Nutrient Solution Concentrations of Na_2SeO_4 Affect the Accumulation of Sulfate and Selenate in *Brassica oleracea* L.

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Abstract. Dietary sources of selenium (Se) are associated with human health benefits, and *Brassica* species are good sources of Se in human diets. Selenium and S compete for absorption and accumulation in plant tissues; therefore, the ratios of Se to S in the growing environment determine the accumulation of selenium in plants. To determine responses for *Brassica oleracea* L., two levels of Na_2SeO_4 (96 $\text{mg}\cdot\text{L}^{-1}$ SeO_4^{2-} and 0.384 $\text{mg}\cdot\text{L}^{-1}$ SeO_4^{2-}) were added to nutrient solutions with or without $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ (96 $\text{mg}\cdot\text{L}^{-1}$ SO_4^{2-}). The highest plant fresh weight and S and SO_4^{2-} accumulation were found when plants were grown in the medium with a SeO_4^{2-} to SO_4^{2-} ratio of 1 : 250 (0.384 $\text{mg}\cdot\text{L}^{-1}$ SeO_4^{2-} and 96 $\text{mg}\cdot\text{L}^{-1}$ SO_4^{2-}). However, the highest accumulation of Se was found when a low level of selenate (0.384 $\text{mg}\cdot\text{L}^{-1}$ SeO_4^{2-}) was added to nutrient solutions without S. The activity of glutathione peroxidase (GPx) was regulated by Se status; the highest GPx activity was measured when a high level of SeO_4^{2-} (96 $\text{mg}\cdot\text{L}^{-1}$) was supplied to nutrient solutions without S supplementation. The lowest concentration of total glucosinolates was found when adding SeO_4^{2-} to nutrient solutions without S. We saw no difference in plant growth and mineral accumulation when plants were grown with K_2SeO_4 versus Na_2SeO_4 , suggesting that the growth-inhibiting effect of Na_2SeO_4 was the result of the SeO_4^{2-} rather than potentially toxic effects of Na^+ .

It has been proposed that selenium (Se) can be metabolized through the S assimilation pathway (Läuchli, 1993; Pilon-Smits et al., 1999; Terry et al., 2000) and subsequently can be incorporated into Se amino acids and proteins in higher plants (de Souza et al., 2002; Terry et al., 2000), because both S and Se are in Group VI of the periodic table and have similar chemical properties. Several studies showed that in Se accumulators such as *Astragalus*, *Stanleya*, and *Haplopappus* (Brown and Shrift, 1982), Se either is incorporated into nonprotein Se amino acids or that selenocysteine can be methylated by selenocysteine methyltransferase to avoid Se toxicity (Brown and Shrift, 1981, 1982; Shrift, 1969). On the other hand, in non-accumulators, Se amino acids have been shown to substitute for S amino acids non-

specifically in metabolic pathways and can be incorporated into proteins, rendering them nonfunctional (Eustice et al., 1981; Ng and Anderson, 1979). Such abnormal substitutions are considered the cause of Se toxicity in plants.

Selenium is an essential trace nutrient in the human diet. There is a narrow range between Se sufficiency and toxicity (Lemly, 1997). Se can be delivered to the human daily diet through standard food uptake (Gissel-Nielsen et al., 1984; Reilly, 1996). Therefore, the amount of human Se intake depends on the amount of Se in their food, and the amount of Se in plant material depends on the availability of Se in the soil and the ability of plants to take it up. Among vegetables, *Brassica* species are good Se deliverers because of their high accumulation of Se; e.g., Indian mustard (*Brassica juncea* L.) (Bañuelos et al., 1997b) and canola (*Brassica napus* L.) (Bañuelos, 2002) are able to accumulate several hundred milligrams of Se per kilogram dry weight in their shoot tissues when grown with high levels of Se.

Brassica crops are consumed because of their nutritional benefits (Finley, 2003; Stoewsand et al., 1989), whereas many people are attracted to their bitter flavor and distinctive odor (Fenwick et al., 1983a, 1983b). The accumulation of Se and its compounds in *Brassica* species has drawn attention because Se was found to be a component of the antioxidant enzyme, glutathione peroxidase (GPx), which may have human health benefits (Rotruck et al., 1973). The Se-dependent GPx was first found in green alga, *Chlamydomonas reinhardtii* (Fu et al., 2002), but its presence has not been proved in higher plants.

