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Full Length Article

Cadmium Telluride Quantum Dots Application Impaired Seedling Growth and Leaf Protoplasts of Bread Wheat

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Abstract

Nowadays more quantum dots are released into the environment due to large-scale use of artificial nanomaterials (NPs); and plants are considered to be one of many organisms directly affected by NPs. This study was conducted to evaluate the effects of cadmium telluride quantum dots (CdTe QDs), a common form of NPs, on wheat seedlings growth and mesophyll protoplasts. For this, six CdTe QDs dose gradients *i.e*., 0, 50, 100, 200, 500 and 1000 mg/L were applied on wheat seedlings and mesophyll protoplasts. Results revealed that CdTe QDs application inhibited shoot and root length and higher concentrations proved more toxic. For instance, shoot and root lengths of wheat seedlings observed 85.5 and 85.7% decline at 1000 mg/L concentration. Moreover, the CdTe QDs also caused a decrease in soluble sugar contents (88.5 and 93.1% reduction, respectively in shoots and roots at 1000 mg/L) along with an increase in the malondialdehyde (MDA) contents in shoots and roots of wheat seedlings (187.2 and 468.7% increase, respectively in shoots and roots at 1000 mg/L). Nonetheless, the total protein contents of mesophyll protoplast of wheat seedlings decreased with increasing concentration of CdTe QDs. However, protein types in protoplast of wheat seedlings were increased with increasing concentration of applied CdTe QDs. In conclusion, CdTe QDs application impaired wheat growth due to decrease is soluble sugars along with elevated MDA contents. Moreover, CdTe QDs treatments reduced the total protein contents while increased proetin types in mesophyll protoplast of wheat seedlings.

Keywords: Toxicity; Nanoparticles; Quantum dots; Mesophyll protoplasts; Wheat

Introduction

The current large-scale use of artificial nanomaterials has released more quantum dots into the environment and has an unpredictable impact on the environment and human health (Wiesner *et al*. 2006). Recent studies showed that the water environment provides a way for these nanomaterials (NPs) to enter the environment (Kim *et al*. 2010) and will eventually sink and accumulate in the soil (Navarro *et al*. 2010). Plants are one of the many organisms that are directly affected by NPs since these are grown in soil. It is also confirmed that NPs can penetrate different biological barriers, from insect to plant cells (Lin *et al*. 2009).

The QDs are crystalline NPs, first synthesized in the early 1980s and used in the electronics industry (Brus 1984). They are a type of nanocrystalline semiconductor material. The core and shell of a semiconductor nanocrystal are composed of elements of groups III to V or II to VI, and their sizes range from 1 to 10 nm. At such a size, these NPs exhibit unique photoluminescence (Chandan *et al*. 2018). The widely studied QDs are CdTe QDs, cadmium selenide (CdSe) QDs and cadmium sulfide (CdS) QDs (Ensafi *et al*. 2017).

The CdTe is one of the most important II-VI semiconductors and has been extensively applied in the fields of biomedicine, optoelectronic devices and photonic crystals (Wang *et al*. 2018). As a new type of nanomaterial, CdTe QDs showed toxicity *in vitro* and *in vivo* (Schneider *et al*. 2018). Liver cancer cells showed reduced metabolic activity, increased apoptotic cells, increased intracellular reactive oxygen species (ROS) contents along with reduced glutathione (GSH) and catalase (CAT) contents and glutathione thiol transferase (GST) activity after exposure to CdTe QDs (Nguyen *et al*. 2013). After treating with CdTe QDs and daunorubicin in HepG2 cells, the proportion of apoptosis after synergistic action was at the highest level (Zhang *et al*. 2011). The CdTe QDs and CdTe/CdS QDs decreased the survival rate of human epithelial cells in 24 h, and most of the cells died in 48 h (Su *et al*. 2009). While in plants, CdTe-QDs and enhanced UV-B radiation triggered antioxidant enzyme metabolism and programmed cell death in wheat (*Triticum aestivum* L.) seedlings (Chen *et al*. 2014).

