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MAGNESIUM AND NITROGEN CO-DOPED CARBON DOTS ALLEVIATE UV-B RADIATION DAMAGE IN WHEAT SEEDLINGS

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Abstract

Ultraviolet B radiation (UV-B) has a negative effect on crops seedling vigor and germination. Magnesium and nitrogen co-doped carbon dots (Mg-N-CDs) are the major molecules participated in various physiological events of seedlings. The effects of various concentrations of exogenous Mg-N-CDs on the wheat seed germination together with the features of early seedling growth under a stress of UV-B were studied. The UV-B stress (10.08 KJ m^{-2} d^{-1}) significantly suppressed the germination index, seed germination potential, the growth of germs and roots and vigor index. The pretreatment of Mg-N-CDs attenuated the effect of UV-B stress with a dose-dependent mode, as exhibited by increasing the features of early seedling growth parameters and seed germination, the mitigation effect of 0.04 mg ml^{-1} Mg-N-CDs was the most significant. Mg-N-CDs pretreatment activated the effective antioxidant systems and then effectively enhanced the peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) activities in roots and germs, so as to prevent the oxidative damage caused by UV-B stress. In addition, Mg-N-CDs pre-treatment significantly increased the increase the expression of other related genes to improve the antioxidant capacity of roots and germs, leading to the up-regulated *ATPS*, *CS*, and *GS* level. The results demonstrated that applying Mg-N-CDs to the wheat seeds probably is an excellent choice. It can enhance the seed germination together with seedling growth under a stress of UV-B by regulating physiological response, so as to improve the seed germination along with seedling growth.

Key words: Mg-N-CDs; UV-B radiation; Alleviation; Oxidative stress; Gene regulation.

Introduction

In recent years, more and more serious air pollution has exacerbated the ozone layer destruction, leading to an increasing UV-B (from 280 to 350 nm) radiation on the surface of earth, which has enhanced the attention of the damage UV-B causes to ecosystems (Eichholz *et al*., 2011). Due to the fixed life style of plants, they cannot move freely like animals, so as to avoid the long-term radiation of solar UV-B (Choudhary & Agrawal, 2014). As the highest energy in the solar spectrum, UV-B may damage some macromolecules (including DNA) of plants, and generate the reactive oxygen species (ROS), and finally affect the cellular process (Han *et al*., 2002; Éva *et al*., 2013). After UV-B irradiation, the lipid peroxidation of wheat seedlings was enhanced, and the activities of antioxidant enzymes containing POD, SOD and CAT were changed (Chen *et al*., 2019). Flavonoids are known as UV-B filters because they can absorb UV-B light, have antioxidant and ultraviolet absorption functions, and possess a protective effect in the reaction of plant to UV-B (Agati & Tattini, 2010). Nevertheless, UV-B radiation can cause flavonoids synthesis to appear high hydroxylation, showing strong antioxidant capacity, and effectively reduce the ROS concentration in the plant cells (Agati *et al*., 2012). Plants can employ various defense mechanisms to resist the harmful ROS effects and oxidative stress. In fact, ROS is a kind of chemical marker for the prediction of the nanoparticle's toxicity. When the balance between the antioxidant defense and ROS is destroyed, oxidative stress occurs (Lee *et al*., 2013). Therefore, it indirectly affects the physiological reactions of plants by affecting the synthesis of secondary metabolites under UV-B radiation.

These reactions may affect the growth, biomass, photosynthetic system, antioxidant system and other physiological phenomena in plants, and cause to cell impair (Schreiner *et al*., 2012; Chen *et al*., 2019). With the aim of improving the phytoremediation efficiency, researchers have proposed to apply nano materials to plants to alleviate the UV-B stress (Wu *et al*., 2017; Xiao *et al*., 2019). Carbon dots (CDs), as a novel kind of the nanomaterials, are extensively employed in biosensor, biological imaging, drug carrier and photocatalysis due to their small size, good fluorescence performance and low toxicity (Liu *et al*., 2017). Such as, it has been shown that carbon nanoparticles can be absorbed by plants from the roots and transported through the plant interior to the leaves (Chen *et al*., 2016). Wheat treated with water-soluble carbon nanoparticles could promote root and stem elongation under dark and light conditions (Tripathi & Sarkar, 2015). Carbon nanoparticles can penetrate the plant cell walls along with cell membranes, and transport different substances (for example fertilizers, ions and water) to the plant organelles (Liu *et al*., 2009; He *et al*., 2018). Carbon based nanomaterials CDs have attractive application prospects in bioremediation because of their good fluorescence, low toxicity and biocompatibility.

When studying the biological effects of metal carbon nanoparticles, it is found that carbon quantum dots doped with appropriate concentrations of metal oxides can have a positive effect against the plants growth along with development, mainly in promoting the plant roots and shoots growth, and improving biomass (Mukherjee *et al*., 2016). For example, N-CDs can stimulate the germination of *Arabidopsis* seeds, increase biomass accumulation, promote root growth, increase the antioxidant enzymes activity and the content of total chlorophyll (Chen *et al*., 2020); CDs, N-CDs, magnetic TiO_2 and Fe_3O_4 nanoparticles can decrease

the toxicity of cadmium to plant seedlings via enhancing the antioxidant enzymes activity (Ji *et al*., 2016; Alexandre *et al*., 2017; Xiao *et al*., 2019); N-CDs alleviate the toxicity of cadmium to *Arabidopsis thaliana* through enhancing the antioxidant enzymes activity (Chen *et al*., 2020). The phytoxicity of chromium (VI) to wheat seedlings was reduced by adding silicon nanoparticles (SiNp) (Tripathi *et al*., 2015). FeO nanoparticles, ZnO nanoparticles, biochar and carbon nanotubes, as well as lots of other nanomaterials have been confirmed to decrease the ROS damage to plants via inducing higher activity of antioxidant enzyme (Hussain *et al*., 2018). Despite lots of research that has reported the protective influence of CDs on the plant heavy metal toxicity, the biochemical and physiological response of plants to the effect of UV-B radiation and CDs is still not clear.

Wheat (*Triticum aestivum* L.) is a cereal crop widely grown around the world. Which has important economic value and ecological significance (Chen *et al*., 2014; Tripathi & Sarkar, 2015; Chen *et al*., 2019). In this paper, wheat was used as the research object of phytoremediation. Mg and N-co doped CDs was synthesized by hydrothermal method using citric acid, urea and magnesium acetate as raw materials. In some practical applications, doping heteroatoms (Mg, N, S and P) into CDs can improve the distinctive electronic and optical performances of carbon quantum dots (Mohapatra *et al*., 2015; Wang *et al*., 2015; Li *et al*., 2017; Han *et al*., 2018). Therefore, the physiological response indexes of wheat seedlings treated by UV-B radiation and Mg-N-CDs alone or combined treatment will be compared. The outcomes will clarify the potentiality of Mg-N-CDs in decreasing UV-B damage to plants, which contributes greatly to the application of Mg-N-CDs in agriculture.

