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Comparison of foliar silicon and selenium on cadmium absorption, compartmentation, translocation and the antioxidant system in Chinese flowering cabbage

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ABSTRACT

Silicon (Si) and selenium (Se) are beneficial for many higher plants when grown on stress conditions. However, the mechanisms underlying the differential effects between foliar Si and Se in alleviation of plant toxicity exposed to cadmium (Cd) stress are remained unclear. In this study, we investigated the discrepant mechanisms of foliar Si and Se on Cd absorption and compartmentation by roots, its translocation in xylem, and the antioxidant system within Chinese flowering cabbage (*Brassica campestris* L. ssp. *chinensis* var. *utilis*) under low and high Cd stress. Results showed that plant growth was significantly enhanced by foliar additions of Si or/and Se according to an increased plant tissue biomass at high Cd exposure. In addition, the foliar coupled addition of Si and Se showed little effects on the concentrations of Si or Se in plant tissues in comparison with the single addition of foliar Si or Se respectively. The foliar Si alone or combined with Se markedly reduced the Cd concentrations in plant shoots under two Cd treatments. This might be explained by the lower Cd concentrations in symplast and apoplast and the higher Cd concentrations in cell walls of plant roots, and the lower Cd concentrations in xylem sap. However, no great changes in these values were observed under the treatments of foliar Se alone. Moreover, the foliar additions of Si or/and Se all increased the antioxidant enzyme activities of SOD, CAT and APX in plant tissues, especially at high Cd dosage. No significant differences in the increasing degrees of these three antioxidant enzymes were found between the foliar Si and Se treatments. However, only the foliar Se alone or combined with Si markedly promoted the antioxidant enzyme activities of GR and DHAR in plant tissues. Our findings demonstrate that the alleviation of Cd toxicity by foliar Si maybe mainly responsible for inhibition of Cd absorption and its translocation to plant shoots, reinforcing its compartmentation into root cell walls, whilst enhancing the antioxidant enzyme system may be employed by foliar Se.

1. Introduction

Cadmium (Cd) is a very widespread heavy-metal pollutant in agricultural ecological environments. Due to its high mobility, water solubility and non-degradability, Cd is easily taken up and accumulated by many plant species (Gallego et al., 2012). When Cd accumulation in plants exceeds their resistance values, it causes serious phyto-toxicity, including reduction of plant growth, disorder in physiological and molecular metabolism, inhibition of uptake and translocation of mineral elements, excess accumulation of reactive oxygen species (ROS), and even plant death (Wu et al., 2015b, 2017).

Many strategies have been suggested for the reduction of phytotoxicity when grown in Cd-contaminated areas. These mainly include using of soil conditioners, addition of mineral and non-mineral elements, and other chemical materials (Fargasova et al., 2006; Ahmad et al., 2017; Khan et al., 2017). Recent decades, more and more literatures are focused on the effects of silicon (Si) and selenium (Se) applied into nutrient media or their foliar addition in promoting plant

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resistance to Cd stress.

Silicon comprises a major proportion of the earth's crust just after oxygen. However, its bioavailable species to plant utilization in the soil environment such as silicic acid are frequently limiting. Numerous previous researchers believed that Si has many positive effects for the growth of various plant species by relieving the negative effects of exposure to adverse conditions. One of these strategies for stimulating plant tolerance/resistance to heavy-metal stress by Si addition is based on the reduction of Cd absorption by roots or/and transport within plants, leading to lower Cd accumulation in plant shoot tissues. Liu et al. (2013) found that Si addition to solution media brought about a significant decline of root net Cd influx at the single-cell level in rice plant, indicating lower Cd entrance into root cells. Wu et al. (2016) believed that exogenous addition of Si to nutrient solutions not only inhibits Cd uptake by roots through apoplastic and symplastic routes, but it also decreased xylem Cd translocation to shoots in *B. campestris* plant. Enhancement of Cd compartmentation into areas of low metabolic activity in plant tissues such as cell walls or vacuoles is also an important mechanism for heavy metal tolerance/resistance in plants. Zhang et al. (2008) reported that a Si-induced increase in compartmentation of Cd in the rice roots cell walls, and decreased Cd accumulation in shoots. Similar results were also observed in root tissue of *Kandelia obovata* L. under Cd stress (Ye et al., 2012). Several other researchers have also pointed out that Si addition can reduce oxidative stress in plants by enhancing the activities of antioxidant enzymes when under Cd stress, including in cucumber plants (Khodarahmi et al., 2012), cotton plants (Farooq et al., 2013) and rice plants (Tripathi et al., 2013). Apart from these literatures involved in the effects of Si addition to plant roots, several findings indicated that foliar Si could also alleviate plant toxicity to Cd stress. It was reported that foliar Si alleviated the toxicity and accumulation of Cd in grains of rice by the probable Cd sequestration into shoot cell walls. Foliar Si application alleviated Cd toxicity in rice by decreasing Cd accumulation and Cd partitioning in shoots, and malonaldehyde (MDA) levels by increasing contents of some mineral elements (Wang et al., 2015). Whereas, the previous studies on this field almost involved in the effects of Si addition on Cd distribution and accumulation, and partial antioxidant enzyme activities, the researches on the alleviation mechanisms by foliar addition of Si or combined with other elements (such as Se) and their coupled effects within leafy vegetable plants are still rarely reported.

