JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Assessment of the Anticancer Compounds *Se*-Methylselenocysteine and Glucosinolates in Se-Biofortified Broccoli (*Brassica oleracea* L. var. *italica*) Sprouts and Florets

Fabricio William Ávila,[†,‡,∥] Valdemar Faquin,[‡] Yong Yang,[†] Silvio Junio Ramos,[§] Luiz Roberto G. Guilherme,[‡] Theodore W. Thannhauser,[†] and Li Li*[,†,∥]

[†]Robert W. Holley Center for Agriculture and Health, USDA-ARS, Cornell University, Ithaca, New York 14853, United States

[‡]Departamento de Ciência do Solo, Universidade Federal de Lavras, Lavras, MG 37200-000, Brazil

[§]Instituto Tecnológico Vale, Belo Horizonte, MG 30140-130, Brazil

[∥]Department of Plant Breeding and Genetics, Cornell University, Ithaca, New York 14853, United States

ABSTRACT: Broccoli (*Brassica oleracea* L. var. *italica*) is a rich source of chemopreventive compounds. Here, we evaluated and compared the effect of selenium (Se) treatment on the accumulation of anticancer compounds *Se*-methylselenocysteine (SeMSCys) and glucosinolates in broccoli sprouts and florets. Total Se and SeMSCys content in sprouts increased concomitantly with increasing Se doses. Selenate was superior to selenite in inducing total Se accumulation, but selenite is equally effective as selenate in promoting SeMSCys synthesis in sprouts. Increasing sulfur doses reduced total Se and SeMSCys content in sprouts treated with selenate, but not in those with selenite. Examination of five broccoli cultivars reveals that sprouts generally have better fractional ability than florets to convert inorganic Se into SeMSCys. Distinctive glucosinolate profiles between sprouts and florets were observed, and sprouts contained approximately 6-fold more glucoraphanin than florets. In contrast to florets, glucosinolate content was not affected by Se treatment in sprouts. Thus, Se-enriched broccoli sprouts are excellent for simultaneous accumulation of chemopreventive compounds SeMSCys and glucoraphanin.

KEYWORDS: *selenium, Se-methylselenocysteine, glucoraphanin, glucosinolates, broccoli sprout, broccoli floret, chemopreventive agent*

INTRODUCTION

Selenium (Se) is an essential trace element for mammals. It is estimated that between 500−1000 million people worldwide are deficient in Se.[1] Se in the form of selenocysteine constitutes 25 selenoproteins in mammals and plays both structural and enzymatic roles such as glutathione peroxidases in antioxidant functions, thioredoxin reductases in thioredoxin system, and iodothyronine deiodinases in thyroid hormone metabolism.[2] According to the Institute of Medicine of the National Academy, the RDA (recommended dietary allowance) value for Se is 55 μg day^{-1} for adults.[3] Deficiency in Se results in a dramatic reduction in activity of selenoproteins in humans, causing various diseases and disorders such as thyroid gland dysfunction, irreversible brain injury, Keshan disease (cardiomyopathy), decreased immune responses to viral infections, and increased risk to various cancers.[2] Apart from selenoproteins, other (nonprotein) selenocompounds have also been implicated to have important health benefits. Interest has been focused on the anticancer property of monomethylated selenoamino acids.[4−7] Although some selenoproteins may also act as cancer preventive agents,[2,4] studies have shown that monomethylated Se forms, especially *Se*-methylselenocysteine (SeMSCys) that serves as precursor to methylselenol, possess potent anticancer activity.[4,6]

Ingestion of Se-biofortified plant foods has been assumed to be important to decrease Se deficiency in the population.[1] In general, plants take up inorganic forms of Se (selenate, Se^{6+}, or selenite, Se^{4+}) applied in soil, nutrient solution (in hydroponic culture system), or leaves (through foliar spray) and metabolize them. Given that Se is an analogue of sulfur (S), Se uptake and assimilation in plants follow S metabolic pathways.[8,9]

Glucosinolates are a group of S-containing phytochemicals largely found in plants of the order *Brassicales*. More than 120 different types of glucosinolates are known. They are classified as aliphatic (derived from methionine, alanine, leucine, isoleucine, or valine), indole (derived from tryptophan), or aromatic (derived from phenylalanine or tyrosine) glucosinolates.[10] Many of these phytochemical compounds have attracted great attention because of their beneficial effects on human health.[11,12] Glucoraphanin (4-methylsulfinylbutyl glucosinolate) is an aliphatic glucosinolate that is being widely studied due to its superior anticancer activity. This important glucosinolate is metabolized to isothiocyanate sulforaphane, which acts as potent monoinducer of phase II enzymes in inactivating carcinogenic metabolites.[13,14]

Broccoli (*Brassica oleracea* L. var. *italica*) vegetable is known to contain a large amount of glucoraphanin besides other chemopreventive glucosinolates.[14−17] Furthermore, broccoli is part of a small group of agricultural crops that accumulate considerable amounts of SeMSCys when grown in Se-containing soils,[18,19] thereby increasing its anticancer property.[4,20] Florets of mature broccoli plants constitute the main

Received: April 18, 2013
Revised: June 11, 2013
Accepted: June 13, 2013
Published: June 13, 2013

dx.doi.org/10.1021/jf4016834 | *J. Agric. Food Chem.* 2013, 61, 6216−6223

edible part of this crop consumed worldwide. Broccoli sprouts have also been consumed because it is a rich source of bioactive compounds, in particular, glucoraphanin.[15,21] Early works have shown that there is an inverse relationship between Se and glucosinolate accumulation in mature broccoli plants,[19,22−25] but little information is available on broccoli sprouts. In this work, we investigated the possibility to simultaneously enrich SeMSCys and glucoraphanin in broccoli sprout and floret, the two edible tissues. For this, we evaluated the total Se accumulation, SeMSCys synthesis, and glucosinolate profiles in sprouts and florets of broccoli exposed to different forms of inorganic Se (selenate and selenite), as well as the interaction between Se and S. To obtain a general trend of changes in broccoli, five broccoli cultivars were used in this study.