Material and Methods

Synthesis of Mg-N-CDs: 1.92 g of citric acid, urea (1.2 g) and magnesium acetate (2.14 g) were mixed into 30 ml of ultra-pure water, respectively, and dissolved by stirring. The dissolved mixture was transferred into lined autoclave, which was then heated for fourteen hours at 180℃. Subsequently, cooling the sample to RT and centrifuging it for 30 min (10000 r min^{-1}). Then the supernatant was filtered by 0.22 μm filter element. The filtrate was collected and frozen at -20℃ for 24 h, ultimately, the soiled of Mg-N-CDs was acquired via freeze-drying. The synthesis process of Mg-N-CDs is reflected in S1 (Supplementary materials, S1). The morphology of Mg-N-CDs was characterized by transmission electron microscopy (TEM) and X-ray photoelectron spectroscopy (XPS). Fourier transform infrared (FTIR) spectra were obtained by Nicolet 380 infrared spectrometer (Varian, USA).

Cultivation and treatment of wheat: First, the water was absorbed by the wheat seed in sterile water through an imbibition process, followed by disinfection with 1.5% (V/V) NaClO for 10 min. Then, the seeds were washed with sterilized water, and the seeds in the culture dish were covered with soaked gauze. The seeds were cultured in a 25 ℃ incubator in the dark for 24 h to germinate (subject to the seeds being white), finally, the germinated seeds were seeded in a Petri dish covered with gauze.

Two groups of wheat were cultured with different concentrations of Mg-N-CDs (CK: 0 mg ml^{-1}, C1: 0.02 mg ml^{-1}, C2: 0.04 mg ml^{-1} and C3: 0.12 mg ml^{-1}) every day, and one group was added with UV-B radiation (10.08 kJ $m^{-2}d^{-1}$) at the same time, Optical instrument factory) were treated with compound treatment (UV, U+C1, U+C2, U+C3) and put into the intelligent light incubator with the temperature of 22℃ and the Photoperiod of 16 h/8 h for 5 days, and Mg-N-CDs aqueous solution exposed to sunlight and ultraviolet as shown in S2.

Cytotoxicity of Mg-N-CDs: The toxicity of Mg-N-CDs to HeLa cells was determined via CCK-8. HeLa cell suspension was placed in culture plate (96 well), cultivated in a 37℃ incubator with 5% CO_2 for one day, and subsequently cultured with Mg-N-CDs solution of different concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 mg ml^{-1}) in complete culture medium (90% DMEM+10% fetal serum) for 12 h. The absorbance of the hole was measured by microplate reader. Cell viability was calculated according to the formula:

$$\text{Cell viability} = A/A_0 \times 100\ \%$$

A and A_0 represent the absorbance of experimental and control groups. Each measurement was repeated 3 times.

Measurement of biomass and length of wheat seedlings: After 5 days, we remove the wheat seedlings from hydroponic system. Use filter paper to absorb the water from the root. The fresh weight of single seedling was measured by BT25S electronic balance. The steel ruler was applied to determine plant height and root length and recorded.

Absorption and observation of Mg-N-CDs in wheat seedlings: The 3-day-old wheat seedlings in 0 and 0.02 mg ml^{-1} Mg-N-CDs treatment groups were employed for the observation of the distribution and absorption of Mg-N-CDs. Olympus laser confocal microscope was utilized to observe the leaf epidermis and root tip. Image acquisition was used FV-1000 software. At 405 nm, Mg-N-CDs could be excited.

Determination of chlorophyll content in wheat seedling leaves: The method of Lichtenthaler was modified to measure chlorophyll content (Lichtenthaler, 1987). 0.1 g leaves were placed in the test tube, and 10 ml 95% ethanol was added into the test-tube. The leaves were soaked in the dark at 25℃ for 1 day until all the leaves were chlorotic. The values of absorbance at 663, 645 and 470 nm were observed on the UV spectrophotometer, and the calculation of chlorophyll content was implemented in accordance with absorbance values. The calculation formula is as follows:

$\text{Chl a} = 12.7A_{663}\text{-}2.69A_{645}$;
$\text{Chl b} = 22.9A_{645}\text{-}4.68A_{663}$;
Total chlorophyll = Chla + Chl b

Determination of MDA, flavonoids and antioxidant enzymes: The leaves and roots in each treatment group were cut and mixed, and then 0.1 g leaves and roots were weighed to determine the physiological and biochemical indexes of plant tissue. Lipid peroxidation is an abiotic stress indicator, which can be indirectly detected via the generation of malondialdehyde (MDA). The activities of MDA, flavonoids, SOD (EC 1.15.1.1), POD (EC 1.11.1.7) and CAT (EC 1.11.1.6) were detected by ELISA kit.

Detection of RT qPCR: With the aim of measuring the antioxidant regulatory genes expression level in wheat seedlings, total RNA was extracted from 5-day-old seedlings by TRIzol method, and then reverse transcripted by reverse transcription kit to obtain cDNA strand. The changes of antioxidant associated genes in the wheat seedlings were tested with real-time PCR. The genes relative expression could be calculated through comparing $2^{-\Delta\Delta Ct}$. The internal reference gene is *Actin*. The primers are listed in the table below (Table 1).

Statistical analysis

All of the research were conducted for 3 times. One-way ANOVA on the basis of Newman Keuls multiple comparison test was utilized to analyze the data ($p \leq 0.05$). Origin 8, Graphpad prism 5 and SPSS 17.0 were used for data analysis and mapping.

Results and Discussion

Structural characterization of Mg-N-CDs: The morphology and size of Mg-N-CDs were observed via transmission electron microscopy (TEM). Mg-N-CDs have good dispersion (Fig. 1a). HRTEM images (Fig. 1b) clearly show that the lattice spacing of Mg-N-CDs is 0.23 nm, which corresponds to the graphite (100) crystal plane (Zheng *et al*., 2015). The surface functional groups of Mg-N-CDs can be preliminarily analyzed by FTIR, and the infrared spectrum can be obtained (Fig. 1c). Fig. 1c shows that there are wide absorption bands at 3406 cm^{-1} and 3213 cm^{-1}, which are caused by the vibration of N-H and O-H bond. At 1581 cm^{-1}, the detected peak corresponds to the C-O bond vibration. The vibration of the C-O-C bond forms a peak at 1077 cm^{-1}. FTIR outcomes exhibit that the Mg-N-CDs surface was rich in the hydrophilic functional groups (carboxyl and hydroxyl), which was in accordance with the excellent water solubility of Mg-N-CDs. The elements and surface structure of Mg-N-CDs were in-depth investigated via XPS. Four peaks are clearly shown in Fig. 1d, indicating the presence of Mg1s (1303.48 eV), O1s (529.81 eV), N1s (400.81 eV) and C1s (285.35 eV) on the Mg-N-CDs surface. The optical performances of the Mg-N-CDs were determined through fluorescence spectrometer. Fig. 1e reveals the emission and excitation spectra of Mg-N-CDs. The maximum emission peak of Mg-N-CDs is 410 nm with 320 nm excitation wavelength. The illustration in Fig. 1e shows a picture of Mg-N-CDs aqueous solution under sunlight and ultraviolet. Fluorescence emission spectra for the Mg-N-CDs at the excitation wavelength of 300 ~ 400 nm was shown in Figure 1f, indicating that Mg-N-CDs have excellent fluorescence properties. XPS results show that there are carbon, nitrogen, oxygen and magnesium on the Mg-N-CDs surface, which is consistent with the results of single element analysis (Fig. 2).

