Foliage application of chitosan alleviates cadmium toxicity in wheat seedlings (*Triticum aestivum* L)

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Research Article

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Abstract

Excessive cadmium (Cd) causes toxic effects on crops. The effects of chitosan (CTS) with different molecular weight (Mw) (5 kDa, 3kDa, and 1 kDa) on the growth and biochemical parameters, as well as Cd concentrations in Cd-treated wheat plants were examined in a pot experiment. The results demonstrated that foliar spraying with CTS significantly improve the wheat growth, reduce malondialdehyde (MDA) content in leaves and decrease Cd concentrations in roots and shoots of wheat seeding under Cd stress. The alleviation of Cd toxicity by CTS is probably related with the activity of antioxidant enzymes, osmotic adjustment matter and root morphology. The application of CTS enhanced the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in Cd-stressed wheat seedling leaves by 6.6%–13.1%, 17.2%–33.0%, and 19.6%–25.5%, respectively. Besides, exogenously applied CTS also increased the soluble protein and soluble sugar contents by 17.6%–33.8% and 30.1%–36.1% in the leaves of wheat under Cd stress. Furthermore, CTS with a molecular weight of 1 kDa was the most effective in mitigating Cd toxicity in wheat seedlings, which indicates that the activity of CTS is depend on its molecular weight. It can be concluded that the use of foliar spraying, especially with 1 kDa CTS, could have potential in reducing the damage of Cd stress.

1. Introduction

Cadmium (Cd) is a non-essential chemical element that can enter the environment through agricultural and industrial activities. It is toxic to both humans and plants (Piacentini et al. 2020). Cd is easily absorbed and accumulates in plant tissues because of its high solubility and mobility in agricultural soils (Wu et al. 2020). Elevated Cd concentrations in plants results in chlorosis, photosynthesis inhibition, biomass reduction, and poor quality of products (Rizwan et al. 2017; Liu et al. 2020; Wu et al. 2020). Moreover, the accumulation of Cd in edible parts of plants can result in a detrimental effect on human health (Bernard 2008; Sebastian and Prasad 2014). Wheat is a dominant cereal and is considered the staple food for most of the world's population (FAO, 2014). Excessive Cd content has been widely reported (Wu et al. 2020; Rizwan et al. 2017). Therefore, it is particularly important to develop measures to decrease Cd uptake in wheat growing in a Cd contaminated environment. Many techniques, including site selection, agricultural practices, and soil amendment, have been developed to reduce Cd accumulation in plants (Huang et al. 2019). However, these strategies are often too expensive to apply at a large-scale in the field (Liu et al. 2019). Recently, applying chemical regulators has been regarded as an effective strategy for reducing Cd accumulation in crops (Liu et al. 2019; Zong et al. 2017a).

Chitosan (CTS), a degradation product of chitin, is predominantly obtained from the waste of shellfish and crustaceans. CTS has been found to possess diverse biological activity, such as antimicrobial, antitumor, and antioxidant activity (Rendina et al. 2019; Jia et al. 2019; Song et al. 2020). These bioactive properties make CTS a potential contender for uses in medicine, food, and agriculture (Mukhtar Ahmed et al. 2020; Malerba and Cerana, 2016). In agriculture, CTS could effectively elicit plant innate immunity, promote plant growth, and stimulate the biosynthesis of secondary metabolites (Zhang et al. 2018; Jia et al. 2019; Vosoughi et al. 2018). Moreover, research has identified that CTS and its oligosaccharides could improve plant tolerance to adverse conditions including stresses induced by salinity, high temperature, and cold (Zhang et al. 2020; Zong et al. 2017b; Sen et al. 2020; Zou et al. 2017). It has been reported that CTS can regulate reactive oxygen species (ROS) by enhancing the activity of the antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), and consequently alleviate abiotic stress in plants. Qu et al. (2018) observed that the application of CTS reduces the negative impact of Cd on maize by enhancing SOD and CAT activity. Our previous study also indicated that a CTS foliar spray could increase the tolerance of edible rape to Cd through stimulating the activity of SOD, POD, and CAT as well as altering Cd subcellular distribution (Zong et al. 2017a). Furthermore, molecular weight (MW) is a key factor influencing the physicochemical properties of CTS (Mukhtar Ahmed et al. 2020; Song et al. 2020). However, to our knowledge, there are no studies that have researched the effect of CTS with different MW on the resistance of wheat to Cd stress.

Therefore, we exposed wheat seedlings to Cd stress and investigated the influence of CTS with different MW on the growth of wheat seedlings in this study. Furthermore, the possible benefits of applying CTS were also investigated by determining antioxidant enzyme activity and root morphological parameters.

2. Materials And Methods

2.1. Material

CTS with an average MW of 1, 3, and 5 kDa were purchased from Jinan Haidebei Co. Ltd. (Shandong, China). The deacetylation degree of all three CTS was approximately 80%.