Glucosinolates (GLs) are not only related to anticarcinogenic effects, but also are the dominant flavor precursors in *Brassicaceae*. They are hydrolyzed by myrosinase (E.C. 3.2.3.1) when plant tissue is damaged to produce their unique flavor (Fenwick et al., 1983a, 1983b; MacLeod, 1976). Glucosinolates in *Brassicaceae* are amino acid-derived secondary products containing a sulfur element (Fenwick et al., 1983a, 1983b; Halkier and Du, 1997; Rosa et al., 1997). Mailer (1989) and Booth et al. (1991) showed that increasing SO_4^{2-} concentrations in the soil solution not only influenced S concentrations in *Brassica*, but also enhanced glucosinolate content. However, when *Brassica* plants were exposed to different sodium selenate concentrations, high selenium levels decreased GLs content (Charron et al., 2001). Bailey et al. (1995) found different SeO_4^{2-} uptake rates under high and low SO_4^{2-} conditions in *Ruppia maritima* L., indicating SeO_4^{2-} and SO_4^{2-} competition. When the ratio of SeO_4^{2-} to SO_4^{2-} was below 1:125 (on a weight basis), onions grown with a low amount of Se exhibited more SO_4^{2-} uptake and accumulation than plants grown without Se (Kopsell and Randle, 1997).

Selenium has been associated with antioxidant activity when present at appropriate levels in human diets; however, it can become toxic at higher levels (Raisbeck, 2000; Vinceti et al., 2001). Because *Brassica* species are good candidates to supply both Se and GLs to the human diet (Finley et al., 2005; Sigrid-Keck and Finley, 2004), changes in Se accumulation and glucosinolate amounts in *Brassica* species need more evaluation when plants are grown in areas contaminated with selenium. Therefore, this study was conducted 1) to examine the effects of SeO_4^{2-} and SO_4^{2-} ratios on plant growth; 2) to measure the accumulation patterns of S, SO_4^{2-} , and SeO_4^{2-} Se in different tissues (i.e., roots, stems, and leaves); and 3) to determine GPx activity and flavor intensity (GLs) in *B. oleracea* under different ratios of SeO_4^{2-} and SO_4^{2-} .

Because Na_2SeO_4 was used as the SeO_4^{2-} source, we performed a second study to determine whether treatment effects were caused by different concentrations of Na^+ or SeO_4^{2-} . To do this, we used two different SeO_4^{2-} salts (K_2SeO_4 and Na_2SeO_4) to examine effects on plant growth and dry matter production of different concentrations of these salts.

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Materials and Methods

Plant culture. On 19 June 2006, seeds of a rapid cycling *Brassica oleracea* L. population (Crucifer Genetics Cooperative, Department of Plant Pathology, University of Wisconsin, Madison, WI) were sown in growing cubes (Smithers-Oasis, Kent, OH), covered with vermiculite and watered as needed, and placed in a growth chamber (model E15; Conviron, Asheville, NC) at a temperature of 24 °C and a 24-h photoperiod with an average photosynthetic flux of 250 $\mu\text{mol}^{-1}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Basic Quantum Meter; Spectrum Tech., Plainfield, IL). Seeds germinated in 7 d and seedlings were fertilized with 100 mL of a 20N–8.8P–16.6K fertilizer solution with a nitrogen concentration of 200 $\text{mg}\cdot\text{L}^{-1}$ (Peter's 20–20–20; The Scotts Co., Marysville, OH). On 1 July 2006, the first true leaves emerged and plants were transferred to 270-mL crystallizing dishes (89000-288; VWR, Atlanta, GA) containing 100 mL modified half-strength Hoagland's solution (Hoagland and Arnon, 1950) for 1 week. Thereafter, plants were rinsed in deionized water and groups of 10 seedlings were subsequently transferred to 270-mL crystallizing dishes with fresh half-strength nutrient solution and different treatments were applied. The dishes were placed back into the growth chamber at 24 °C and a 16-h photoperiod for 72 h with a photosynthetic photon flux of 250 $\mu\text{mol}^{-1}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Treatments. Different levels of SO_4^{2-} and SeO_4^{2-} were included in the nutrient solutions as follows: 1.0 mM $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ (96 $\text{mg}\cdot\text{L}^{-1}$ of SO_4^{2-}) as the control treatment, 0.6 mM Na_2SeO_4 (96 $\text{mg}\cdot\text{L}^{-1}$ SeO_4^{2-}) + 1.0 mM $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ (SeO_4^{2-} : SO_4^{2-} ratio of 1 : 1, w/w), 2.68 μM Na_2SeO_4 (0.384 $\text{mg}\cdot\text{L}^{-1}$ SeO_4^{2-}) + 1.0 mM $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ (SeO_4^{2-} : SO_4^{2-} ratios of 1:250, w/w), 0.6 mM Na_2SeO_4 + 0 mM $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, and 2.68 μM Na_2SeO_4 + 0 mM $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ (also see Table 1 for a summary of SO_4^{2-} and SeO_4^{2-} concentrations in each treatment). After 72 h, all 10 plants from a single dish were harvested and separated into leaves, stems, and roots. Plant fresh weights were recorded. The plant tissues were washed using deionized water and frozen in liquid N_2 , placed in plastic bags, and put into a freeze drier (model 79480; Lab-conco Corporation, Kansas, MI) at -40°C for 72 h. Tissue dry weights were recorded, and tissue was stored at -80°C for later analysis.