MATERIALS AND METHODS

Plant Materials and Treatments. Seeds of broccoli (*Brassica oleracea* L. var. *italica*) cv. GYPSY were obtained commercially (Harris Seeds, Rochester, NY). For experiments with sprouts, the seeds were sown on two sheets of filter paper (3 mm, Whatman) soaked with treatment solutions in Magenta boxes (Bio-World, Dublin, OH), and grown in a growth chamber with photoperiod of 16/8 h light/dark period at temperature of 22/18 °C day/night. The treatments consisted of one control (Milli-Q water), five Se doses (10, 25, 50, 75, and 100 μM) of either selenate (Na_2SeO_4) or selenite (Na_2SeO_3), and one treatment with 25 μM of each selenate and selenite. A sulfur dosage treatment consisted of three doses (0.1, 1, and 10 mM) of sulfate (Na_2SO_4) in the presence and absence of 50 μM Se (either selenate or selenite). The experiment was conducted in a completely randomized design, and all treatments were performed with three replicates. During sprout growth, an additional 1 mL of either water or solution corresponding to each treatment was added every 24 h. After 7 days, sprouts were harvested, washed with Milli-Q water, dried with paper towels, and frozen in liquid nitrogen. The samples were ground with mortar and pestle to fine powder in liquid nitrogen and dried for 48 h in a freeze-dry system (Labconco FreeZone, 6 L Benchtop Freeze-Dry System model 77520). The lyophilized samples were stored in a desiccator at 4 °C until use.

In addition, two independent experiments (an experiment with broccoli sprouts and an experiment with mature broccoli plants) also were simultaneously carried out in a completely randomized design. Seeds of five broccoli cultivars (Packman, Diplomat, GYPSY, Marathon, and De Cicco) were obtained either commercially (Harris Seeds, Rochester, NY) or as gifts. The experiment with sprouts was conducted as described above, and treatments consisted of five broccoli cultivars exposed to either Milli-Q water (control) or 25 μM Se (supplied as selenate, Na_2SeO_4). The experiment with mature broccoli plants was conducted as following. Seeds were sown into 23-cm-diameter pots filled with 6 dm^3 of soil mix (Metro-Mix 360, Sun Gro Horticulture) in a greenhouse with photoperiod of 14/10 h light/dark period at temperature of 24 °C. During the plant growth period, soil mix from each pot was fertilized with Osmocote according to nutritional needs of the broccoli crop. Treatments consisted of five broccoli cultivars grown without (control) or with selenate (Na_2SeO_4) supply. When plants just initiated floral primordia, six applications (twice per week for three weeks) of 100 mL of 1.5 mM Na_2SeO_4 solution were performed in each pot, resulting in a final dosage of 25 μM Se in each application (considering 6 dm^3 of soil mix per pot). The application of 25 μM Na_2SeO_4 was chosen on the basis of previous studies[7,22,26] and our preliminary tests for its ability to stimulate SeMSCy synthesis without negative effect on plant growth. Plants were harvested individually when heads were fully formed and at market harvest maturity, and the fresh weights of stem, leaves, and florets were evaluated. Floret samples were collected from each plant at harvesting time, washed with Milli-Q water, dried with paper towels, and immediately frozen in liquid nitrogen. Later, these samples were ground with a mortar and pestle to a fine powder in liquid nitrogen, freeze-dried, and stored as described above.

Analysis of Total Se and S Contents by ICP. Total Se and S contents were determined using an ICP trace analyzer emission spectrometer (model ICAP 61E trace analyzer, Thermo Electron, San Jose, CA). Freeze-dried samples of 200 mg were weighed into glass digestion tubes, acid-digested, and measured for their total Se and S levels as described previously.[7] Each sample was analyzed in triplicate.

Quantification of SeMSCys by UPLC. SeMSCys content in broccoli samples was analyzed following the method essentially as described previously.[19,27] SeMSCys from 25 mg of freeze-dried tissues was extracted in 50 mM HCl (20:1, v/w) at 4 °C overnight with continuous shaking. Following centrifugation at 12 000*g* for 12 min for three times to remove cell debris, the extracted SeMSCys was tagged with AccQ·Tag using the AccQ·Tag Ultra UPLC derivatization kit according to the manufacturer's protocol (Waters Corp., Milford, U.S.). The AccQ·Tag derivatives were analyzed on an Acquity UPLC system (Waters Corp., Milford, U.S.) using an AccQ·Tag Ultra column (100 mm × 2.1 mm). The concentration of SeMSCys in the samples was calculated on the basis of peak areas and a calibration curve generated from the commercial standard (Sigma-Aldrich).

Analysis of Glucosinolates by UPLC. Extraction and analysis of glucosinolates were carried out following the protocol described by Ramos et al.[19] Approximately 25 mg of freeze-dried tissues was mixed in 1.4 mL of 80% MeOH preheated to 75−80 °C and vortexed for 10 s. The mixtures were incubated in a water bath for 15 min at 80 °C and centrifuged at 12 000*g* for 12 min twice. The supernatants (0.8 mL) were transferred to DEAE Sephadex A-25 columns. To each column was added 140 μL of sulfatase (15 U, Sigma), and then it was incubated at room temperature overnight in the dark. Desulfo-glucosinolates were then eluted with 0.2 mL of 80% MeOH followed by 0.2 mL of water. The eluents were combined, speedvac-dried, and dissolved in 600 μL of water. The samples were analyzed on an Acquity UPLC system (Waters) using an HSS T3 column (1.8 μm, 100 mm × 2.1 mm) and eluted with a mobile phase consisting of solvent A (water) and solvent B (100% acetonitrile) at a flow rate of 0.65 mL/min for a total of 6 min. Quantification of individual glucosinolate from samples was achieved on the basis of peak areas and a calibration curve constructed from commercial sinigrin standard (Sigma-Aldrich).

Identification of Individual Glucosinolate by LC−MS/MS. LC−MS/MS was performed on an Acquity UPLC system coupled to a Xevo G2 QTof mass spectrometer with a LockSpray source (Waters Corp., Milford, MA, USA). The desulfo-glucosinolates from both sprouts and florets of broccoli were separated on a HSS T3 column (1.8 μm, 2.1 mm × 100 mm, Waters) and then detected by PDA at UV absorbance of 229 nm and the Xevo G2 QTof using a standard ion source. The Xevo G2 QTof was operated in positive ion, data-dependent acquisition (DDA) mode. The MS and MS/MS data were postacquisition lock mass corrected using the monoisotopic mass at m/z 566.2771 Da of the single charged ion of leucine enkephalin. Identification of individual glucosinolate was carried out following the methods as reported.[28,29] Each desulfo-glucosinolate was identified on the basis of the protonated precursor ion masses $(M + H)^+$ and its group-specific fragment ions including the ion with the loss of a sugar group $(M + H - C_6H_{10}O_5)^+$ and the observed metal ion adducts: $(M + Na)^+$ and $(M + K)^+$.

Statistical Analyses. Statistical analyses of the data were performed using variance analysis (ANOVA) at 5% probability level to test for significant difference between treatment means. The values obtained were expressed as means of three replicates with corresponding standard deviations (±SD).