Selenium is not an essential element for higher plants, but its protective roles against toxic heavy metal stress have been reported in several papers. Recent study showed that Se addition into plant roots could mitigate Cd-induced oxidative stress in tomato plants by modulating chlorophyll fluorescence, osmolyte accumulation, and antioxidant systems (Alyemeni et al., 2018). Ahmad et al. (2016) found that exogenous application of Se into nutrient media mitigated the negative effects of Cd stress in mustard plants through the regulation of osmoprotectants, antioxidant enzymes, and secondary metabolites. Apart from these positive effects above, Se can also affect the accumulation and distribution of Cd in plants and subsequent mitigate Cd toxicity. It has previously been demonstrated that Se significantly inhibited Cd uptake and its translocation from roots to shoots of rice seedlings (Fargasova et al., 2006), and Cd accumulation in the heads and leaves of broccoli plants (Pedrero et al., 2007). However, Wu et al. (2016) reported that application of Se into nutrient media showed little effects on Cd uptake by roots and its transport in xylem. Several literatures have been pointed out that foliar Se also affected plant tolerance to Cd toxicity by regulation subcellular distribution and chemical forms of Cd in rice seedlings in different sulfur concentrations (Q. Zhang et al., 2014; W. Zhang et al., 2014), as well as Cd accumulation in sweet persimmon (Yang et al., 2013). The different effects of various species to Cd stress by Se might be responsible for plant species, concentrations of Cd, Se used, using mode of Se, and the particular growth conditions. Whereas, the previous studies on this field almost involved in the effects of Se addition in Cd distribution and accumulation in plant tissues or regulation of antioxidant enzymes, the researches on alleviation mechanisms by foliar addition of Se or combined with other elements (such as Si) and their coupled effects within leafy vegetable plants are need further study.

Based on a current review from the relevant literature, we hypothesize that foliar addition of Si or/and Se may mitigate toxic effects of Cd in vegetable crops by improving growth via regulating Cd translocation and distribution, and the antioxidant enzyme system within the plant tissues when exposed to Cd stress. To test this hypothesis, we conducted a hydroponic experiment to explore the different resistance mechanisms between foliar Si and Se, related to Cd compartmentation, its absorption, translocation and the antioxidant enzyme system within Chinese flowering cabbage (*B. campestris* L. ssp. *chinensis* var. *utilis*) exposed to low-and-high Cd stress. Our findings will increase the current knowledge of the differential resistance mechanisms underlying Cd detoxification by foliar Si and Se within leafy vegetable plants under Cd stress, and will afford an effective and practical approach to decrease Cd accumulation risks in edible parts, and at the same time, maintaining plant production of vegetable crop plants grown in a Cd-contaminated environment.

2. Materials and methods

2.1. Plant material, cultivation condition, and treatment design

Seeds of Chinese flowering cabbage (*B. campestris* L. ssp. *chinensis* var. *utilis*) in our research were friendly supplied by institute of vegetable research institute, Guangdong academy of agricultural sciences. After sterilizing in dilute sodium hypochlorite (NaClO) solution for 20 min, the seeds were washed thoroughly with distilled water for five times and subsequently soaked in deionized water for germination at 25 ℃ in dark condition for 6 days. Then the selected morphologically uniform seedlings were transferred to holes in the lids of black plastic boxes (37.8 × 27.8 × 9 cm) through holding by small sponges (12 seedlings per box) for 8 days filled with 1/2-strength Hoagland-Arnon solution. After 8 days pretreatment, the seedlings were exposed to full-strength Hoagland-Arnon solution containing each treatment. The experimental treatments of Cd concentrations were 0, 1 and 5 μM ($CdCl_2$) applied as application into the nutrient solution, and Si and Se were both 0 and 5 μM (Na_2SiO_3 and Na_2SeO_3) applied as the mode of brushing on the top and bottom surfaces of the leaves (brushing once after every 2 days). Each box represented one replicate and every treatment was conducted with four replicates. The pH of each cultivation solution was adjusted close to 6.5 by addition of dilute NaOH or HCl solution for every 2 days. The cultivation solution was changed after every 4 days. The cultivated boxes were kept in a greenhouse with a 16 h photo period and a controlled temperature at 20/25 ℃ (night/day) and were aerated continuously with an air pump.

2.2. Plant tissue biomass, and Cd, Si and Se determination

After 16 days culture exposed to each treatments, fresh plant seedlings were harvested and divided into two parts (roots and shoots), and washed cleanly with distilled water for four to five times. Then the separate roots were immersed in 20 mM Na_2-EDTA for 20 min to remove metal ions from root surfaces. After that, the samples of roots and shoots were placed into a dryer at 105 ℃ for 30 min, then at 75 ℃ for 4 days till reached constant weights. After weighing, the dry materials were ground into powder, and subsequently were digested for the determination of Cd, Si and Se concentrations. The measurements of Cd and Si concentrations in digest solution were according to the procedure described in terms of Wu et al. (2015b) and Liu et al. (2013) by inductively coupled plasma mass spectrometry (Agilent 7900, ICP-MS, Japan), and Se determination was followed the procedure described in Chen et al. (2014) by atomic fluorescence spectrometer (Baode, BAF-3000, China).