Does antioxidant capacity of leaves play a role in growth response to selenium at different sulfur nutritional status?

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ABSTRACT

There are evidences of beneficial effects of selenium (Se) on plant growth. In this work, using alfalfa (*Medicago sativa* L.) and two varieties of *Brassica oleracea* L. (cabbage and kohlrabi), the effect of Se addition (10 and 20μM) on growth and concentration of sulfur and Se was investigated in hydroponic experiments. In order to study the involvement of Se-mediated changes in the antioxidant capacity of plants in growth promotion by Se, the activity of antioxidant enzymes including ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and glutathione reductase (GR) and the concentration of H_2O_2 and glutathione were determined. It was demonstrated that sulfur sufficient cabbage and kohlrabi plants concomitant with growth stimulation in response to Se addition expressed a progressive oxidative stress as judged by a lower activity of antioxidant enzymes and accumulation of oxidants. Results imply that the function of antioxidant system of plants could not explain either the growth stimulatory effect of Se in cabbage and kohlrabi or different response of alfalfa plants to Se supplementation.

Keywords: antioxidant system; *Brassica oleracea* L.; *Medicago sativa* L.; Se supplementation

Selenium (Se) is an essential micronutrient necessary for antioxidation and hormone balance in human and animal cells (Ellis and Salt 2003). However, according to current knowledge, higher plants do not require Se and it is toxic at high concentrations (Marschner 1995).

There are some indications that Se can exert beneficial effects on plants at low concentrations (Hartikainen et al. 2000, Simojoki et al. 2003). Selenium can increase the tolerance of plants to UV-induced stress as well as delay senescence and promote the growth of aging seedlings (Xue et al. 2001). In plants grown under high light intensities, Se counteracted senescence-related oxidative stress and maintained green leaf color longer (Xue et al. 2001). Although glutathione peroxidase (GPX) containing Se was not identified in plants, Se supplementation consistently increased GPX activity (Hartikainen et al. 2000, Xue et al. 2001) and inhibited lipid peroxidation (Hartikainen et al. 2000). In contrast, the activity of superoxide dismutase (SOD) diminished in response to Se addition (Hartikainen et al. 2000, Xue and Hartikainen 2000). In a pot experiment using low Se soils amended with increasing dosage of H_2SeO_4 it was observed that at low concentrations of added Se it acted as an antioxidant, whereas at higher concentrations it was a pro-oxidant. This dual effect of Se coincided with promotion and inhibition of plant growth (Hartikainen et al. 2000). These observations indicate that Se may have particular biological functions in higher plants through alteration of antioxidant defense system.

The beneficial effect of Se for plants can be considered in two ways. First, Se has a beneficial effect on consumers, i.e. animals and human; in some countries with low Se bioavailability in agricultural soils, multinutrient fertilizers for field crops are supplemented with Na-selenate to ensure adequate Se intake in domestic agricultural products by humans (Ekholm et al. 1995). Second, due to the promotion of antioxidative system, Se supplementation could increase plant tolerance to environmental stresses and thereby improve growth and yield. One approach to improve crop the tolerance of plants to environmental stresses is to increase their antioxidant capacity (Bowler et al. 1992).

The main objective of this work was to evaluate the role of inducible or constitutive antioxidative defense capacity in growth of Se supplemented plants having different sulfur nutritional status. Important feed and food crops including two va-

rieties of *Brassica oleracea* (cabbage and kohlrabi) and alfalfa were used in this work because of their importance in Se input to food chain directly to human and/or via animals.

MATERIAL AND METHODS

Plant materials. Two varieties of *Brassica oleracea* L. including var. Sabauda (cabbage) and var. Gongyloides (kohlrabi) and one local cultivar of alfalfa (*Medicago sativa* L. var. Gareh-yondjeh) were used for this study. Seeds were provided by the Agricultural Research Center, Tabriz, Iran.