RESULTS AND DISCUSSION

Se Level in Broccoli Sprouts Increases with Increasing Se Dosage. Broccoli is a Se and S accumulator. To examine meticulously the ability of broccoli to accumulate Se, we first performed the dosage analysis in broccoli sprouts. Sprouts of broccoli cv. GYPSY were germinated and grown under different doses of either selenate or selenite. Se was not detectable in broccoli sprouts germinated in Milli-Q water. In contrast, the

total Se content in the broccoli sprouts increased concomitantly with increasing doses of both selenate and selenite from 10 to 100 μM Se in the growth solution (Table 1). Significantly higher levels of total Se content were observed in those sprouts treated with selenate than selenite ($p \leq 0.0001$). For example, the total Se content in 50 μM selenate and selenite treated sprouts was 179 and 98 μg g^{-1} DW, respectively, showing an over 1.8-fold difference in total Se content. This result is consistent with those reported in leaf and floret of mature broccoli plants[7] and others crops[5,27,30−32] showing that selenate is much more effective than selenite in promoting Se accumulation. Noticeably, while the Se content is much lower in selenite treated leaves and florets than selenate treated tissues,[7] the total Se contents on average were only around 35% lower in selenite treated sprouts than selenate treated ones (Table 1).

Table 1. Total Se and SeMSCys Content in 7-Day-Old Sprouts of Broccoli cv. GYPSY Exposed to Different Forms (Selenate and Selenite) and Various Dosages of Se

Se form	Se dose (μM)	total Se (μg g^{-1} DW)[a]	SeMSCys (μg g^{-1} DW)[a]	conversion (%)[b]
selenate (Na_2SeO_4)	10	31.6 ± 1.4	29.2 ± 3.4	40.1
	25	80.0 ± 0.1	62.1 ± 2.7	33.7
	50	178.9 ± 0.9	105.8 ± 2.3	25.7
	75	214.5 ± 0.9	149.2 ± 5.5	30.2
	100	263.2 ± 6.0	157.3 ± 2.2	25.9
selenite (Na_2SeO_3)	10	19.9 ± 6.3	19.2 ± 0.5	41.8
	25	50.0 ± 0.2	68.9 ± 4.5	59.9
	50	97.8 ± 1.3	112.7 ± 5.9	50.0
	75	146.1 ± 2.0	149.8 ± 3.3	44.5
	100	185.3 ± 2.1	167.4 ± 10.6	39.2
ANOVA[c]	Se form	****	NS	
	Se dose	****	****	
	Se form × Se dose	****	NS	
25 μM selenate + 25 μM selenite	50	124.7 ± 2.5[d]	103.9 ± 3.4	36.2

[a]Values are averages of three replicates ± SD (standard deviation). [b]Calculated using only the Se (atomic weight = 79) from SeMSCys (molecular weight = 182). [c]NS and "****" indicate nonsignificance and significance at $p \leq 0.0001$, respectively. [d]Significant difference ($p \leq 0.05$) between 25 μM selenate + 25 μM selenite treatment and 50 μM Se (either selenate or selenite) treatment.

The total Se content in broccoli sprouts simultaneously treated with 25 μM of each selenate and selenite was about intermediary to the values obtained for treatments with 50 μM of selenate and 50 μM of selenite (Table 1). These data suggest that there was no interaction between selenate and selenite in affecting total Se content in the broccoli sprouts. In contrast, previous studies in leaf tissue of broccoli show that supplementation of selenite to selenate treatments inhibits the selenate promoted Se accumulation.[7]

It is noteworthy that 7-day-old broccoli sprouts exposed to higher dose of selenate at 100 μM and selenite at 75 and 100 μM exhibited toxicity symptoms with decreased root growth and purple cotyledons (data not shown). These symptoms were more evident in treatment with 100 μM selenite, showing that selenite was more toxic than selenate to broccoli sprouts.

Selenate and Selenite Are Equally Effective in Promoting SeMSCys Synthesis in Broccoli Sprouts. SeMSCys is a monomethylated form of Se that has been demonstrated to have strong anticarcinogenic activity.[4,6] Among many crops, broccoli plants are known to have the ability to accumulate high levels of SeMSCys when grown in Se-containing environments.[18,19] SeMSCys was not detectable in broccoli sprouts exposed to water (control treatment). Its content increased concomitantly with increasing doses of Se up to 75 μM in growth solution, and it did not differ greatly between treatments at 75 and 100 μM of Se (Table 1). SeMSCys content correlated well with total Se content (Table 1) at dosage up to 75 μM of Se in 7-day-old broccoli sprouts.

Interestingly, unlike the case for total Se content, SeMSCys content in broccoli sprouts was not influenced by the Se form supplied. Both selenate and selenite promote the same levels of SeMSCys synthesis in broccoli sprouts (Table 1). Thus, although selenate treated broccoli sprouts accumulated higher amounts of total Se when exposed to the same doses of selenate and selenite, this additional Se accumulation did not convert into SeMSCys. This result disagrees with that reported for mature broccoli plants in previous studies, where selenate is found to be much effective in stimulating SeMSCys accumulation in leaves and florets than selenite.[7] The mechanisms involved in plant Se metabolism are not similar between the two Se forms. Selenate is taken up by roots and largely transported to shoot where it is assimilated predominantly in leaf chloroplasts, while selenite is largely and rapidly assimilated in roots.[8,9,33] The different localization of metabolism plus that SeMSCys is synthesized in young tissues may explain the equal capacity of broccoli sprouts to synthesize SeMSCys when treated with selenate or selenite as sprouts are young tissues that contain both shoot and root tissues.

Broccoli sprouts exposed to 25 μM of each selenate and selenite synthesized SeMSCys at the level similar to that obtained in either 50 μM of selenate or 50 μM of selenite treatment (Table 1), indicating that there was no interaction between selenate and selenite in promoting SeMSCys accumulation in the broccoli sprouts.

Although both selenate and selenite promoted similar levels of SeMSCys accumulation when sprouts were exposed to same dosage of Se treatment, a much higher rate of conversion of total Se into SeMSCys was observed in selenite-treated sprouts than selenate-treated sprouts (Table 1). The conversion rates in 50 μM selenite and selenate treated sprouts were 50% and 25.7%, respectively, showing that selenite is more efficient than selenate in converting total Se into SeMSCys in broccoli sprouts.