In a second experiment, we determined whether the Na^+ in the Na_2SeO_4 could have caused the plant growth responses seen in the first study. Two levels of K_2SeO_4 and Na_2SeO_4 were applied, resulting in five treatments: 1.0 mM $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ as the control, 1.0 mM Na_2SeO_4 + 1.0 mM $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, 0.004 mM Na_2SeO_4 + 1.0 mM $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, 1.0 mM K_2SeO_4 + 1.0 mM $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, and 0.004 mM K_2SeO_4 + 1.0 mM $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ (see Table 2 for a summary of the treatments). The four treatments that included both Se and S provided SeO_4^{2-} : SO_4^{2-} ratios of 1 : 1 and 1 : 250 on a molar basis.

Mineral analysis. Total S in leaves, stems, and roots was measured using a Leco Sulfur

Table 1. Fresh and dry weight of different parts of *B. oleracea* grown with different concentrations of SeO_4^{2-} and SO_4^{2-} in the nutrient solutions.^z

SeO_4^{2-} : SO_4^{2-} ($\text{mg}\cdot\text{L}^{-1}$)		Leaf ^y	Stem	Root
		Fresh wt (g)		
0	96	1.48 ab	1.22 b	0.77 a
96	96	1.30 b	0.82 c	0.58 b
0.384	96	1.55 a	1.51 a	0.78 a
96	0	1.06 c	0.72 c	0.51 c
0.384	0	1.40 ab	1.45 a	0.78 a
		Dry wt (g)		
0	96	0.15 a	0.12 b	0.07 ab
96	96	0.12 b	0.08 c	0.05 c
0.384	96	0.14 ab	0.14 a	0.07 b
96	0	0.09 c	0.06 c	0.05 c
0.384	0	0.13 ab	0.14 a	0.08 a

^zValues represent the total of 10 plants.

^yMeans within a column followed by the same letter are not significantly different from each other ($P = 0.05$).

Table 2. Fresh and dry weight of different parts of *B. oleracea* grown with different concentrations of SeO_4^{2-} and SO_4^{2-} by adding K_2SeO_4 and Na_2SeO_4 in the nutrient solutions.^z

MgSO_4	K_2SeO_4	Na_2SeO_4	Leaf ^y	Stem	Root
			Fresh wt (g)		
1.0	0	0	2.32 a	1.39 b	0.88 b
1.0	1.0	0	1.77 b	0.45 c	0.25 c
1.0	0.004	0	2.29 a	1.45 a	0.97 a
1.0	0	1.0	1.69 b	0.41 c	0.20 c
1.0	0	0.004	2.21 a	1.42 ab	0.91 ab
			Dry wt (g)		
1.0	0	0	0.228 a	0.129 a	0.082 a
1.0	1.0	0	0.169 b	0.040 b	0.021 b
1.0	0.004	0	0.225 a	0.128 a	0.078 a
1.0	0	1.0	0.152 b	0.038 b	0.017 b
1.0	0	0.004	0.224 a	0.126 a	0.085 a

^zValues represent the total of 10 plants.

^yMeans within a column followed by the same letter are not significantly different from each other ($P = 0.05$).

Determinator (model SC-232; Leco Corp., St. Joseph, MO). Twenty milligrams of dried tissue was combined with a vanadium pentoxide accelerator (Leco Corp.) and combusted at 1300 °C with O_2 . Total S was quantified against S standards. To determine total SO_4^{2-} , another 20 mg of dried tissue was put into a 125-mL flask with 50 mL high-performance liquid chromatography grade (18 Ω) water. Suspensions were shaken at 100 rpm overnight and injected into 1-mL plastic vials (National Scientific Company, Lawrenceville, GA) through a 0.22- μm nylon syringe filter (Fisher Scientific, Pittsburgh, PA). Forty microliters of sample solution was analyzed with a Waters 2690 Separations Module using a Waters 432 Conductivity Detector (Waters Corp., Milford, MA). The peak, representing SO_4^{2-} , was quantified and integrated by Millennium Chromatography software (version 3.05; Waters Corp., Milford, MA) against a 30 ppm SO_4^{2-} standard.

