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EFFECTS OF CADMIUM-ADDED ON ASCORBATE-GLUTATHIONE CYCLE OF YOUNG CITRUS SEEDLINGS UNDER SELENITE OR SELENOMETHIONINE-ENRICHED SOIL

XIEPING SUN[1,2], GUOQIANG HAN[1], YOUJIN LUO[2*], GUANGWEN ZHOU [2] AND YU WANG[1]

[1]Life Science and Technology Institute, Yangtze Normal University, P.R. China
[2]Fruit Research Institute, Chongqing Academy of Agricultural Sciences, Chongqing, P.R. China
[*]Corresponding author's emails: luoyoujin1984@163.com

Abstract

In order to study the effect of cadmium (Cd)-added on citrus growth under selenite or selenomethionine (SeMet)-enriched soil, the concentration of leaf pigments, H_2O_2, antioxidants and antioxidases in ascorbate glutathione **(**AsA-GSH) cycles were detected in 'Daya' citrus seedlings (*Citrus sinensis* L.). The results showed that chlorophyll a, b, and a+b were significantly enhanced by cadmium-added under SeMet-enriched treatment, while leaf pigments showed little influence cadmium under selenite-enriched treatment. Meanwhile, cadmium-added significantly increased H_2O_2 concentration under both selenite and SeMet-enriched soils. In AsA-GSH cycle, the concentration of AsA, dehydroascorbate (DHA), and AsA/(AsA+DHA) ratio were increased, while the levels of GSH, oxidized glutathione (GSSG), and GSH/(GSH+GSSG) were decreased by cadmium-added under selenite or SeMet-enriched soil. Meanwhile, the activities of ascorbate peroxidase (APX), monodehydroascorbate reductase (DHAR), and glutathione reductase (GR) increased, and the activities of glutathione peroxidase (GPX) decreased under selenite or SeMet-enriched soil with cadmium-added. Our results indicated that despite cadmium-added had positive effects on chlorophyll concentrations under Se-enriched soil, leaf was injured by increased H_2O_2 concentration. Furthermore, the AsA-GSH cycle efficiency was increased to defend cadmium stress under two selenium types-enriched soils.

Key words: 'Daya' orange, Selenium, Cadmium, AsA-GSH cycle.

Introduction

Heavy metals known in nature are divided into two main groups: 1-the essential heavy metals, 2-non-essential heavy metals for plants' growth (Emamverdian, & Ding, 2018). Cadmium (Cd) is a highly toxic non-essential heavy metal, which reduces plant growth and alter metabolic processes (Hassan & Mansoor, 2017). Citrus plants possess a remarkable tolerance to cadmium. For instance, citrus could exhibit no leaf damage or plant death under 300 μM cadmium treatment (Aggarwal *et al.*, 2011). The plants typically accumulated cadmium are able to maintain the intracellular redox balance and defend the oxidative stress. Plants employ antioxidant enzymes and the small molecular antioxidants to cope with the oxidative stress. Ascorbate-glutathione (AsA-GSH) cycle system involved in scavenging of H_2O_2 contains GSH, glutathione reductase (GR), oxidized glutathione (GSSG), ascorbic acid (AsA), ascorbate peroxidase (APX), dehydroascorbate (DHA), and monodehydroascorbate reductase (MDHAR) (Ríos *et al.*, 2009; Chao *et al.*, 2010; López-Climent *et al.*, 2014). Plant maintaining high ratios of AsA/(AsA+DHA) as well as GSH/(GSH+GSSG) increased the toxic reactive oxygen species defense (Wang *et al.*, 2012).

