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Selenium restricts cadmium uptake and improve micronutrients and proline concentration in tomato fruits

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ABSTRACT

In order to identify the beneficial effects of Selenium (Se) in tomato (*Solanum lycopersicum*), genotypes MT and *hp1*, under Cadmium (Cd) toxicity, the following analyses were performed: Cd and Se concentration and translocation index in roots, leaves and fruits as well as the macro and micronutrients quantification, proline concentration, fruits dry weight, chlorophyll and carotenoids concentration in fruits. Se reduced the Cd concentration in roots of both genotypes. The Cd translocation to above ground parts were restricted by Se only in the MT genotype. Fruit proline concentration increased either in genotype *hp1* when Se was applied alone or in the MT genotype, by the mixture of Se and Cd. When Se was applied alone the concentration of Mn and Zn in *hp1* fruits and Fe in MT fruits were enhanced. The beneficial mechanisms of Se to tomato plants under Cd stress could be partially related to the restriction of Cd uptake and translocation, enhancing proline concentration and micronutrient improvements in fruits.

1. Introduction

Different strategies have been used to alleviate the harmful effects of heavy metal contamination in plants, and selenium (Se), is extensively investigated due to its substantial antioxidant characteristics. Se is an essential micronutrient for humans and animals at low levels, because it is part of enzymes responsible for the cellular redox homeostasis (Catania et al., 2009), the absence or inadequate ingestion of this nutrient in the diet can cause health problems related to malnutrition.

Despite not being recognized as a nutrient to plants, the supply of low Se concentrations appear to stimulate the cellular defense systems (Djanaguiraman et al., 2011), improve the accumulation of starch and sugars, delay the senescence process and enhance photosynthesis (Feng et al., 2013). Different studies have shown the beneficial effect of Se against the oxidative stress caused by heavy metals in plants (Pereira et al., 2018; Yu et al., 2019), however, the role of Se in tomato plants under cadmium (Cd)contamination have not been fully elucidated.

Cadmium is the most toxic among the heavy metals. It can cause a strong oxidative stress in plants, due to its chemical similarity with some nutrients and its misincorporation in the active site of antioxidant enzymes (Cuypers et al., 2010). In humans, Cd is known to be carcinogenic and cause physiological problems to kidney, liver and other organs (Cuypers et al., 2010). This heavy metal cannot be degraded and the uptake and translocation to edible tissues of plants are an immediate concern due to its accumulation within the food chain. .

This study aimed to analyze the beneficial aspects of Se (Na_2SeO_3) over Cd ($CdCl_2$) stress in tomato plants, through physiological, biochemical and nutritional analyses. Therefore, the Se and Cd concentration, as well as the translocation indexes were analyzed in fruits, leaves and root tissues. Furthermore, nutritional and physiological aspects of fruits were also investigate, by means of nutrients and proline quantification, pigments concentration, in addition to fruit growth.

2. Materials and methods

Seeds of tomato (*Lycopersicum esculentum* Mill.) cv. Micro-Tom (MT) and the genotype *high pigment*-1 (*hp1*), were cultivated in separate boxes containing a mixture of 1:1 (by volume) of commercial pot mix (Plantmax HT-Eucatex®, Brazil) and medium size vermiculite,

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Table 1
Selenium and Cadmium concentration in fruits, leaves, roots and translocation index (TI). Values are the mean of three replicates and standard deviation (± SD). Note detectable values are shown as n.d. Lower case letters indicate statistically significant differences (P < 0.05).

