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Comparative responses to silicon and selenium in relation to antioxidant enzyme system and the glutathione-ascorbate cycle in flowering Chinese cabbage (*Brassica campestris* L. ssp. *chinensis* var. *utilis*) under cadmium stress

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ABSTRACT

Silicon (Si) and selenium (Se) are generally considered as contributing elements for plant resistance to abiotic stresses, especially for those in heavy-metal stressed environments. However, the mechanisms underlying the different roles of Si and Se in mitigating cadmium (Cd) stress in flowering Chinese cabbage (*Brassica campestris* L. ssp. *chinensis* var. *utilis*) are still poorly understood. Here, we investigated the comparative responses to Si and Se in relation to antioxidant enzyme system and the glutathione-ascorbate cycle in flowering Chinese cabbage plants under Cd stress. Addition of Si and/or Se at equivalent concentrations alleviated Cd toxicity as demonstrated by increasing plant tissue (shoots and roots) biomass and reducing plant tissue (leaves and roots) concentrations of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) in plants exposed to high Cd stress. Additionally, in comparison with the high Cd-alone treatment, the application of Si and/or Se significantly increased the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), while no differences in the size of these effects between Si and Se were observed at equal concentrations, suggesting that these three antioxidant enzymes were not the key factors involved in differences of Cd detoxification between Si and Se. Furthermore, the single addition of Se or in combined with Si markedly stimulated the efficiency of the GSH-AsA cycle by increasing the concentrations of glutathione (GSH) and ascorbate (AsA) as well as the activities of glutathione reductase (GR) and dehydroascorbate reductase (DHAR) in plant tissues (leaves and roots), especially at high dose, while little changes were observed in the Si-alone treatment, indicating that Se has the greater ability of increasing the efficiency of GSH-AsA cycle rather than of Si exposed to Cd stress. Overall, our results reveal that Se-mediated alleviation of Cd toxicity is due to increasing antioxidant enzyme activities and the GSH-AsA cycle efficiency. However, Si mitigation may involve other mechanisms apart from increasing antioxidant enzyme activities.

1. Introduction

Cadmium (Cd) is one of the most toxic environmental elements without any known beneficial function in plants to date (Wang et al., 2007). Due to weathering of parent material and atmospheric deposition as well as anthropogenic sources, such as mining, industrial emissions, application of sewage sludge, and fertilizer and pesticide use, there is a growing concern related to Cd pollution issues worldwide in agricultural soils (Nagajyoti et al., 2010), and consequent Cd accumulation in crop plant tissues (Lima

Abbreviations: Cd, cadmium; Si, silicon; Se, selenium; ROS, reactive oxygen species; SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; GSH, glutathione; AsA, ascorbate; GR, glutathione reductase; DHAR, dehydroascorbate reductase.

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et al., 2006; Wu et al., 2015a). Excessive Cd accumulation in plant tissues causes a series of severe phyto-toxicities including growth inhibition, leaf chlorosis, decreases in nitrogen metabolism, photosynthesis, respiration and mineral nutrition, accumulation of reactive oxygen species (ROS), protein denaturation, and even plant death (Smeets et al., 2008; Shah et al., 2010; Wu et al., 2015b).

Cadmium stress has been shown to induce the disturbance of cellular redox balance leading to the accumulation of ROS, and causes severe damages to plant cells. Fortunately, plants have evolved a range of protective systems to minimize the occurrence of oxidative damage. There are antioxidative defense systems in all subcellular compartments which react with ROS directly or indirectly via enzyme catalysis and maintain them at a very low level such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and dehydroascorbate reductase (DHAR) (Nahakpam and Shah, 2011) as well as antioxidants such as glutathione (GSH) and ascorbate (AsA) (Cuypers et al., 2010). Within plants, $O_2^{\bullet -}$ can first be catalyzed to hydrogen peroxide (H_2O_2) by SOD with remarkably high reaction rates, and further degraded into H_2O by CAT and APX. GR and DHAR play important roles in keeping the metabolic balance between GSH and AsA contents in the GSH-AsA cycle (Sharma and Dietz, 2009). GSH functions as a stress indicator of oxidative stress, can react directly with ROS and promo#te the regeneration of AsA which also plays a crucial role in protecting cells against oxidative stress in plants (Aravind and Prasad, 2005).