Selenium (Se) is termed to be a beneficial element for numerous plants. Low-dose selenium enhanced plant growth, while high-dose hindered plant growth (Jiang *et al.*, 2015; Sun *et al.*, 2018). Low dose selenium induced antioxidant production, stimulating plant growth (Ríos *et al.*, 2009). In addition, selenium plays a protective function against cadmium stress (Elguera *et al.*, 2014). For example, low-dose selenium had beneficial effect on rice growth, together with mitigating the toxicity of cadmium (Ding *et al.*, 2014). Adverse effect of cadmium stress was mitigated by regulating the osmoprotectants, the antioxidant enzymes, and the secondary metabolites with application of selenium (Ahmad *et al.*, 2016). Selenium considerably induced the biosynthesis of melatonin, and melatonin can also reduce cadmium uptake together with mitigating cadmium toxicity (Li *et al.*, 2016) .

Plants growing on the seleniferous soil produce the selenium-enriched foods (Yasin *et al.*, 2015). Some Se-enriched soil is inevitable to be polluted by cadmium, or has a risk of cadmium pollution. In our work, AsA-GSH cycle systems of 'Daya' orange were compared, which grown under cadmium-polluted or not polluted Se^{4+} (from selenite) and selenomethionine (SeMet)-enriched soil.

Materials and Methods

The experiment was carried out in a greenhouse at Baishiyi Town (E 106°21'44", N29° 27'24"), Jiulongpo District, Chongqing city of China. Uniform grafted seedlings of 'Daya' orange (*Citrus sinensis* L.) were planted in 10.0 L plastic pots filled with garden soil, turf soil, and puerile (10:10:1) mixture. Plants were allowed to acclimate for one month under well planted conditions. On Aug 24th 2015, healthy seedlings of uniform size were selected as materials. Four treatments were applied, via (1) pots contained 3.0 mg/L selenium from selenite; (2) pots contained 3.0 mg/L selenium from selenomethionine (SeMet); (3) pots contained 3.0 mg/L selenium (from selenite) + 0.6 mg/L cadmium (from cadmium chloride); (4) pots contained 3.0 mg/L selenium (from selenomethionine (SeMet)) + 0.6 mg/L cadmium (from cadmium chloride). The experiments ended on April 18th, 2016. Leaves were collected and ground at a temperature of -80°C prior to the detection.

Leaf pigments were extracted using 95% ethyl alcohol in dark, determined by spectrophotometry in accordance with the method of Lichtenhaler & Wellburn (1983). The concentration of H_2O_2 was determined as a H_2O_2-titanium complex raised from the reaction of tissue-H_2O_2 with titanium tetrachloride (Zhang *et al.,* 2012).

The activity of APX, DHAR, GPX, GR, and the content of AsA, DHA, GSH, GSSG were measured by enzyme linked immunosorbent assay (ELISA)-sandwich approach by Rayto RT-6100 and ELISA detection kits. And then, the sum of AsA+DHA and GSH+GSSG, and the ratios of AsA/(AsA+DHA) and GSH/(GSH+GSSG) were calculated.

All data were statistically analyzed with Origin 8.0 software. The data was displayed as mean ± standard deviation (SD) performing of four replications. One-way analysis of variance (ANOVA) was used to assess the treatment effect for comparing significance among treatments. Significance levels were expressed at $p<0.05$ level.

Results

Pigment contents: 'Daya' pigment contents were not significantly influenced by cadmium-added under selenite-enriched soil. While chlorophyll a, chlorophyll b, carotenoid and chlorophyll a+b increased 18.3%, 14.8%, 15.3% and 17.2% by cadmium-added under SeMet-enriched soil, respectively(Fig. 1). Furthermore, higher concentrations of chlorophyll a (8.9%), chlorophyll b (3.0%), carotenoid (8.6%), and chlorophyll a+b (7.1%) were found under selenite-enriched soil compared to those under SeMet-enriched treatment. The contents of the chlorophyll a, chlorophyll b, carotenoid, and chlorophyll a+b were 11.5%, 15.5%, 10.2%, 12.4% decreased by cadmium-added under SeMet-enriched treatment, respectively.