Treatment		Cadmium (mg kg^{-1} DW)			TI (%)	Selenium (mg kg^{-1} DW)			TI (%)
		Fruits	Leaves	Root		Fruits	Leaves	Root	
Micro-Tom	Control Plants	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Se 50 μM	n.d.	n.d.	n.d.	n.d.	4.3 ± 0.67 a	2.8 ± 0.93 b	358.3 ± 0.60 a	1.9
	Cd 0.5 mM	30.2 ± 0.88 c	596.3 ± 0.53 a	6340.4 ± 0.74 a	9	n.d.	n.d.	n.d.	n.d.
	Cd 0.5 mM + Se 50 μM	9.4 ± 0.69 d	258.9 ± 0.93 d	3117.8 ± 0.28 c	7.9	n.d.	n.d.	310.9 ± 0.18 b	n.d.
hp1	Control Plants	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Se 50 μM	n.d.	n.d.	n.d.	n.d.	5.4 ± 0.59 a	5.3 ± 0.44 a	210.4 ± 0.48 c	4.9
	Cd 0.5 mM	44.8 ± 0.77 a	503.9 ± 0.91 b	4092.1 ± 0.05 b	11.8	n.d.	n.d.	n.d.	n.d.
	Cd 0.5 mM + Se 50 μM	41.8 ± 0.34 b	324.5 ± 0.65 c	2002.7 ± 0.92 d	15.4	n.d.	n.d.	193.0 ± 0.05 d	n.d.

supplemented with 1 g L^{-1} of 10:10:10 NPK and 4 g L^{-1} of lime. After the first true leaves appeared, seedlings were transplanted to 1 L Leonard pots (Gratão et al., 2012) (1 seedling per pot) filled with sand and polystyrene (4:3) and supplied with Hoagland's nutrient solution (Hoagland and Arnon, 1950). Subsequently, twenty-one-day old plants were selected and further grown in the same solution spiked with either 0.5 mM CdCl$_2$ or 50 μM Na$_2$SeO$_3$ applied either individually or simultaneously. The experiment was designed to have the following treatments: *T1*: Control (nutrient solution); *T2*: Se 50 μM (MT, *hp1*); *T3*: Cd 0.5 mM (MT, *hp1*); *T4*: Cd 0.5 mM + Se 50 μM (MT, *hp1*). After a period of 95 days post germination, corresponding to 74 days of exposure to CdCl$_2$ and/or Na$_2$SeO$_3$, samples of fruits, leafs and roots were harvested, rinsed and frozen immediately in liquid N$_2$, and stored at −80 °C for further analyses.

Quantitative Cd, Se and nutrient analysis was carried out using energy dispersive X-ray fluorescence spectrometry (EDXRF) as described by Tezzoto et al. (2013). Samples of fruits were dried at 70 °C for 7 days, and 0.2 g DW of fine powder, obtained following grinding with mortar and pestle, were microwave digested with 2 mL of 70% HNO$_3$, 2 mL of H$_2$O$_2$ and 2 mL of Milli-Q water (18.2 MΩ cm at 25 °C) at a controlled pressure of 2 MPa, concentrated acids on a digestion block heated gradually to 203 °C. Biological samples with increased concentrations of Cd, Se and nutrients were used to establish standard calibration curves.

Free proline concentration in fruits was measured according to Gratão et al. (2012). Samples (0.5 g) of fruit pulp were ground with 3% sulphosalicylic acid. The homogenate was centrifuged at 10.000 g for 15 min at 4 °C, and 2 mL of the supernatant was held for 1 h in boiling water by adding 2 mL ninhydrin acid and 2 mL of glacial acetic acid, to which cold toluene (4 mL) was added. The absorbance was recorded at 520 nm and proline concentration calculated as mmol g^{-1} FW using a proline standard calibration curve.

Samples of fruits (total fruit weight per plant) were placed in a thermal convection laboratory oven (Fanem® SP, BR – model 330) at 70 °C for a week. Later, these fruits were ground in an electric mill (Marconi®, model 048) and the dry measurements were performed on an analytical balance (Denver instruments company®, model AA-200) accurate to 1.10^{-18} mg. Fruit production was quantified as biomass (g).