Total Se Levels Are Reduced by High Dosage of Sulfate in Sprouts Treated with Selenate But Unaffected with Selenite. As an analogue of selenate (SeO_4^{2-}), sulfate (SO_4^{2-}) affects Se accumulation in plants.[8,9] To gain a better understanding of the interaction between S and Se, we investigated whether supply of S as sulfate influenced the responses of the sprouts of broccoli cv. GYPSY to selenate and selenite treatments. Supply of S in growth solution improved root development of the 7-day-old broccoli sprouts, which could be verified visually. Increasing S doses affected total Se content of the broccoli sprouts differently depending on the Se form applied (Table 2). A low S dose (0.1 mM) did not dramatically altered total Se content in sprouts treated with 50 μM selenate, but medium (1 mM) and high (10 mM) S dose significantly decreased total Se contents in these sprouts by 20% and 67%, respectively ($p \leq 0.0001$), when compared to sprouts treated only with 50 μM selenate (without S supply)

Table 2. Total Se and SeMSCys Content in 7-Day-Old Sprouts of Broccoli cv. GYPSY Exposed to Different Se Forms (Selenate and Selenite) and Various S Dosages

Se form	S dose (mM)	total Se (μg g^{-1} DW)[a]	SeMSCys (μg g^{-1} DW)[a]	conversion (%)[b]
selenate (50 μM)	0.0	178.9 ± 0.9	105.8 ± 2.3	25.7
	0.1	168.6 ± 2.4	99.5 ± 1.8	25.6
	1.0	142.5 ± 0.3	64.3 ± 3.8	19.6
	10.0	59.9 ± 0.4	30.3 ± 1.8	21.9
selenite (50 μM)	0.0	97.8 ± 1.3	112.7 ± 5.9	50.0
	0.1	112.7 ± 3.1	110.8 ± 3.1	42.7
	1.0	116.7 ± 2.0	114.9 ± 8.6	42.8
	10.0	129.1 ± 2.2	114.3 ± 5.3	38.4
ANOVA[c]	Se form	***	****	
	S dose	****	****	
	Se form × S dose	****	****	

[a]Values are averages of three replicates ± SD (standard deviation). [b]Calculated using only the Se (atomic weight = 79) from SeMSCys (molecular weight = 182). [c]"***" and "****" indicate significance at $p \leq 0.001$ and 0.0001, respectively. Data of treatment without S (0 μM S dose) were copied from the Table 1 (50 μM Se dose; selenate or selenite) for comparison, because both Tables 1 and 2 are part of the same experiment.

(Table 2). The inhibitory effect of S on Se accumulation is also reported in mature broccoli plants treated with selenate.[7] This antagonistic interaction between sulfate and selenate seems to be mostly due to competition of uptake and assimilation system, which has been well documented.[8,9,34−36] On the other hand, no inhibition of total Se accumulation in sprouts grown in 50 μM selenite was observed with increased levels of S supply (Table 2). Increasing S doses slightly increased total Se content. This increase might be due to better root development of sprouts grown under S supplement, which enabled a slight increase in selenite uptake from growth solution. Furthermore, because sulfate transporters do not mediate selenite uptake in plants, S did not compete for transport to interfere with Se uptake. Selenite is taken up by plants through passive diffusion and may use phosphate transporters.[9,35]

SeMSCys Levels Are Reduced by High Dosage of Sulfate in Sprouts Treated with Selenate But Unaffected with Selenite. Sprouts treated with 50 μM of selenate contained 35% and 70% lower SeMSCys content when exposed to medium (1 mM) and high (10 mM) S dose, respectively (Table 2), showing an inhibition of SeMSCys synthesis in selenate-treated sprout. In contrast, SeMSCys content in selenite-treated sprouts was not influenced by increasing S dosage from 0.1 to 10 mM and remained similar between control and 10 mM S supply. The effect of S dosage on SeMSCys accumulation in the 7-day-old broccoli sprouts was linked to that of total Se accumulation. Consistently, a general much higher rate of conversion of total Se into SeMSCys was observed in selenite-treated sprouts than selenate-treated sprouts (Table 2).

Increasing S dosage was also reported to decrease SeMSCys content in leaves of selenate-treated broccoli plant.[7] The decreased SeMSCys synthesis of selenate-treated sprouts in response to high S dose treatments was most likely to be linked with the reduction of Se uptake, which diminished the Se availability in the cells.

Different Capacity of Total Se and SeMSCys Accumulation in Sprouts and Florets of Broccoli. To investigate the general response of broccoli to Se, we examined the total Se content in sprouts and florets of five broccoli cultivars exposed to Se supply. In this experiment, only selenate was used as Se source, because this form increased total Se and SeMSCys content in both broccoli sprouts (Table 1) and mature broccoli plants[7] more effectively than selenite. Both broccoli sprouts and mature broccoli plants were exposed to 25 μM of selenate as this concentration of selenate supplement did not show any toxicity symptom in comparison to control, which was verified by visual inspection of the sprouts and plants, and also by fresh weight of root, shoot, and floret of the mature plants (data not shown). Slight variable values of total Se content in sprouts and florets were found among these broccoli cultivars studied (Table 3). Variation in total Se content among accessions of

Table 3. Total Se and SeMSCys Content in 7-Day-Old Sprouts and Florets of Five Broccoli Cultivars Exposed to 25 μM of Selenate

edible part	cultivars	total Se (μg g^{-1} DW)[a]	SeMSCys (μg g^{-1} DW)[a]	conversion (%)[b]
sprout	Packman	79.1 ± 5.3	67.5 ± 1.8	37.0
	Diplomat	71.7 ± 8.3	73.2 ± 2.9	44.3
	GYPSY	87.5 ± 5.2	72.3 ± 3.0	35.9
	Marathon	85.6 ± 6.7	70.8 ± 4.7	35.9
	De Cicco	58.4 ± 4.6	64.6 ± 5.1	48.0
	average	76.5	69.7	40.2
	ANOVA[c]	*	NS	
floret	Packman	472.4 ± 64.3	102.6 ± 16.0	9.4
	Diplomat	531.1 ± 58.5	126.8 ± 20.2	10.4
	GYPSY	401.1 ± 50.8	87.8 ± 5.4	9.5
	Marathon	483.2 ± 80.0	88.5 ± 11.3	8.0
	De Cicco	557.6 ± 82.6	137.1 ± 24.7	10.7
	average	489.1	108.6	9.6
	ANOVA	NS	*	

[a]Values are averages of three replicates ± SD (standard deviation). [b]Calculated using only the Se (atomic weight = 79) from SeMSCys (molecular weight = 182). [c]NS and "*" indicate nonsignificance and significance at $p \leq 0.05$, respectively.

mature broccoli plants was reported in a previous study.[19] In general, florets (average value of 489 μg g^{-1}) of mature broccoli plants had approximately 6.5-fold higher total Se content than 7-day-old broccoli sprouts (average value of 76 μg g^{-1}) under the treatment conditions. Exposure time, architecture of the root system, and other factors may have contributed to the difference noted in the Se accumulation between broccoli sprouts and florets. Broccoli plants, as well as other brassica crops, are classified as Se secondary accumulators because they can accumulate hundreds μg Se g^{-1} DW when exposed to Se-enriched growth media.[19,37]

SeMSCys content in sprouts and florets of various broccoli cultivars was examined. SeMSCys level was linked to that of total Se content, with some exceptions. SeMSCys content of the sprouts (average value of 70 μg g^{-1} DW) did not vary greatly among cultivars (Table 3). However, florets of both cv. GYPSY and cv. Marathon (average value of 88 μg g^{-1} DW) had lower SeMSCys content than florets of cv. De Cicco (average value of 137 μg g^{-1} DW), while florets of cv. Packman and cv. Diplomat exhibited intermediary SeMSCys content (Table 3). In general, a much high conversion rate of total Se into SeMSCys was observed in sprouts than florets under the current treatment conditions.