2.2. Plant material and experimental design

Wheat (*Triticum aestivum* L. Jimai-22) seeds were used in this study. After being surface sterilized with a solution of 10% NaClO, the seeds were covered with moist gauze for germination for 24 h at 25 °C in the dark. Thirty similar seedlings were selected and transferred into Petri dishes (11.5 cm in diameter) with nylon mesh containing a half-strength Hoagland nutrient solution (Arnon and Hoagland, 1940). The plants were grown in an illuminating incubator, 25/18 °C (day/night) temperature, 14/10 h (light/dark) light period, 800 mol m^{-2} s^{-1} light intensity, 70% humidity. After the second leaf was developed fully, 50 μM Cd was provided in solution as $CdCl_2·2.5H_2O$. The Cd concentration was based on our previous studies (Zong et al. 2017a). The nutrient solution containing Cd was renewed every other day. After 7 days of Cd application, the different CTS treatments were sprayed in 50 mg/L solution with 0.1% Tween-20. The control treatment was a spray of distilled water with 0.1% Tween-20. Each treatment was repeated three times randomly. The wheat seedlings were sprayed with 20 mL distilled water or CTS every other day for 1 week, plants were collected for analysis after spraying three times with CTS.

2.3 Growth parameter and Cd concentration determinations

The wheat plants were separated into roots and shoots after harvest. The fresh weight (FW) of root and shoot is used as an index to evaluate the growth of wheat. Then the plant tissues (i.e. shoots and roots) were dried at 105 °C to obtain dry samples which were digested by a HNO_3 + $HClO_4$ mixture, (4/1, v/v). The Cd concentration was measured by inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima, 7000). Quality assurance and quality control (QA/QC) were measured with duplicates, method blanks, and standard reference material GBW 10048 (GSB-26).

2.4 Measurement of lipid peroxidation content

The level of lipid peroxidation in fresh leaves was determined by estimating malonaldehyde (MDA) content using 0.1% thiobarbituric acid (Zou et al. 2017). The MDA content was expressed as ug MDA g^{-1} FW.

2.5 Determination of chlorophyll soluble protein, soluble sugar, and proline contents

Samples were extracted from fresh leaves with 95% ethanol. The total chlorophyll (a+b), chlorophyll a (*Chl a*), and chlorophyll b (*Chl b*) contents were measured by a spectrophotometer at 665 nm and 649 nm (Sedmak and Grossberg 1977).

The determination of soluble protein was based on a method by Zou et al. (2017). Proteins were extracted from fresh leaves (0.5 g) with 5 ml cold potassium phosphate buffer (pH 7.8). The extract was mixed with Coomassie brilliant blue G250 staining and the absorbance of the mixture was read at 595 nm. Protein concentration was quantified by comparison with a standard curve using bovine serum albumin. Soluble sugar was assessed using a method by Zou et al. (2017). Soluble sugar concentration was quantified by comparison with a standard curve using the criterion of glucose.

Proline content was measured based on the method of Zong et al. (2017a). About 0.2 g of fresh leaf was homogenized in 5 ml of 3% sulfosalicylic acid and then heated for 10 min at 100 °C. The extract was cooled on ice and measured at 520 nm by using a spectrophotometer.

2.6 Analysis of antioxidant enzyme activities

Antioxidant enzymes (SOD, POD, and CAT) activities were measured spectrophotometrically. Fresh leaf samples (0.5 g) were ground in liquid nitrogen and homogenized in 5 ml cold potassium phosphate buffer (pH 7.8). The extract was centrifuged at 12,000 × *g* for 15 min at 4 °C. The supernatants were transferred for further analysis. SOD activity was measured according to the methods detailed by Paoletti et al. (1986). POD activity was measured using guaiacol as a substrate according to a method by Chance and Maehly (1955). One unit of POD is defined as the change in absorbance/min at 470 nm. The determination of CAT activity was based on the consumption of H_2O_2 measured at 240 nm, which were detailed in reference of Chance and Maehly (1955).

2.7 Statistical analyses

All data were analyzed using SPSS 22.0 software, providing the means ± S.D. (standard deviation) of three replicates. Significant differences were detected by one-way analysis of variance (ANOVA) using

Duncan’s multiple range test ($P < 0.05$).

3. Results

3.1 plant growth and chlorophyll content

Cd has a significant negative effect on wheat seedling biomass (Fig. 1). Compared with the control, the root and shoot biomass decreased by 28.9% and 42.85%, respectively. While, CTS with different MW (5, 3, and 1 kDa) application significantly increased the growth parameters of wheat seedling compared with the single Cd treatment. The increases in the root and shoot biomass were 33.7%–64.9% and 19.9%–50.4%, respectively. The heaviest FW of root and shoot was found in the Cd with 1 kDa CTS treatment. Moreover, there was no significant difference in root and shoot FW between Cd + CTS(1k) treatment and the Cd-free control. The results indicated that spraying CTS on leaves could alleviate the toxic effect of Cd on wheat seedlings.

Alterations in chlorophyll content in the wheat leaves following different treatments are illustrated in Table 1. Cd stress caused a 21.4%, 46.3%, and 28.8% decrease in the contents of *Chl* a, *Chl* b and total chlorophyll compared to control (Table 1). Additionally, foliar application of CTS with (1, 3, and 5 kDa MW) significantly increased *Chl* a, *Chl* b, and total chlorophyll content by 3.90%–20.7%, 18.2%–27%, and 7.07%–32.3%, respectively. Moreover, chlorophyll content in Cd with 1 kDa CTS treatment was significantly higher than that in Cd with 3 kDa CTS treatment and there was no difference between Cd with 1 kDa CTS treatment and the Cd-free control. The results indicate that CTS could prevent chlorophyll degradation in wheat leaves under Cd stress, and a close connection exists between the level of protection offered by CTSs and their MWs.