Stability of FL of Mg-N-CDs: To further study the FL stability and subsequent biological application of Mg-N-CDs, the stability of Mg-N-CDs under different conditions was detected. S3 shows the FL stability of Mg-N-CDs at 320 nm under different pH, ionic strength and time. Multiple pH gradients (4-9) were set to detect FL of Mg-N-CDs at distinct values of pH. In accordance with S3a, the FL of Mg-N-CDs does not fluctuate greatly when the pH value is 4.0-7.0, so the neutral environment with pH value of 7.0 is selected for subsequent biological experiments. In order to test the stability of FL of Mg-N-CDs at high salinity, they were dissolved in different concentrations of NaCl solution, and FL intensity was recorded (S3b). The results showed that FL of Mg-N-CDs changed little when NaCl concentration was from 0.2 to 1.0 m, which indicated that FL of Mg-N-CDs was not affected by salt concentration, and it had good stability. Besides, in addition, the effect of different time (1, 10, 20 and 30 h) on FL intensity in PBS solution with pH 7.0 is shown in S3c, indicating that time has no effect on the fluorescence of Mg-N-CDs.

Cytotoxicity of Mg-N-CDs: Cytotoxicity is an important factor to determine the safety of biomaterials. Therefore, before Mg-N-CDs were applied in the bio-researches, the Mg-N-CDs toxicity was detected through CCK-8 method (Zhang *et al*., 2019). In this study, HeLa cells were utilized for the exploration of the cytotoxicity of Mg-N-CDs. HeLa cells were cultured in 8 concentration gradients. The results showed that with the Mg-N-CDs concentration of 0.8 mg/ml, the cell survival rate remained above 85%, indicating that Mg-N-CDs had no obvious cytotoxicity and could be used in subsequent biological experiments (S4).

Table 1. Primer sequences for qRT-PCR (Xiao *et al*., 2019).

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
ACTIN	TGCTGGAATCGGAATAGTTGAG	ACTACGCAGGCTCATCAAACAG
ATPS	CTGGAAACTGGCTGATTGGTG	TGGAACGCAAATACAGCATCA
CS	CACCATAGAGCCAGAAACAAGG	GCAAAGACACCCAACTCATACAT
GS	TGAGCGAACTTCACAGGACC	AACCCATGCTTTGCCCAAT

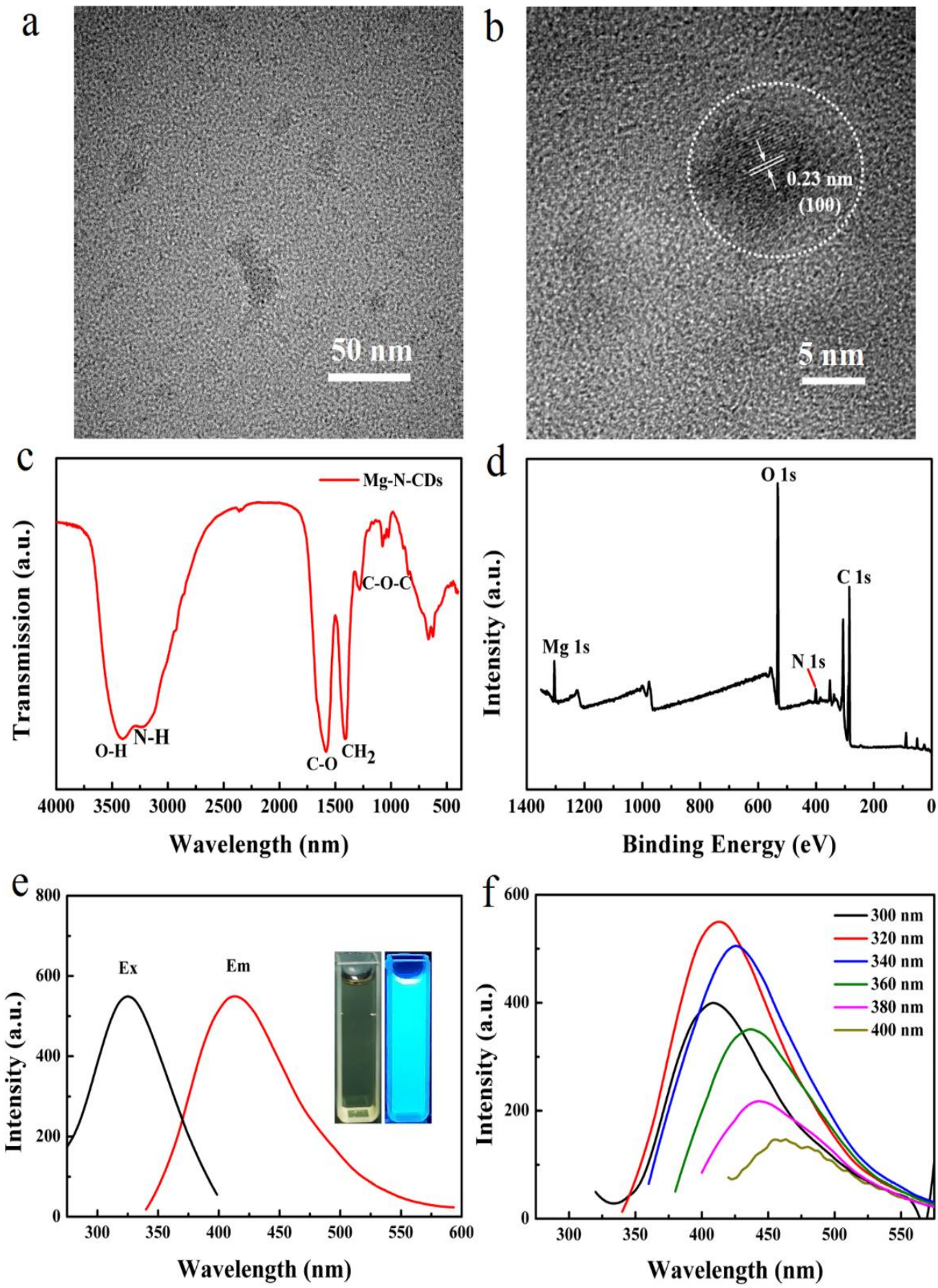

Fig. 1. (a) TEM of Mg-N-CDs; (b) HRTEM of Mg-N-CDs; (c) FTIR of Mg-N-CDs; (d) XPS of Mg-N-CDs; (e) The emission and excitation spectra of Mg-N-CDs. The inset shows the solution of Mg-N-CDs in sunlight and ultraviolet light; (f) Excitation-dependent emission spectra of Mg-N-CDs.