H_2O_2 contents: There was no significant difference in leaf H_2O_2 concentration of 'Daya' orange between selenite and SeMet-enriched soil. Leaves H_2O_2 concentrations were increased by 125.8% and 45.3% with cadmium additions of under selenite and SeMet-enriched soil (Fig. 2).

AsA-GSH cycle efficiency: GSH and AsA are low molecular antioxidants, meanwhile GSSG and DHA are their oxidation states, respectively. APX and GPX are major H_2O_2-scavenging enzymes. GR and DHAR are two key enzymes to maintain high concentration of GSH and AsA. AsA was increased 31.7% (selenite-enriched) and 8.6% (SeMet enriched soil) by cadmium addition. Meanwhile, DHA concentration was also increased about 23.0% (selenite-enriched) and 10.0% (SeMet enriched soil). GSH concentration was decreased 12.3% and 14.3% by cadmium addition under selenite-enriched and SeMet-enriched soil, respectively. GSSG concentration was also reduced to 6.1% and 7.2% by application of cadmium under selenite-enriched and SeMet-enriched soil, respectively. A slight increase of AsA/(AsA+DHA) ratio and a slight decrease of GSH/(GSH+GSSG) ratio were found with cadmium addition under two types enriched soil APX, GR and DHAR activities in leaf were decreased by 28.0%, 17.4% and 64.2% by addition of cadmium under selenite-enriched soil, respectively. While APX, GR and DHAR activities in leaf were increased by 23.1%, 13.1% and 59.7% by cadmium addition under SeMet-enriched soil, respectively. GPX activities were decreased 10.0% and 13.4% by cadmium addition under selenite-enriched and SeMet-enriched soil, respectively.

Discussion

Reactive oxygen species are increased in the plant under abiotic stress, damaging the structure as well as function of the cells. Chlorophyll pigments is one of the key factors of cadmium injury in plants, whereas, different selenium type also affects plant pigments (Muradoglu *et al.*, 2015, Garous *et al.*, 2016). In our study, 'Daya' orange had higher pigments under selenite-enriched soil than that under SeMet-enriched soil, indicating that plant pigments were affected with selenium type. 'Daya' pigment concentration had no significant change with cadmium addition under selenite-enriched soil, while it was significantly increased by cadmium addition under SeMet-enriched soil (Fig. 1). The results were consistent with report by Aggarwal *et al.,* (2011) that citrus is resistant to cadmium. Cadmium-added increased H_2O_2 concentration under selenium-enriched soils (Fig. 2). High level of H_2O_2 damaged cell structures, causing severe consequences (Ozyigit *et al.*, 2016). 'Daya' orange showed more sensitive to cadmium stress under selenite-enriched soil than that under SeMet-enriched soil. It may be due to much more selenium gained by seedlings leaves under SeMet condition than under selenium-enriched soil (data not shown). Selenium, especially SeMet related with GPX, has a very special biological function, including protection against oxidative damage, defenses against infection, and modulation of growth and development (EI-Ramady, *et al*., 2014).

A large number of studies indicated that exogenous selenium mitigated cadmium toxicity through the regulation of antioxidative system (Ahmad *et al.*, 2016; Luo *et al.*, 2017). Wu *et al.*, (2017) reported that application of selenium increased the contration of GSH and AsA, activities of GR and DHAR, improving the AsA-GSH cycle efficiency. The activities of GR and GPX were increased, the oxidative stress was reduced, and the plant growth was improved by selenium addition under cadmium stress (Khan *et al.,* 2015). Studies on physiological and biochemical reaction of plants under cadmium-contaminated selenite-enriched soil were few. In our study, AsA-GSH cycle efficiency was analyzed with cadmium addition under selenium-enriched soil (Figs. 2, 3 and 4). The higher ratios of AsA/(AsA+DHA) and GSH/(GSH+GSSG) indicated the stronger antioxidant capacity (Fotopoulos, *et al.*, 2010; Wang *et al.*, 2012). But in our study, a higher AsA/(AsA+DHA) ratio and a lower GSH/(GSH+GSSG) ratio were found by addition of cadmium under selenium-enriched soil (Figs. 3 and 4). Inconsistent changes of the two ratios may be due to GSH redox cycle efficiency (GPX was reduced by cadmium-added) which was reduced by cadmium-added. In AsA-GSH cycles, GPX and DHAR consumed same enzyme substrate GSH. GSH is a molecular antioxidant that directly scavenging reactive oxygen species. Moreover, GSH was combined with cadmium to alleviate the toxicity of cadmium (Brouwer *et al.*, 1993; Yadav, 2010). Thus, it explains why cadmium-added treatments reduce GSH/(GSH+GSSG) ratio.