Table1 Effects of CTS on chlorophyll contents of wheat seedlings under Cd stress

	Chl-a (mg g^{-1} FW)	Chl-b (mg g^{-1} FW)	Chl-(a+b) (mg g^{-1} FW)
CK	0.98±0.05 d	0.41±0.03 c	1.39±0.08 c
Cd	0.77±0.03 a	0.22±0.01 a	0.99±0.05 a
Cd+ CTS(5k)	0.80±0.06 ab	0.26±0.02 a	1.06±0.07 a
Cd+ CTS(3k)	0.86±0.05 bc	0.37±0.02 b	1.23±0.09 b
Cd+CTS(1k)	0.93±0.07 d	0.38±0.03 bc	1.31±0.08 bc

* Different letters within the same column mean significant differences at $P < 0.05$ level. Data are presented as the means ± SE (n = 3).

3.2 Effects of CTSs on lipid peroxidation

To further analyze the influence of CTS on oxidative damage of wheat caused by Cd, MDA content, a directly indicator of lipid peroxidation, was investigated. The results indicated that a dramatic increase in lipid peroxidation was found in Cd-stressed wheat seedlings leaves, and this oxidative damage could be decreased by the foliar application of CTS. Compared with single Cd treatment, CTS with 1, 3, and 5 kDa

MW appeared to reduce MDA content by 48.2%, 43.6%, and 21.4%, respectively. It seemed that 1 kDa CTS demonstrated the best activity in alleviating the degree of lipid peroxidation.

3.3 Effects of CTS on soluble protein, soluble sugar, and proline contents

The osmotic adjustment of soluble protein, soluble sugar, and proline contents in wheat seedlings are illustrated in Table. 2. Cd exposure significantly reduced content of soluble protein by 12.9% and soluble sugar by 9.7% in the wheat leaves compared with those in the Cd-free wheat plants (Table 2). However, foliar application of CTS (5, 3 and 1 kDa) significantly elevated content of soluble protein by 17.6%–33.8% and 30.1%–36.1% in the leaves of wheat exposed to Cd stress alone. Furthermore, 1 kDa CTS was found to be relative more effective in enhancing the soluble protein and soluble sugar content.

Table 2 Effects of CTS on soluble protein, soluble sugar, and proline contents of wheat seedlings under Cd stress

	proline ▯ug g^{-1} FW▯	soluble protein ▯mg g^{-1} FW▯	soluble sugar ▯mg g^{-1} FW▯
CK	9.45±0.10a	24.8±0.23b	18.4±0.09b
Cd	26.9±0.14d	21.6±0.35a	16.6±0.12a
Cd+CTS(5k)	19.9±0.26c	25.4±0.78b	21.9±0.33c
Cd+CTS(3k)	18.6±0.48b	27.1±0.47c	22.5±0.16c
Cd+CTS(1k)	9.88±0.11a	28.9±0.34d	22.6±0.25c

* Different letters within the same column mean significant differences at $P < 0.05$ level. Data are presented as the means ± SE (n = 3).

Proline accumulation is a potential indicator of stress tolerance. As shown in Table 2, a dramatic increase in proline content (184.7%) occurred in the leaves of wheat plants treated with Cd compared with the control (Table 2). While foliar applications of 5, 3, and 1 kDa CTS significantly decreased the proline content by 25.9%, 30.9%, and 63.3%, respectively, in the leaves of wheats exposed to Cd stress alone, and no significant difference was observed between Cd plus 1 kDa CTS treatment and the control, suggesting that foliar spraying with 1 kDa CTS brought the proline content down to a normal level in Cd-stressed wheat seedling leaves.

3.4 Activity of antioxidant enzyme in wheat seedling leaves

Cd toxicity was generally closely related to the production of ROS. Increasing antioxidant enzymes activities could deplete the excess ROS and effectively maintain a redox balance. To further understand how CTS exerted its protective action toward the oxidative damage caused by Cd stress, the activity of key antioxidant enzymes (SOD, POD, and CAT) in wheat leaves was investigated in this study. Results showed that compared with the control, Cd stress significantly increased the total activity of SOD, POD, and CAT by 28.4%, 22.3% and 22.3%, respectively (Fig. 3A–C). Application of CTS had a significantly synergetic effect on SOD, POD and CAT activity and increased their activity by 6.6%–13.1%, 17.2%–33.0% and 19.6%–25.5%, respectively, compared with single Cd treatment. Additionally, the three enzymes activity in Cd with 1 kDa CTS treatment was significantly higher than that in Cd with the 3 and 5 kDa CTS treatments, indicating that of all the CTS samples, low MW CTS was the most effective in inducing the activity of antioxidant enzymes.

