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Comparative responses to silicon and selenium in relation to cadmium uptake, compartmentation in roots, and xylem transport in flowering Chinese cabbage (*Brassica campestris* L. ssp. *chinensis* var. *utilis*) under cadmium stress

Zhichao Wu[a,b,c], Fuhua Wang[a,b,c,*], Shuai Liu[a,b,c,d], Yingqiong Du[a,b,c], Furong Li[a,b,c], Ruiying Du[a,b,c], Dian Wen[a,b,c], Jie Zhao[a,b,c]

[a] *Public Monitoring Center for Agro-Product, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China*
[b] *Key Laboratory of Testing and Evaluation for Agro-product Safety and Quality, Ministry of Agriculture, Guangzhou 510640, China*
[c] *Laboratory of Quality and Safety Risk Assessment for Agro-product (Guangzhou), Ministry, Guangzhou 510640, China*
[d] *College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, China*

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ABSTRACT

Silicon (Si) and selenium (Se) are generally considered as beneficial elements for the growth of higher plants, especially for those grown in heavy-metal stressed environments. However, the mechanisms underlying the 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 Cd uptake, compartmentation in roots, and xylem transport in flowering Chinese cabbage plants under Cd stress. Addition of Si or/and Se efficaciously alleviated the toxicity of Cd as demonstrated by increasing of tissue (shoots and roots) biomass of plants exposed to high Cd stress, especially for their coupling treatments with high doses. In compare with the Cd-alone treatment, the application of Si alone or in combination with Se greatly decreased plant shoot Cd concentrations as well as its translocation factor (TF), though the Cd concentrations in roots and the total Cd accumulation of whole plants showed increasing trends (especially for the treatments of high Si or coupling with Se), while no marked differences were found in plants exposed to the Se-alone treatment. Additionally, the application of Si alone or in combination with Se considerably reduced the Cd concentrations and its proportions in symplast and apoplast root saps and increased them in cell wall fragments, while little changes were observed for the Se-alone treatment. Furthermore, a greatly decreased tendency was also displayed for the Cd concentrations in xylem saps exposed to the treatments containing Si alone or in combination with Se. Overall, our results reveal that Si-mediated alleviation of Cd toxicity may be due to decreasing Cd concentrations and its proportions in symplasts and apoplasts, enhancing adsorption of Cd on cell walls, and restriction root-to-shoot Cd translocation. However, Se mitigation may involve other mechanisms.

1. Introduction

Cadmium (Cd) is a highly toxic element without any known beneficial physiological function in plants to date. It enters the environment mainly through industrial, urban, and agricultural activities, and causes several biochemical, structural and physiological disorders in plants, even when plants are grown under moderately Cd-polluted conditions. Cadmium accumulation in vegetables and other edible parts of crops poses a considerably potential health problem for human healthy safety, and increased dietary intake of Cd has been highly correlated with an increased

Abbreviations: Cd, cadmium; Si, silicon; Se, selenium; FAO, Food and Agriculture Organization; WHO, World Health Organization; SIET, scanning ion-selective electrode technique; ROS, reactive oxygen species; Conc., concentration; Accum., accumulation; TF, translocation factor.

* Corresponding author at: Public Monitoring Center for Agro-Product, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China.
E-mail address: wfhwqs@163.com (F. Wang).

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consumption of vegetables cultivated in Cd-contaminated areas (Yang et al., 2009). To reduce the potential risks caused by excessive Cd intake, it is necessary to restrict Cd concentrations in edible parts of crops below the threshold values established by the Codex Alimentarius Commission of FAO/WHO (CODEX, 2006).

So far, various chemical, physical and biological approaches are adopted for decreasing Cd concentrations in edible parts of crops grown in Cd-polluted soils. Of all these strategies, exogenous application of nutrients such as silicon (Si) and selenium (Se) has gained considerable attentions as an effective strategy to mitigate the adverse effects from Cd pollution (Liang et al., 2007; Lin et al., 2012).