A wet-acid digestion was applied to measure total Se from each tissue (Kopsell and Randle, 1997). Twenty milligrams of dried tissue was placed into a 125-mL flask with 4 mL concentrated nitric acid (70% HNO_3) and incubated overnight. Then, flasks were set on a hot plate (model 2200; Thermolyne, Dubuque, IA) at 120 °C for 1 h. A funnel test tube filled with water was put into each flask as a condenser to reduce sample loss during digestion. Flasks were removed and allowed to cool to room temperature, and 4.0 mL of

30% hydrogen peroxide (H_2O_2) was added. Flasks were placed back on the hot plate with an additional 4.0 mL H_2O_2 . The flasks were cooled to room temperature and adjusted to a final volume of 10.0 mL with deionized water. The solutions were filtered through a 0.22- μm nylon syringe filter (Fisher Scientific). Total Se was measured by graphite furnace atomic absorption spectrophotometry (GFAA; model 300; Perkin Elmer Corp., Norwalk, CT).

Glutathione peroxidase activity. Total GPx activity was measured from 100 mg of fresh leaves using Total Glutathione Peroxidase Assay Kits (ZeptoMetrix Corp., Buffalo, NY). Changes in absorbance were recorded every 15 s by spectrophotometer at 340 nm for 1 min. The first 40 s were not taken into consideration. The GPx activity was calculated by subtracting a blank from the measurements of each sample.

Glucosinolate analysis. The method of Raney and McGregor (1990) was used to measure GLs. Briefly, 20 mg of dried leaf tissues was extracted with 0.25 mL of benzyl GLs solution (1 mM, added as internal standards), 0.5 mL of methanol, and 0.075 mL of barium lead acetate (0.6 M) in a 16 mm $\times$ 100-mm tube and shaken at 60 rpm for 1 h. Samples were centrifuged at 2000 g for 10 min. Two hundred microliters of supernatant was desulfated on a 1.0-mL column containing 0.3 mL preswollen DEAE Sephadex A-25. The separation of desulphoglucosinolates

was carried out on a high-performance liquid chromatograph (Hewlett-Packard, Palo Alto, CA) using a C-18 ODS reverse-phase column (250×4.6 mm i.d., 5 μ m) and detected by an ultraviolet detector at a wavelength of 230 nm. A water–acetonitrile mobile phase gradient was used for separation of desulfoglucosinolates. Desulphoglucosinolates were identified by comparison with retention times of standards. Desulfonated forms of glucobrassicin (GI; 3-methylsulfinylpropyl GS), glucobrassicin (GB; indol-3-ylmethyl GS), gluconapin (GNP; 3-butenyl GS), 4-methoxyglucobrassicin (4MGB; 4-methoxyindol-3-ylmethyl GS), neoglucobrassicin (NGB; 1-methoxyindol-3-ylmethyl GS), progoitrin (PRO; 2-hydroxybut-3-enyl GS), and sinigrin (SN; 2-propenyl GS) were used as internal standards (Sandro Palmieri of the Istituto Sperimentale Industriali, Bologna, Italy). Gluconasturtiin (GNS; 2-phenylethyl GS) was purchased from LKT Laboratories (LKT, St. Paul, MN). Response factors for the quantification of the desulfoglucosinolates were from the International Organization for Standardization Method 9167–1.

Statistical analyses. The experimental design for both experiments was a randomized complete block with three replications and 10 plants per experimental unit. To test the effects of various treatments on plant growth, mineral accumulation, and GPx activity and GLs amount, data were analyzed by the GLM procedure using SAS statistical software (version 9.1.3; SAS, Cary, NC).

Results

Plant growth. Some *Brassica* species are classified as Se accumulators. However, Se toxicity was observed when SeO_4^{2-} was applied to the nutrient solutions at concentrations of $96 \text{ mg} \cdot \text{L}^{-1}$. *B. oleracea* grown with this concentration of SeO_4^{2-} showed chlorotic spots on old leaves. In addition, a pinkish orange color appeared on roots.

Compared with the control (with $96 \text{ mg} \cdot \text{L}^{-1} \text{SO}_4^{2-}$), decreases in fresh and dry weight of leaves, stems, and roots ($P \leq 0.001$) occurred when plants were grown with $96 \text{ mg} \cdot \text{L}^{-1} \text{SeO}_4^{2-}$ and no SO_4^{2-} (Table 1), indicating that SeO_4^{2-} could not replace SO_4^{2-} . Similarly, the inclusion of both $96 \text{ mg} \cdot \text{L}^{-1} \text{SeO}_4^{2-}$ and $96 \text{ mg} \cdot \text{L}^{-1} \text{SO}_4^{2-}$ in the nutrient solution decreased stem and root fresh weight as well as leaf, stem, and root dry weight as compared with the control treatment without SeO_4^{2-} .

