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Improvement in cadmium tolerance of edible rape (*Brassica rapa* L.) with exogenous application of chitooligosaccharide

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HIGHLIGHTS

- COS alleviated the toxic effect of Cd and improved the morpho-physiology of plants.
- COS significantly decreased the Cd concentrations both in shoots and roots of plants.
- Foliar application with COS under Cd tress enhanced antioxidant enzymes activities.
- COS modified Cd subcellular distribution and enhanced Cd fixation to vacuoles.

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ABSTRACT

Cadmium (Cd) is one of the most toxic heavy metals, which is readily taken up by plant roots and has deleterious effects on crop yield and quality. The study investigated the potential cross-protection roles of chitooligosaccharide (COS) in alleviating Cd toxicity in edible rape (*Brassica rapa* L.) under greenhouse conditions. The results demonstrated that spraying COS onto the leaves of edible rape could promote the plant growth and leaf chlorophyll contents, decrease the malondialdehyde (MDA) level in leaves as well as the Cd^{2+} concentration in shoots and roots of edible rape under Cd stress. Moreover, exogenous COS could obviously enhance the activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) in edible rape leaves under Cd-toxicity. The alleviation effect of COS on Cd stress was concentration-dependent and COS of 50–100 mg L^{-1} showed the best activity. Subcellular distribution experiments further revealed that COS of 50 mg L^{-1} decreased the proportion of Cd in the organelle fraction of leaves by 40.1% while increased the proportion of Cd in the soluble fraction by 13.2%. These results indicated that COS had a potential to enhance plant resistance to Cd through promoting antioxidant enzyme activities and altering Cd subcellular distribution.

1. Introduction

Cadmium (Cd), one of the toxic heavy metals, has become a major pollutant due to its wide range application of pesticides, herbicides, fertilizers and irrigation with industrial wastewater. Cd has high solubility and mobility in agricultural soils and is readily taken up by roots and transported to the vegetative and reproductive organs, which has deleterious effects on plant yield and quality (Greger and Landberg, 2015; Zhao et al., 2015). The accumulation of Cd in food may cause severe adverse effects on human health, such as renal dysfunction, bone demineralization, and cancer (Bernard, 2008; Sebastian and Prasad, 2014). Excess Cd in plants can interfere with many metabolic processes, such as photosynthesis, transpiration, respiration or nutrients homeostasis (Ali et al., 2014; Hernández et al., 1998). Subsequently, visible symptoms of Cd-induced toxicity in plants would be observed including plant growth inhibition, chlorosis and leaf epinasty and finally death (Ali et al., 2014; Zhu et al., 2013). Therefore, the development of effective techniques to reduce the Cd concentration in plants is urgent. Conventional methods to reduce Cd accumulation in plants is to decrease the concentration of Cd in the soil solution by reduction of Cd influx into the soil system, site selection, and management practices (Li et al., 2016a). However, these

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techniques can be very costly and consume a lot of energy (Qiu et al., 2011). Therefore, new efficient methods become obligatory to be developed for enhancing plant resistance to Cd. Numerous studies have demonstrated that chemical regulators were able to induce plant tolerance to Cd-stress as well as increase crop yield and quality (Ali et al., 2014; Liu et al., 2016), which were considered to be a potential strategy to alleviate Cd toxic symptoms in plants.

Chitosan is a naturally occurring compound that can be easily obtained from crab and shrimp shells waste. It is proved that chitosan possessed various potential applications in medicine, industries and food fields due to its excellent biocompatibility, biodegradability and bioactivity (Katiyar et al., 2015; Malerba and Cerana, 2016). Chitooligosaccharide (COS), the hydrolysis product of chitosan (Li et al., 2012), attracts much interest due to its elicitor activity in plant immunity in recent years. It has been reported that COS could induce defense response by NO pathways in tobacco (Zhang et al., 2011), promote the wheat growth (Zhang et al., 2016) and increase the biosynthesis of secondary metabolites in various plants (Bhaskara Reddy et al., 1999; Lei et al., 2011; Malerba and Cerana, 2016). Furthermore, COS is reported to enhance the plants tolerance to abiotic stress including drought stress, salinity stress and toxic metal stress via increasing antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT) and peroxide (POX) (Yang et al., 2009; Zou et al., 2015). Ma et al. (2010) reported that soaking the seeds in COS solution for 6 h could enhance the SOD and CAT activity in wheat seedlings under Cd stress and alleviate the toxicity of Cd. Similar results were reported by Xiao et al. (2012), who observed that the seed of Chinese cabbage treated with COS for 24 h could alleviate the harmful effects of Cd. However, soaking seed with COS is limited by the fact that the duration of soaking seed could affect the priming effects of COS (Cho et al., 2008; No et al., 2003). COS application as foliar sprays may avoid this disadvantage and is considered as a more feasible way, but so far little information is known about whether foliar application with COS modulates Cd-induced toxicity in plants. The metal detoxification processes of plants treated with COS also remain unclear.

