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Protective Effect of Selenium in Broccoli (*Brassica oleracea*) Plants Subjected to Cadmium Exposure

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The protective effect of selenium against the cadmium-induced oxidative effect in broccoli (*Brassica oleracea*) plants was studied. Plants grown in hydroponic culture were supplied with selenium [as Se(IV)] and cadmium [as Cd(II)], individually or simultaneously. Cadmium accumulation in roots was noticeably higher than in the aerial parts of the plants, and this effect was even more acute when selenium was simultaneously added. Cadmium phytotoxicity was evidenced by an increase in the malondialdehyde (MDA) concentration in the roots and a decrease of photosynthetic pigment and tocopherol concentration in the aerial parts of the plant. The simultaneous addition of selenium alleviated cadmium-induced stress in the roots after 40 days of exposition. In the leaves, a more remarkable decrease of tocopherol and chlorophyll concentration was observed in the cadmium-enriched plants after 10 days of exposure. The results provided evidence that selenium supplementation helps the plant to minimize the cadmium oxidant effect. Tocopherol concentration in broccoli fruit of cadmium-supplied plants was not affected in comparison to control. However, the proportion of α-tocopherol increases with the addition of selenium. This response is important not only for the protective effect against oxidative damage in the plant but also in terms of human nutrition.

KEYWORDS: Selenium; antioxidant protective effect; cadmium; broccoli plants

INTRODUCTION

Cadmium is an important environmental pollutant, which is emitted from natural and anthropogenic sources. Its phytotoxicity depends on different factors such as the exposure time, concentration, and the plant species (*1*). This nonessential element induces stress symptoms, including the appearance of chlorosis and growth reduction (*2, 3*). Cadmium also can replace (*1*) and interfere with the uptake and translocation of elements essential to the plant metabolism such as zinc, iron, and manganese (*4*).

It is well-known that cadmium causes oxidative stress on plants although the mechanisms are not completely clear. It is hypothesized that it induces generation of free radicals and toxic oxygen species that cause lipid peroxidation, membrane damage, and inactivation of enzymes, detrimentally affecting the plant (*5, 6*).

Despite the fact that cadmium is a nonessential element for plants, they can readily uptake and accumulate it in their tissues. This feature makes cadmium a serious problem since the cadmium-enriched plants can be incorporated in the food chain. Therefore, this toxic element could be incorporated into the human diet through edible plants, causing toxicity (*7, 8*). The cadmium-induced adverse effect on human health is well documented, affecting mainly the kidney and renal functions (*9, 10*).

Selenium, in contrast, is an essential nutrient for humans (*11, 12*), which has been related with immune functions (*13*). The antioxidant and anticarcinogenic properties attributed to some seleno compounds (*14, 15*) justify the increasing interest in growing selenium-enriched vegetables, which represent an important source of this element in the human diet (*16*).

Selenium has not been recognized as an essential element in plants, but several studies demonstrate that it has antioxidant effects (*17, 18*). Some vegetal species grown in selenium-enriched media are shown to enhance the photooxidative stress tolerance of the plant (*19, 20*). Another beneficial effect has been observed in potato plants (*Solanum tuberosum* L.), where the tuber mass is found to increase due to enhancement of carbohydrate metabolism when grown in the presence of selenium (*21*).

The protective effect of selenium against toxic elements such as mercury (*22–24*) and cadmium (*25*) in animals has also been widely studied. Some studies carried out in vegetal species reveal the capacity of selenium to reduce the availability of toxic metals and alleviate their oxidative effect (*26, 27*), but the literature is limited. The beneficial effect of added selenium on

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Table 1. Experimental Conditions for Total and Se Species Determination by ICP-MS and LC-ICP-MS, Respectively

ICP-MS Instrumental Parameters	
rf power	1250 W
plasma gas flow	15.0 L min^{-1}
auxiliary gas flow	0.73 L min^{-1}
carrier gas flow	0.7 L min^{-1}
isotopes monitored	^{77}Se, ^{78}Se, ^{82}Se, ^{111}Cd, ^{112}Cd, ^{114}Cd
LC Conditions	
chromatographic column	PRP-X100
mobile phase	10 mM ammonium citrate, pH 5
flow rate	1.0 mL min^{-1}
injection volume	100 μL

the plant has been related with the form of the chemical added to the plant, the most effective form being selenite (*27, 28*), preferably at low concentration to avoid selenium-induced toxicity (*26*). However, this mechanism has not yet been fully elucidated, and further experiments are required.