In addition to studying the toxic effects of CdTe QDs on cells, researchers have also conducted preliminary investigations into the mechanism of toxic effects (Whiteside *et al*. 2009). The CdTe QDs exposure is found to be associated with ROS production, mitochondrial damage, protein and DNA damage, and apoptosis (Wang *et al*. 2017). Comet tests showed that CdTe QDs damaged cellular DNA in a dose-dependent manner (Zhang *et al*. 2015) and it affected some processes of mitochondrial biosynthesis (Li *et al*. 2014). Genetic research showed that the tail DNA content of the CdTe QDs treatment was significantly higher than the control. The possible reason is the released content of Cd^{2+} (Nguyen *et al*. 2015).

Although previous studies confirmed that Cd^{2+} released from the core of CdTe QDs are transported into the cells causes cytotoxicity (Wang *et al*. 2010). Fewer studies have evaluated the toxicity of CdTe QDs in plants (Chen *et al.* 2014), especially in food crops such as wheat, rice (*Oryza sativa* L.) and corn (*Zea mays* L.). Therefore, this study was designed to evaluate the effects of CdTe QDs on wheat seedlings and mesophyll protoplasts. Moreover, the results of this study will provide a foundation to further study the toxicity and biological safety of CdTe QDs in plants.

Materials and Methods

Plant materials

The wheat variety Lin Y8198 donated by Shanxi Wheat Research Institute was used as experimental material.

Preparation of CdTe QDs

The CdTe QDs were configured with distilled water (DW), M standard solution (5 mmol/L $CaCl_2{\cdot}2H_2O$, 0.5 mol/L mannitol, 0.5 mmol/L KH_2PO_4, 2 mmol/L $MgSO_4$, 3 mmol/L 2-(N-Morpholino) ethanesulfonic acid 4-Morpholineethanesulfonic acid, MES, pH= 5.6), Phosphate Buffered Saline (PBS) (NaCl 137 mmol/L, KCl 2.7 mmol/L, Na_2HPO_4 4.3 mmol/L, KH_2PO_4 1.4 mmol/L, pH= 7.2), and PEM (50 mmol/L PIPES, 5 mmol/L EGTA, 5 mmol/L $MgSO_4$, 0.225 mol/L Sorbitol, pH= 6.9). The CdTe QDs had different stability and fluorescence intensity in different solvents (Fig. 1a) and the CdTe QDs configured with PBS were more effective. The PBS configured CDTe QDs were suitable for living environment of leaf protoplasts and alkaline environment of the solvent. This made the CdTe QDs more stable and the fluorescence intensity stronger. In order to investigate the effect of different concentrations of CdTe QDs on protoplasts, different concentrations of CdTe QDs were configured with PBS as a solvent (Fig. 1b) and then the Image J software was used to calculate the fluorescence intensity of the same area in different treatment groups.

Six different concentrations of CdTe QDs solutions configured with PBS solution as 0, 50, 100, 200, 500 and 1000 mg/L were used to observe the fluorescence intensity in the gel imager system. Compared with control (CK) group, the fluorescence intensity of the CdTe QDs in the concentrations 50–500 mg/L increased with increasing concentration, but the fluorescence intensity of the Q5 treatment group (1000 mg/L) did not increase further (Fig. 2).

Wheat cultivation

Wheat seeds with full and uniform grains without damage and mildew were selected, washed with tap water 2 to 3 times and then sterilized with 1.5% NaClO for 10 min. The disinfection solution was washed with distilled water, and then was added an appropriate amount of distilled water. Thirty seeds were cultured per Petri dish in a growth chamber at 25°C with 55% relative humidity and were watered daily. From the day the seeds germinated, six treatments were performed, each repeated three times. Briefly, seeds were soaked with either CdTe QDs (0, 50, 100, 200, 500 and 1000 mg/L) all day long. After 7 days of growth, the plant height and root length were measured with a ruler. Eighty seedlings per replicate per treatment were randomly selected for analysis.