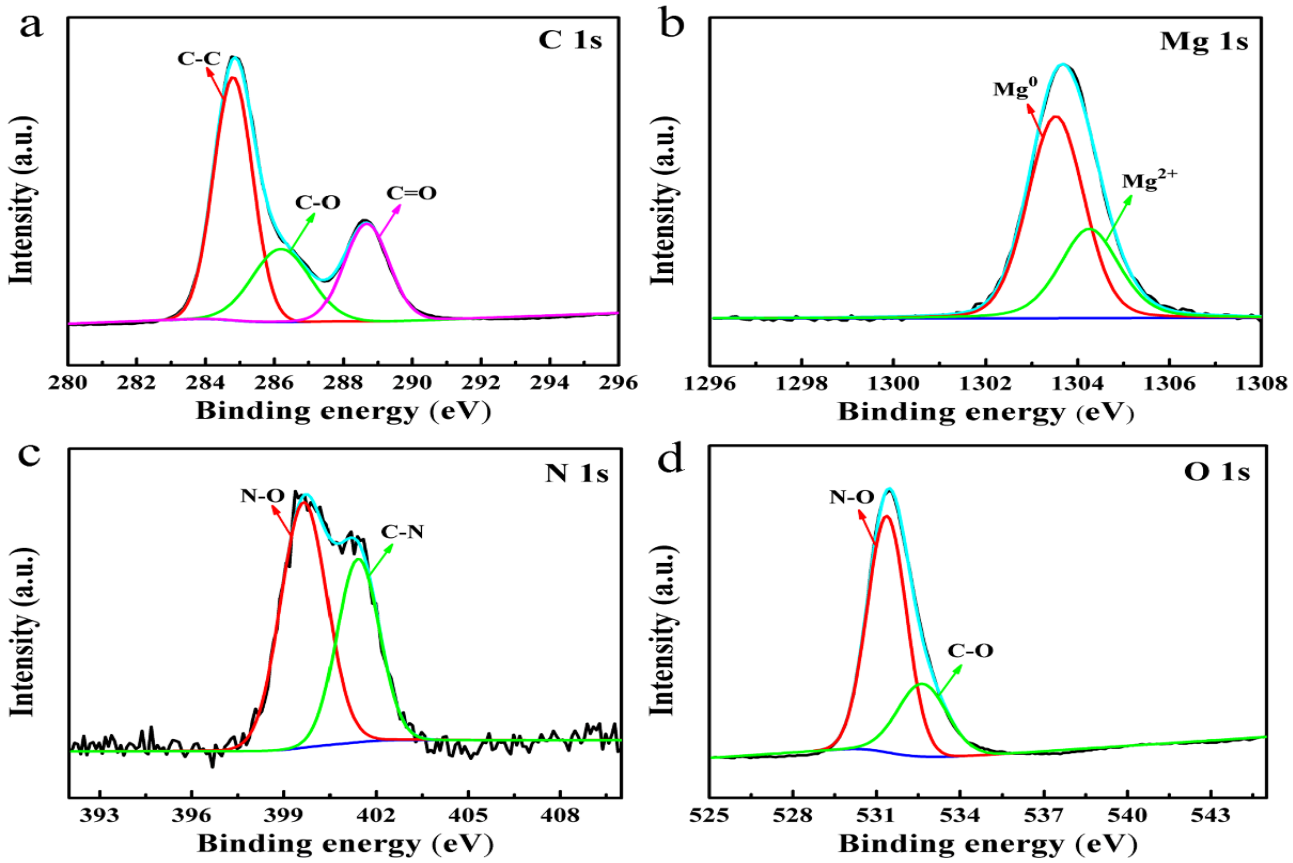

Fig. 2. XPS single element map of Mg-N-CDs: (a) C 1s map; (b) Mg 1s map; (c) N 1s map; (d) O 1s map.