The capacity of the plants to tolerate and accumulate metals is strongly related to the vegetal species, among other factors. Broccoli (*Brassica oleracea*) belongs to the Cruciferae vegetal family, which has been reported to be quite tolerant to different metals (*29, 30*). In recent studies on this plant species carried out in our laboratory, it has been observed that inorganic selenium supplied as selenite can be easily transformed by the plant to selenoamino acids (*31*). Its distribution within the broccoli plants justifies the high selenium accumulation capacity and could be related with the tolerance toward this element.

Therefore, the main aim of this study was to investigate the protective effect of selenium on cadmium-induced oxidative stress in the *B. oleracea* plant. In this order some biochemical markers of oxidative stress such as chlorophyll, malondialdehyde, and tocopherol concentration (*19, 32*) were measured in plants exposed to selenium and cadmium supplied individually or simultaneously. The uptake and translocation of selenium and cadmium in the plants were also studied. Furthermore, selenium speciation analyses were performed to find out the impact of cadmium on selenium metabolism.

MATERIALS AND METHODS

Plant Growth Conditions. Broccoli (*B. oleracea*) seeds were germinated in coconut fiber moistened with deionized water. Two weeks after germination, the seedlings were grown in hydroponic culture (20 plants pre vessel) in vessels containing 0.1 strength Hoagland's solution (*33*) using perlite as substrate. After 5 weeks, Na_2SeO_3 and $CdCl_2$ (1 mg L^{-1} each) were added separately and/or in combination to the culture media. The control group of plants were grown in a parallel hydroponic culture unexposed to selenium and cadmium.

Plants were harvested after 10 and 40 days of exposition to selenium and cadmium, and their root and leaves were separated. In the later harvesting (40 days after exposition), the fruits were also collected.

Total Selenium and Cadmium Determination. Approximately 250 mg of fresh sample of the different parts of the plants harvested after 10 and 40 days of exposition were digested with 2.5 mL of 14 M HNO_3 (Merck) and 0.5 mL of 30% H_2O_2 (Sigma) in a microwave oven (CEM, Mattheus) for total selenium and cadmium determination. The resulting solutions were appropriately diluted with Milli Q water and analyzed by ICP-MS (Agilent HP 4500) according to the conditions given in **Table 1**.

Selenium Speciation. Selenium species extraction from roots, leaves, and fruits of plants collected after 40 days of exposition to selenium and cadmium and selenium simultaneously supplied was performed by 2 min of sonication by using an ultrasonic homogenizer (Sonoplus Bandelin) in 3 mL of water and 10 mg of protease XIV (Sigma) added to 250 mg of fresh sample. Species separation by anion-exchange chromatography-ICP-MS was carried out under conditions compiled in **Table 1**.

MDA Determination. The concentration of malondialdehyde (MDA), a lipid peroxidation marker, was determined to investigate the oxidative effect induced by cadmium in roots by spectrophotometric determination following the procedure described by Kumar et al. (*34*).

Chlorophyll Analysis. Chlorophyll concentration was determined in leaves collected after 10 and 40 days of exposition to selenium and/or cadmium. Frozen samples (200 mg) were ground, and the pigments were extracted for 30 min in 4 mL of methanol. The extracts were centrifuged at 3000 rpm (4 °C), and chlorophyll concentration was spectrophotometrically determined as described by Lichtenthales (*35*).