Plants cultivation and treatments. Seeds were surface-sterilized using 5% (v/v) sodium-hypochlorite, then were germinated in the dark. Six-day-old seedlings were pre-cultured for 16 days in 50% nutrient solution, thereafter plants were transplanted to 2 l dark pots and were pre-cultured for another 2 days. After the preculture, plants were transferred to treatment solutions with full strength nutrient solution (Hoagland and Arnon 1950) and two levels of sulfur including 0.05 (cabbage and kohlrabi) or 0.25 (alfalfa), and 1mM and three levels (0, 10 and 20μM) of Se (Na_2SeO_4.10 H_2O, Fluka). Alfalfa plants died in the treatment combinations of 0.05mM sulfur and 10 or 20μM Se as observed in the first experiment. Therefore, the lowest sulfur treatments for this plant species for experiments on the activity of enzymes and concentration of metabolites was set at 0.25mM. The application of sulfur treatments lower than the complete nutrient solution (1.0mM) was realized by a change in the concentration of $MgSO_4$ and addition of equimolar amounts of $MgCl_2$ to the medium. Nutrient solutions were changed completely every 3 days and pH of nutrient solutions was adjusted every day using 1% HCl. Plants were grown under controlled environmental conditions with a temperature regime of 25/18°C day/night, 14/10 h light/dark period, a relative humidity of 70/80% and at a photon flux density of about 400 μmol/m^2/s. In a preliminary experiment we showed that the highest effect of Se treatments could be observed during the first week of treatment and that differences between +Se and –Se treatments reduced during the second week. Hence, in this work plants were treated for one week and then were harvested. Leaves were washed with double-distilled water, fresh weight and after drying at 70°C for 1 day, dry weight was determined.

Analysis of sulfur and selenium. Samples were digested in perchloric acid (1:15 w/v) on heating plate for 5–6 h. Sulfur analysis was carried out according to the turbidimetry method (Chesnin and Yien 1950). Selenium was determined by atomic absorption spectrometry (Shimadzu, AA 6500) with an automated hydride generation accessory (HVG-1). Preparation of samples and standards for Se determination as well as instrumental set up was carried out according to the method of Norheim and Haugen (1986).

Enzyme assays. Fresh leaf samples were used for enzyme assays and measurements of protein and metabolites. Samples were ground in extraction buffer using pre-chilled mortar and pestle. Each enzyme assay was tested for linearity between the volume of crude extract and the measured activity. The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was measured using a procedure modified from that described in Boominathan and Doran (2002). One unit of APX oxidizes ascorbic acid at the rate of 1 μmol/min at 25°C. Catalase (CAT, EC 1.11.1.6) activity was assayed spectrophotometrically by monitoring the decrease in absorbance of H_2O_2 at 240 nm (Simon et al. 1974). Unit activity was taken as the amount of enzyme that decomposes 1M of H_2O_2 in one min. Peroxidase (POD, EC 1.11.1.7) activity was determined using the guaiacol test (Chance and Maehly 1955). The enzyme unit was calculated as enzyme protein required for the formation of 1μM tetraguaiacol for 1 min. Total superoxide dismutase (SOD, EC 1.15.1.1) activity was determined according to Giannopolitis and Ries (1977). One unit of SOD was defined as the amount of enzyme required to induce a 50% inhibition of NBT (p-nitro blue tetrazolium chloride) reduction as measured at 560 nm, compared with control samples without enzyme aliquot. The activity of glutathione reductase (GR, EC 1.6.4.2) was assayed by following the oxidation of NADPH at 340 nm as described by Foyer and Halliwell (1976). The unit activity of enzyme was calculated as enzyme protein required for oxidation of one μM NADPH in 1 min.

Other assays. Total glutathione was measured according to a modification of the method described by Hermsen et al. (1997). The concentration of H_2O_2 was determined using methods described by Patterson et al. (1984). The non-protein thiol content of plant extracts and concentration of selenium in protein fraction were measured according to the methods of Galli et al. (1996) and Pilon-Smits et al. (1999), respectively. Soluble proteins were determined as described by Bradford (1976) using a commercial reagent (Sigma) and BSA (Merck) as standard.