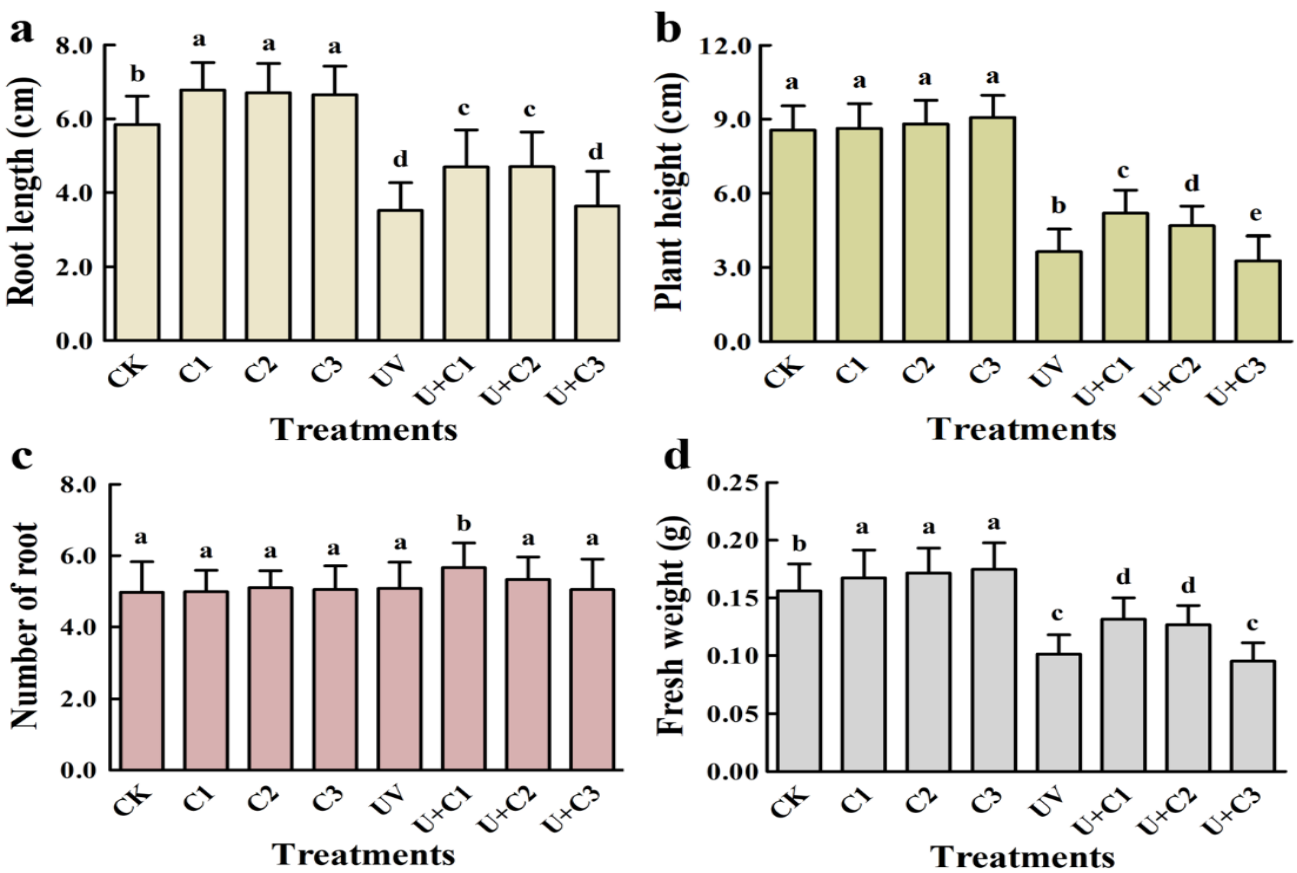

Fig. 3. Root length (a); plant height (b); root coefficient (c) and fresh weight (d) of wheat in different treatment groups. The different letters represent significant differences (p<0.05).

Effects of Mg-N-CDs on wheat growth: With the aim of observing the effects of UV-B radiation or/and Mg-N-CDs on the growth and development of wheat seedlings, the preliminary analysis began with the evaluation of physiological growth indicators, such as root length, plant height, root coefficient and biomass accumulation (measured by fresh weight). Fig. S5 shows the growth of wheat seedlings. In this study, UV-B showed damage to wheat and caused serious damage to its growth indicators. As exhibited in (Fig. 3), the plant height, root length and fresh weight of wheat exposed to UV-B radiation decreased significantly (as high as 39.8%, 57.4% and 35.3%), indicating that high dose UV-B could seriously inhibit the wheat growth and influence various physiological indexes, which also indicates that UV-B radiation is negatively correlated with wheat growth traits (Chen *et al*., 2014). However, Mg-N-CDs can facilitate the plants growth. The root length (Fig. 3a) and fresh weight (Fig. 3d) of wheat reach significant levels after Mg-N-CDs cultivation, but the plant height has no significant difference compared with the control group (Fig. 3b). This is similar to previous research that CDs can facilitate the growth of the mung bean (Wang *et al*., 2018). When Mg-N-CDs and UV-B was utilized to treat wheat seedlings, the root length, plant height and fresh weight of Mg-N-CDs were markedly higher than treated with UV-B alone, which indicated that Mg-N-CDs could alleviate the inhibition of UV-B on the growth of plant. This may be due to the degradation of CDs after entering the plant body and the production of plant hormone analogues, thus promoting the growth of plants (Li *et al*., 2019). It may also be that nano carbon enters into root cells and changes the metabolic rate, which leads to the increase of auxin content in Root. Moreover, the increase of root activity will make the root absorb more water and facilitate the growth of plants faster (Li *et al*., 2019), which indicates that Mg-N-CDs can facilitate the plant growth.

Absorption and observation of Mg-N-CDs in Hela cell and wheat seedlings: The distribution and absorption of the Mg-N-CDs in the Hela cell and roots and leaves of wheat were found through confocal laser scanning. It was found that Mg-N-CDs could cross the membrane and enter the cytoplasm, but no fluorescence signal was found in the nucleus (S6), which is similar to previous research (Zhang *et al*., 2019). So, can Mg-N-CDs cross the wall of plant cells? In accordance with Fig. 4, the fluorescence intensity in root tips of wheat was stronger than that in leaves, and no fluorescence was detected in the control group without Mg-N-CDs (Fig. 4a). Fluorescence was mainly distributed in the cell membrane and cytoplasm of root tip cells (Fig. 4e). However, fluorescence was also observed in root hairs (Fig. 4g), indicating that Mg-N-CDs had been absorbed by roots. Besides, fluorescence was also detected in the leaf epidermis (Fig. 4j), mainly located in the leaf epidermis and vascular bundles, indicating that Mg-N-CDs have been transported to the leaves, which is consistent with the report that fluorescence was detected in the roots and leaves of *Arabidopsis thaliana* by laser confocal microscopy (Chen *et al*., 2020). Carbon quantum dots can penetrate through the cell wall of plants and enter into cells, which can be absorbed and enriched in the cell cytoplasm and cell membrane (Dong *et al*., 2012; Zhang *et al*., 2013). There are stomata on the cell wall of plant cells, which can regulate the transport of substances (Jasmina *et al*., 2010; Ran *et al*., 2010). The pore size of cell wall is about 10 nm (Kruk & Jaroniec, 1999; Berestovsky, 2001). Therefore, Mg-N-CDs (less than 5 nm) can enter the cell through cell wall aperture. This experiment proved that Mg-N-CDs did penetrate plants, and the absorption order was from root to leaf.

Effects of Mg-N-CDs on photosynthetic pigments of wheat seedlings: Chlorophyll is an important physiological parameter during the plant growth together with development, and various conditions of stress can cause changes in photosynthetic pigment content (Xin *et al*., 2015). In Fig. 5b, the total content of chlorophyll of the Mg-N-CDs treated plants was remarkably higher than the content in control group, but the total chlorophyll content decreased significantly after UV-B irradiation. After Mg-N-CDs and UV-B treatment, the content of total chlorophyll increased, but not to the level of the control group. It shows that CDs could also increase the content of chlorophyll (Wang *et al*., 2018), and both multi-walled and single-walled carbon nanotubes evidently up-regulated the expression level of the genes associated with chloroplast development (Zhang *et al*., 2017), indicating that Mg-N-CDs could alleviate the decrease of chlorophyll content resulted from the radiation of UV-B to a certain extent.

Determination of MDA: The accumulation of high level of MDA can be explained by the presence of toxic ROS (Li *et al*., 2006; Cao *et al*., 2013). Damage may occur when production of reactive oxygen species exceeds its ability to protect itself (Li *et al*., 2006; Zhang *et al*., 2013). In Fig. 6, MDA activity in leaves increased after C1, C2 and C3 treatment, indicating that the cells were subjected to oxidative stress, but they could still carry out normal biosynthesis. However, when exposed to the radiation of UV-B, the activity of MDA in leaves reduced significantly, indicating that the cells were seriously damaged and could not be biosynthesized normally, resulting in malondialdehyde could not continue to be produced, or even degraded, so the activity decreased. This may also be because the leaves are directly exposed to UV-B, so the leaf cells are seriously damaged. After UV-B and Mg-N-CDs treatment, MDA activity in U+C1 and U+C2 groups increased by about 18% in contrast to treated with UV-B alone, suggesting that the damage degree of leaf cells was alleviated.