Tocopherol Analysis. Tocopherols were determined in leaves and broccoli fruit fresh samples by HPLC-UV (292 and 395 nm) as reported by Ryynänen et al. (*36*) by external calibration method.

RESULTS AND DISCUSSION

Cadmium and Selenium Distribution in Broccoli Plants. The influence of the presence of selenium on cadmium uptake and its distribution within broccoli plants was studied. Analyses of these elements along the plants were carried out 40 days after the addition of cadmium and/or selenium, when broccoli fruits were collected.

The total concentrations of cadmium and selenium determined in roots, leaves, and fruits of broccoli plants are given in **Table 2**. They show that in the cadmium-supplied plants the cadmium concentration in the roots was about 4–5 times higher than in the aerial parts (leaves and fruits), which is in good agreement with the results reported for other plant species (*2, 4, 5, 37*). This response implies a low translocation of cadmium within the plant, which could be a mechanism of this vegetal species to defend itself toward this toxic metal. When cadmium was supplied simultaneously with selenium, its accumulation in roots was even more pronounced than when supplied separately. This enhanced accumulation in roots coincided with a reduction of 30% and 55% in leaves and fruits, respectively, which clearly illustrates that the presence of selenium could enhance the plant's tolerance against cadmium and leads to a considerable reduction of cadmium concentration on the edible part of the plants. In contrast, added selenium to plants was efficiently translocated from roots to fruits, and its concentration neither in roots nor in leaves was affected by the presence of cadmium (**Table 2**). However, when plants were simultaneously exposed to selenium and cadmium, the selenium concentration in fruits decreased.

The selenium species in roots, leaves, and fruits of the selenium-supplied broccoli grown with and without cadmium addition were compared in order to determine the effect of cadmium on the transformation of the uptaken selenium. The chromatograms in **Figure 1** show that distribution of seleno species in the different parts of the cadmium−selenium-supplied plants was quite similar to those obtained for the plants exposed to selenium, suggesting that cadmium did not affect it. The main difference found between the selenium species distribution in plants exposed to both treatments (Se and Se + Cd) was the Se species concentration in the fruits. In the plants grown in the presence of selenium and cadmium, selenomethylselenocysteine was the major species, as in selenium-enriched plants (**Table 3**). However, since the total selenium concentration was reduced in the presence of cadmium, the species concentration was also diminished. It is negative from a nutritional point of view, since the main species in the fruit is selenomethylselenocysteine, which is one of the most effective chemopreventive selenoamino acids (*38*).

Table 2. Concentration of Cadmium and Selenium (mg kg^{-1} Fresh Weight) in Fruit, Leaves, and Roots of Broccoli Plants after 40 Days of Exposition to Selenium and Cadmium[a]

	control		supplied with Se		supplied with Cd		supplied with Se + Cd	
	Se (mg kg^{-1})	Cd (mg kg^{-1})	Se (mg kg^{-1})	Cd (mg kg^{-1})	Se (mg kg^{-1})	Cd (mg kg^{-1})	Se (mg kg^{-1})	Cd (mg kg^{-1})
fruit	0.24 ± 0.01	0.27 ± 0.01	27 ± 2	0.40 ± 0.02	0.27 ± 0.02	9.3 ± 0.1	10 ± 1	4.8 ± 0.1
leaves	0.13 ± 0.01	0.14 ± 0.01	2.0 ± 0.1	0.20 ± 0.01	0.14 ± 0.01	9.9 ± 0.8	1.54 ± 0.08	7.5 ± 0.2
roots	0.27 ± 0.01	0.34 ± 0.03	20 ± 1	0.36 ± 0.02	0.28 ± 0.02	39 ± 2	21 ± 1	53 ± 3

[a] Results are expressed as the mean value ± standard deviation ($n = 3$). LOD: Se, 0.5 μg L^{-1}; Cd, 0.1 μg L^{-1}.