Samples of fruit pulp were subjected to spectrophotometric pigment quantification in triplicate. Pigment extraction was carried out in 80% acetone and the extract was filtered through filter paper using a vacuum pump. Measurement of pigments was performed in a spectrophotometer (Beckman®, model DU-640) at the following wavelengths: chlorophyll-*a* in 663 nm, chlorophyll-*b* in 647 nm and carotenoids (carotene[c] + xanthophyll [x]) in 470 nm. The total chlorophyll (chl) and carotenoids (car) contents were calculated as described by Lichtenthaler (1987):

$$\text{Chl } a = 12.25 \times A663 - 2.79 \times A647 \quad (A.1)$$

$$\text{Chl } b = 21.50 \times A647 - 5.10 \times A663 \quad (A.2)$$

$$\text{Chl } a+b = 7.15 \times A663 + 18.71 \times A647 \quad (A.3)$$

$$\text{Car } c + x = (1000 \, A470 - 1.82 \, \text{Chl}a - 85.02 \, \text{Chl}b)/198 \quad (A.4)$$

Pigment concentration was expressed in micrograms of pigment per gram of tissue fresh weight (μg g^{-1} FW).

The Cd and Se translocation indexes (TI, %) were calculated by dividing their concentration in the shoot (fruits, leaves) by their total plant concentration (root, leaves, fruits), and multiplying the quotient by 100, as described by Abichequer and Bohnen (1998). The statistical analysis was performed using the Assistat software version 7.7 (Silva and Azevedo, 2009). ANOVA followed by a multiple comparison between means by the Tukey test for each character, at a 0.05 level of significance (5%), was performed.

3. Results

MT and *hp1* tomato plants showed higher Cd concentration in the roots tissue compared to leaves and fruits. However, roots of the MT plants concentrated 35% more Cd compared to the *hp1* roots when exposed to 0.5 mM of Cd (Table 1). The Cd translocation index (TI) was higher in the *hp1* genotype compared to MT, 11.8% and 9% respectively (Table 1), when exposed to the same treatment, 0.5 mM of CdCl$_2$.

Higher values of Se concentration were found in roots compared to all the other tissues analyzed for both genotypes. The MT genotype showed 41% more Se in the root system compared to the *hp1* when it received only Se, 358 mg kg^{-1} DW and 210 mg kg^{-1} DW, respectively (Table 1). Moreover, the *hp1* showed higher Se TI compared to the MT, 4.9% and 1.9%, respectively. The Se concentration decreased in the roots of both genotypes in the same condition, and its presence in the shoot was not detectable (Table 1).

The application of Cd decreased the zinc (Zn) and iron (Fe) concentration in the MT fruits and manganese (Mn) and Zn in *hp1* fruits, compared to the control plants. The Se treatment induced a decrease of Zn and an increase of Fe in the MT fruits (Table 2). The same treatment also induced an increase of Mn and Zn in *hp1* fruits; however, the Fe concentration was lower compared to the control. When Se and Cd were applied simultaneously, the concentration of Fe and Zn decreased in the fruits for both genotypes, and Mn decreased only in *hp1* compared to the control plants (Table 2) though no increases in nutrient absorption were observed in any of these genotypes under this treatment (Table 2). However, the Se alone application increased the Mn and Zn concentration in the fruits of the *hp1* genotype and Fe in the fruits of the MT genotype.

When Se was applied alone, the proline concentration increased in the *hp1* fruits compared to the control (Fig. 1). The Cd alone treatment, on the other hand, showed a strong decrease in proline concentration in the MT fruits compared to the control (Fig. 1).

There were no significant differences in fruit biomass between the treatments for both genotypes (Fig. 2), and the supply of 0.5 mM of Cd
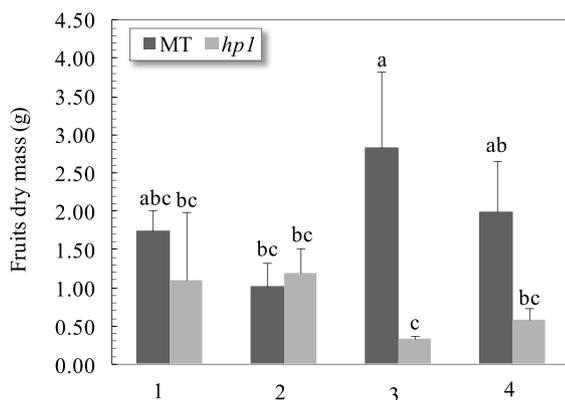
Table 2
Fruits nutrient concentration. 1. Control/2. Na$_2$SeO$_3$ 50 μM/3. CdCl$_2$ 0.5 mM/4. CdCl$_2$ 0.5 mM with Na$_2$SeO$_3$ 50 μM. Values are the mean of three replicates. Lower case letters indicate statistically significant differences ($P < 0.05$).