3.5 Cd concentration in shoots and roots

Cd was not detected in the shoots and roots of wheat seedlings grown in Cd-free conditions. However, Cd concentration increased to 321.8 mg kg^{-1} (Fig. 4) and 2500 mg kg^{-1} (Fig. 4) in the shoots and roots of wheat seeding grown under Cd stress, respectively. Compared with Cd-stressed plants, spraying 1 and 3 kDa CTS appeared to suppress Cd uptake in both shoots and roots of wheat seeding. In particular, 1 kDa CTS reduced shoot and root Cd^{2+} concentration by 22.2% and 11.6%, respectively. Furthermore，the Cd concentrations in shoot showed significantly lower in the Cd with 1 kDa CTS treatment than that in the Cd with 3 and 5 kDa CTS treatments, suggesting that 1 kDa CTS had the greatest potential to decrease shoot Cd accumulation in wheat.

3.6 The root morphological parameters of wheat seedlings

The root is the first organ in contact with the various components of the rhizosphere. The root morphology responds rapidly to changes in the environment. In this study, compared with the control, the presence of Cd in the growth medium reduced root length, root volume, and total root tips by 44.4%, 68.1%, and 43.8%, respectively (Table 3). Spraying 3 and 1 kDa CTS improved the root system of wheat seedlings under Cd stress and increased root length, root volume, and total root tips by 20.4%–35.8%, 8.5%–46.8%, and 12.6–46.7%, respectively (Table 3). Moreover, root length, root volume, and total root tips in Cd with 1 kDa CTS treatment were significantly higher than that with 3 and 5 kDa CTS treatment, indicating that the mitigated Cd toxicity depends to some extent on the MW of the CTS.

Additionally, Cd stress increased the average root diameter of wheat seedlings by 66.7% compared to control (Table 3). However, foliar Spraying of CTS with 1 and 3 kDa significantly decreased the average root diameter by 31.4% and 20%, respectively, compared to the Cd-stressed control (Table 3).

Table 3 Effect of CTS on root morphological parameters of wheat seedling

Treatment	Total root length（cm）	Root volume（cm^3）	Root diameter（mm）	Number of root tips
CK	900±21.1d	0.79±038 d	0.21±0.014 a	2670±122.7 d
Cd	500±13.2 a	0.47±0.021 a	0.35±0.15 d	1500±88.2 a
Cd+CTS(5k)	511±17.9 a	0.49±0.022 ab	0.34±0.017 d	1550±90.3 a
Cd+CTS(3k)	602±18.1 b	0.51±0.023 b	0.28±0.014 c	1689±70.6 b
Cd+CTS(1k)	679±16.5 c	0.69±0.36 c	0.24±0.013 b	2200±98.2 c

* Different letters within the same column mean significant differences at $P < 0.05$ level. Data are presented as the means ± SE (n = 3).

4. Discussion

CTS is a natural non-toxic and biodegradable polymer (Mukhtar Ahmed et al. 2020). Foliage sprayed CTS has positive effects on plant growth and improves their tolerance to a variety of abiotic stresses (Malerba and Cerana 2016; Zhang et al. 2018; Jia et al. 2019). A large number of studies have shown that CTS can reduce the negative impact of drought, salinity, and extreme temperatures (Zou et al. 2017), however, whether CTS can enhance plant resistance to Cd stress is uncertain.

This study evaluated the effects of foliar sprays of different MW CTS on alleviating Cd toxicity in wheat seedlings and analyzed physiological and biochemical parameters. The results indicated that Cd toxicity inhibited the growth of wheat seedlings and decreased its biomass. While, foliar spraying CTS diminished the negative effects of Cd on wheat seedlings and significantly promoted biomass accumulation. CTS-mediated growth promotion may be due to its effect on stimulating the expression of several genes that participate in CTS-induced enhancement of growth and plant defense (Zhang et al. 2018). Furthermore, after 1 kDa CTS treatment, the biomass of seedlings under Cd stress recovered to a level similar to that of the Cd-free control (Fig. 1). Biomass is used as a direct index to measure the toxicity of heavy metals in plants. These results suggested that the foliar spray of low MW (3 and 1 kDa) CTS could significantly alleviate the toxic effect of Cd on wheat seedlings. The stimulatory effects that CTS has on plant growth have been found in edible rape under Cd stress (Zong et al. 2017b), wheat under salt stress (Zou et al. 2015) and wheat under drought stress (Zeng and Luo 2012).

MDA is a product of peroxidation under stress and the levels in plants are often used as biomarkers to evaluate the damage to plant cells due to stress. In the present study, Cd stress significantly increased the MDA content of wheat seedlings (Fig. 2), suggesting that Cd stress exacerbated the generation of ROS in wheat leaves, changing the structure and functions of the cell membranes. Foliar application of 1 and 3 kDa CTS reduced MDA to normal levels, suggesting that the exogenous application of CTS with lower MW could reduce the level of lipid peroxidation caused by Cd stress. These results were in accordance with our previous research in which lowest MW of CTS (1 kDa CTS) markedly reduced the MDA content in Cd-stressed edible rape leaves (Zong, et al. 2017a). Similar result was observed by Zou et al. 2015, who found that CTS application could decrease MDA concentrations in wheat under salt stress. These results indicated that CTS could protect the plants from membrane damage by excess ROS produced under Cd stress, possibly by the structure of CTS. It was reported that the functional groups like hydroxyl and amino groups on the surface of CTS enabled ROS to form relatively nontoxic macromolecular radicals (Mukhtar Ahmed et al. 2020; Song et al. 2020). Another possible mechanism of CTS reduced MDA level in wheat seedlings might be the activation of ROS scavenging enzymes.