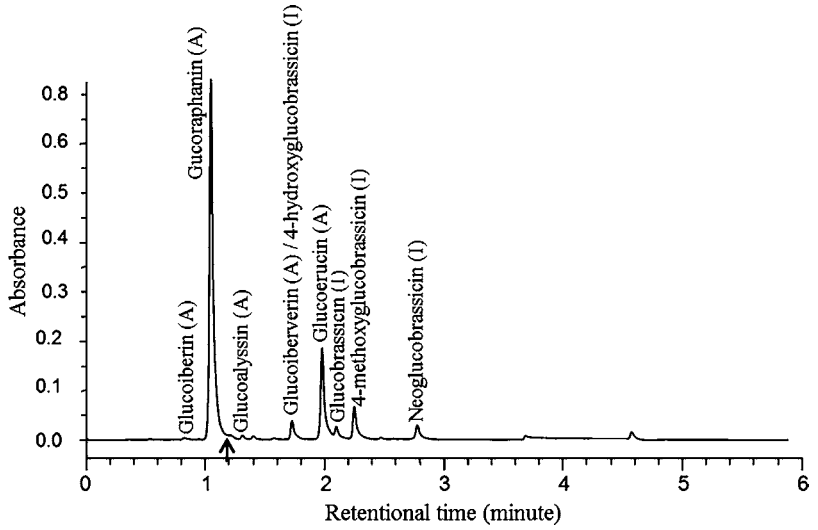

Figure 1. Typical UPLC elution chromatogram of individual glucosinolates (as desulpho-glucosinolates) that were found in the 7-day-old sprouts of broccoli cv. GYPSY. The arrow indicates elution position of the internal standard (sinigrin). Glucoiberverin was eluted slightly earlier than 4-hydroxyglucobrassicin, and their peaks were overlapped. Letters (A) or (I) indicate aliphatic or indole glucosinolates, respectively.

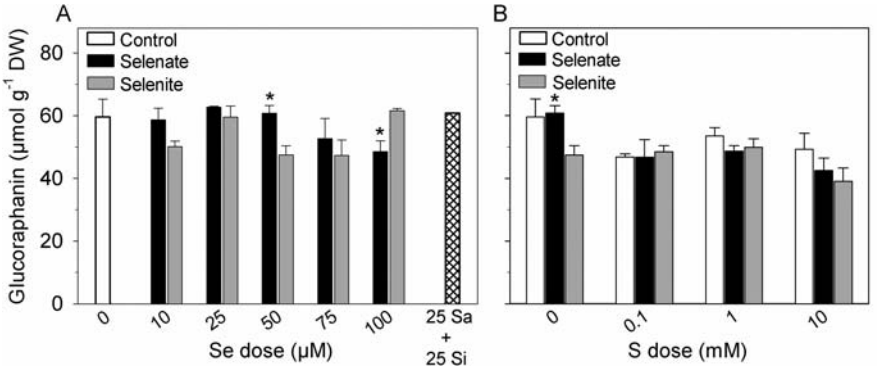

Figure 2. Glucoraphanin content in 7-day-old broccoli sprouts exposed to treatments with various Se dosage (A) and S dosage in the absence (control) and presence of 50 μM Na_2SeO_4 (selenate) or Na_2SeO_3 (selenite) (B). Error bars indicate standard deviation (n = 3). Values marked by asterisks indicate significant difference between selenate and selenite treatments ($p \leq 0.05$). In (A), 25 Sa + 25 Si represents treatment with 25 μM selenate + 25 μM selenite.

Variation in SeMSCys accumulation among accessions of mature broccoli plants was also reported previously.[19] The ability of broccoli specie to synthesize and accumulate considerable amounts of the potent anticancer SeMSCys is related to the activity of several enzymes from the Se transport and assimilation pathway.[5,7−9,19] In this study, florets of mature broccoli plants contained around 1.6-fold higher SeMSCys content than did 7-day-old broccoli sprouts. On the other hand, total Se content of florets was 6.5-fold higher than that of sprouts, indicating a better fractional ability of sprouts to convert inorganic Se into SeMSCys.

Glucosinolate Profile in Sprouts. The fact that ingestion of broccoli reduces the incidence of certain cancer in humans is most likely to be linked to the presence of glucosinolates.[38] In this study, glucosinolates from broccoli were separated by UPLC and identified by MS/MS. A total of nine individual glucosinolates were identified in the 7-day-old sprouts of broccoli cv. GYPSY, which included five aliphatic glucosinolates and four indole glucosinolates. The aliphatic glucosinolates were glucoiberin (3-methylsulfinylpropyl), glucoraphanin (4-methylsulfinylbutyl), glucoalyssin (5-methylsulfinylpentyl), glucoiberverin (3-methylthiopropyl), and glucoerucin (4-methylthiobutyl). The indole glucosinolates were 4-hydroxyglucobrassicin (4-hydroxyindol-3-ylmethyl), glucobrassicin (indol-3-ylmethyl), 4-methoxyglucobrassicin (4-methoxyindol-3-ylmethyl), and neoglucobrassicin (1-methoxyindol-3-ylmethyl) (Figure 1).