Changes of flavonoid and antioxidant enzyme activities: The latent damage resulted from Mg-N-CDs and UV-B at wheat cell level is shown in Fig. 7a, which describes the effect of UV-B radiation and Mg-N-CDs particles on the cells to induce the oxidative stress with generation of ROS (Éva *et al*., 2013). Flavonoid is an antioxidant, which is used as a defense against ROS damage in plants, and is also a secondary metabolite produced by plant stress response (Ma *et al*., 2014). Fig. 7b represents the flavonoid activity of leaf and root, which was improved under Mg-N-CDs treatment. After the radiation of UV-B, the flavonoids activity in roots decreased significantly, suggesting that higher UV-B intensity could restrain the production of flavonoids (Gao *et al*., 2019). However, the flavonoid activity of UV-B combined with Mg-N-CDs was obviously higher than the activity of UV-B alone, indicating that Mg-N-CDs activated the production of flavonoid and improved the antioxidant activity.

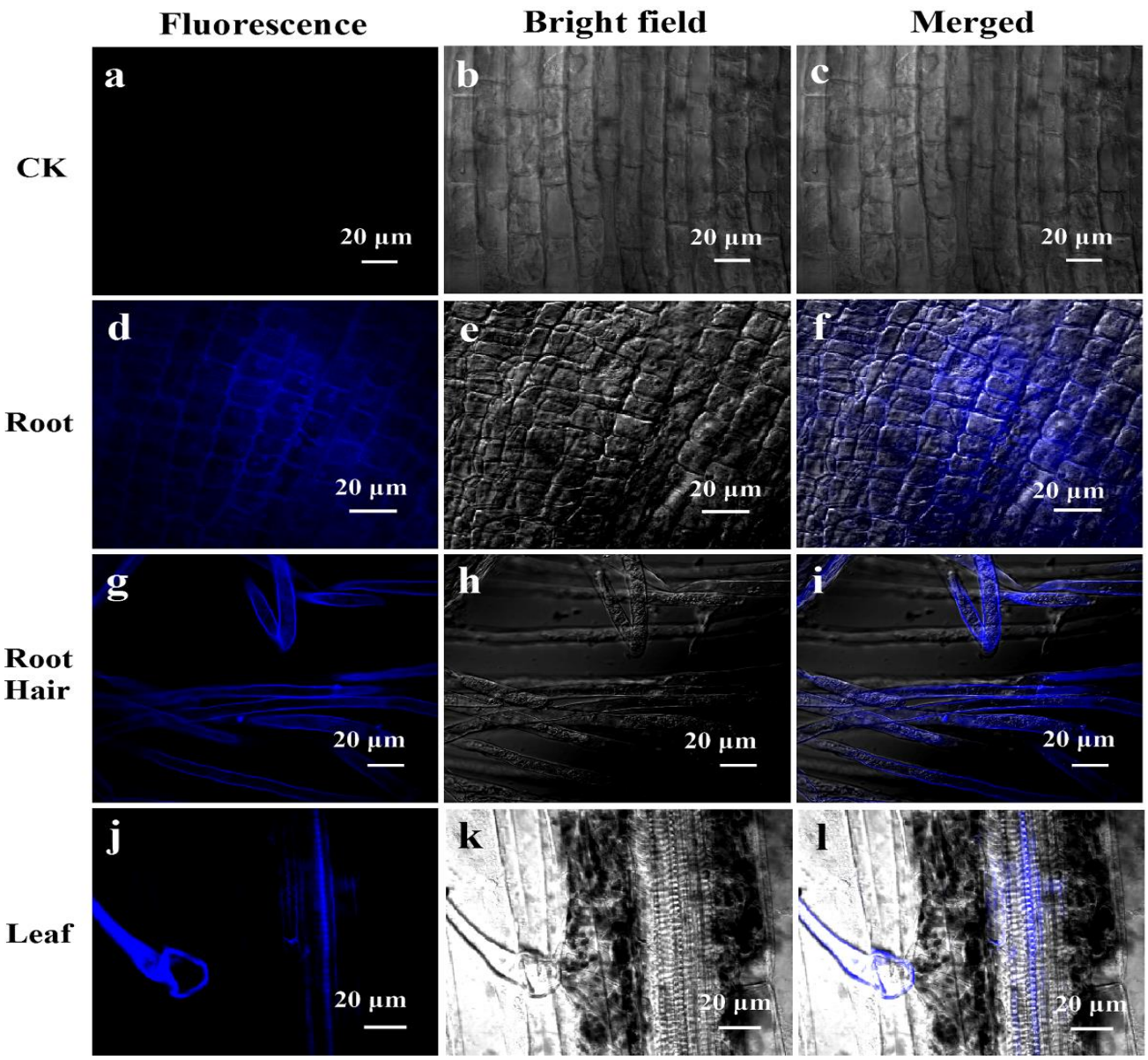

Fig. 4. Laser confocal images of wheat root tip and leaf epidermis; a-c was the apical control group; d-f were apical; g-i were root hair; j-l were leaf epidermis.

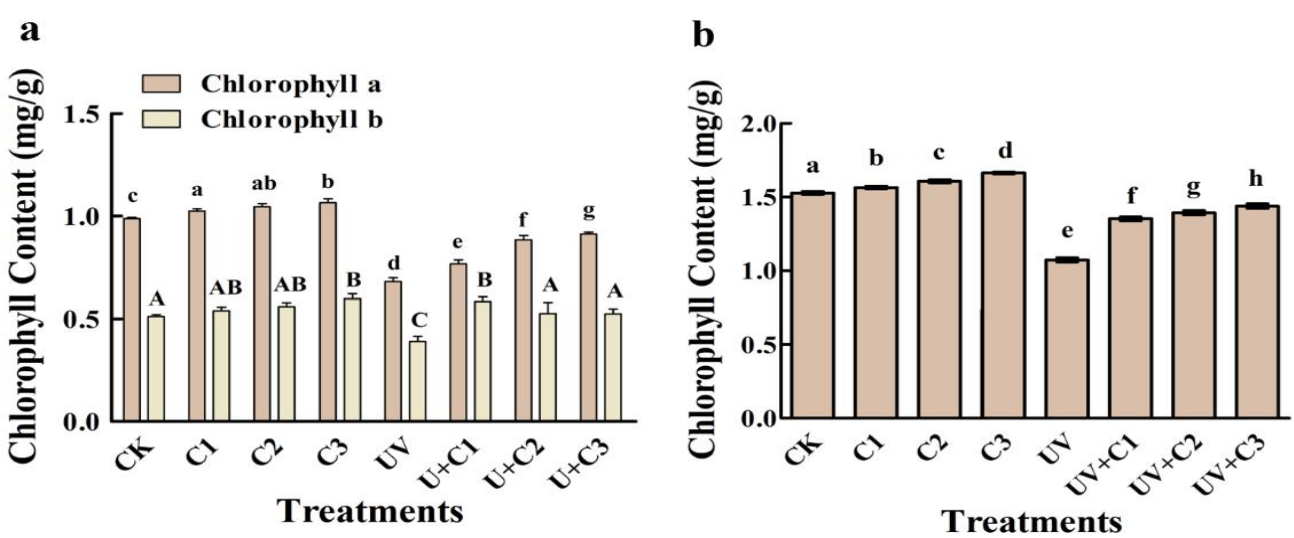

Fig. 5. Chlorophyll content of wheat seedlings in different treatment groups. (a) Chlorophyll a and b; (b) Total Chlorophyll content. The different letters represent significant difference (p<0.05).