Silicon is the second most abundant mineral element in the soil after oxygen. Although Si has not been considered as an essential element for higher plants, there is increasing evidence that Si has many beneficial functions for plant growth and development and can improve stress resistance against heavy metals in many plant species, including *Zea mays* L. (Liang et al., 2005; Vaculík et al., 2012), *Solanum nigrum* L. (Liu et al., 2013a), *Gossypium* spp. (Farooq et al., 2013), *Avicennia marina* (Zhang et al., 2014), *Triticum turgidum* L. cv. *Claudio* (Rizwan et al., 2012), *Oryza sativa* L. (Gong et al., 2006). One of these benefits is that Si application would enhance Cd-detoxification by reducing uptake and translocation of Cd within plants. It has been reported that Si at least partially blocked the apoplastic bypass flow across roots and restrained apoplastic transport of Cd (Shi et al., 2005). A direct evidence was provided that Si treatments in Si-accumulator (+Si) cells markedly inhibited net Cd^{2+} influx in compared with that in Si-limiting (-Si) cells in roots by scanning ion-selective electrode technique (SIET) (Liu et al., 2013b). Ma et al. (2015) suggested that a hemicellulose-bound of Si with net negative charges was responsible for inhibition of Cd uptake and subsequent co-deposition on cell walls, suggesting a plausible explanation for detoxification of Cd in rice. In addition, Si application increased xylem sap flow in rice plants under Cd stress and decreased Cd concentrations in xylem saps which might be due to the dilution of metals in saps, indicating that Si addition inhibited Cd translocation by xylem flow (Liang et al., 2005). Another mechanism of Si-mediated amelioration to Cd stress is alteration of subcellular compartmentation of Cd in plants. Liu et al. (2009) reported that Si foliar application increased Cd concentrations and its percentages bound to cell walls of shoots but did not affect the Cd distribution in the cell walls of roots. Similarly, Ye et al. (2012) reported that Si enhanced the compartmentalization of Cd to root cells and decreased the distribution of Cd in symplasts. However, most of research on this field has been mainly focused on the effects of applications of Si alone on the Cd detoxification in fruit or grain crops, while the coupling effects of Si with other beneficial elements such as Se on Cd detoxification in vegetables have been less studied, especially for leafy vegetables.

Selenium is an essential micro-nutrient and has important benefits for human and animal health. It has been identified that Se at low concentrations reduces Cd toxicity by increasing antioxidative capacity, reducing lipid peroxidation, promoting growth, and enhancing the accumulation of starch and sugars, since it is incorporated in the active center of antioxidant seleno-enzymes (glutathione peroxidase and thioredoxin reductase) (Turakainen et al., 2004; Zwolak and Zaporowska, 2012; Lin et al., 2012). In rape and wheat, Se showed a capacity to counterbalance Cd-induced changes in nutrition and reduced lipid peroxidation (Zembala et al., 2010). Hasanuzzaman et al. (2012) demonstrated that Se at low concentrations increased the tolerance of rapeseed seedlings exposed to Cd by enhancing their antioxidant defence and methylglyxoal detoxification systems. Lin et al. (2012) suggested that Cd toxicity alleviated by Se was associated with the reduction of Cd uptake and the elimination of reactive oxygen species (ROS). However, apart from the related research outlined above, little information is available on the mechanisms underlying the effects of Se on Cd detoxification in relation to the uptake and compartmentation in roots and root-to-shoot transport of Cd within plants, especially for leafy vegetables.

The objectives of the present study were to investigate the comparative responses and the coupling effects of Si and Se against Cd stress in relation to the modification of the Cd binding properties of cell walls, the Cd concentrations in apoplasts and the symplasts, and the root-to-shoot translocation of Cd in flowering Chinese cabbage. The results from this study will improve our understanding of the alleviating phenomenon of Si-Se mediated resistance to Cd stress in crop plants and will provide a basis for developing strategy to reduce the risks 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 each 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 two weeks after the treatments were applied, the plants were harvested. The separated roots and shoots were thoroughly washed with deionized water, and the roots were soaked in 20 mmol L^{-1} EDTA-Na_2 for 20-min to remove metal ions from the root surfaces, and used for the determination of the various parameters described below.

2.2. Determination of Cd in plant tissues

Cadmium in plant tissues (roots and shoots) was determined according to the method as reported by Wu et al. (2015b). Separated samples of shoots and roots were dried at 68 °Cfor 96 h. After weighing, dried plant materials were digested with a mixture acid of HNO_3 and $HClO_4$ (4:1, v/v) at a controlled temperature of 160 °C. The ratio of the volume (mL) of the mixture acid/the mass of tissue (g) was 20:1, and Cd concentrations in the digests were subsequently determined by inductively coupled plasma mass spectrometry (Agilent 7900, ICP-MS, Japan).

2.3. Determination of Cd in symplast, and apoplast saps and in cell walls

Symplastic and apoplastic saps of root segments were collected according to the method as reported by Zhang et al. (2014) with some modifications. In brief, fresh samples of roots (1 g) excised 2 cm from the root tips were arranged in 10-mL needle tubes with the cut ends facing down. The root segments were infiltrated three times with 50 mmol L^{-1} MES-Tris buffer solution (pH 6.5) in a suction flask with the pressure of 0.5 kPa for 20 min. Then the needle tubes were transferred to plastic centrifuge tubes and