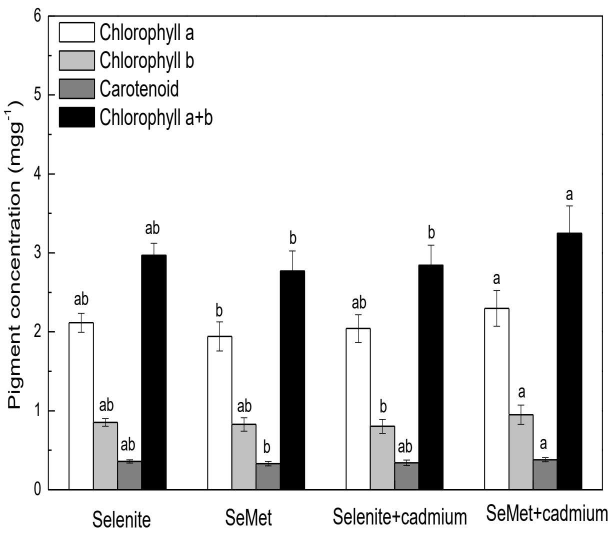

Fig. 1. Effect of cadmium-added treatment on the concentration of chlorophyll a, chlorophyll b, carotenoid, and chlorophyll a+b of 'Daya' orange under selenite or SeMet soil. The data shown are the averages of four replicates, with the standard errors indicated by the vertical bars. The means demoted by the same letter do not significantly differ at a $p<0.05$ (Fisher LSD test)

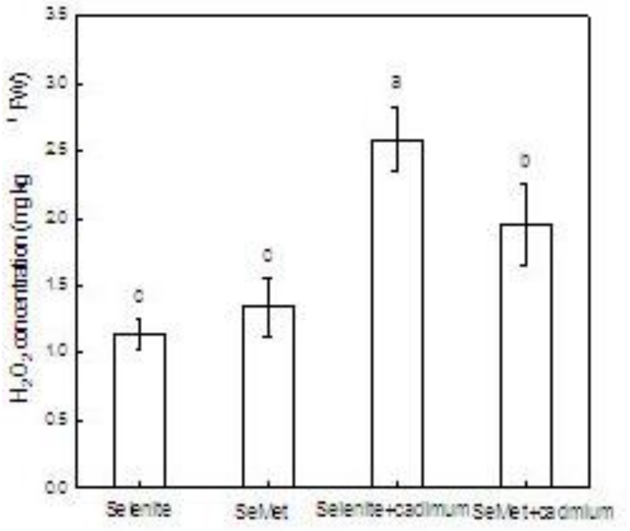

Fig.2. Effect of cadmium-added treatment on the concentration of H_2O_2 of 'Daya' citrus under selenite or SeMet soil. The data shown are the averages of four replicates, with the standard errors indicated by the vertical bars. The means demoted by the same letter do not significantly differ at a $p<0.05$ (Fisher LSD test).