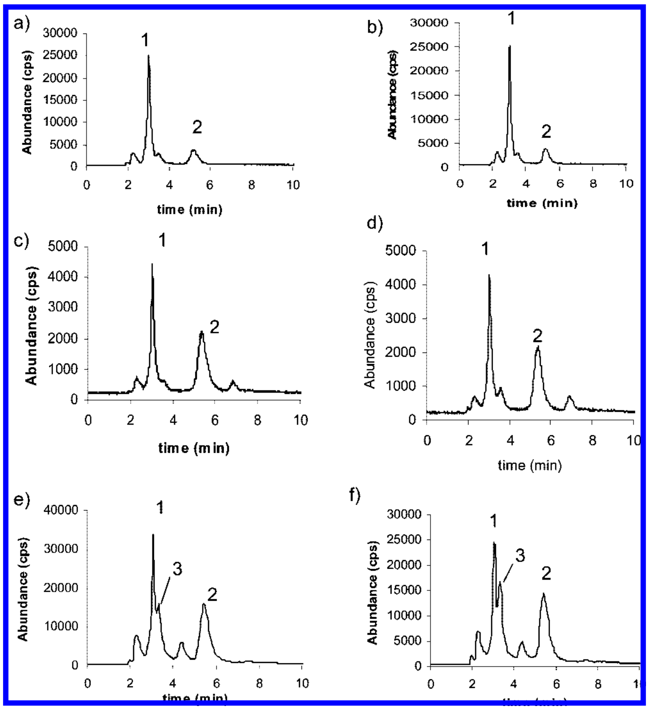

Figure 1. LC-ICP-MS chromatograms monitored at $m/z = 82$ corresponding to fruit (**a**, **b**), leaves (**c**, **d**), and roots (**e**, **f**) of broccoli pants subjected to selenium and selenium and cadmium, respectively: (1) selenomethylselenocysteine, (2) selenomethionine, and (3) selenite.

Lipid Peroxidation. The first contact of the plant with cadmium and selenium occurs through the roots that act as a barrier. Therefore, the concentration of malondialdehyde (MDA), an oxidation product of polyunsaturated fatty acids, in the roots can be taken to indicate the level of oxidative damage caused by cadmium or selenium in growth media. **Figure 2** shows that 10 days after the exposition of the plants to these elements added separately or in combination MDA in the roots increased about 50%. Interestingly, 30 days later the plants showed a different reaction pattern depending on the treatment. In the roots of the cadmium-supplied plants the MDA concentration was the highest, being 20% higher than in control roots. This response provides evidence that cadmium causes oxidative stress in broccoli plants. However, when plants were exposed simultaneously to selenium and cadmium, the MDA level noticeably decreased to the level found in the control. In the plants supplied only with selenium, the level of MDA was the lowest. These findings can be attributed to the antioxidative effect of selenium reported in previous studies (*18, 39*).

Chlorophyll. Chlorophyll is important not only in edible plants from a biological point of view but also for the food quality. Being responsible for the green color of the vegetable leaves, it is a symbol of quality (*40*). This photosynthetic pigment in leaves has been suggested to be negatively affected by Cd (*5*).

The chlorophyll (chlorophylls *a* and *b*) concentrations in broccoli leaves 10 and 40 days after exposure to cadmium and selenium are given in **Table 4**. They show that after 10 days the addition of cadmium diminished the chlorophyll concentration in comparison with control plants. This response can be attributed to lipid damage in chloroplast membranes (*2*). The decrease was more noticeable in the cadmium-supplied plants, which indicates a higher oxidative effect of cadmium. It could be attributed to interaction of cadmium with the −SH group (*5*) or the induction of modifications in chloroplasts (*2, 41*).