The aliphatic glucosinolates glucoraphanin and glucoerucin were the first and second most abundant, constituting approximately 72% and 13% of the total glucosinolate content, respectively, in broccoli sprouts. The sum of the other seven glucosinolates consisted of approximately only 15% of the total glucosinolates. A previous study also reported that glucoraphanin and glucoerucin represented more than 70% and 20% of the glucosinolates found in 3-day-old broccoli sprouts, respectively.[21] Glucoraphanin is a direct precursor of the isothiocyanate sulforaphane, a very potent monoinducer of phase II enzymes that metabolically inactivate carcinogens.[13] Consequently glucoraphanin as well as sulforaphane has been recently largely investigated in broccoli specie.[14,16,39] Glucoerucin also plays an important role for human health,[11] and it may also exhibit chemopreventive properties.[14]

Glucoraphanin Content in Sprouts Is Not Affected by Se and S Treatment. Considering that glucoraphanin is the dominant and most important glucosinolate in broccoli sprouts, we examined the effects of Se and S dosage on the

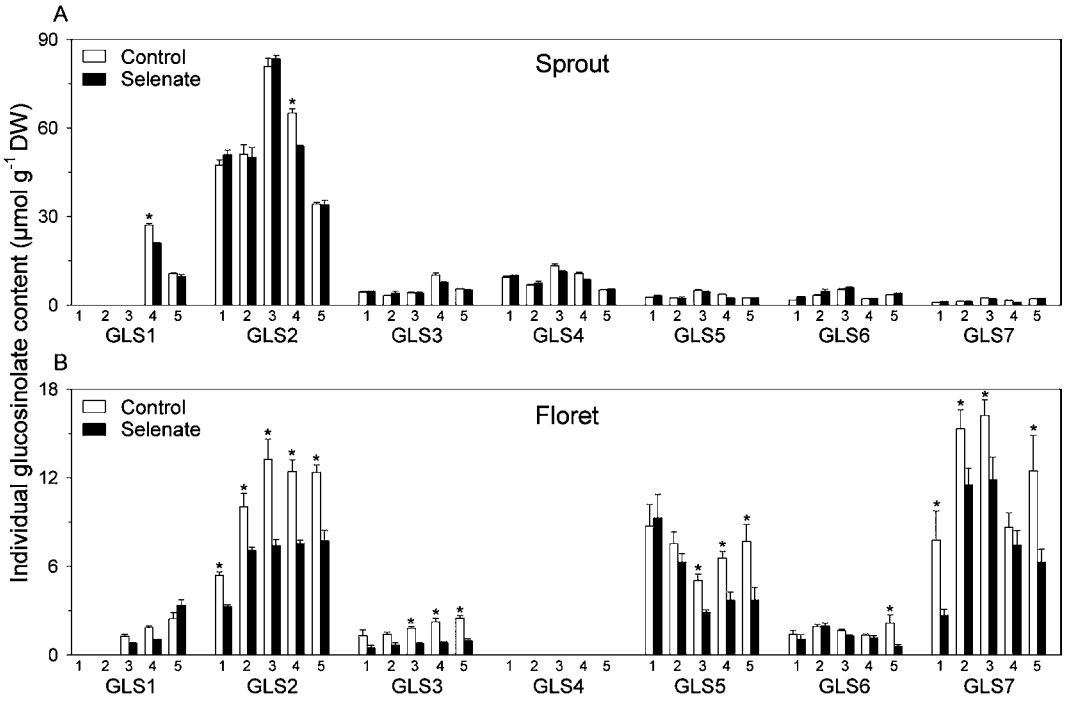

Figure 3. Contents of seven main individual glucosinolates quantified in 7-day-old broccoli sprouts (A) and broccoli floret (B) exposed to 25 μM of selenate. Error bars indicate standard deviation (n = 3). Values marked by asterisks indicate significant difference between control and selenate treatments ($p \leq 0.05$). In the selenate treatments, average Se contents in tissues were approximately 75 μg g^{-1} DW in sprouts and 490 μg g^{-1} DW in florets as detailed in Table 3. Individual glucosinolate: glucoiberin (GLS 1), glucoraphanin (GLS 2), glucoiberverin/4-hydroxyglucobrassicin (GLS 3), glucoerucin (GLS 4), glucobrassicin (GLS 5), 4-methoxyglucobrassicin (GLS 6), and neoglucobrassicin (GLS 7). Numbers 1−5 represent the five broccoli cultivars (Packman, Diplomat, GYPSY, Marathon, and De Cicco, respectively).

glucoraphanin content in 7-day-old broccoli sprouts. In general, glucoraphanin content was not considerably affected by increasing Se dosage in growth solutions (Figure 2A). Similar tendencies were observed for the content of the other eight glucosinolates (data not shown), with a few minor exceptions. Increasing S dosage caused slight (not significant) variations in glucoraphanin content (Figure 2B). The literature has shown that S fertilization may favor glucoraphanin accumulation,[16] although an early study reports negative effects in broccoli sprouts.[15] Glucoerucin content also had variation similar to that of glucoraphanin, while content of glucobrassicin and neoglucobrassicin (both are indole glucosinolates) was significantly ($p \leq 0.05$) increased under high S dose (10 mM) as compared to control (data not shown).

Distinctive Glucosinolate Profiles in Sprouts and Florets of Broccoli. The glucosinolate profiles in sprouts and florets of various cultivars were examined. A similar pattern of individual glucosinolate composition was found among these five broccoli cultivars, and the glucosinolate profiles were substantially different between sprouts and florets (Figure 3). In 7-day-old sprouts of the studied broccoli cultivars, glucoraphanin was the most abundant glucosinolate, accounting an average over 65% of the total glucosinolates accumulated (Figure 3A). On the other hand, florets of mature broccoli plants contained glucoraphanin, glucobrassicin, and neoglucobrassicin as major glucosinolates, accounting on average approximately 30%, 22%, and 34% of the total glucosinolates (Figure 3B). Although glucobrassicin is metabolized to indole-3-carbinol that is proven to be a chemopreventive agent,[40] breakdown products of neoglucobrassicin seem to inhibit the anticancer activity of the glucoraphanin hydrolysis products.[41] Thus, broccoli sprouts appear to be a better source for accumulating glucosinolates with high chemopreventive properties. Further, total glucosinolates accumulated (value that summarizes the accumulation of all individual glucosinolates) were much higher in sprouts than in florets (data not shown).

Selenate Exerts Minimal Effect on Glucosinolate Level in Sprouts But Suppresses Glucosinolate Accumulation in Florets. The effect of Se on glucosinolate accumulation was examined in sprouts and florets of these broccoli cultivars. In general, the individual glucosinolates in the 7-day-old sprouts of the five broccoli cultivars were not affected by 25 μM selenate treatment (Figure 3A). The fact that Se exerts no effect on glucosinolate content in sprouts could be due to the fact that those glucosinolates are pre-existed and not newly synthesized. Indeed, glucosinolates are rich in broccoli seeds and sprouts.[16,38]

In contrast, florets of selenate-treated plants exhibited approximately 36% (an average value among the five cultivars) lower total glucosinolate content than florets of nontreated plants (Figure 3B). This floret result is consistent with previous studies showing suppression of total glucosinolate levels by Se treatment in broccoli and rapid-cycling *Brassica oleracea* plants.[19,22−25] Thus, it appears that Se exerts a considerable effect in reducing the general accumulation of glucoraphanin in florets (Figure 3B). The molecular mechanism that underlies the inhibition of the glucosinolate accumulation in response to Se treatment is not clarified because the metabolic pathways that regulate the biosynthesis of indole and aliphatic glucosinolates in plants are complex.[10] In florets, Se competes with or affects sulfur metabolism that influenced glucosinolate synthesis and accumulation, although supplementation of 25 μM of selenate was found to slightly enhance total S

accumulation in florets of these broccoli cultivars (data not show).