Measurement of MDA and soluble sugar concentrations

Trichloroacetic acid (TCA) method was used to determine MDA concentration. Fresh tissues (1.0 g) were ground with SiO_2 in 2 mL 10% TCA. After centrifugation at 4000 rpm for 10 min, the supernatant was removed, 2 mL of 0.6% (w/v) thiobarbituric acid was added, and the mixture was incubated in a 100°C water bath for 15 min. After centrifugation at 4000 rpm for 15 min, the supernatant was measured at 532 and 450 nm, respectively. The total sugar concentration was determined by anthrone colorimetry. Dry plant tissues (50 mg) ware triturated with 4 mL of 80% ethanol. The supernatant was collected after continuous stirring in a water bath at 80°C for 40 min. Activated carbon (10 mg) was used to decolorize the solution for 30 min, after which 5 mL anthrone was added and samples were incubated in a water bath at 100°C for 10 min. Samples were then cooled for 5 min before spectrophotometric absorbance assessment at 625 nm. The concentration was determined using standard curves.

Isolation and identification of protoplasts

The 7-day-old leaves of wheat seedlings without being soaked with CdTe QDs were taken. The wheat mesophyll protoplasts were prepared by enzymatic hydrolysis, and the centrifuge tubes containing the prepared protoplasts were numbered 0, 1, 2, 3, 4, 5; then 3 mL of CdTe QDs of different concentrations were taken and added to the numbered centrifuge tube, finally the centrifuge tube was covered and shake up and down gently to mix well, and incubated at 37°C in the dark overnight.

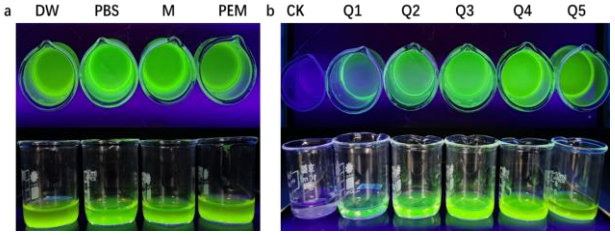

Fig. 1: Comparison of fluorescence intensity of CdTe QDs in different solvents (**a**) and with different concentrations in PBS (**b**). The red box indicates the area used to calculate the fluorescence intensity

Here DW= Distilled water; PBS= Phosphate buffered saline; M= M standard solution; PEM= PIPES, EGTA, MgSO; CK= 0 mg/L; Q1= 50 mg/L; Q2= 100 mg/L; Q3= 200 mg/L, Q4= 500 mg/L; Q5= 1000 mg/L

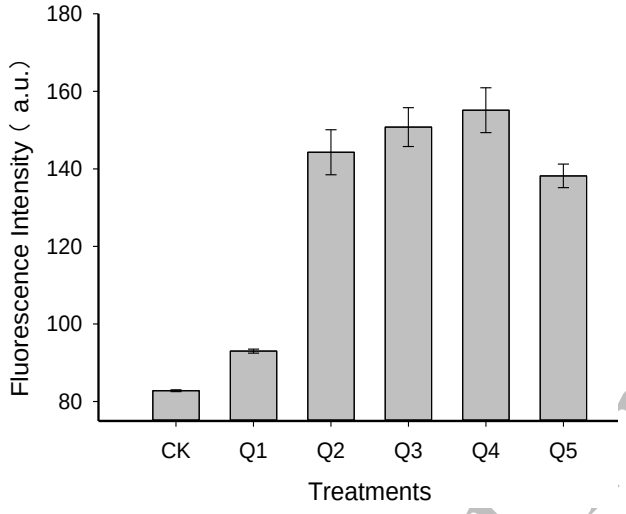

Fig. 2: Comparison of fluorescence intensity of CdTe quantum dots with different concentrations in PBS

Here CK= 0 mg/L; Q1= 50 mg/L; Q2= 100 mg/L; Q3= 200 mg/L, Q4= 500 mg/L; Q5= 1000 mg/L

Leaf protoplast suspension (0.2 mL) was taken and FDA staining solution (0.2 mL) was added (Cheng and Belanger 2000). A small amount of the mixed solution was taken on a glass slide and fluorescence microscope was used to take photos of protoplasts under a 565 nm fluorescence filter to observe and count live (yellow) protoplasts and dead (red) protoplasts (Silva and Menendezyuffa 2006).

Extraction of leaf protoplast protein

The treated leaf protoplasts were centrifuged and the supernatant was discarded. Aprotinin (50 μL) and cell extract (2 mL) were added to each centrifuge tube and mixed thoroughly and an appropriate amount of quartz sand was added to the mortar. The treated leaves in the centrifuge tube were ground separately. After grinding, aliquot was transferred into a new centrifuge tube and marked accordingly. Then the supernatant was centrifuged to obtain the protein extraction solution, stored at-80°C for future use.