It has been reported that SOD, POD, and CAT are key enzymes involved in the removal of ROS (Zhang et al. 2017). Figure 3 shows that the total activity of SOD found in wheat seedlings treated with Cd alone was significantly increased, which could be the result of a self-defense mechanism. When the wheat seedlings were treated with Cd in combination with CTS, the SOD activity significantly increased compared with samples treated with Cd alone (Fig. 3A). It has been well known that SOD is a key enzyme responsible for the elimination of $O_2\cdot^-$ in cells (Takahashi and Asada 1983). These results imply that the wheat seedlings treated with CTS have a better ability to scavenge $O_2\cdot^-$ to improve plant protection against oxidative stress under Cd conditions. CAT and POD are also play an important role in defense system against Cd stress in plants, which can catalyze H_2O_2 to H_2O and O_2 (Agami and Mohamed 2013). The results of this study showed that CTS application can further increase CAT and POD activities in the leaves of wheat seedlings compared to the single Cd treatment (Fig.3B, Fig.3C), implying that CTS could regulate antioxidant defenses. As a result, this stimulatory action has enabled plants to resist Cd stress. Moreover, these results explain the lower MDA level found in CTS treatment. In addition, the antioxidant enzyme activity in the Cd with 1 kDa CTS treatment was significantly higher than that in the Cd with 3 and 5 kDa CTS treatments, indicating that CTS with low MW had better antioxidant properties than that with high MW. Similar phenomena were reported by Kulikov et al. (2006), who found that CTS fractions with MWs of 1.2, 2.2, 10.1, 30.3, and 40.4 kDa effectively inhibited virus accumulation and systemic propagation, and the degree of CTS-induced antiviral resistance increased as the CTS MW decreased. Tomida et al. (2009) studied the relationships between some different MW CTS (2.8, 17.0, 33.5, 62.6, 87.7, 604, and 931 kDa) and found that 2.8 kDa CTS was found to be the most effective in diminishing oxidative stress among all the CTS samples.

Soluble proteins and soluble sugars are important osmotic regulators in plants and the contents of soluble sugar and soluble protein also were considered as good indicators to the oxidative stress in the plant cells under metal stress (Ismaiel and Said,2018). In general, Cd stress may lead to ROS overproduction in plant cell. Those changes might have a negative effect on ion exchange capacity of plasma membrane and metabolic processes. As a result, the soluble sugar and soluble protein contents are affected and decreased (Chia et al., 2017, Ismaiel and Said,2018). This was supported by current research on Cd. While all CTS treatments (5, 3, and 1 kDa) significantly increased the contents of soluble sugar and soluble protein in Cd-stressed wheat leaves (Table 2). These findings suggested that CTS could regulate plant cellular homeostasis and thus alleviate the toxic effect of Cd on wheat plants. This was in agreement with the findings of Zhang et al. (2017) who revealed that CTS play a key role in improving tolerance to NaCl-stressed wheat plants thereby inducing synthesis of soluble sugar and soluble protein. Furthermore, spraying 1 kDa CTS showed the best activity in increasing the contents of soluble protein and soluble sugar in this study (Table 2), suggesting that the regulation ability of CTSs on contents of osmotic regulators in leaves of wheat plants under Cd stress was dependent on their MW.

In addition to improving antioxidant defenses, exogenous application of 3 and 1 kDa CTS reduced the roots and shoots Cd concentrations of wheat seedlings (Fig. 4). These findings indicated that low MW CTS can suppress the Cd uptake in roots and thereby reduce root-to-shoot Cd translocation and stimulate promote plant growth, which may better explain the reduction of Cd toxicity and oxidative stress with CTS treatment. Furthermore, many studies have indicated that there is a significantly negative relationship between root or shoot Cd concentrations and the average root diameter (Huang et al. 2015; Yu et al. 2017; Qin et al. 2018). Cd stress enhanced the average root diameter of wheat seedlings compared with the control in this study (Table 3). While foliar spraying with 1 and 3 kDa CTS significantly reduced the average root diameter compared to the Cd-stressed control (Table 3). This might suggest that the decrease in root Cd^{2+} concentration in wheat seedlings treated with CTS was mainly attributed to the inhibited root diameter.

5. Conclusions

This study demonstrated that a foliar application of CTS provided an effective method to alleviate the toxic effect of Cd on wheat seedlings, which could improve the growth, chlorophyll content as well as soluble protein, soluble sugar in the leaves of wheat seedling under Cd stress. Exogenously CTS also reduced MDA level and Cd concentrations in roots and shoots of wheat seedling. Besides, wheat seedlings treatment with CTS enhanced the activity of SOD, POD, and CAT. Meanwhile, it increased the root length, root volume, total root tips and decreased the root average diameter of wheat seedling. Furthermore, 1 kDa CTS had the greatest potential to alleviate the harmful effects of Cd stress.

Declarations

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Authors contributions Jun Liu performed the experiment, Ying Xu performed the data analyses, Haiying Zong wrote the manuscript. All authors read and approved the final manuscript.

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approvalandConsent to participate Not applicable

Consent to publish Not applicable.