In conclusion, the present results show that Se-enriched broccoli sprouts might be expected to have greater anticancer activity due to the synthesis of SeMSCys without negatively affecting chemopreventive glucosinolate accumulation. Distinctive glucosinolate profiles between broccoli sprouts and florets were observed, and sprouts contained approximately 6-fold higher content of the potent anticancer glucoraphanin than floret of mature broccoli plants. Noticeably, a recent work indicates that *Brassica* crops supplied with selenate have the potential to incorporate Se into selenoglucosinolates.[42] The synthetic Se-containing isothiocyanates are reported to be more potent anticancer inhibitors than their sulfur counterparts.[43] Under the conditions of our experiments, when broccoli sprouts and plants were treated with 25 μM selenate, a serving size of 7.20 g of fresh sprouts or 1.12 g of fresh florets (considering an average moisture content of 90% for both edible parts) will meet the daily requirement of Se, 55 μg day^{-1} for adults.[3] This amount of sprouts provides approximately 50.18 μg of SeMSCys and 17.12 mg of glucoraphanin (molecular weight = 437), and this amount of florets offers approximately 12.16 μg of SeMSCys and 0.32 mg of glucoraphanin. Thus, Se-biofortified broccoli that provides adequate levels of anticancer agents could be an excellent source of chemopreventive compounds.

AUTHOR INFORMATION

Corresponding Author

*Phone: (607) 255-5708. Fax: (607) 255-1132. E-mail: ll37@cornell.edu.

Funding

F.W.Á. is grateful to the CNPq, CAPES, and FAPEMIG from Brazil for financial support and scholarships. This work was supported in part by the USDA-ARS base fund.

Notes

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

The authors declare no competing financial interest.

ABBREVIATIONS USED

Se, selenium; S, sulfur; SeMSCys, *Se*-methylselenocysteine

REFERENCES

(1) Combs, G. F. Selenium in global food systems. *Br. J. Nutr.* **2001**, *85*, 517−547.

(2) Rayman, M. P. Selenium and human health. *Lancet* **2012**, *379*, 1256−1268.

(3) Bendich, A. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. *Nutrition*; National Academy Press: Washington, DC, 2001; Vol. 17, p 364.

(4) Abdulah, R.; Miyazaki, K.; Nakazawa, M.; Koyama, H. Chemical forms of selenium for cancer prevention. *J. Trace Elem. Med. Biol.* **2005**, *19*, 141−150.

(5) Brummell, D. A.; Watson, L. M.; Pathirana, R.; Joyce, N. I.; West, P. J.; Hunter, D. A.; McKenzie, M. J. Biofortification of tomato (*Solanum lycopersicum*) fruit with the anticancer compound methylselenocysteine using a selenocysteine methyltransferase from a selenium hyperaccumulator. *J. Agric. Food Chem.* **2011**, *59*, 10987−10994.

(6) Medina, D.; Thompson, H.; Ganther, H.; Ip, C. Se-methylselenocysteine: a new compound for chemoprevention of breast cancer. *Nutr. Cancer Int. J.* **2001**, *40*, 12−17.

(7) Lyi, S. M.; Heller, L. I.; Rutzke, M.; Welch, R. M.; Kochian, L. V.; Li, L. Molecular and biochemical characterization of the selenocysteine Se-methyltransferase gene and Se-methylselenocysteine synthesis in broccoli. *Plant Physiol.* **2005**, *138*, 409−420.

(8) Pilon-Smits, E. A. H.; Quinn, C. F. Selenium metabolism in plants. In *Cell Biology of Metals and Nutrients*; Hell, R., Mendel, R. R., Eds.; Plant Cell Monographs: Heidelberg, 2010; Vol. *17*, pp 225−241.

(9) Sors, T. G.; Ellis, D. R.; Salt, D. E. Selenium uptake, translocation, assimilation and metabolic fate in plants. *Photosynth. Res.* **2005**, *86*, 373−389.

(10) Sonderby, I. E.; Geu-Flores, F.; Halkier, B. A. Biosynthesis of glucosinolates - gene discovery and beyond. *Trends Plant Sci.* **2010**, *15*, 283−290.

(11) Barillari, J.; Canistro, D.; Paolini, M.; Ferroni, F.; Pedulli, G. F.; Iori, R.; Valgimigli, L. Direct antioxidant activity of purified glucoerucin, the dietary secondary metabolite contained in rocket (*Eruca sativa* Mill.) seeds and sprouts. *J. Agric. Food Chem.* **2005**, *53*, 2475−2482.

(12) Traka, M.; Mithen, R. Glucosinolates, isothiocyanates and human health. *Phytochem. Rev.* **2009**, *8*, 269−282.

(13) Li, Y. Y.; Zhang, T.; Korkaya, H.; Liu, S. L.; Lee, H. F.; Newman, B.; Yu, Y. K.; Clouthier, S. G.; Schwartz, S. J.; Wicha, M. S.; Sun, D. X. Sulforaphane, a dietary component of broccoli/broccoli sprouts, inhibits breast cancer stem cells. *Clin. Cancer Res.* **2010**, *16*, 2580−2590.

(14) Abbaoui, B.; Riedl, K. M.; Ralston, R. A.; Thomas-Ahner, J. M.; Schwartz, S. J.; Clinton, S. K.; Mortazavi, A. Inhibition of bladder cancer by broccoli isothiocyanates sulforaphane and erucin: characterization, metabolism, and interconversion. *Mol. Nutr. Food Res.* **2012**, *56*, 1675−1687.

(15) Aires, A.; Rosa, E.; Carvalho, R. Effect of nitrogen and sulfur fertilization on glucosinolates in the leaves and roots of broccoli sprouts (*Brassica oleracea* var. *italica*). *J. Sci. Food Agric.* **2006**, *86*, 1512−1516.

(16) Gu, Z. X.; Guo, Q. H.; Gu, Y. J. Factors influencing glucoraphanin and sulforaphane formation in Brassica plants: a review. *J. Integr. Agric.* **2012**, *11*, 1804−1816.

(17) Schonhof, I.; Blankenburg, D.; Müller, S.; Krumbein, A. Sulfur and nitrogen supply influence growth, product appearance, and glucosinolate concentration of broccoli. *J. Plant Nutr. Soil Sci.* **2007**, *170*, 65−72.