The combination of $0.384 \text{ mg} \cdot \text{L}^{-1} \text{SeO}_4^{2-}$ and $96 \text{ mg} \cdot \text{L}^{-1} \text{SO}_4^{2-}$ in the nutrient solution increased stem fresh and dry weight compared with the control plants and similar results were found for the $0.384 \text{ mg} \cdot \text{L}^{-1} \text{SeO}_4^{2-}$ treatment without SO_4^{2-} (Table 1).

In the second experiment, high concentrations of either K_2SeO_4 or Na_2SeO_4 (1.0 mM) decreased fresh and dry weight of the leaves, stems, and roots compared with those of control plants ($P \leq 0.001$), but there were no significant differences between the fresh and dry weights in the 1.0 mM K_2SeO_4 and

Na_2SeO_4 treatments (Table 2). Similarly, there were no differences in weight between plants grown with $0.004 \text{ mM} \text{K}_2\text{SeO}_4$ or Na_2SeO_4 . The $0.004 \text{ mM} \text{K}_2\text{SeO}_4$ treatment resulted in small but significant increases in stem and root fresh weight compared with the control treatment, but there were no differences in dry weight.

Total sulfur, sulfate, and selenium. The accumulation of S in *B. oleracea* varied among plant tissues and adding Na_2SeO_4 to nutrient solutions affected S accumulation. The highest leaf, stem, and root S concentrations were found when *B. oleracea* plants were grown in S-sufficient nutrient solutions ($96 \text{ mg} \cdot \text{L}^{-1}$) with a low concentration of SeO_4^{2-} ($0.384 \text{ mg} \cdot \text{L}^{-1}$) (Table 3). A significant decrease in S accumulation was observed in both leaf and stem tissues ($P \leq 0.001$), but not in root tissues, when plants were transferred to a nutrient solution with low concentrations of SeO_4^{2-} ($0.384 \text{ mg} \cdot \text{L}^{-1}$) without SO_4^{2-} as compared with control plants. In addition, a decrease ($P \leq 0.001$) was found in leaf, stem, and root S when plants were grown with $96 \text{ mg} \cdot \text{L}^{-1}$ of both SeO_4^{2-} and SO_4^{2-} in the solution as compared with the control plants. Furthermore, S concentrations in stem and root tissues were lower than in control plants when *B. oleracea* plants were grown with a high concentration of SeO_4^{2-} ($96 \text{ mg} \cdot \text{L}^{-1}$) without SO_4^{2-} , whereas no significant difference was seen in the S concentration of the leaf tissues (Table 3).

The concentrations of SO_4^{2-} in leaves, stems, and roots was highest ($P \leq 0.001$) when plants were grown in a S-sufficient solution with a low concentration of SeO_4^{2-} ($0.384 \text{ mg} \cdot \text{L}^{-1}$). As expected, a high concentration of SeO_4^{2-} decreased SO_4^{2-} accumulation in leaves, stems, and roots compared with control plants. When $0.384 \text{ mg} \cdot \text{L}^{-1} \text{SeO}_4^{2-}$ was added to the nutrient solution without S, it resulted in a lower SO_4^{2-} concentration in plant tissues compared with the control treatment (Table 3).

When *B. oleracea* plants were grown in Se-containing nutrient solutions, tissue Se concentrations increased ($P \leq 0.001$). The concentration of Se in different plant tissues differed depending on the SeO_4^{2-} and SO_4^{2-} concentrations in the nutrient solution (Table 3). Concentrations of Se in control plants, which were grown without Na_2SeO_4 , were below the limits of GFAA detection. The concentrations of Se in leaf and root tissues were similar with 1 : 1 and 1 : 250 ratios of SeO_4^{2-} and SO_4^{2-} , respectively, but the 1 : 250 ratio resulted in higher Se concentrations in the stem than the 1 : 1 ratio. There was no difference in Se accumulation in stem tissues between the treatments with $96 \text{ mg} \cdot \text{L}^{-1}$ of both SeO_4^{2-} and SO_4^{2-} ratios and $96 \text{ mg} \cdot \text{L}^{-1} \text{SeO}_4^{2-}$ without SO_4^{2-} .