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Figures

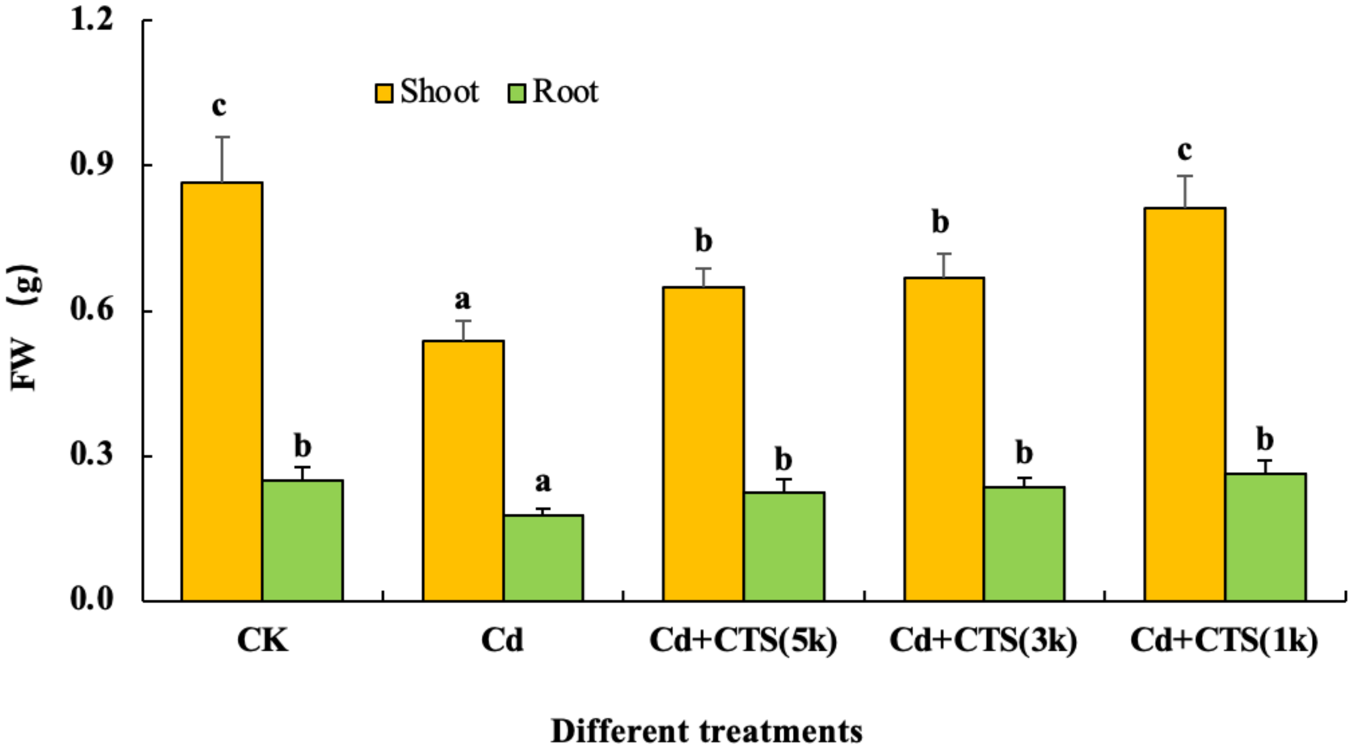

Figure 1

Effects of CTS on the root and shoot of fresh weight in wheat seedlings under Cd stress. Data are presented as the means ± SE (n = 3). Different letters mean significant differences at P < 0.05 level.