During the past years, many approaches have been conducted to mitigate Cd toxicity in plants by applying exogenous materials such as salicylic acid, proline, hydrogen sulfide and nitric oxide (Metwally et al., 2003; Xu et al., 2009; Sun et al., 2013; Gill et al., 2013). Recent studies show that, under Cd stress, exogenous silicon (Si) and selenium (Se) stimulate increases in antioxidants including both enzymatic and non-enzymatic antioxidants and appears to be a mechanism that accounts for increased Cd tolerance in plants. This Si stimulation effect on antioxidants under Cd stress has been observed in *Arachis hypogaea* L. (Shi et al., 2010), *Brassica chinensis* L. (Song et al., 2009), *Oryza sativa* L. (Tripathi et al., 2012), and *Zea mays* L. (Lukačová et al., 2013). Similar results have also been found in treatments containing Se, including for *Brassica chinensis* L. (Hasanuzzaman et al., 2012), and *Triticum aestivum* L. (Khan et al., 2015). On the other hand, several studies have reported that Si or Se reduced antioxidant enzymes activities in the presence of Cd. For example, the activities of SOD, CAT, POD and APX significantly decreased in functional tissues of plants in the presence of Si or Se under Cd stress as compared to Cd treatments alone. (Liu et al., 2013). These different responses to Si or Se treatments in activities of both enzymatic and non-enzymatic antioxidants of various Cd-treated species might be due to differences in plant species, age, concentrations of Cd, Si and Se used, duration of treatment and experimental conditions (Sharma and Dietz, 2009; Shi et al., 2010; Wu et al., 2016). However, apart from the research outlined above, little information is available on the mechanisms underlying the different effects of Si and/or Se on Cd detoxification in relation to antioxidant enzyme system and the ascorbate-glutathione cycle within plants, especially for leafy vegetable plants.

The objectives of the present study were to investigate the comparative responses and the combined effect of Si and Se in relation to antioxidant enzyme systems and the ascorbate-glutathione cycle in Cd stressed flowering Chinese cabbage. The results from this study will improve our understanding of the alleviating phenomenon of Si-Se in related to antioxidant systems to Cd stress in crop plants and will provide a means for developing a strategy for mediating phyto-toxicity associated with growing crop plants in Cd-polluted areas.

2. Materials and methods

2.1. Plant culture and experimental treatments

Seeds of flowering Chinese cabbage were germinated by immersing in deionized water at 28 °C in the dark. After one week, 12 morphologically uniform seedlings per container were selected and placed (held by small sponges) in holes in the lids of black polyethylene boxes (378 × 278 × 90 mm) containing 6-L 1/4-strength Hoagland-Arnon solution for 5 days and subsequently half-strength for another 5 days. The pH of the solution was adjusted to 6.7 with 1.0 mol L^{-1} NaOH. The containers were kept in a greenhouse with conditions maintained with a 16-h photo period and a temperature controlled at 20/25 °C (night/day). The nutrient solution was aerated continuously with an air pump and renewed once every three days. After 10 days of growth to adapt to these conditions, the following treatments were applied: three Cd concentrations of 0, 1, and 5 μmol L^{-1} applied as $CdCl_2$, three Si concentrations of 0, 1, and 5 μmol L^{-1} applied as Na_2SiO_3 and three Se concentrations of 0, 1, and 5 μmol L^{-1} applied as Na_2SeO_3. Each treatment was replicated four times and 20 days after the treatments were applied, the plants were harvested. Subsequently, the shoots and roots were separated and washed thoroughly with deionized water. The separated samples of shoots and roots were used for the determination of the various parameters described below.

2.2. Determination of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) concentrations

Fresh tissue samples (leaves or roots 0.5 g) were homogenized with 5-mL 0.1% (w/v) cold trichloroacetic acid (TCA) in an ice bath. After centrifugation at 12,000*g* for 15 min, the supernatants were used for the determination of H_2O_2 and MDA concentrations. MDA concentrations in tissue were determined according to Zhang and Kirkham (1994). The reaction mixture contained 1-mL of supernatant and 4-mL of thiobarbituric acid (TBA) reagent (0.5% of TBA in 20% TCA). After heating at 95 °C for 30 min in a water bath, the reaction mixture was quickly cooled in an ice bath and centrifuged at 12,000*g* for 15 min, the MDA concentration was calculated from the difference between the absorbance values at 532 and 600 nm with a extinction coefficient of 155 mmol cm^{-1} expressed as millimicromoles per gram (nmol g^{-1}). H_2O_2 determination was described by Alexieva et al. (2001). The reaction mixture contained 0.5-mL of supernatant, 0.5-mL of 100 mmol L^{-1} potassium-phosphate buffer (pH 6.8) and 2-mL reagent (1 mol L^{-1} KI w/v in fresh double-distilled water). 0.1% TCA solution without extracted material was used as the blank. The mixture was incubated for 1 h in the dark and the H_2O_2 concentration was measured at 390 nm expressed as micromoles per gram (μmol g^{-1}). Absorbances of both MDA and H_2O_2 reaction mixtures were measured using a spectrophotometer (TU-1901, Beijing, China).

2.3. Determination of glutathione (GSH) and ascorbate (AsA) concentrations

Fresh tissue samples (leaves or roots 0.5 g) were homogenized in 3-mL ice-cold acidic extraction buffer (5% potassium-phosphoric acid containing 1 mmol L^{-1} ethylenediaminetetraacetic acid, EDTA). After centrifugation at 12,000*g* for 20 min at 4 °C, the supernatant was collected for analysis of GSH and AsA. GSH was estimated following the method reported by Anderson (1985) and AsA was determined by a previous reported method by Law et al. (1983) with expression as micromoles per gram (μmol g^{-1}).