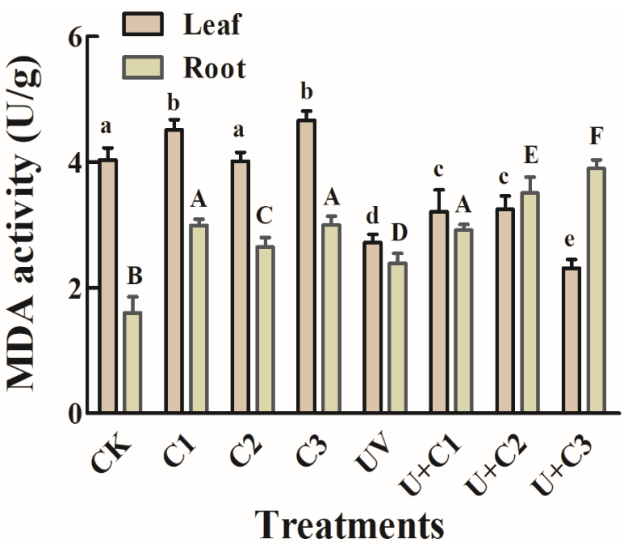

Fig. 6. MDA activity of wheat roots and leaves under different treatments. The different letters represent significant difference (p<0.05).

Plant cells possess the antioxidant defense systems formed by enzymes, for example CAT, POD and SOD. The enzymatic effect of SOD can produce H_2O_2 and O_2, and H_2O_2 can be removed by cat and pod, which can be transformed into water and oxygen, relieving the poisoning of H_2O_2 (Mostafa, 2009; Du *et al*., 2011; Hazani *et al*., 2013). Plants can resist the harmful effects of oxidative stress and ROS by using a variety of defense mechanisms, oxidative stress will appear when the balance between antioxidant defense and ROS is destroyed (Lee *et al*., 2013). According to research, for instance, ZnO Fe_2O_3 nanoparticles, nanoparticles, biochar and carbon nanotubes, etc, have been demonstrated to decrease the injury of ROS to the plants via causing higher activities of antioxidant enzyme (Hussain *et al*., 2018). Compared with the control group, Fig. 7c reveals that the activity of SOD in wheat roots and leaves after UV-B treatment reduced by approximately 12%, which is in accordance with the outcomes reported in literature that high-intensity radiation of UV-B can lessen the activity of SOD in wheat seedlings (Chen *et al*., 2019). When the function of SOD in scavenging excess ROS was weakened, the SOD activity was also suppressed via a combined treatment of Mg-N-CDs and UV-B. POD activity in wheat seedling leaves and roots enhanced with the increasing concentration of Mg-N-CDs (Fig. 7d). After treating with UV-B, the activity of POD in roots and leaves was remarkably decreased by 22.3% and 12.1% compared with the control group. However, after UV-B combined with C1 and C2 groups, the activity of POD in leaves was obviously enhanced, which in roots was evidently increased in all groups. It has been proved that N-CDs can markedly up-regulate the pod activity to improve the antioxidant capacity of plants, indicating that Mg-N-CDs can enhance the antioxidant effect of plants and alleviate the damage caused by UV-B (Chen *et al*., 2020). In contrast to control group, the activity of CAT in all root treatment groups was significantly increased, and after UV-B and Mg-N-CDs combined treatment, the CAT activity was dramatically higher than that of UV-B treatment alone (Fig. 7e), indicating that Mg-N-CDs activated CAT activity. The activity of CAT in leaves decreased after treating by UV-B, which was similar to the previous studies (Chen *et al*., 2019). The CAT activity increased by 17.3% and 20.8% after UV-B combined with C1 and C2 groups, respectively, indicating that Mg-N-CDs relieved the injury of UV-B to plants (Chen *et al*., 2019). The CAT activity of leaves under UV-B treatment was similar to the activity of control, however, the combination between UV-B, C1 and C2 activated some antioxidant functions in wheat leaves. UV-B radiation may cause excessive production of ROS, which will be eliminated by antioxidant enzymes (Li *et al*., 2006). However, if ROS is not removed timely, it will lead to certain damage to the cell membrane of plant and destroy the plant antioxidant defense system. In conclusion, Mg-N-CDs can partially alleviate the antioxidant reaction induced by UV-B. These enzymatic antioxidants give the essential protective mechanisms to decrease the oxidative injury to plants, indicating the potential value of Mg-N-CDs in phytoremediation (Zhang *et al*., 2007).

Expression of antioxidant related genes: Glutathione is one of the most significant antioxidants in cells (Alscher, 2010), which synthesizes several important genes in the process (*ATPS*, *CS*, *GS*) that have been screened to study the response of UV-B to plants at the molecular level (Lu, 1999). Real time PCR was employed for the analysis of the three genes relative expression. Fig. 8a shows that after UV-B radiation, the relative expression level of *ATPS* is 31.6% lower than the level in control group, leading to the reducing content of adenosine 5'-phosphosulfate (APS). Nevertheless, the major pathway is the reduction of APS to sulfite and subsequently applied to produce cysteine, which is the glutathione biosynthesis precursor (Wiedemann *et al*., 2010). If the expression of *ATPS* is inhibited, glutathione synthesis will be reduced (Leustek, 1999). However, after Mg-N-CDs and UV-B combined treatment, the *ATPS* expression level in all the treatment groups was higher than the level in UV-B only treatment group, indicating that Mg-N-CDs can indirectly reduce the inhibitory influence of UV-B against glutathione synthesis. Under the radiation of UV-B, *GS* and *CS* expression levels were remarkably up-regulated in comparison with control group (Fig. 8b and Fig. 8c). In this study, in order to efficiently synthesize glutathione to resist oxidative stress, wheat seedlings increased the expression of related genes except *ATPS*. The results showed that Mg-N-CDs could indirectly improve the antioxidant capacity at the molecular level, which was similar to the previous research of plant physiology. More importantly, this study explained the potential of Mg-N-CDs to protect plants from the damage of UV-B on the molecular level.