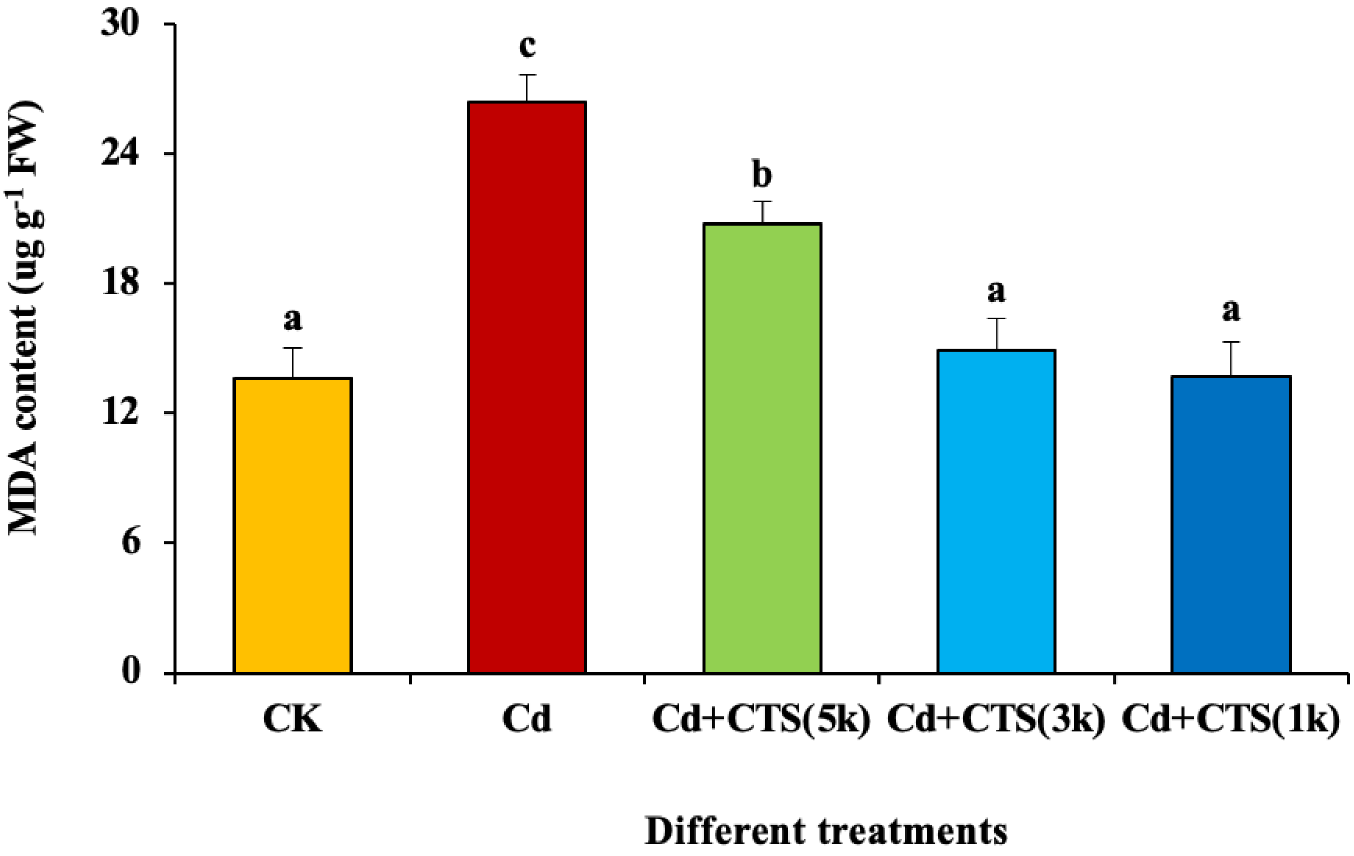

Figure 2

Effect of CTS on MDA content in leaves of wheat seedlings under Cd Stress. Data are presented as the means ± SE (n = 3). Different letters mean significant differences at $P < 0.05$ level.