Analysis of data was carried out using the Tukey test ($n = 4$, $P < 0.05$).

RESULTS AND DISCUSSION

Effect of Se treatment on growth, sulfur and Se concentration

Selenium addition exerted a dual effect on the growth of plants depending on their sulfur nutritional status. In plants supplied with adequate sulfur, Se showed a growth-promoting effect; for cabbage and kohlrabi shoot dry weight increased up to 59% and 35%, respectively, in alfalfa this stimulation was only 25%. In contrast to adequate sulfur supply, in low sulfur plants, Se showed toxic effect; the strongest effect was observed in alfalfa, which died at sulfur treatment of 0.05mM in the presence of Se concentration of 10 and 20µM (Figure 1). The toxic effect of Se for plants results mainly from interferences of Se with sulfur metabolism (Mikkelsen et al. 1989) and from replacing S-amino acids by corresponding Se-amino acids and their subsequent incorporation into proteins. The mechanisms of positive growth response to Se at low concentrations will be discussed in the following sections of this paper.

Sulfur concentration of leaves increased in response to Se treatment at both sulfur deficient and sufficient plants, but the opposite was observed on the Se concentration in response to sulfur treatment. Selenium concentration was higher in sulfur deficient plants and particularly two varieties of *Brassica* accumulated large amounts of Se, e.g. 9 mg/g dry weight for kohlrabi (Table 1). Reduction of Se uptake in sulfur sufficient plants could be the result of a reduced activity of selenate ion in the presence of sulfate and/or competition between sulfate and selenate for carriers (White et al. 2004) in favor of sulfate. A high Se accumulation capacity of some *Brassica* species found applications in phytoextraction of Se-contaminated soils (Simon et al. 2006).

Se concentration in the protein fraction was influenced both by sulfur and Se treatments. As expected, Se concentration in protein increased with increasing Se concentration in the medium. In sulfur deficient plants, Se concentration in the protein fraction was higher than in sulfur sufficient plants for all three studied species/varieties (Table 1). It may be the main cause of different responses of sulfur deficient and sufficient plants to Se addition, e.g. Se toxicity. Incorporation of Se-amino acids into proteins can lead to dysfunctional enzymes with altered tertiary structure and strongly modified catalytic activity, and is therefore considered a major cause of Se toxicity (Terry et al. 2000). Although Se accumulation in dry matter of leaves was higher in cabbage and kohlrabi than in alfalfa, the amount of Se in protein fraction was