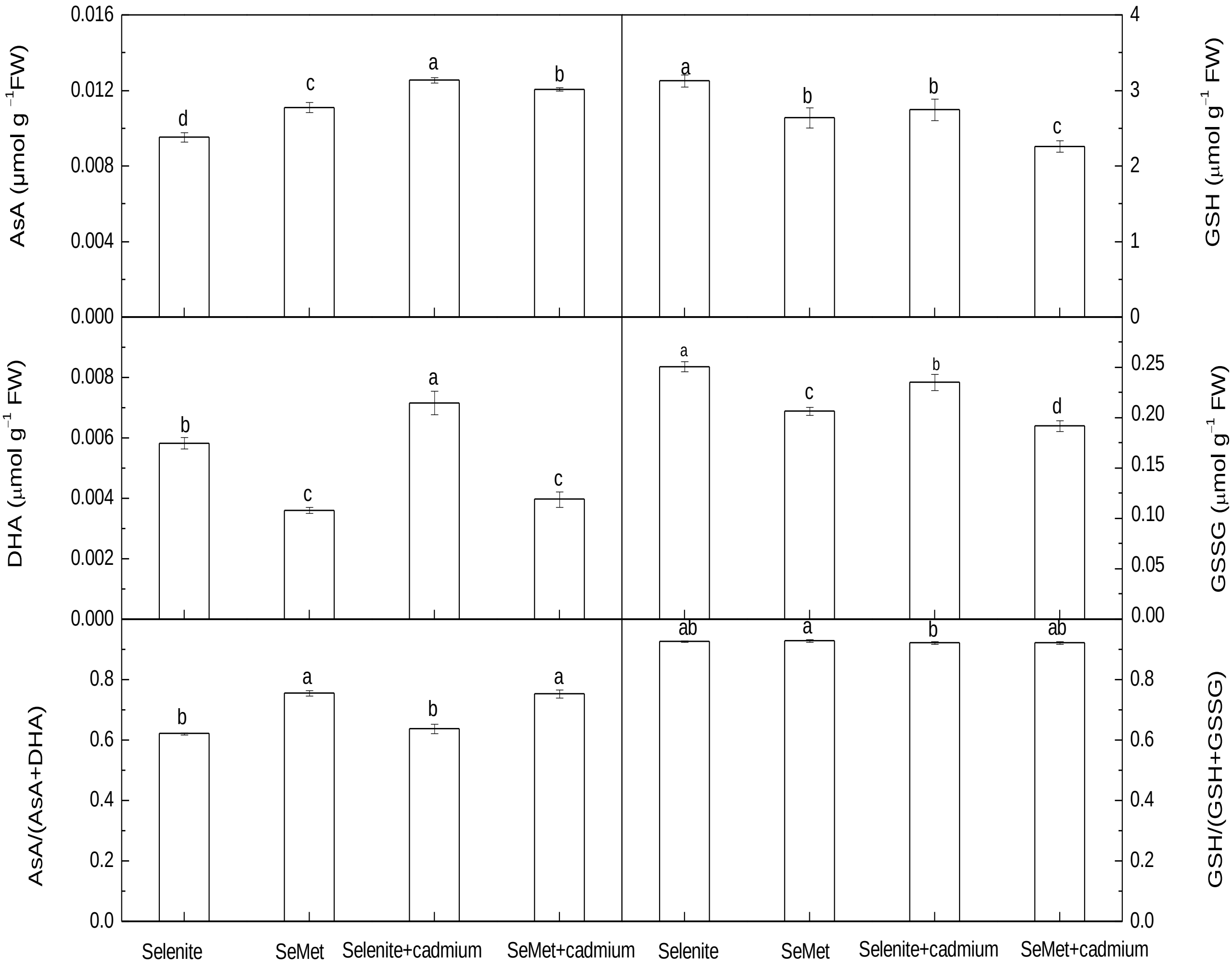

Fig. 3. Effect of cadmium-added treatment on the concentration of AsA, DHA, GSH, GSSG, and radios of AsA/(AsA+ DHA), GSH/(GSH+ GSSG) of 'Daya' citrus under selenite or SeMet soil. The data shown are the averages of four replicates, with the standard errors indicated by the vertical bars. The means demoted by the same letter do not significantly differ at a $p<0.05$ (Fisher LSD test).