Treatment		Macronutrients (g kg^{-1} DW)					Micronutrients (mg kg^{-1} DW)			
		Mg	P	S	K	Ca	Mn	Fe	Cu	Zn
MT	1	1.5 ± 0.14 a	3.8 ± 0.06 ab	2.1 ± 0.04 a	24.0 ± 1 bc	3.6 ± 0.10 a	33.2 ± 0.56 bc	88.0 ± 1 c	4.6 ± 0.33 a	25.3 ± 0.06 c
	2	1.5 ± 0.03 a	3.2 ± 0.08 ab	2.2 ± 0.10 a	25.2 ± 0.36 b	3.4 ± 0.40 a	36.0 ± 2 b	113.0 ± 1.8 b	5.7 ± 0.20 a	21.3 ± 0.38 de
	3	1.4 ± 0.11 a	3.5 ± 0.31 ab	2.6 ± 0.10 a	25.2 ± 0.95 b	3.7 ± 0.10 b	34.7 ± 0.16 b	43.7 ± 0.11 f	5.4 ± 0.16 a	18.1 ± 0.22 f
	4	1.3 ± 0.11 a	2.7 ± 0.51 b	2.1 ± 0.02 a	22.2 ± 0.10 c	3.1 ± 0.10 a	30.4 ± 0.20 c	41.9 ± 0.05 f	4.8 ± 0.20 a	19.6 ± 0.55 ef
hp1	1	1.4 ± 0.07 a	3.6 ± 0.26 ab	2.4 ± 0.12 a	24.2 ± 1.1 bc	3.8 ± 0.17 a	33.6 ± 0.13 b	110.6 ± 0.46 b	5.2 ± 0.11 a	34.3 ± 1.1 b
	2	1.2 ± 0.08 a	4.7 ± 0.28 ab	2.4 ± 0.10 a	24.0 ± 1 bc	4.0 ± 0.87 a	46.0 ± 1 a	66.7 ± 0.58 d	5.7 ± 0.06 a	39.5 ± 0.52 a
	3	1.3 ± 0.02 a	5.8 ± 0.05 a	2.8 ± 0.38 a	29.6 ± 0.44 a	3.0 ± 0.50 a	17.7 ± 0.84 e	141.8 ± 0.96 a	4.3 ± 0.04 a	24.0 ± 1 cd
	4	1.3 ± 0.08 a	3.0 ± 0.21 b	2.1 ± 0.02 a	22.2 ± 0.17 c	3.6 ± 0.30 a	21.5 ± 0.47 d	54.6 ± 0.67 e	3.8 ± 0.29 a	18.6 ± 0.54 ef
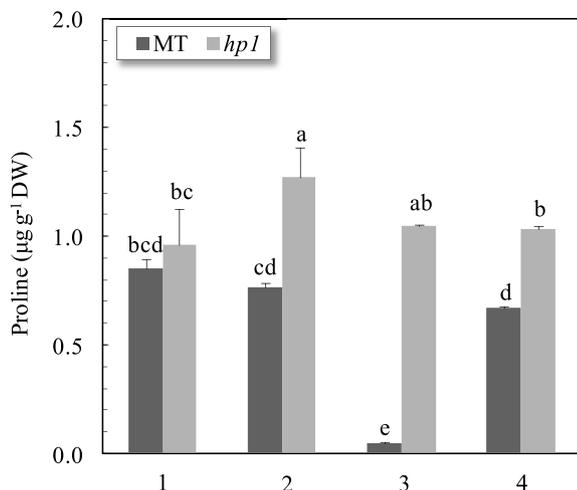
Fig. 1. Fruits proline concentration. 1. Control/2. Na$_2$SeO$_3$ 50 μM/3. CdCl$_2$ 0.5 mM/4. CdCl$_2$ 0.5 mM with Na$_2$SeO$_3$ 50 μM. Values are the mean of three replicates and standard deviation (± SD). Lower case letters above bars indicate statistically significant differences ($P < 0.05$).

