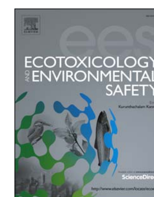
Ecotoxicology and Environmental Safety 138 (2017) 271–278

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Protective effect of chitosan on photosynthesis and antioxidative defense system in edible rape (*Brassica rapa L.*) in the presence of cadmium

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A R T I C L E I N F O

Keywords:
Cd
Chitosan
Edible Rape (*Brassica rapa L* .)
Photosynthetic characteristics
Antioxidant defense system

A B S T R A C T

Chitosan (CTS) induces plant tolerance against several abiotic stresses, including salinity and drought exposure. However, the role of CTS in cadmium (Cd)-induced stress amelioration is largely unknown. In the present study, a hydroponic pot experiment was conducted to study the roles of CTS with different molecular weight (Mw) (10 kDa , 5 kDa and 1 kDa) in alleviating Cd toxicity in edible rape (*Brassica rapa L* .). The results showed that Cd stress significantly decreased plant growth, leaf chlorophyll contents and increased the malondialdehyde (MDA) level in rape leaves. Foliar application of CTS promoted the plant growth and leaf chlorophyll contents, and decreased the malondialdehyde (MDA) level in edible rape leaves under Cd stress. The alleviation effect of CTS on toxicity was depended on its Mw and CTS with Mw of 1 kDa showed the best activity. Spraying 1 kDa CTS onto the leaves of edible rape under Cd-toxicity could decrease shoot Cd^{2+} concentration and improve photosynthetic characteristics of edible rape. Moreover, 1 kDa CTS also significantly enhanced non-enzymatic antioxidants (ascorbic acid) and enzyme activities (superoxide dismutase, catalase and guaiacol peroxidase) under Cd stress. Based on these findings, it can be concluded that application of exogenous CTS could be an effective approach to alleviate the harmful effects of Cd stress and could be explored in an agricultural production system.

1. Introduction

Cadmium (Cd) is a toxic heavy metal common in the environment. Given its high mobility and water solubility, Cd is easily absorbed and accumulated by plants (J. Liu et al., 2013, X. Liu et al., 2013). Since Cd is a non-essential chemical element for normal functioning and does not perform any physiological functions in plants, it can be characterised as the most toxic of heavy metals (Shakirova et al., 2016). Plant responses to Cd are characterised by an unbalanced nutritional status (Espanany et al., 2016; Guo et al., 2007; Wang et al., 2007), inhibition of photosynthesis and transpiration (Agami and Mohamed, 2013; Shi et al., 2010) and disturbance of membrane structure integrity (Ekmekçi et al., 2008; Shi et al., 2010). This is due to the generation of reactive oxygen species (ROS), which not only decrease the plant yield but also deteriorate plant quality (Shakirova et al., 2016). Moreover, if Cd is allowed to accumulate in crop plants, toxic levels pose a severe threat to human health through food chains (Järupa and Åkessonb, 2009).

A number of studies have shown that Cd toxicity in plants is related to a stimulated generation of ROS, like singlet oxygen ($^1O_2$), superoxide ($O^{\bullet -}{}_2$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (·OH) (Mittler et al., 2004). These ROS are able to react with lipids, proteins and pigments, resulting in membrane damage and enzyme inactivation (Agami and Mohamed, 2013). To survive such oxidative damage, plants have evolved a complex antioxidant system (i.e. enzymatic system and non-enzymatic components) to increase their tolerance and survival under such extreme environmental conditions. The enzymatic system includes superoxide dismutase (SOD), guaiacol peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione peroxidase (GPX), whereas non-enzymatic components comprise reduced glutathione (GSH), ascorbic acid (ASA) and alkaloids (Pandey et al., 2015). These antioxidant systems can scavenge or remove different ROS.