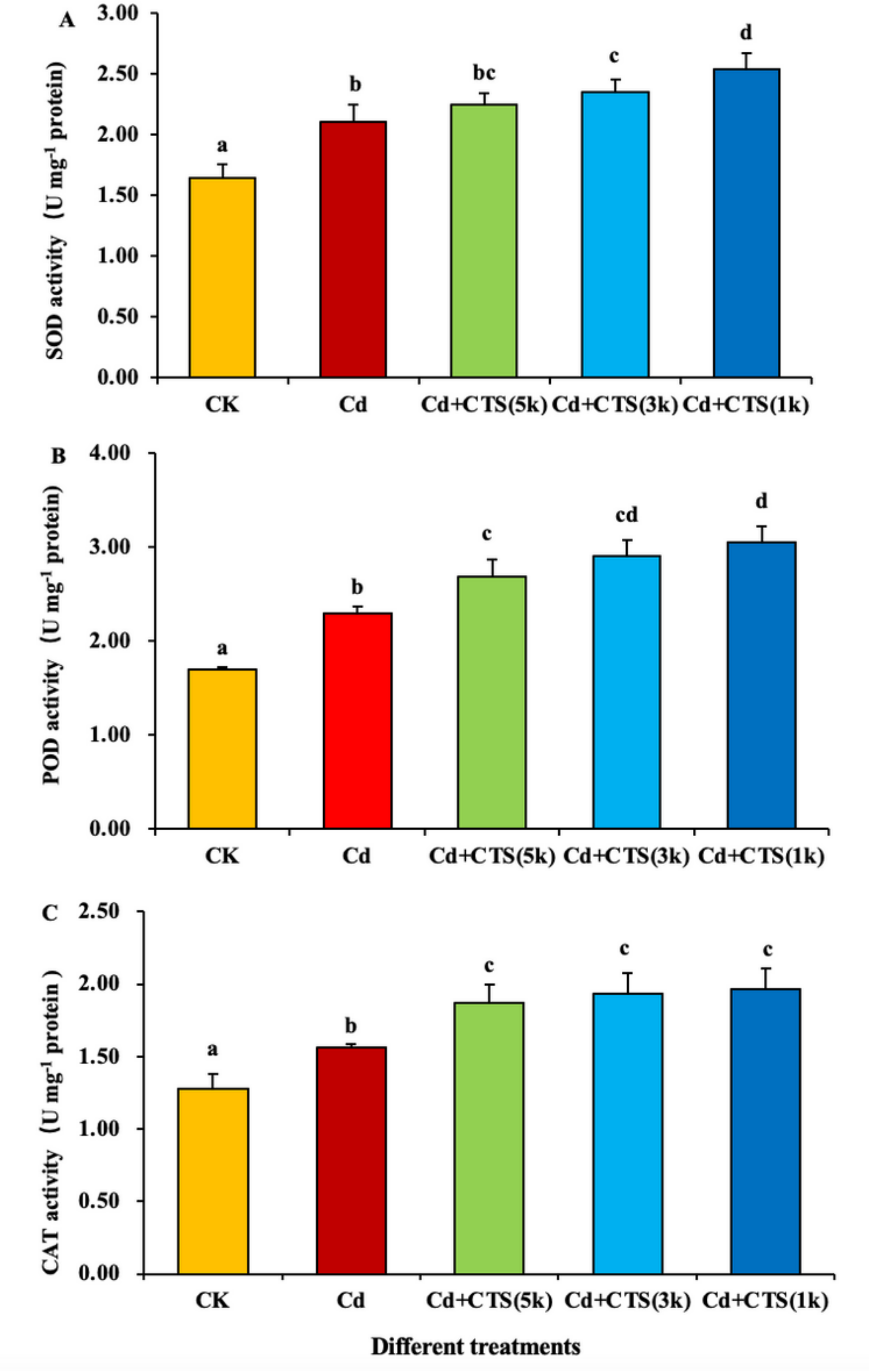

Figure 3

Effects of CTS on SOD (A), POD (B) and CAT (C) activities in wheat seeding leaves under Cd stress. Data are presented as the means ± SE (n = 3). Different letters mean significant differences at $P < 0.05$ level.

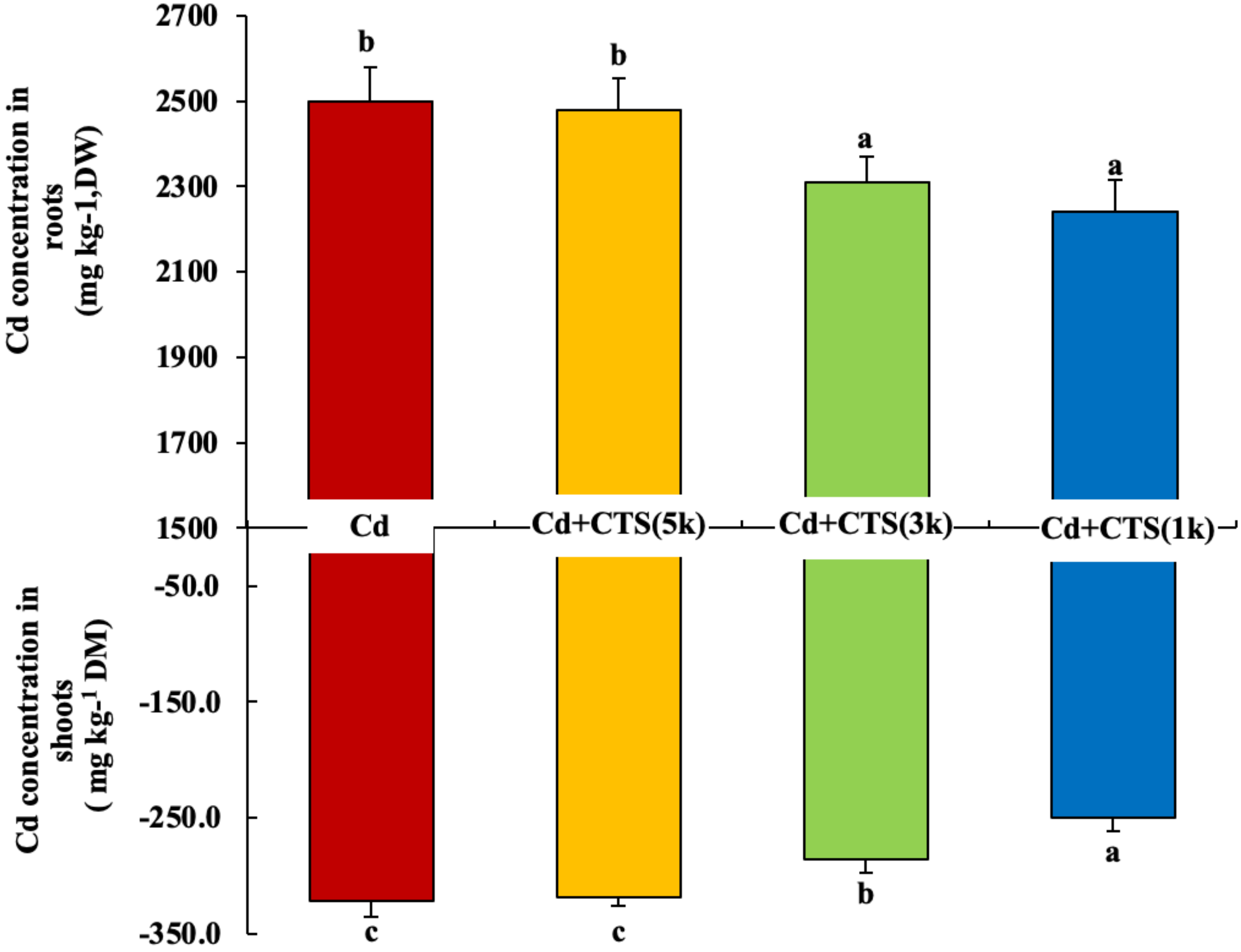

Figure 4

Effects of CTS on Cd concentration in shoots(A) and roots(B) of wheat seedlings. Data are presented as the means ± SE (n = 3). Different letters mean significant differences at P < 0.05 level.