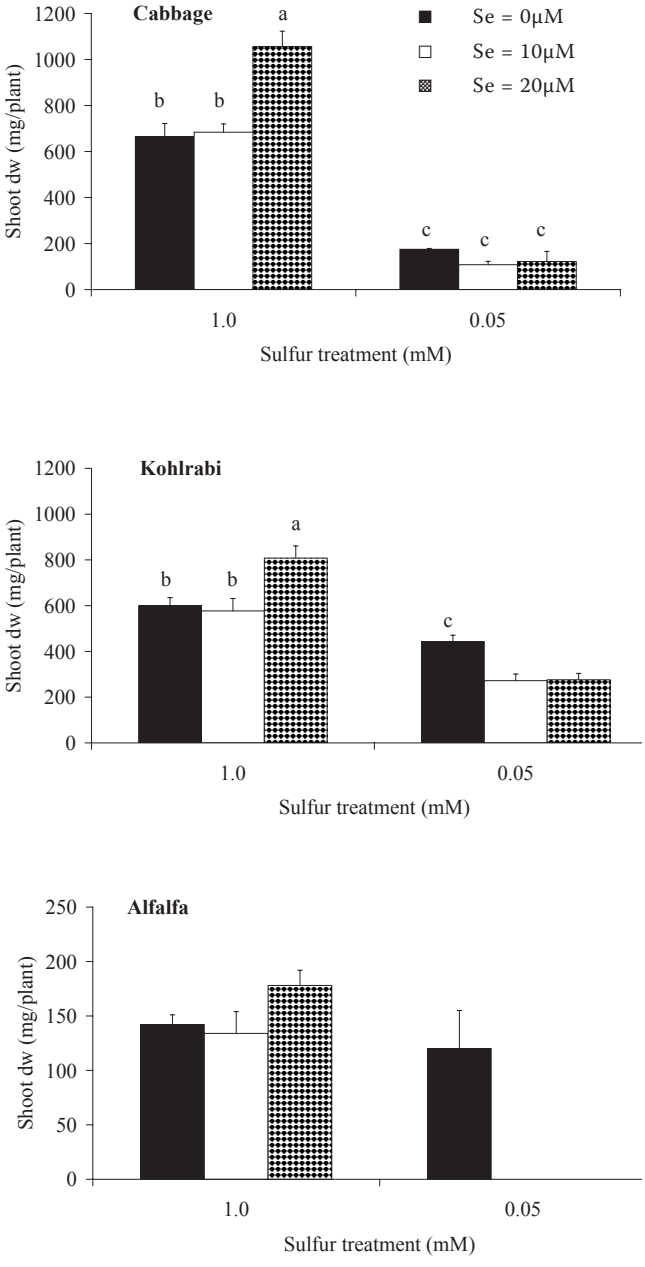

Figure 1. Effect of Se treatment on shoot biomass of cabbage (*Brassica oleracea* L. var. Sabauda), kohlrabi (*B. oleracea* L. var. Gongyloides) and alfalfa (*Medicago sativa* L. var. Garehyounjeh) supplied with low (0.05mM) or adequate (1.0mM) sulfur. Bars indicated by the same letter are not significantly different ($P < 0.05$)

Table 1. Effect of Se treatment on the shoot concentration of Se (μg/g dw) and S (mg/g dw), Se concentration in protein fraction (μg Se/g Pro.) and protein concentration (mg/g fw) at different sulfur nutritional status in cabbage (*Brassica oleracea* L. var. Sabauda), kohlrabi (*B. oleracea* L. var. Gongyloides) and alfalfa (*Medicago sativa* L. var. Garehyounjeh)

Se (μM)	S (mM)	S concentration	Se concentration	Se in protein fraction	Protein concentration
Cabbage					
0	1	17.6 ± 3.2^{b}	52 ± 31^{d}	3.1 ± 0.9^{e}	180 ± 3.1^{a}
	0.05	9.7 ± 1.4^{bc}	1.8 ± 0.4^{e}	3.7 ± 2.1^{e}	178 ± 3.2^{a}
10	1	22.2 ± 1.9^{a}	211 ± 29^{c}	38.3 ± 9.1^{d}	179 ± 3.6^{a}
	0.05	6.4 ± 0.4^{c}	1129 ± 90^{a}	77.3 ± 17.5^{c}	167 ± 2.4^{b}
20	1	16.4 ± 10.7^{b}	446 ± 69^{b}	125.2 ± 20.1^{b}	164 ± 4.7^{b}
	0.05	5.5 ± 1.9^{c}	1002 ± 145^{a}	200.3 ± 20.2^{a}	159 ± 6.2^{b}
Kohlrabi					
0	1	13.8 ± 0.4^{b}	12 ± 1^{f}	2.5 ± 1.1^{d}	180 ± 3.3^{a}
	0.05	3.7 ± 0.9^{c}	20 ± 4^{e}	3.8 ± 1.6^{d}	163 ± 4.8^{b}
10	1	17.7 ± 3.4^{a}	1565 ± 273^{c}	31.7 ± 10.1^{c}	145 ± 2.1^{c}
	0.05	4.9 ± 1.7^{c}	4477 ± 184^{b}	62.7 ± 10.1^{b}	142 ± 3.5^{c}
20	1	21.2 ± 2.4^{a}	771 ± 14^{d}	84.5 ± 12.8^{b}	152 ± 2.7^{c}
	0.05	2.6 ± 0.8^{c}	9051 ± 227^{a}	158.2 ± 15.1^{a}	113 ± 4.4^{d}
Alfalfa					
0	1	2.5 ± 0.5^{cd}	23 ± 5^{e}	6.1 ± 3.2^{e}	192 ± 5.0^{a}
	0.25	1.2 ± 0.1^{d}	7 ± 2^{f}	7.7 ± 2.1^{e}	168 ± 6.5^{b}
10	1	5.9 ± 1.0^{b}	247 ± 52^{d}	48.7 ± 25.2^{d}	175 ± 3.9^{b}
	0.25	3.7 ± 0.7^{c}	1627 ± 112^{b}	95.3 ± 12.6^{c}	147 ± 4.2^{c}
20	1	8.7 ± 0.8^{a}	883 ± 167^{c}	192.3 ± 9.5^{b}	168 ± 2.2^{b}
	0.25	4.9 ± 1.3^{b}	4316 ± 167^{a}	250.2 ± 12.3^{a}	147 ± 2.9^{c}