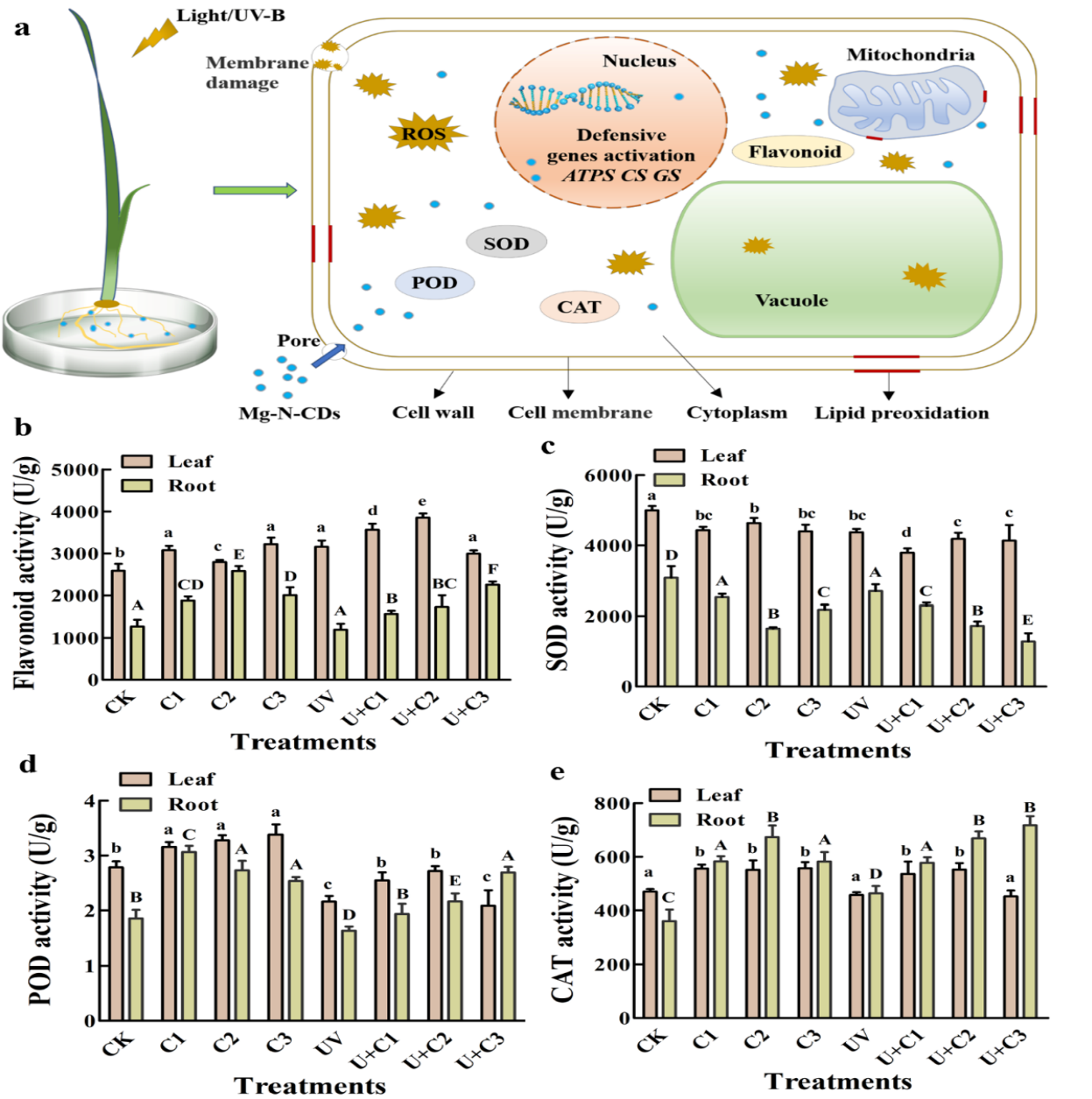

Fig. 7. (a) The potential damage caused by UV-B and Mg-N-CDs at wheat cell level. The activities of flavonoid (b), SOD (c), POD (d) and CAT (e) of wheat seedlings in different treatments were studied. The different letters represent significant difference (p<0.05).

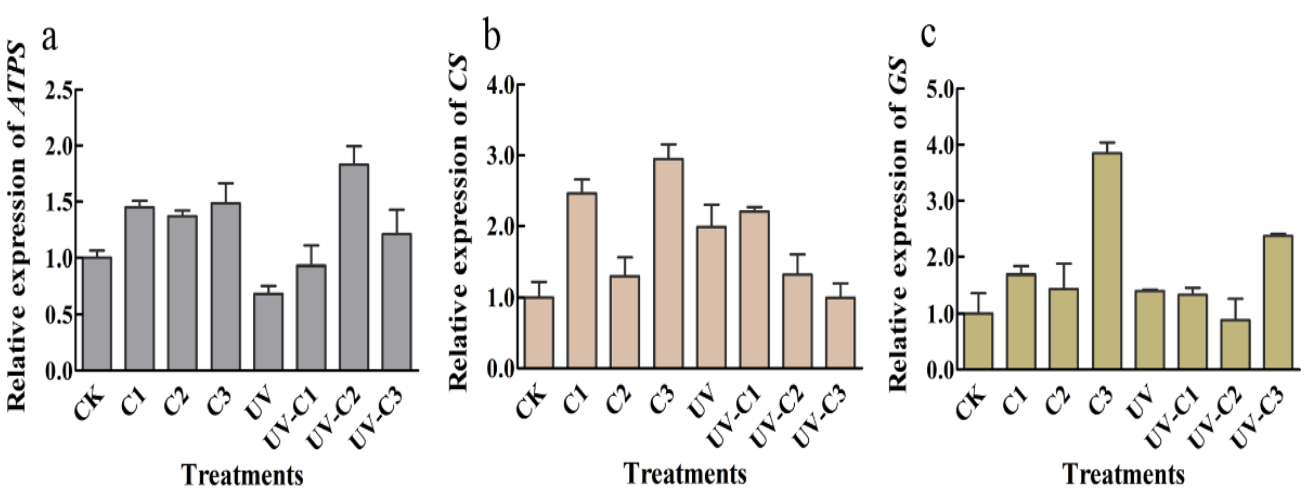

Fig. 8. The relative expression of antioxidant gene in wheat seedlings in different treatment groups. (a) The relative expression of *ATPS*; (b) The relative expression of *CS*; (c) The relative expression of *GS*.

Conclusions

In conclusion, Mg-N-CDs can reduce root inhibition, biomass reduction and oxidative damage of Wheat Seedlings under the exposure of UV-B. The application of Mg-N-CDs to wheat after UV-B treatment can activate defense enzymes, enhance antioxidants, reduce plant damage and promote the increase of chlorophyll content. Besides, glutathione related genes were also analyzed, the outcomes indicated that the Mg-N-CDs could enhance the antioxidant capacity of plants by regulating gene expression and acted a positive part in improving the UV-B radiation damage of wheat. This gives a new way to boost the tolerance of plants to UV-B radiation, but further research is essential for the explanation of the latent molecular mechanism of the tolerance of Mg-N-CDs to plants under UV-B stress.

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