Determination of protein contents

The protein was quantified using the Bradford protein assay; bovine serum albumin at different dilutions was measured using the standard curve method, and the absorbance was measured colorimetrically at 595 nm, repeated 3 times, and recorded; using spectrophotometry determine the absorbance of the sample extract under different processing conditions formed at 595 nm, repeated 3 times, and recorded. The total protein content of leaf protoplasts was measured according to the formula.

Analysis of leaf protoplast proteins by SDS-PAGE

After cleaning, Vaseline was used to prepare the rubber sheet. After checking for leakage with distilled water, it was wiped clean with filter paper. The glue was made according to the method of separating the glue and pour the glue. After pouring the glue, N-butanol was used to press the surface immediately. After the gel is solidified, upper layer of N-butanol was poured off. The concentrated gel was configured, poured to full. The comb was inserted into the gel for gelation; the electrophoresis tank was prepared, the electrode buffer was poured, the bottom rubber strip was remove, the comb was pulled out, then the gel plate was inserted into the electrophoresis tank, and air bubbles were eliminated with a syringe. Spot the sample and plug in the power. Initially, the constant pressure of the concentrated gel is 80 v and the constant pressure of the separation gel is 120 v. Stain for 3 h, then decolorizes and observe.

Statistical analysis

Data results are expressed as means ± standard error (SE). Statistical significance of data were assessed using one-way analysis of variance (ANOVA) tests using General Linear Model and Tukey test was performed using the S.P.S.S. 21.0 and Sigma-plot 12.5 to compare the treatments means.

Results

Effects of CdTe-QDs on wheat seedlings growth

Compared with control the shoot and root length of wheat seedlings gradually decreased with increasing CdTe QDs concentrations (Fig. 3). Shoot length of wheat seedlings at 50 (Q1), 100 (Q2), 200 (Q3), 500 (Q4) and 1000 mg/L (Q5) concentration groups decreased by 5.0, 10.1, 20.1, 73.8 and 85.5%, respectively compared with control (Fig. 3a). The root length at Q1 ~ Q5 treatment groups was decreased by 13.2, 22.9, 41.1, 71.1 and 85.8%, respectively over control (Fig. 3d). The soluble sugar contents in shoots and roots of wheat seedlings were gradually decreased with the increasing concentrations of CdTe QDs. Compared with control, the Q1 ~ Q5 treatment groups reduced soluble sugar contents by 7.1, 13.7, 21.8, 77.9 and 88.5% in shoots and by 21.1, 30.1, 53.4, 79.3 and 93.1 in roots (Fig. 3c, f).

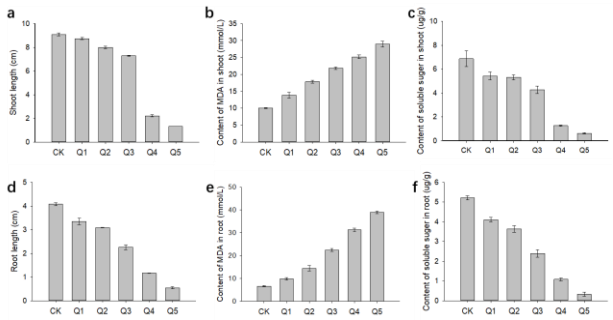

Fig. 3: Effects of different concentration of CdTe QDs on 7-day-old wheat seedlings
Here CK= 0 mg/L; Q1= 50 mg/L; Q2= 100 mg/L; Q3= 200 mg/L, Q4= 500 mg/L; Q5= 1000 mg/L

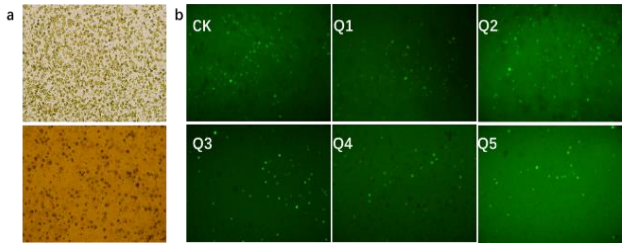

Fig. 4: Morphology of unpurified (upper) and purified (lower) wheat leaf protoplasts without FAD staining (**a**) and purified leaf protoplasts stained with FAD under 10x magnification (**b**)
Here CK= 0 mg/L; Q1= 50 mg/L; Q2= 100 mg/L; Q3= 200 mg/L, Q4= 500 mg/L; Q5= 1000 mg/L