Data in each column followed by the same letter are not significantly different ($P < 0.05$)

higher in alfalfa. It could be the cause of higher susceptibility of alfalfa plants to Se toxicity under sulfur deficiency. Hence, a greater proportion of Se was excluded from protein fraction in cabbage and kohlrabi than in alfalfa. The accumulation of non-toxic species of Se-amino acids was suggested to be the basis of Se tolerance in some species (Neuhierl et al. 1999). Another explanation is that in cabbage and kohlrabi the majority of Se taken up was associated with organic molecules other than proteins, e.g. glucosinolates, therefore remaining out of protein fraction. Members of Crucifereae contain high concentrations of glucosinolates. There are indications on the association of Se with glucosinolates in *Brassica* species. For example, an effect of aqueous extracts of Se-fertilized broccoli on animals against oxygen free radicals was hypothesized to be due to ingestion of bioactive isothiocyanate derived from glucosinolates (Keck and Finley 2006). Similarly, onion (*Allium cepa*) and garlic (*Allium sativum*) were shown to have the ability to readily uptake Se from the soil and new analytical methods indicated the presence of Se-alliins, a class of second metabolites in genus *Allium* (Arnault and Auger 2006).

A general reduction of protein concentration in response to sulfur deprivation particularly in Se treated plants (Table 1) could be the result of limitation of supply of proper amino acids to proteins or the result of higher destruction, i.e. as a result of Se-induced oxidative stress, which was documented also in this work (see below). Se-methionine was shown to be less effective as a substitute for peptide bond formation during

translation than methionine. This could reduce the rate of protein synthesis and also contribute to Se toxicity (Eustice et al. 1981).

Effect of sulfur deficiency on antioxidant capacity of leaves

Activity of APX, CAT and SOD decreased at low compared with adequate sulfur supply in all three tested species/varieties. In contrast, sulfur deficiency increased the activity of POD and GR at all Se treatments in all three tested plants (Table 2). Increase in the activity of POD is reported as the result of deficiency of other nutrients (Marschner 1995). A depletion of the glutathione pool due to sulfur deficiency in our work may be the main cause of increased GR activity, which helps to keep the GSSG-to-GSH ratio in balance.

Sulfur deficiency caused a dramatic increase in H_2O_2 concentration in all three studied species/varieties. The highest amount of H_2O_2 was observed in sulfur deficient plants treated by 20μM Se (Table 3). The amount of glutathione and non-protein -SH groups decreased in response to sulfur deficiency at all Se treatments and in all three studied plants. Reduction of activities of APX, CAT and SOD and the subsequent increase in H_2O_2 concentration and reduction of glutathione in sulfur deficient plants could be an important cause of higher susceptibility of sulfur deficient plants to Se addition. It means that sulfur deficiency per se induced an oxidative stress as a result of reduction of antioxidant capacity of plants, which

Table 2. Effect of Se on the specific activity (Unit/g Pro./min) of ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and glutathione reductase (GR) at different sulfur nutritional status in cabbage (*Brassica oleracea* L. var. Sabauda), kohlrabi (*B. oleracea* L. var. Gongyloides) and alfalfa (*Medicago sativa* L. var. Garehyounjeh)