In Expt. 2, S, SO_4^{2-} , and SeO_4^{2-} accumulation in leaves, stems, and roots was unaffected by SeO_4^{2-} source, K_2SeO_4 versus Na_2SeO_4 . An increase ($P \leq 0.001$) in the S concentration was found in root and leaf tissues and a decrease ($P \leq 0.001$) in the S concentration of the stem tissue when plants were grown in nutrient solutions with $0.004 \text{ mM} \text{SeO}_4^{2-}$ compared with control plants. Lowest S concentrations were found in leaves, stems, and roots when plants were grown with $1.0 \text{ mM} \text{SeO}_4^{2-}$ (Table 4).

Sulfate concentrations were lowest in all tissues when the solution contained $1.0 \text{ mM} \text{SeO}_4^{2-}$ and highest with $0.004 \text{ mM} \text{SeO}_4^{2-}$, irrespective of whether the SeO_4^{2-} was applied as Na_2SeO_4 or K_2SeO_4 .

An increase ($P \leq 0.001$) in the Se concentration in different parts of plant tissue was seen when SeO_4^{2-} was added to the nutrient solutions. There was more accumulation of Se in leaves and roots when $0.004 \text{ mM} \text{SeO}_4^{2-}$ was added than with $1.0 \text{ mM} \text{SeO}_4^{2-}$ (Table 4).

Glutathione peroxidase activity. All treatments containing Se had higher GPx activity than the control treatment with the highest activity in the treatment with $96 \text{ mg} \cdot \text{L}^{-1} \text{Se}$

Table 3. Sulfur, sulfate, and selenium concentrations (on a dry weight basis) in different parts of *B. oleracea* grown with different ratios of SeO_4^{2-} and SO_4^{2-} in the nutrient solutions.

$\text{SeO}_4^{2-} : \text{SO}_4^{2-}$ ($\text{mg} \cdot \text{L}^{-1}$)		Leaf ^a	Stem	Root
		Sulfur ($\text{mg} \cdot \text{g}^{-1}$)		
0	96	5.77 b	4.04 b	4.37 b
96	96	4.11 d	2.38 d	4.09 c
0.384	96	6.22 a	4.69 a	5.38 a
96	0	5.47 b	2.78 c	4.11 c
0.384	0	4.85 c	2.24 d	4.66 b
		Sulfate ($\text{mg} \cdot \text{g}^{-1}$)		
0	96	2.86 b	1.97 b	1.79 b
96	96	1.39 d	1.01 c	0.89 c
0.384	96	4.21 a	3.74 a	2.74 a
96	0	0.44 e	0.41 d	0.24 d
0.384	0	1.89 c	1.24 c	0.78 c
		Selenium ($\mu\text{g} \cdot \text{g}^{-1}$)		
0	96	ND ^b	ND	ND
96	96	53 c	92 c	23 c
0.384	96	104 c	186 b	47 c
96	0	226 b	116 c	206 a
0.384	0	284 a	202 a	144 b

^aMeans within a column followed by the same letter are not significantly different from each other ($P = 0.05$).

^bND = below detection limits.

and no S (Table 5). No significant differences were found between treatments with 0.384 mg·L⁻¹ Se with and without S (Table 5).

Glucosinolates. A high concentration (96 mg·L⁻¹) of SeO₄²⁻ in the nutrient solution (with or without S) and a low concentration (0.384 mg·L⁻¹) of SeO₄²⁻ with high SO₄²⁻ (96 mg·L⁻¹) resulted in the lowest concentrations of GLs (Table 5). When 0.384 mg·L⁻¹ SeO₄²⁻ was applied, a difference was found in total GLs between nutrient solutions with S and without S ($P \leq 0.05$) with lower levels of GLs in the absence of S. The highest total GLs concentration was found when plants were grown with 96 mg·L⁻¹ SO₄²⁻ and 0 (control) or 0.384 mg·L⁻¹ SeO₄²⁻.

Discussion

There were changes in plant growth, S and Se accumulation, GPx activity, and total GLs in response to Se supplementation. Decreases in plant fresh and dry weight in response to Se addition also were found in alfalfa (*Medicago sativa* L.), clover (*Trifolium repens*) (Broyer et al., 1966), *Brassica juncea* (Bañuelos et al., 1997b), rice (*Oryza sativa* L.) (Zhou, 1990), and wheat (*Triticum* spp) (Peng et al., 2000). In this study, a high concentration of SeO₄²⁻ (96 mg·L⁻¹) reduced the growth of *B. oleracea*. This is considered to be Se toxicity because retardation of plant growth is one of the symptoms noted when plants were grown

with high levels of Se (Bañuelos et al., 1997a). In the initial study, Na₂SeO₄ was used as the source of SeO₄²⁻, making it impossible to distinguish between potentially toxic effects of Na⁺ and SeO₄²⁻. However, our second study showed that K₂SeO₄ and Na₂SeO₄ affected plant growth similarly, indicating that SeO₄²⁻ rather than Na⁺ was the cause of the growth reduction. Although Brassica species are able to accumulate abundant Se, the threshold of Se accumulation before growth reductions occur may differ based on species, growth stage, and sulfate concentrations in the medium. In this case, an overdose of Se in the early vegetative stage resulted in growth inhibition.