However, when cadmium and selenium were simultaneously supplied, a noticeable increase of chlorophyll concentration was observed, in comparison with plants grown in the presence of cadmium. This response found at the early exposition stage illustrates the role of selenium alleviating the cadmium-induced oxidative stress in chloroplasts (*19*) obviously through scavenging of reactive oxygen species that can affect the chlorophyll (*18, 42*). At the later stage (after 40 days of exposition) the behavior was the same, but differences between treatments were less accused. Microlocalization studies of cadmium in leaves of *Salix viminalis* L. carried out by Vollenweider et al. reveal that leaf age is an important factor for response to cadmium stress. The allocation capacity of cadmium in the cell wall in younger leaves is reduced in comparison to older ones (*43*). Therefore, the differences found between the leaf samplings at days 10 and 40 could be attributed to their differences in the cadmium storage capacity. It implies that younger leaves are more affected by cadmium stress.

Tocopherols. Tocopherols are synthesized by photosynthetic organisms such as plants, algae, and some cyanobacteria (*44*). They have several essential functions in plants and are recognized as antioxidants that prevent lipid peroxidation by scavenging of reactive oxygen species (*45*). Tocopherols are classified in four types (α, *β*, *γ*, and *δ*) depending on the number and position of methyl substituents.

In this study, three forms of tocopherol (α, *β*, and *γ*) were identified in all samples. Total tocopherol concentrations and the percentage of each form are shown in **Figure 3** for the leaves of the plants exposed to selenium and cadmium for 10 and 40 days and in the fruits for the plants exposed to these elements for 40 days. As expected, α-tocopherol, which has been reported as the main tocopherol form in the green parts of the plant (*45*), was the major form in both leaves and fruits. In general, a decrease of total tocopherol concentration in the leaves was observed when the plants were grown in cadmium-enriched culture media. This decrease was most remarkable in the plants supplied separately with cadmium for 10 days. On the contrary, the simultaneous addition of selenium counteracted the reduction in tocopherols by about 40%.

The proportion of α-tocopherol was similar in the control plants and in those supplied with Se separately or in combination with cadmium. The lowest values were found in the plants supplied only with cadmium. This cadmium-induced decrease was associated with an increase of *γ*-tocopherol species which

Table 3. Selenium Species Concentration (mg kg^{-1} Fresh Weight) in Different Parts of the Plant after 40 Days of Exposition[a]

	supplied with Se (mg kg^{-1})			supplied with Se + Cd (mg kg^{-1})		
	root	leaves	fruit	root	leaves	fruit
SeMetSeCys	3.6 ± 0.3	0.71 ± 0.02	12.3 ± 0.1	3.4 ± 0.4	0.65 ± 0.02	6.5 ± 0.1
Se(IV)	1.4 ± 0.3	0.082 ± 0.004	1.89 ± 0.05	1.6 ± 0.2	0.09 ± 0.02	0.039 ± 0.001
SeMet	6.1 ± 0.2	0.59 ± 0.01	7.2 ± 0.3	5.4 ± 0.6	0.62 ± 0.01	0.24 ± 0.02

[a] Results are expressed as the mean value ± standard deviation ($n = 3$).

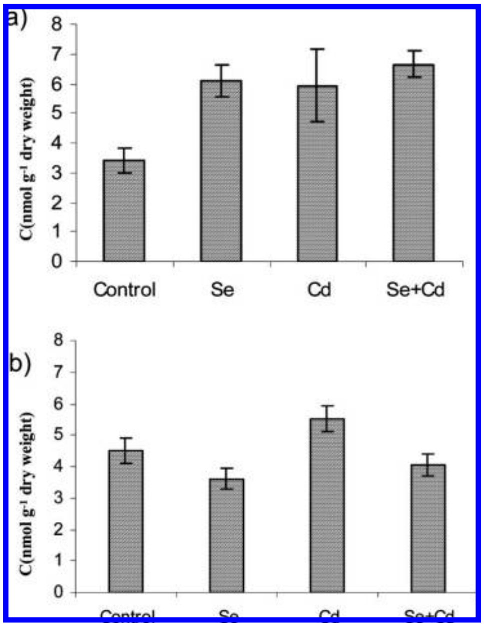

Figure 2. Malondialdehyde concentration in roots of broccoli after (**a**) 10 and (**b**) 40 days of exposition to treatments with Se and Cd.