Se (μM)	S (mM)	APX	CAT	POD	SOD	GR
Cabbage						
0	1	18.4 ± 1.3^{a}	2504 ± 277^{b}	19.8 ± 2.1^{a}	930 ± 66^{a}	24.6 ± 2.4^{c}
	0.05	15.4 ± 0.8^{b}	1907 ± 168^{c}	24.3 ± 3.8^{a}	893 ± 50^{a}	29.1 ± 2.3^{c}
10	1	11.1 ± 1.0^{c}	2930 ± 143^{b}	12.8 ± 1.3^{b}	757 ± 31^{b}	42.2 ± 8.0^{b}
	0.05	4.1 ± 0.7^{de}	2651 ± 163^{b}	19.9 ± 2.3^{a}	548 ± 38^{c}	105.6 ± 2.3^{a}
20	1	5.3 ± 0.4^{d}	3944 ± 275^{a}	16.1 ± 2.5^{b}	449 ± 49^{c}	10.9 ± 1.5^{d}
	0.05	2.6 ± 0.7^{e}	3118 ± 234^{b}	22.4 ± 2.9^{a}	317 ± 27^{d}	30.8 ± 2.8^{b}
Kohlrabi						
0	1	59.8 ± 1.9^{a}	825 ± 56^{c}	30.7 ± 1.0^{a}	1454 ± 81^{a}	8.7 ± 2.0^{c}
	0.05	51.6 ± 0.8^{b}	625 ± 66^{c}	44.6 ± 3.2^{a}	1384 ± 130^{a}	22.4 ± 2.7^{b}
10	1	36.6 ± 1.1^{c}	2056 ± 92^{b}	21.2 ± 7.7^{b}	1048 ± 70^{b}	18.3 ± 0.8^{b}
	0.05	28.7 ± 0.9^{d}	818 ± 89^{c}	33.6 ± 8.0^{ab}	1088 ± 60^{b}	27.1 ± 0.1^{a}
20	1	5.4 ± 0.47^{e}	2861 ± 63^{a}	20.0 ± 5.3^{b}	707 ± 39^{c}	12.6 ± 1.9^{c}
	0.05	3.4 ± 0.34^{e}	2002 ± 181^{b}	38.2 ± 8.3^{a}	407 ± 51^{d}	21.1 ± 3.5^{b}
Alfalfa						
0	1	29.8 ± 1.7^{a}	2325 ± 232^{a}	5.5 ± 0.8^{c}	1360 ± 77^{a}	28.5 ± 4.9^{d}
	0.25	26.5 ± 1.2^{b}	2142 ± 113^{a}	9.7 ± 1.3^{b}	1152 ± 52^{b}	64.6 ± 9.4^{a}
10	1	23.5 ± 1.3^{c}	1699 ± 37^{b}	6.6 ± 0.6^{b}	1037 ± 101^{b}	18.8 ± 2.0^{d}
	0.25	22.5 ± 0.7^{c}	1244 ± 88^{c}	7.6 ± 1.8^{b}	929 ± 90^{b}	41.6 ± 3.2^{c}
20	1	20.8 ± 1.6^{c}	1551 ± 80^{b}	3.7 ± 0.8^{c}	550 ± 47^{c}	33.5 ± 3.5^{d}
	0.25	11.4 ± 1.3^{d}	1155 ± 98^{d}	13.5 ± 1.8^{a}	535 ± 35^{c}	49.5 ± 2.9^{b}

Data in each column followed by the same letter are not significantly different ($P < 0.05$)

Table 3. Effect of Se on concentration of hydrogen peroxide (μM H_2O_2/g fw), glutathione (nM/g fw) and non-protein -SH groups (nM/g fw) at different sulfur nutritional status in cabbage (*Brassica oleracea* L. var. Sabauda), kohlrabi (*B. oleracea* L. var. Gongyloides) and alfalfa (*Medicago sativa* L. var. Garehyounjeh)