Chitosan (CTS) is produced from chitin, an important component of crustacean shells, such as crab, shrimp and crawfish, and is mainly made up of (1 − 4)-2-amino-2-deoxy-β-D-glucan. It has been proved that CTS possesses various bioactivities, including antifungal (Ma et al., 2013), antibacterial (Li et al., 2013) and antiviral (Davydova, et al., 2011). It has also been demonstrated that CTS has a broad-spectrum antimicrobial activity against a variety of bacteria and fungi, and that it improves the growth and development of plants. Nowadays, there has been renewed interest on the effect of CTS on plant physiology,

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http://dx.doi.org/10.1016/j.ecoenv.2017.01.009
Received 17 October 2016; Received in revised form 3 January 2017; Accepted 3 January 2017
Available online 11 January 2017
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especially under stressed conditions. It was observed that CTS decreased electrolyte leakage and MDA level in apple seedlings under drought stress (Yang et al., 2009). Similarly, Jabeen and Ahmad, (2013) and Zou et al. (2015) reported a rise in antioxidants' enzymes activities under salinity in safflower and wheat seedlings, respectively. Lately, adding CTS into soil were also reported to increase the capacity of radish (Farouk et al., 2011) and rapeseed (*Brassica napus*) plants to survive under Cd stress. However, applying CTS into soil was limited by the fact that CTS was easily degraded by soil microbial (Sawaguchi et al., 2015). Foliar application may avoid this disadvantage, but little information is known about whether foliar application with CTS modulates Cd-induced toxicity in plants. Additionally, it was reported that the antiviral activity of CTS depends on the structure of the CTS, in particular, its molecular weight (Mw) (Kulikov et al., 2006). However, the roles of CTS with different Mws in alleviating cadmium toxicity in plants still remain unclear.

Leafy vegetables easily absorb Cd from the contaminated soil (J. Liu et al., 2013, X. Liu et al., 2013). Daily intake of these leafy vegetables containing high Cd levels results in a significant health hazard to the residents and consumers (J. Liu et al., 2013, X. Liu et al., 2013). In this study, Edible rape (*Brassica rapa L.*), one of the most widely consumed leafy vegetables, was selected as an experimental mode plant. We examined the effect of foliar application with CTS with different Mws on the growth of edible rape under Cd stress. Furthermore, the study investigated some physiological and biochemical events induced by foliar applied CTS, followed by Cd stress in edible rape. We aimed to provide a basis for developing strategies to reduce risks associated with Cd toxicity to maintain sustainable plant production.

2. Material and methods

2.1. Preparation of different CTS Mws

CTS with a deacetylation degree of 80% was purchased from Qingdao Yunzhou Biochemistry Co., Ltd., Shandong Province, China. Different CTS Mws were obtained according to the method reported by Li et al. (2012), with some modifications. CTS powder (5 g) was introduced in 250 mL 2% acetic acid. Then, 5 mL 30% H_2O_2 was added to the CTS aqueous solution. Degradation was assisted with microwave radiation carried out at 600 W at 70 °C for 45, 60 and 120 min, respectively. When cooled to room temperature, the reaction mixture was adjusted to pH 7.0 and then dialysed to remove the salts and remaining H_2O_2. The dialysis fluid was lyophilised to yield powdered products.

The average Mw of the degraded CTS was measured by high performance liquid chromatography (HPLC), as described in our previous studies (Li et al., 2012; Zou et al., 2015). The Mw of degraded products with 45, 60 and 120 min were 9800 Da, 5500 Da and 1600 Da, hereafter referred to as 10 kDa, 5 kDa and 1 kDa, respectively.

2.2. Plant material and experimental design

Edible rape seeds were surface sterilised with 3% H_2O_2 for 10 min, thoroughly rinsed with sterile deionised water and then germinated in rolls of wet filter paper for 72 h at 25 °C in the dark. Uniform seedlings were selected and transferred into plate holes on plastic pots (12 plants per pot) containing a half-strength Hoagland nutrient solution. After 2 d of cultivation, the medium was changed to full-strength Hoagland solution. Seedlings were grown in an illuminating incubator (14 h light with a light intensity of 800 mol m^{-2} s^{-1} at 25 ± 1 °C, and 10 h dark at 18 ± 1 °C , relative humidity was approximately 70%.). The solutions were changed every 2 d.