When plants are grown in a medium with a mixture of SeO₄²⁻ and SO₄²⁻, the ratio of SeO₄²⁻ to SO₄²⁻ plays a role in the regulation of sulfate uptake (Bell et al., 1992; Ferrari and Renosto, 1972; Kopsell and Randle, 1997, 1999; Mikkelsen and Wan, 1990; White et al., 2004). Differences in preference for SeO₄²⁻ and SO₄²⁻ were found between accumulators and nonaccumulators. Ferrari and Renosto (1972) reported that under high ratios (1.4 to 1.0) of SeO₄²⁻ to SO₄²⁻, SO₄²⁻ is taken up more than SeO₄²⁻ in nonaccumulators. On the other hand, Se accumulators are able to take up Se preferentially even when high SO₄²⁻ is supplied (Bell et al., 1992). Onions and brassicas are Se-enriched vegetables (Ip and Lisk, 1994; Ip et al., 1992).

Kopsell and Randle (1997) reported that when the SeO₄²⁻ to SO₄²⁻ ratio was lowered to 1:125 or 1:500, SeO₄²⁻ increased SO₄²⁻ uptake and accumulation in onions. Moreover, S concentrations increased in leaf tissues of *B. oleracea* when the SeO₄²⁻ to SO₄²⁻ ratio was increased from 1 : 42 to 1 : 14 (Kopsell and Randle, 1999). In this study, we saw higher accumulation of SO₄²⁻ in response to a 1 : 250 ratio of SeO₄²⁻ : SO₄²⁻ as compared with a 1 : 1 ratio. Additionally, SO₄²⁻ concentrations in leaves, stems, and roots decreased dramatically when plants were transferred to nutrient solutions without SO₄²⁻ regardless of the SeO₄²⁻ concentration (Table 3). Other studies have shown that application of Na₂SeO₄ leads to S starvation, which triggers sulfate reduction to keep plants alive (Chen and Leustek, 1995; Leustek et al., 2000). In the current study, the accumulation of S in leaf tissues was reduced under S deprivation (Table 3). In addition, similar results were seen from different parts of plant tissue when a high concentration of either K₂SeO₄ or Na₂SeO₄ was added to nutrient solutions (Table 4).

The accumulation of Se in different parts of plant tissue varies depending on plant species, growth stage, and fertilizer, especially sulfate. In our experiments, substantial amounts of Se accumulated in young leaves even when no further SO₄²⁻ was applied (Tables 3 and 4). It is possible that S deficiency results in upregulation of sulfate transporters, which leads to increasing SeO₄²⁻ uptake and further accumulation in plants (Bolchi et al., 1999; Zayed and Terry, 1992). This hypothesis is based on the assumption that SO₄²⁻ and SeO₄²⁻ share the same transporters (Abrams et al., 1990; Arvy, 1993). High concentrations of SO₄²⁻ directly inhibit SeO₄²⁻ uptake by plants (Barak and Goldman, 1997; Bell et al., 1992; Wu and Huang, 1991). Indeed, we also found higher Se concentrations in the plant tissue in treatments without SO₄²⁻ as compared with treatments with 96 mg·L⁻¹ SO₄²⁻. Conversely, the accumulation of Se under high levels of SO₄²⁻ in the nutrient solutions was reported when organic Se compounds such as selenomethionine were applied to wheat seedlings (Abrams et al., 1990). Interestingly, we also found that high levels of SeO₄²⁻ in the nutrient solution reduced accumulation of Se in the stem and leaves. There generally was more Se in the leaves and stems in treatments with 0.384 mg·L⁻¹ SeO₄²⁻ than in those with 96 mg·L⁻¹ SeO₄²⁻.