Moreover, the contents of MDA increased with the increase of CdTe QDs concentration (Fig. 3b, e). Compared with control group, Q1 ~ Q5 treatment groups increased MDA contents in shoot by 40.4, 75.7, 111.8, 146.5 and 187.2% (Fig. 3b) while in root MDA contents the increase was 40.8, 115.6, 229.6, 355.5 and 468.7, respectively over control (Fig. 3e).

Determination of protoplast viability

Before staining with FAD, the purified protoplasts were round and almost free of impurities (Fig. 4a). The protoplasts in the control group showed bright green fluorescence after FDA staining, indicating that their protoplasts were more active. Compared with the control group, the number of protoplasts in the leaves of the Q1 ~ Q5 treatment groups gradually decreased with the increase of CdTe QDs concentrations, which may be due to membrane rupture and cells death (Fig. 4b).

Determination of protein content

As shown in Fig. 5a, the total protein contents of protoplast of wheat seedlings of each group were calculated according to the standard curve. Compared with control, the total protein contents of protoplast of wheat seedlings of the Q1 treatment group was significantly reduced and the total protein contents in the Q2 group were significantly

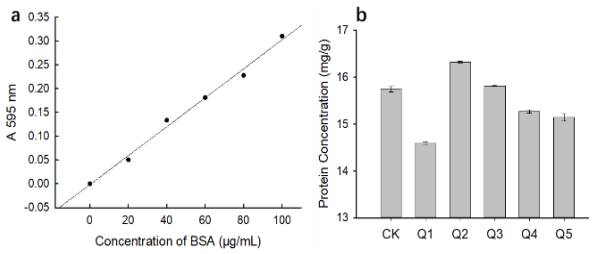

Fig. 5: Standard curve: bovine serum albumin content at different dilutions (**a**) and total protoplast protein content of wheat leave protoplasts in different treatments (**b**)
Here CK= 0 mg/L; Q1= 50 mg/L; Q2= 100 mg/L; Q3= 200 mg/L, Q4= 500 mg/L; Q5= 1000 mg/L

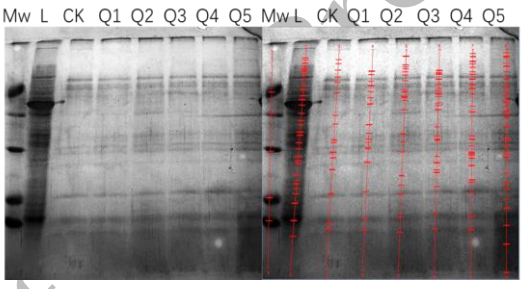

Fig. 6: SDS-PAGE electrophoresis profiles of total protein in wheat leaf protoplasts treated with different concentrations of CdTe QDs. The red lines in the above figure are automatically added by the Quantity One software which indicated the protein bands
Here L = Leaves of the CK group; CK= 0 mg/L; Q1= 50 mg/L; Q2= 100 mg/L; Q3= 200 mg/L, Q4= 500 mg/L; Q5= 1000 mg/L

increased. From the Q2 ~ Q5 treatment groups, the total protein contents of protoplast of wheat seedlings decreased significantly with increase of CdTe QDs concentrations. The protein content of the Q3 treatment group and the control did not change significantly, and the total protein contents of the Q4 and Q5 groups were both lower than the control group (Fig. 5b).

Analysis of leaf protoplast protein by SDS-PAGE

In order to studying the relationship between the concentration of CdTe QDs and the total protein content of wheat protoplasts, the effect of QDs concentration on protein types was further explored. The results showed that the Mw, CK, Q1, Q2, Q3, Q4, and Q5 treatment groups contained 5, 16, 17, 21, 23, 26 and 31 bands, respectively (Fig. 6), which means the protein types increased with the increasing concentration of CdTe QDs. From Fig. 5, it could be seen that these increased protein bands were mainly distributed between 97.4 kD and 66.2 kD.