Fig. 2. Fruits dry mass. 1. Control/2. Na$_2$SeO$_3$ 50 μM/3. CdCl$_2$ 0.5 mM/4. CdCl$_2$ 0.5 mM with Na$_2$SeO$_3$ 50 μM. Values are the mean of three replicates and standard deviation (± SD). Lower case letters above bars indicate statistically significant differences ($P < 0.05$).

also did not reduce fruit biomass of both genotypes, as compared to the control plants (Fig. 1).

Under the concentration and form used, Se did not significantly affected pigment concentration in fruits of both genotypes when it was applied alone or together with Cd, as compared to the control plants (Fig. 3). There were few significant differences in pigment concentrations between the treatments when Cd was supplied alone (Fig. 3). However, it is noteworthy that Se-treated plants had slightly higher

levels of different pigments and the Cd-treated plants showed slightly lower levels of these pigments, so the chlorophyll-*a* concentration in fruits of the *hp1* genotype was higher when Se was applied alone in comparison to the Cd treatment (Fig. 3A, C).

4. Discussion

The beneficial effects of selenium (Se) in tomato (*Solanum lycopersicum*), genotypes Micro-Tom (MT) and *high pigment*-1 (*hp1*), under cadmium (Cd^2) toxicity was studied. Cadmium and Se concentration and translocation index in roots, leaves and fruits as well as the macro and micronutrients quantification, proline concentration and fruits dry weight analysis were performed.

According to the results, the differences in Cd concentration between genotypes (Table 1) were related to the plant growth, since the *hp1* fruits dry mass was lower compared to the MT under the Cd treatment (Fig. 1). Se is not well translocated to shoot when applied in selenite form, due to its assimilation into organic forms and the local incorporation into proteins, which could explain the higher Se concentration in the roots (Malagoli et al., 2015).

Furthermore, an evidence of Se restriction on the heavy metal uptake and translocation from root to shoot was notable for both genotypes. The simultaneous application of Cd and Se led to a lower concentration of Cd in the roots and leaves tissue compared to the treatment that received only the heavy metal (Table 1).

Free Cd radical shows high affinity to the thiol groups of proteins such as the reduced glutathione (GSH) and cysteine (Cuypers et al., 2010). Additionally, Se can enter the sulfate pathway due to its chemical similarity with sulfur (S), which may cause its incorporation into amino acids, like selenocysteine (SeCys) and other proteins (for a review see Malagoli et al., 2015). Under these circumstances, the competition between Cd and Se for the thiol group into the amino acid cysteine and proteins can result in a lower uptake and translocation of one or both minerals (Lin et al., 2012).

Additionally, Cd can be stored in vacuoles at the site of the metal uptake, i.e. in the roots due to chelates activity (Gallego et al., 2012), while this process can be improved by Se and the reactive oxygen species (ROS) scavenging in the cells (Gratão et al., 2015). Likewise, when Se and Cd were applied simultaneously, the heavy metal TI (Translocation Index) demonstrated to be much lower when compared to the Cd treatment in the MT genotype, 7.9% and 9% respectively (Table 1).