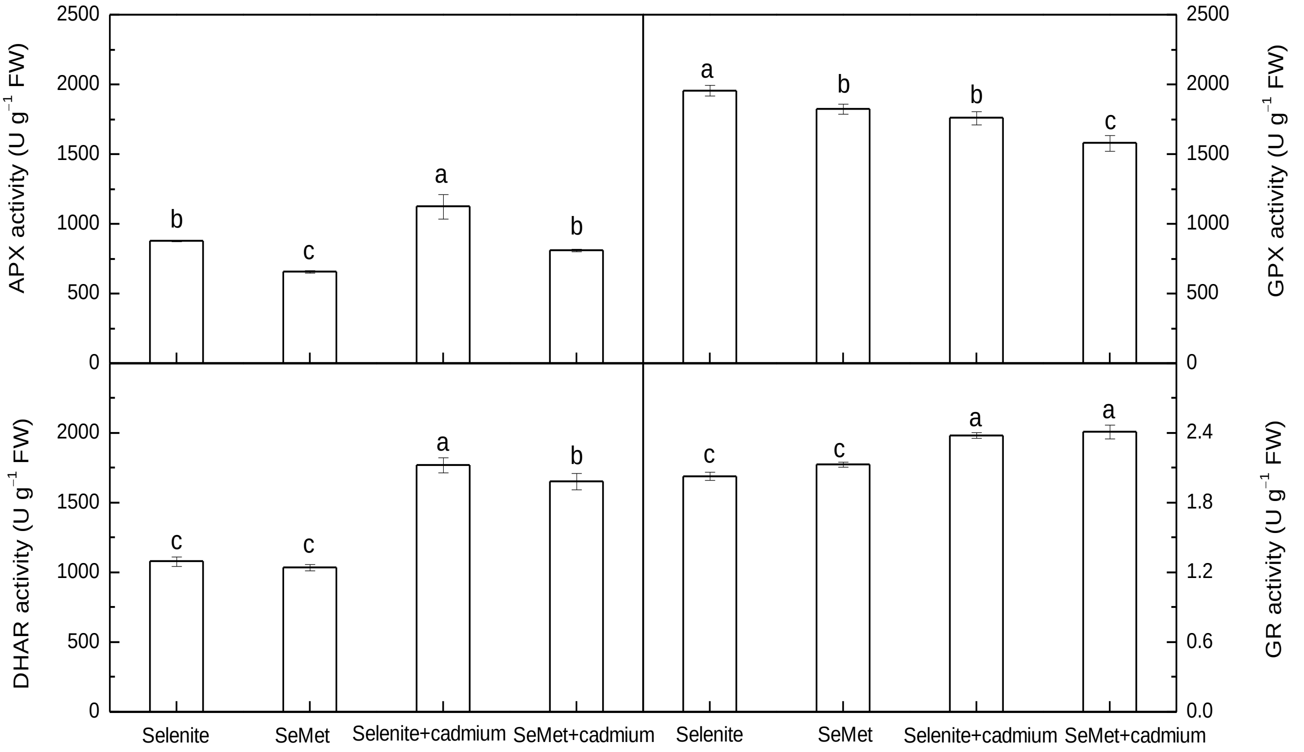

Fig. 4. Effect of cadmium-added treatment on the activates of APX, DHAR, GPX, GR of 'Daya' citrus under selenite or SeMet soil. The data shown are the averages of four replicates, with the standard errors indicated by the vertical bars. The means demoted by the same letter do not significantly differ at a $p<0.05$ (Fisher LSD test)

Conclusion

In our study, Cd-addition showed positive effects on 'Daya' orange leaf pigments under selenium-enriched soil, especially under SeMet-enriched soil. However, adding cadmium significantly increased leaf H_2O_2 under selenium-enriched soil. The AsA-GSH cycle efficiency was increased to defend cadmium stress.

Acknowledgments

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