Se (μM)	S (mM)	H_2O_2	Glutathione	Non-protein -SH groups
Cabbage				
0	1	0.11 ± 0.02^{e}	72.3 ± 5.4^{a}	1036 ± 77^{a}
	0.05	0.68 ± 0.05^{c}	54.4 ± 6.4^{b}	937 ± 72^{a}
10	1	0.09 ± 0.01^{e}	46.3 ± 3.9^{bc}	701 ± 47^{b}
	0.05	0.41 ± 0.03^{d}	37.8 ± 3.7^{c}	574 ± 24^{c}
20	1	0.89 ± 0.05^{b}	17.7 ± 4.1^{d}	354 ± 10^{d}
	0.05	1.17 ± 0.21^{a}	11.7 ± 6.5^{d}	282 ± 24^{d}
Kohlrabi				
0	1	0.17 ± 0.02^{e}	72.5 ± 3.0^{a}	1693 ± 140^{a}
	0.05	0.60 ± 0.07^{c}	38.6 ± 6.6^{b}	699 ± 59^{d}
10	1	0.17 ± 0.02^{e}	38.2 ± 4.9^{b}	1276 ± 147^{b}
	0.05	0.47 ± 0.01^{d}	25.3 ± 3.4^{c}	516 ± 30^{d}
20	1	0.74 ± 0.09^{b}	20.7 ± 5.4^{c}	820 ± 72^{cd}
	0.05	1.04 ± 0.06^{a}	6.2 ± 2.4^{d}	305 ± 40^{e}
Alfalfa				
0	1	0.12 ± 0.02^{c}	70.3 ± 2.7^{a}	954 ± 63^{a}
	0.05	0.75 ± 0.06^{a}	54.8 ± 1.8^{b}	876 ± 56^{ab}
10	1	0.09 ± 0.01^{c}	51.9 ± 3.0^{b}	783 ± 27^{b}
	0.05	0.40 ± 0.04^{b}	40.5 ± 3.2^{c}	487 ± 36^{c}
20	1	0.74 ± 0.12^{a}	21.3 ± 3.7^{d}	599 ± 80^{c}
	0.05	0.79 ± 0.06^{a}	13.9 ± 3.9^{e}	272 ± 36^{d}

Data in each column followed by the same letter are not significantly different ($P < 0.05$)

caused a higher susceptibility to other stresses, including Se treatment. Under sulfur deficiency, plants may undergo an oxidative stress caused by the shortage of GSH (Asada 1999).

Effect of Se on antioxidant capacity of leaves

Se treatment inhibited the specific activity of APX, this reduction was 71%, 91% and 30% in sulfur sufficient, and 83%, 93% and 57% in sulfur deficient cabbage, kohlrabi and alfalfa, respectively. Therefore, the lowest reduction was observed in alfalfa and the highest in kohlrabi (Table 2). In two varieties of *Brassica*, Se supplementation increased the activity of CAT both in sulfur deficient and sufficient plants. The stimulation of CAT activity was 63% and 220% in cabbage and kohlrabi, compared to control and Se = 20μM, respectively. In alfalfa, the inhibition of CAT in response to 20μM Se was 33% and 42% in sulfur sufficient and deficient plants, respectively (Table 2). In response to Se addition, POD activity was changed depending on sulfur nutritional status and plant species. In cabbage and kohlrabi, the activity of POD decreased in response to Se addition in sulfur sufficient and remained unchanged in sulfur deficient plants. However, the activity of POD in alfalfa increased at Se = 10μM but decreased in response to 20μM Se addition in sulfur sufficient plants. In sulfur deficient plants, POD activity increased in response to 20μM Se addition (Table 2). The addition of 20μM Se resulted also in inhibition of SOD activity, which was 66%, 72%

and 60% in sulfur sufficient and 50%, 49% and 54% in sulfur deficient cabbage, kohlrabi and alfalfa plants, respectively (Table 2). The activity of GR increased in response to Se supplementation at 10μM, but decreased again at 20μM in cabbage and kohlrabi in both sulfur treatments; in alfalfa, the opposite tendency was observed, after a reduction at 10μM Se, an increase in GR activity was observed at 20μM (Table 2).