Table 4. Chlorophyll Concentration (mg kg^{-1} Dry Weight) in Leaves after 10 and 40 Days of Exposition to Selenium and Cadmium[a]

	control (mg kg^{-1})	Se (mg kg^{-1})	Cd (mg kg^{-1})	Se + Cd (mg kg^{-1})
10 days	200 ± 26	155 ± 12	120 ± 8	177 ± 25
40 days	181 ± 23	148 ± 20	118 ± 12	175 ± 29

[a] Results are expressed as the mean value ± standard deviation ($n = 3$).

is less reactive than the α form (*19*). However, the percentage of α-tocopherol concentration increased to the level found in control and Se-enriched plants when selenium was added simultaneously with cadmium. It has been reported that an increase of α-tocopherol favors the stress tolerance of plants as it favors the scavenging of singlet oxygen species in chloroplasts (*44, 45*). Therefore, the increase of α-tocopherol in plants exposed to selenium and cadmium simultaneously, in comparison to those grown only in cadmium, shows evidence that selenium assists the plants in the adaptation.

In broccoli fruit a noticeable increase of total (and α-tocopherol) concentration was observed for plants exposed to selenium when compared to the control plant and those exposed to other treatments. Although the presence of selenium in the culture media did not raise the tocopherol concentration in those supplied with cadmium, the proportions of tocopherol species show a higher α-tocopherol percentage when it was supplied (separately or with cadmium). The increase of tocopherol concentration in fruits of the selenium-enriched broccoli is important not only for the protective effect against oxidative damage in the plant but also from the human nutritional point of view.

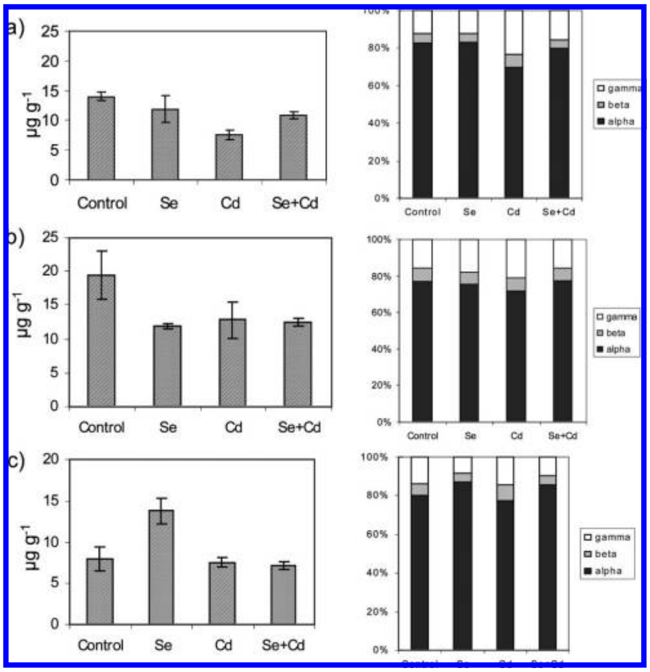

Figure 3. Total tocopherol concentration (μg g^{-1} dry weight) and percentage of α-, β-, and γ-tocopherol in leaves after (**a**) 10 days and (**b**) 40 days of exposition and (**c**) in the fruit.

It should be mentioned that the biomass production was not altered by the presence of selenium and/or cadmium in the different parts of the plants.

In conclusion, the results obtained illustrate the antioxidant properties of selenium against cadmium effect in *B. oleracea*. Oxidative stress induced by cadmium was characterized by an increase of malondialdehyde concentration in roots and the decrease of photosynthetic pigment and tocopherol concentration in the aerial parts of the plant. The simultaneous addition of selenium leads to higher concentration of cadmium in roots, reducing translocation of this toxic element from root to fruit.

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