After 3 weeks, plants were exposed to 50 μM Cd by adding $CdCl_2$ to the Hoagland nutrient solution. Each pot was supplemented every 2 d with Cd^{2+} containing Hoagland's nutrient solution. The Cd concentration used in this experiment was based on our preliminary studies with the same rape cultivar. After a Cd treatment of 7 d, five treatments were performed: (1) control (CK), foliar spray with distilled water; (2) Cd, 50 μM Cd + foliar spray with distilled water; (3) Cd + 10 K CTS, 50 μM Cd + foliar spray with 50 mg/L 9800 Da CTS; (4) Cd + 5 K CTS, 50 μM Cd + foliar spray with 50 mg/L 5500 Da CTS; (5) Cd + 1 K CTS, 50 μM Cd + foliar spray with 50 mg/L 1600 Da CTS. Plants were sprayed every other day for 1 week with 50 mL distilled water or CTS. The plants were collected to assess all attributes after exposing the seedlings to Cd stress for 14 d.

2.3. Plant growth analysis and Cd (Cd^{2+}) determination

Plants from each treatment were harvested and separated into roots and shoots. The root length was measured using a meter scale. Fresh root and shoot weights (FW) were determined. The shoot material was oven-dried for 30 min at 105 °C then at 70 °C for a constant weight. The powdery dried shoots were used to determine the Cd^{2+} content using inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima, 7000) after being digested with mixed acid [HNO_3 + $HClO_4$ (4/1,v/v)]. The amount of Cd was expressed based on dry weight (J. Liu et al., 2013, X. Liu et al., 2013).

2.4. photosynthetic pigments, fluorescence and photosynthetic characters

Pigment content of chlorophyll a, b, and carotenoids were determined by the method of Lichtenthaler and Wellburn (1983), with some modifications. Fresh leaf tissue was ground in a mortar with some sand and $MgCO_3$, and photosynthetic pigments were extracted with 95% ethanol. The suspension was centrifuged at 4 °C for 5 min at 5000 *g*. The chlorophyll a (Chl *a*), chlorophyll b (Chl *b*), total chlorophyll (a + b) and carotenoid (Car) content were determined by a spectrophotometer at 665 nm, 649 nm and 470 nm. The pigment amount was calculated by equation:

$$\text{Chl } a = 13.95A_{665} - 6.88A_{649}$$

$$\text{Chl } b = 24.96A_{649} - 7.32A_{665}$$

$$\text{Car} = (1000A_{470} - 2.05\ \text{Chl } a - 114.8\ \text{Chl } b)/245$$

The photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs) and intercellular CO_2 concentration (Ci) were measured with a portable photosynthesis system (L.MAN-LCProSD, BioScientific Ltd., UK). Atmospheric conditions were a temperature of 25 ± 2 °C, gas flow rate of 200 mol s^{-1}, photosynthetic photon flux density of 800 mol m^{-2} s^{-1} and a CO_2 concentration of 395 ± 5 mol^{-2} s^{-1}. Chlorophyll fluorescence was measured using a portable fluorometer (PAM-2100, Walz, Germany). All parameters [the maximum quantum yield of PSII (Fv/Fm)], quantum yield of PSII (PSII), maximum primary yield of PSII photochemistry (Fv/Fo), photochemical quenching coefficient (qP) and non-photochemical quenching coefficient (NPQ) were measured after a dark adaptation for 30 min.

2.5. Lipid peroxidation degrees, proline, AsA and total GSH contents

Lipid peroxidation was measured by estimating MDA, a product of lipid peroxidation, using a thiobarbituric acid reaction (Ali et al., 2014b). The MDA content was expressed as μg MDA g^{-1} FW.

The leaf proline content was measured by the rapid colorimetric method, as suggested by Agami and Mohamed, (2013), with some modifications. Fresh samples (0.5*g*) were homogenised in 3% sulphosalicyclic acid and the extract was then centrifuged at 10000 *g* for 10 min. Next, the supernatant was mixed with ninhydrin and glacial acetic and phosphoric acids, incubated at 90 °C for 30 min and then cooled on ice. The reaction mixture was extracted with toluene. The absorbance was read at 520 nm by a spectrophotometer. The proline concentration in the sample was calculated as μg g^{-1} FW.

AsA was assayed according to the method of Nahar et al. (2016),

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