Discussion

In this study, high-concentration CdTe QDs significantly inhibited the growth of wheat roots and shoots. The impacts of NPs on crop plants are unavoidable because plants cannot

move but only acclimate to environmental changes. More and more literature has confirmed that CdTe QDs have a significant toxic effect on wheat growth and development, and have been studied in terms of physiology and biochemistry (Su *et al*. 2010; Chen *et al*. 2014). When CdTe QDs were absorbed by plants, they can reduce the amount of antioxidants in cells or increase the production of reactive oxygen species (ROS) (Santos *et al*. 2012), increased ROS will inhibit wheat root length (Marmiroli *et al*. 2015). It is well reported that CdTe QDs can reduce the expression of intracellular proteins involved in auxin transport (Marmiroli *et al*. 2016), thereby reducing auxin synthesis, inhibiting cell elongation and shoot length.

Results unveiled that the exogenous CdTe QDs could be absorbed and gathered in cell vacuoles in wheat and displayed inhibitory effects on wheat seedlings growth. Since some acid enzymes were in the vacuole which made CdTe QDs released Cd^{2+} in the vacuole; thereby deepening the toxic effects on wheat (Hassan *et al*. 2016). At the concentration of Q4 (500 mg/L), shoot growth of wheat plants were significantly inhibited (Fig. 3a). It might be due to the CdTe QDs absorbed from wheat roots and entered to shoots through vascular tissue; the CdTe QDs dissociated into more Cd^{2+} in the plant and showed more toxicity (Wang *et al*. 2010). This needs to be taken seriously, because the effects of CdTe QDs on plants may be twofold, namely the effects of CdTe QD particles and the toxic effects of Cd^{2+} on plants.

Externally added CdTe QD particles caused the decline and death of wheat mesophyll protoplasts (Fig. 4b); which indicated that plant cells without cell walls are more susceptible to the toxicity of CdTe QDs. Since, the plant cells were directly treated with CdTe QDs *in vitro*, which avoided the degradation effects of plants on the CdTe QDs during transportation from plant roots to shoots. Therefore, *in vitro* treatment of mesophyll protoplasts with CdTe QDs can more accurately reflect the response of mesophyll cells to CdTe QDs. This system has not been reported yet, but it is necessary to directly reflect the impact of NPs on plant cells at the cellular level.

From the effect of CdTe QDs concentration on the protein content of wheat leaf protoplasts (Fig. 5), low concentration of CdTe QDs can inhibit protein synthesis, but within a certain concentration range, QDs can promote protein synthesis. The CdTe QDs may inhibit the activity of proteolytic enzymes and activate the expression of wheat resistance genes at this concentration. When the concentration of CdTe QDs continued to increase, its toxicity exceeded the resistance of wheat itself, destroying wheat genes and some life-active substances such as enzymes, leading to the death of wheat leaf protoplasts.

Significant changes have taken place in the protein expression pattern in wheat mesophyll protoplasts treated with CdTe QDs. As the concentration of treatments increased, more and more different types of proteins were detected (Fig. 6). Based on previous studies conducted by Marmiroli *et al*. (2015) combined with the growth phenotype analysis of the seedlings, it is speculated that the increased protein types should be related to oxidative stress response, auxin synthesis and transport, and cell metabolism. CdTe QDs not only affect the synthesis of intracellular proteins, but also cause cellular DNA damage and inhibit DNA repair, but the mechanism of DNA damage is still insufficiently explored. Therefore, future research directions should focus on the interconnections between various mechanisms, studying the response mechanism of crop plants to environmental CdTe QDs at the molecular level, and continue to explore the potential mechanisms of NPs toxicity in plants.

Conclusion

Results obtained revealed that CdTe QDs application inhibited wheat seedlings growth and show toxicity to mesophyll protoplasts. The CdTe QDs application led to a decrease in soluble sugar concentration along with simultaneous increase in MDA contents in shoots and roots. Also, CdTe QDs changed the total protein pattern of the mesophyll protoplasts. More *in vitro* experiments are needed to study the effect of CdTe QDs on crop plants.

Acknowledgments

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