Some reports have shown the synergetic effect of Cd by stimulating the absorption of specific nutrients, whereas the concentration of Fe, for example, increased after Cd contamination (Kumar et al., 2014). In our study, the application of 0.5 mM of CdCl$_2$ caused an increase in potassium (K) and Fe concentration specifically in the *hp1* fruits (Table 2). Contrarily, the negative effect on nutrients concentration in response to the Cd stress can be related to various deleterious mechanisms. This includes an increased lipid peroxidation, decreased plasmalema fluidity, and, as a consequence, harm to the entire cell integrity, which
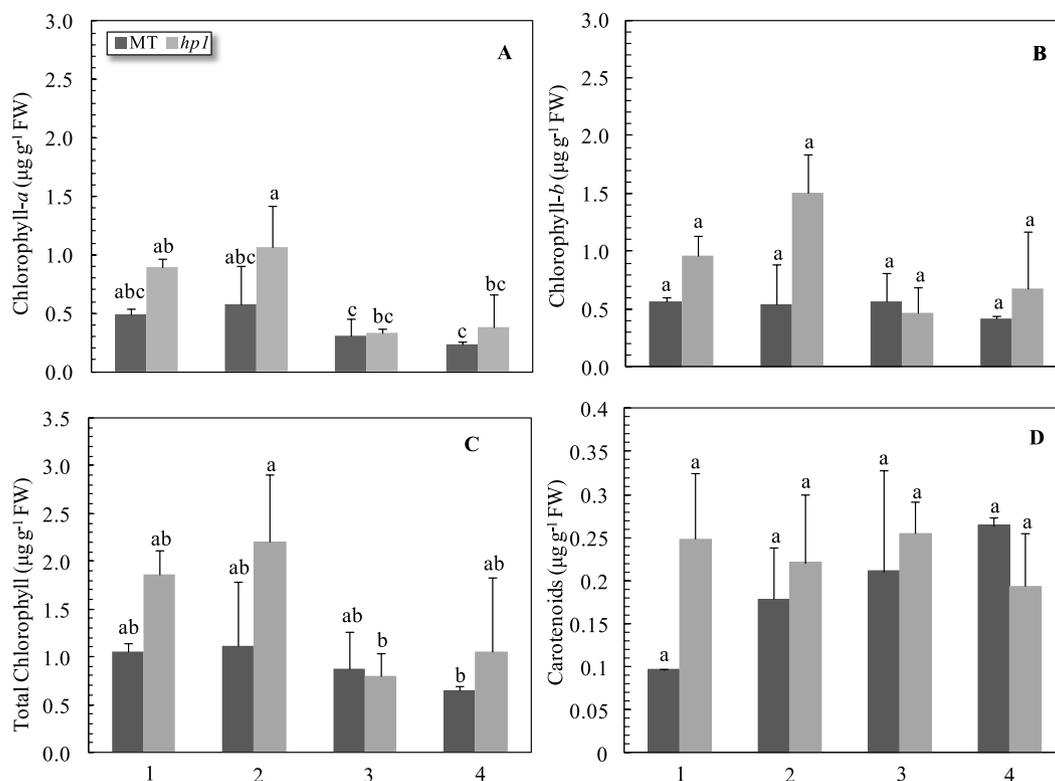
Fig. 3. Fruits pigments concentration. 1. Control/2. Na₂SeO₃ 50 μM/3. CdCl₂ 0.5 mM/4. CdCl₂ 0.5 mM with Na2SeO₃ 50 μM. Values are the mean of three replicates and standard deviation (± SD). Lower case letters above bars indicate statistically significant differences (P < 0.05).

can directly reduce absorption and translocation of nutrients (Cuypers et al., 2010). Furthermore, the form Cd^{2+}, usually concentrated in the organic fraction of the soil, can actively compete with the uptake of those nutrients that have the same valence number, such as Zn^{2+} (Kumar et al., 2014).

When Se was applied alone there was an increase in the Mn and Zn concentration in the fruits of the *hp1* genotype and also Fe in the fruits of the MT genotype. This could confer an efficient mechanism to alleviate the oxidative stress in this tissue, since these micronutrients are in the main active site of different isoforms of the superoxide dismutase (SOD) enzyme, which is the first antioxidant defense in the cell (For review see Gratão et al., 2005).

The amino acid proline is part of the compatible solutes group and plays an important role in the cellular osmotic regulation and plant protection against different abiotic stresses. Its concentration in the control plants was similar for both genotypes (Fig. 1). Interestingly, when Se was applied alone, the proline concentration increased in the *hp1* fruits compared to the control (Fig. 1), however the Cd treatment showed lower proline concentration in MT fruits compared to the control (Fig. 1). This result could be a response for a localized proline synthesis in the root instead of the shoot, related to the higher Cd concentration in this tissue (Table 2). The simultaneous application of Se with Cd increased the proline concentration for the MT genotype, compared to the Cd treatment (Fig. 1).