Like sulfur deficiency, Se treatment caused a dramatic increase in H_2O_2 concentrations in all three studied species/varieties. For example, the concentration of H_2O_2 in response to 20μM Se in sulfur sufficient plants increased up to 8.1, 4.4 and 6.6 folds in cabbage, kohlrabi and alfalfa, respectively (Table 3). Se addition resulted in a significant reduction of glutathione content in plants in both sulfur sufficient and deficient plants with similar extent; this reduction was 76%, 71% and 70% in sulfur sufficient and 78%, 84% and 75% in sulfur deficient cabbage, kohlrabi and alfalfa plants, respectively (Table 3).

Selenium addition reduced non-protein -SH groups in all three studied species/varieties; this reduction was 32%, 24% and 17% at 10μM Se treatment, and 65%, 51% and 37% at 20μM Se treatment in sulfur sufficient cabbage, kohlrabi and alfalfa, respectively. A similar range of reduction was observed in sulfur deficient plants. Therefore, the highest content of -SH groups in each plant studied was observed in sulfur sufficient plants without Se supplementation (Table 3).

As for the highly significant effect of Se on antioxidant capacity of plants in this work and suggestions of other authors (Hartikainen et al. 2000), it is expected that the stimulatory effect of Se as well as different responses of three studied species/varieties to Se toxicity should be explained by function of antioxidant defense system. Se treatment caused a dramatic reduction of APX and SOD activity in all three studied plants. Surprisingly, the reduction of APX activity was much higher in cabbage and kohlrabi than in alfalfa. Therefore, an involvement of APX via induction of antioxidative system in the differential response of studied plants as well as their growth improvement by Se supplementation could be eliminated.

Catalase, POD and GR responded to Se addition differently depending on plants and/or Se concentration. Catalase activity increased in cabbage and kohlrabi but decreased in alfalfa after Se addition. However, such differential changes in the activity of CAT were not reflected in the concentration of H_2O_2. Thus, it is unlikely that CAT has any determining role in the differential response of alfalfa to Se addition and it cannot explain the growth promoting effect of Se in sulfur sufficient plants. The extent of reduction of non protein -SH groups was not in accordance with different response of three studied plants and so it could not explain the growth promoting effect of Se supplementation, either.

The change in the activity of POD in response to Se addition in sulfur sufficient plants coincided with the growth response of plants to Se supplementation. The unspecific POD activity assayed with guaiacol as a universal substrate was considered as total activity and it can exhibit activities of APX, coniferyl alcohol peroxidase, NADH oxidase and IAA oxidase. The individual activities of these enzymes with the exception of APX, were not distinguished from the soluble pool in our extraction procedure. On the other hand, the involvement of PODs in the defense mechanisms of plant tissues, for example against metal-induced damages, remains controversial (Chaoui and El Ferjani 2005). Like CAT, changes in the activity of GR in response to Se addition could not explain growth responses of studied plants and presumably could be considered rather a result of stress than a cause of adaptation either to sulfur deficiency or Se treatment.

In number of previous works, an amelioration of stresses such as UV radiation (Hartikainen and Xue 1999, Xue and Hartikainen 2000), cold injury (Seppänenn et al. 2003) and aging (Xue et al. 2001) by Se was attributed to the activation of antioxidant defense system of plants. However, in our experiment the positive effect of Se was observed in non-stressed plants and in the early growth period. The lack of functional significance of antioxidative capacity of plants in growth response observed in this work suggests that the cause of growth stimulatory effect of Se supplementation most likely lies in the other mechanisms, for example in the availability of reduced cell metabolites or changes of lignification and of auxin catabolism. Such mechanisms were suggested to be involved in the tolerance to metal toxicity (Chaoui and El Ferjani 2005).

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