food Chem.*, 62(40): 9669-9675.
- Rikabad, M.M., L. Pourakbar, S.S. Moghaddam and J. Popović-Djordjević. 2019. Agrobiological, chemical and antioxidant properties of saffron (*Crocus sativus* L.) exposed to tio2 nanoparticles and ultraviolet-b stress. *Ind. Crops Prod.*, 137: 137-143.
- Rong, H., Z. Mei-Ping, W. Xiao-Xia, Y. Ming and W. Xun-Ling. 2007. Mechanism of repairation of he-ne laser irradiation on "root-bending" in wheat seedling induced by enhanced ultraviolet-b. *Acta Bot. Bore.-Occidental. Sinic.*, 27(3): 565-570.
- Schwabe, F., R. Schulin, L.K. Limbach, W. Stark, D. Bürge and B. Nowack. 2013. Influence of two types of organic matter on interaction of ceo2 nanoparticles with plants in hydroponic culture. *Chemosph.*, 91(4): 512-520.
- Shi, D.Q., J. Liu, Y.H. Xiang, D. Ye and W.C. Yang. 2005. Slow walker1, essential for gametogenesis in *arabidopsis*, encodes a wd40 protein involved in 18s ribosomal ma biogenesis. *Plant Cell*, 17(8): 2340-2354.
- Soni, S., A.B. Jha, R.S. Dubey and P. Sharma. 2022. Application of nanoparticles for enhanced uv-b stress tolerance in plants. *Pl. Nano Biol.*, 2: 100014.
- Tripathi, D.K., S. Singh, V.P. Singh, S.M. Prasad, N.K. Dubey and D.K. Chauhan. 2017. Silicon nanoparticles more effectively alleviated uv-b stress than silicon in wheat (*Triticum aestivum*) seedlings. *Plant Physiol. Biochem.*, 110: 70-81.
- Walkey, C., S. Das, S. Seal, J. Erlichman, K. Heckman, L. Ghibelli, E. Traversa, J.F. McGinnis and W.T. Self. 2015. Catalytic properties and biomedical applications of cerium oxide nanoparticles. *Environ. Sci. Nano*, 2(1): 33-53.
- Wan, J., P. Zhang, R. Wang, L. Sun, W. Wang, H. Zhou and J. Xu. 2018. Uv-b radiation induces root bending through the flavonoid-mediated auxin pathway in *arabidopsis*. *Front. Plant Sci*, 9: 618. www.doi.org/10.3389/fpls.2018.00618.
- Wei, J., Y. Zou, P. Li and X. Yuan. 2020. Titanium dioxide nanoparticles promote root growth by interfering with auxin pathways in *Arabidopsis thaliana*. *Int. J. Exp. Bot.*, 89(4): 883-891.
- Wu, H., L. Shabala, S. Shabala and J.P. Giraldo. 2018. Hydroxyl radical scavenging by cerium oxide nanoparticles improves *arabidopsis* salinity tolerance by enhancing leaf mesophyll potassium retention. *Environ. Sci.*, 5(7): 1567-1583.
- Wu, H., N. Tito and J.P. Giraldo. 2017. Anionic cerium oxide nanoparticles protect plant photosynthesis from abiotic stress by scavenging reactive oxygen species. *ACS nano*, 11(11): 11283-11297. www.doi.org/10.1021/acsnano.7b05723.
- Yang, S.-H., L.-J. Wang, S.-H. Li, W. Duan, W. Loescher and Z.-C. Liang. 2007. The effects of uv-b radiation on photosynthesis in relation to photosystem ii photochemistry, thermal dissipation and antioxidant defenses in winter wheat (*Triticum aestivum* L.) seedlings at different growth temperatures. *Fun. Pl. Biol.*, 34(10): 907-917. www.doi.org/doi.org/10.1071/FP07083.
- Zhang, R., G. Huang, L. Wang, Q. Zhou and X. Huang. 2019. Effects of elevated ultraviolet-b radiation on root growth and chemical signaling molecules in plants. *Ecotox. Environ. Safe.*, 171: 683-690. www.doi.org/10.1016/j.ecoenv.2019.01.035.
- Zhao, L., J.R. Peralta-Videa, C.M. Rico, J.A. Hernandez-Viezcas, Y. Sun, G. Niu, A. Servin, J.E. Nunez, M. Duarte-Gardea and J.L. Gardea-Torresdey. 2014. Ceo2 and zno nanoparticles change the nutritional qualities of cucumber (*Cucumis sativus*). *J. Agri. Food Chem.*, 62(13): 2752-2759.
- Zhao, L., L. Lu, A. Wang, H. Zhang, M. Huang, H. Wu, B. Xing, Z. Wang and R. Ji. 2020. Nano-biotechnology in agriculture: Use of nanomaterials to promote plant growth and stress tolerance. *J. Agri. Food Chem.*, 68(7): 1935-1947.

(Received for publication 24 April 2024)