Glutathione peroxidase, which reduces hydroperoxides and lipid peroxides, and protects cells from oxidative damage, was discovered to contain Se as an essential component (Rotruck et al., 1973). The functions and protective mechanisms of GPx have been reported mostly in relation to mammals. Selenocysteine has been shown to be incorporated into GPx in green alga, *Chlamydomonas reinhardtii*, which directly indicates that a Se-dependent GPx has been found in the plant kingdom (Fu et al., 2002). In this study, we did not prove that a Se-dependent

Table 4. Sulfur, sulfate, and selenium accumulation in different parts of *B. oleracea* grown with different ratios of SeO₄²⁻ and SO₄²⁻ by adding K₂SeO₄ and Na₂SeO₄ in the nutrient solutions.

MgSO ₄	K ₂ SeO ₄	Na ₂ SeO ₄	Leaf ^a	Stem	Root
mm			Sulfur (mg·g ⁻¹)		
1.0	0	0	5.16 b	3.09 a	3.39 b
1.0	1.0	0	3.17 c	1.98 c	3.03 c
1.0	0.004	0	6.30 a	2.91 b	4.19 a
1.0	0	1.0	3.21 c	2.04 c	3.04 c
1.0	0	0.004	6.22 a	2.87 b	4.31 a
			Sulfate (mg·g ⁻¹)		
1.0	0	0	2.07 b	1.52 b	1.58 b
1.0	1.0	0	0.75 c	0.55 c	0.57 c
1.0	0.004	0	3.13 a	2.23 a	2.78 a
1.0	0	1.0	0.28 c	0.48 c	0.55 c
1.0	0	0.004	3.12 a	2.14 a	2.86 a
			Selenium (μg·g ⁻¹)		
1.0	0	0	ND ^b	ND	ND
1.0	1.0	0	121 b	123 b	64 b
1.0	0.004	0	135 a	164 a	89 a
1.0	0	1.0	115 b	98 c	65 b
1.0	0	0.004	143 a	161 a	91 a

^aMeans within a column followed by the same letter are not significantly different from each other ($P = 0.05$).

^bND = below detection limits.

Table 5. Glucosinolates (GLs) concentrations and glutathione peroxidase (GPx) activity in leaves of *B. oleracea* grown with different concentrations of SeO₄²⁻ and SO₄²⁻ in the nutrient solutions.

SeO ₄ ²⁻	SO ₄ ²⁻	Aliphatic GLs ^a	Indole GLs	Total GLs	Glutathione peroxidase activity
-----mg·L ⁻¹ -----		-----μmol·g ⁻¹ -----			μmol·g ⁻¹ ·s ⁻¹
0	96	29.56 a	1.46 ab	31.01 a	32.6 d
96	96	19.05 b	0.89 cd	19.94 b	63.3 b
0.384	96	29.42 a	1.67 a	31.09 a	54.8 c
96	0	18.98 b	0.54 d	19.51 b	100.6 a
0.384	0	18.74 b	1.08 bc	19.82 b	59.6 bc

^aMeans within a column followed by the same letter are not significantly different from each other ($P = 0.05$).

GPx is found in higher plants; however, GPx activity increased significantly with increasing Se concentration, especially when Se was added to nutrient solutions without S (Table 5). It is possible that addition of Se increased other isozyme activity such as phospholipid hydroperoxide glutathione peroxidase, which was found in citrus (Eshdat et al., 1997).

It was reported that addition of Se decreased glucosinolates concentrations in *B. oleracea* (Charron et al., 2001). Similar results were found in our study, especially when a high concentration of Se was added to the medium whether with S or without S. Interestingly, in the same study, it was also reported that an increase in S uptake resulted in lower GLs concentrations (Charron et al., 2001). However, in this experiment, Se not only enhanced SO_4^{2-} uptake, but also increased GLs concentrations at low ratios of Se to S (1 : 250 on a weight basis).

Conclusion

When *Brassica oleracea* was grown in a nutrient solution with a 1 : 250 ratio of SeO_4^{2-} to SO_4^{2-} , S, SO_4^{2-} , and Se concentrations in plant tissues were higher than in treatments with a 1 : 1 ratio of SeO_4^{2-} to SO_4^{2-} or without SeO_4^{2-} (Tables 3 and 4). Selenium is important because it is related to antioxidant activity and has human health benefits; therefore, brassica vegetables could be used as a means to provide Se to the human diet. The concentrations of Se in the growing medium also affected the concentrations of glucosinolates because SO_4^{2-} is a component of glucosinolates. The breakdown products of glucosinolates include hot and bitter flavor compounds, which are related to the levels of SO_4^{2-} . Brassicaceae can be used as a Se source to deliver beneficial forms of Se for the mammalian diet. Thus, it is important to have a proper balance among Se content, GLs concentrations, and flavor intensity in such vegetables to satisfy consumer acceptance.

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