Proline concentration in tissues due to stress is widely varied among different plant species and highly dependent on the type and level of stress to which the plant was submitted (Shevyakova et al., 2013). Proline overproduction can function as a mechanism to actively clear the cell ROS during the oxidative stress, culminating in protection and stabilization of cell membranes, proteins and enzymes during severe stresses (Ashraf and Foolad, 2007). Se application may induce proline synthesis through an improved activity of glutamyl kinase (GK), the first enzyme of the proline biosynthetic pathway, and decreased activity of proline oxidase (PROX), responsible for the proline molecule

denaturation (Khan et al., 2015).

The products resulting from the catabolism of proline molecules can be used in the oxidative phosphorylation process in mitochondria as an effort to synthetize molecules of ATP (adenosine 5′-triphosphate), important for the cell recovery processes (Ashraf and Foolad, 2007). The results demonstrate that 50 μM of Se increased the proline concentration in *hp1* fruits and also in MT fruits when it was applied simultaneously with Cd, which could protect the tissue from the induced stress.

There were no significant differences in fruit biomass between the treatments for both genotypes, MT and *hp1* (Fig. 2). The adequate Se concentration to promote plant growth and development under abiotic stress may differ due to different factors, such as the plant species, the type of tissue analyzed, the type of stress, and also how Se is applied to the plant (for review: Feng et al., 2013). It is known that low levels of Se can attenuate oxidative stress, enhance membrane stability, in response to a lower lipid peroxidation, and increase the concentration of starch and sugars (Feng et al., 2013). However, the excess of Se can directly induce an oxidative stress due to its assimilation in the S (sulfur)metabolism, resulting in growth reduction (Capaldi et al., 2015).

The supply of 0.5 mM of CdCl₂ did not reduce fruit biomass on both genotypes when compared to the control plants (Fig. 1). Current reports have shown the detrimental effects of Cd on plant growth and development because of an imbalance of ROS production and the antioxidant system, reduction in water content, altered membrane permeability, lower photosynthesis and mineral deficiency (Irfan et al., 2014; Gratão et al., 2015).

The quantification of carotenoids and chlorophyll in fruits was carried out to investigate whether Se could protect fruit development and maturation during Cd stress. Damaging effects of Cd on photosynthesis and pigment concentration have been reported, predominantly through modifications of the chloroplast structure and the photosynthetic apparatus, as a response to the higher oxidative stress and lipid peroxidation (Gallego et al., 2012). Additionally, the negative effect of Cd specifically to the chlorophyll concentration can be a result

of damages in pigments by ROS and a lower chlorophyll biosynthesis.

According to our results, the chlorophyll-*a* concentration in fruits of the *hp1* genotype was higher when Se was applied alone in comparison to the Cd treatment (Fig. 3A, C). However, this effect cannot be considered a beneficial effect of Se against Cd stress in the fruits, because the pigment concentration was similar between the Cd treated plants and to those plants which received Se and Cd together (Fig. 3A–D).

The *hp1* genotype demonstrates an exaggerated light responsiveness as well as a natural increased fruit pigmentation and vitamin C concentration. These characteristics provide a greater cellular antioxidant metabolism to deal with the increased radiation absorption, energy transformation and the oxidized environment in the chloroplasts (Carvalho et al., 2011). Further analyzes on other tissues would help to elucidate the Se effect on pigments concentration and photosynthesis under the oxidative stress in this genotype.

5. Conclusions

The apparent underlying mechanisms of Se against Cd toxicity in tomato includes the reduction of Cd uptake and translocation, particularly in the MT genotype, improved concentration of Mn (*hp1*), Zn (*hp1*) and Fe (MT) in fruits, as well as the proline concentration enhancement in fruits of both genotypes. This study suggests that Se fertilization may not only create nutritionally enhanced food but also be a means to alleviate Cd toxicity in tomato plants.

Acknowledgements

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