Recent investigations showed that leafy vegetables had the highest capacity of accumulating Cd among various vegetables (Liu et al., 2013; Xu et al., 2013). Daily consumption of these leafy vegetables containing high Cd levels results in a significant health hazard to the human. In this study, edible rape (*Brassica rapa* L.), one of the most widely consumed leafy vegetables, was selected as an experimental mode plant. The effect of foliar application with COS on reducing Cd accumulation in edible rape was investigated. Moreover, Cd subcellular distribution pattern in response to Cd stress in the rape plant sprayed with COS was further tested in order to explore the potential detoxification mechanisms in plants.

2. Materials and methods

2.1. Materials

COS powder (average molecular weights was 1600 Da; degree of deacetylation was 82%) was obtained according to the method reported by Li et al. (2012). In brief, the chitosan powder (5 g) was introduced into 250 mL 2% acetic acid, and then 5 mL 30% H_2O_2 was added to the chitosan aqueous solution. The degradation assisted with microwave radiation was carried out with the power of 600 W at 70 °C for 2 h.

2.2. Plant material and treatments

Edible rape (*Brassica rapa* L.) seeds were germinated in rolls of wet filter paper for 72 h at 25 °C in the dark. Then the seedlings with uniform size were selected and transferred into plate holes on plastic pots (12 plants per pot) containing a half-strength Hoagland nutrient solution (Arnon and Hoagland, 1940). The nutrient solution was replaced every 2 d. 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%.).

After acclimatization period of 24 d, plants were exposed to 50 μM Cd by adding $CdCl_2$ to the Hoagland nutrient solution. The concentration of Cd was often found in soils of old industrial zone in China (Li et al., 2014; Zhao et al., 2009), and it did not seriously inhibit the growth of rape based on our preliminary studies. Each pot was supplemented every 2 d with Cd^{2+} containing nutrient solution. After Cd treatment of 7 d, plants were sprayed with different concentrations of COS making six treatments: (1) control (CK), foliar spray of distilled water; (2) Cd, 50 μM Cd + foliar spray of distilled water; (3) Cd+ 25COS, 50 μM Cd + foliar spray of 25 mg/L COS; (4) Cd+ 50COS, 50 μM Cd + foliar spray of 50 mg/L COS; (5) Cd+ 100COS, 50 μM Cd + foliar spray of 100 mg/L COS; (6) Cd+ 200COS, 50 μM Cd + foliar spray of 200 mg/L COS. Plants were sprayed every other day for 1 week with 50 mL distilled water or COS. The experiments were laid out in a completely randomized design with three replicates. The plants were collected to assess all attributes after spaying the COSs three times.

2.3. Growth traits, Cd and chlorophyll content determinations

Plants from each pot were harvested and separated into roots and shoots. The growth of rape plants was evaluated by root length and fresh weight (FW). With the exception of the fresh shoot for the analysis of MDA, chlorophyll content, the subcellular distribution and antioxidant enzyme activities, all other parts and roots were oven-dried for 30 min at 105 °C then at 70 °C for a constant weight.

The powdery dried samples (i.e. shoots and roots) were used to determine their 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 chlorophyll content was determined by the method of Sedmak and Grossberg (1977). Fresh leaf tissues were homogenized with 95% ethanol and centrifuged. The chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and total chlorophyll (a + b) contents were determined by a spectrophotometer at 665 nm and 649 nm.

2.4. Analysis of lipid peroxidation content

The oxidative damage to lipids was determined by estimating MDA, a product of lipid peroxidation, using a thiobarbituric acid reaction (Hodges et al., 1999). The MDA content was expressed as μmol MDA g^{-1} fresh weight (FW).

2.5. Measurement of antioxidant enzyme activities

For enzyme activity, fresh leaves were homogenized in sodium 50 mM phosphate buffers (pH 7.8) under ice cold conditions. The suspension was centrifuged at 12,000 g for 15 min at 4 °C, and then the following enzyme activities were determined. The SOD activity was determined using the method of Paoletti et al. (1986) following inhibition of the photochemical reduction by nitro blue tetrazolium (NBT). One SOD unit was defined as the amount of enzyme needed to produce a 50% inhibition of NBT at 560 nm. The CAT activity was determined as the consumption of H_2O_2 (extinction coefficient 39.4 mM cm^{-1}) measured at 240 nm for 3 min at 25 °C. CAT activity was expressed as H_2O_2 reduced min^{-1} mg^{-1} of protein (Chance and Maehly, 1955). POD activity was determined by the method of Chance and Maehly (1955) and assayed using guaiacol as the