(18) Roberge, M. T.; Borgerding, A. J.; Finley, J. W. Speciation of selenium compounds from high selenium broccoli is affected by the extracting solution. *J. Agric. Food Chem.* **2003**, *51*, 4191−4197.

(19) Ramos, S. J.; Yuan, Y. X.; Faquin, V.; Guilherme, L. R. G.; Li, L. Evaluation of genotypic variation of broccoli (*Brassica oleracea* var. *italic*) in response to selenium treatment. *J. Agric. Food Chem.* **2011**, *59*, 3657−3665.

(20) Irion, C. W. Growing alliums and brassicas in selenium-enriched soils increases their anticarcinogenic potentials. *Med. Hypotheses* **1999**, *53*, 232−235.

(21) Fahey, J. W.; Zhang, Y. S.; Talalay, P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 10367−10372.

(22) Kim, H. S.; Juvik, J. A. Effect of selenium fertilization and methyl jasmonate treatment on glucosinolate accumulation in broccoli florets. *J. Am. Soc. Hortic. Sci.* **2011**, *136*, 239−246.

(23) Charron, C. S.; Kopsell, D. A.; Randle, W. M.; Sams, C. E. Sodium selenate fertilisation increases selenium accumulation and decreases glucosinolate concentration in rapid-cycling *Brassica oleracea*. *J. Sci. Food Agric.* **2001**, *81*, 962−966.

(24) Barickman, T. C.; Kopsell, D. A.; Sams, C. E. Selenium influences glucosinolate and isothiocyanates and increases sulfur uptake in *Arabidopsis thaliana* and rapid-cycling *Brassica oleracea*. *J. Agric. Food Chem.* **2013**, *61*, 202−209.

(25) Toler, H. D.; Charron, C. S.; Sams, C. E.; Randle, W. R. Selenium increases sulfur uptake and regulates glucosinolate metabolism in rapid-cycling *Brassica oleracea*. *J. Am. Soc. Hortic. Sci.* **2007**, *132*, 14−19.
(26) Lee, J.; Finley, J. W.; Harnly, J. M. Effect of selenium fertilizer on free amino acid composition of broccoli (*Brassica oleracea* cv. Majestic) determined by gas chromatography with flame ionization and mass selective detection. *J. Agric. Food Chem.* **2005**, *53*, 9105−9111.
(27) Ramos, S. J.; Rutzke, M. A.; Hayes, R. J.; Faquin, V.; Guilherme, L. R. G.; Li, L. Selenium accumulation in lettuce germplasm. *Planta* **2011**, *233*, 649−660.
(28) Mellon, F. A.; Bennett, R. N.; Holst, B.; Williamson, G. Intact glucosinolate analysis in plant extracts by programmed cone voltage electrospray LC/MS: performance and comparison with LC/MS/MS methods. *Anal. Biochem.* **2002**, *306*, 83−91.
(29) Zimmermann, N. S.; Gerendas, J.; Krumbein, A. Identification of desulphoglucosinolates in Brassicaceae by LC/MS/MS: comparison of ESI and atmospheric pressure chemical ionisation-MS. *Mol. Nutr. Food Res.* **2007**, *51*, 1537−1546.
(30) Ramos, S. J.; Faquin, V.; Guilherme, L. R. G.; Castro, E. M.; Avila, F. W.; Carvalho, G. S.; Bastos, C. E. A.; Oliveira, C. Selenium biofortification and antioxidant activity in lettuce plants fed with selenate and selenite. *Plant Soil Environ.* **2010**, *56*, 584−588.
(31) Sharma, S.; Bansal, A.; Dhillon, S. K.; Dhillon, K. S. Comparative effects of selenate and selenite on growth and biochemical composition of rapeseed (*Brassica napus* L.). *Plant Soil* **2010**, *329*, 339−348.
(32) Chen, L. C.; Yang, F. M.; Xu, J.; Hu, Y.; Hu, Q. H.; Zhang, Y. L.; Pan, G. X. Determination of selenium concentration of rice in China and effect of fertilization of selenite and selenate on selenium content of rice. *J. Agric. Food Chem.* **2002**, *50*, 5128−5130.
(33) Pilon-Smits, E. A. H.; Hwang, S. B.; Lytle, C. M.; Zhu, Y. L.; Tai, J. C.; Bravo, R. C.; Chen, Y. C.; Leustek, T.; Terry, N. Overexpression of ATP sulfurylase in Indian mustard leads to increased selenate uptake, reduction, and tolerance. *Plant Physiol.* **1999**, *119*, 123−132.
(34) Hawkesford, M. J.; Zhao, F.-J. Strategies for increasing the selenium content of wheat. *J. Cereal Sci.* **2007**, *46*, 282−292.
(35) Li, H. F.; McGrath, S. P.; Zhao, F. J. Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. *New Phytol.* **2008**, *178*, 92−102.
(36) Chang, P.-T.; van Iersel, M. W.; Randle, W. M.; Sams, C. E. Nutrient solution concentrations of Na_2SeO_4 affect the accumulation of sulfate and selenate in *Brassica oleracea* L. *HortScience* **2008**, *43*, 913−918.
(37) Terry, N.; Zayed, A. M.; de Souza, M. P.; Tarun, A. S. Selenium in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **2000**, *51*, 401−432.
(38) Bellostas, N.; Kachlicki, P.; Sorensen, J. C.; Sorensen, H. Glucosinolate profiling of seeds and sprouts of *B. oleracea* varieties used for food. *Sci. Hortic.* **2007**, *114*, 234−242.
(39) Kensler, T. W.; Ng, D.; Carmella, S. G.; Chen, M. L.; Jacobson, L. P.; Munoz, A.; Egner, P. A.; Chen, J. G.; Qian, G. S.; Chen, T. Y.; Fahey, J. W.; Talalay, P.; Groopman, J. D.; Yuan, J. M.; Hecht, S. S. Modulation of the metabolism of airborne pollutants by glucoraphanin-rich and sulforaphane-rich broccoli sprout beverages in Qidong, China. *Carcinogenesis* **2012**, *33*, 101−107.
(40) Brew, C. T.; Aronchik, I.; Kosco, K.; McCammon, J.; Bjeldanes, L. F.; Firestone, G. L. Indole-3-carbinol inhibits MDA-MB-231 breast cancer cell motility and induces stress fibers and focal adhesion formation by activation of Rho kinase activity. *Int. J. Cancer* **2009**, *124*, 2294−2302.
(41) Haack, M.; Lowinger, M.; Lippmann, D.; Kipp, A.; Pagnotta, E.; Iori, R.; Monien, B. H.; Glatt, H.; Brauer, M. N.; Wessjohann, L. A.; Brigelius-Flohe, R. Breakdown products of neoglucobrassicin inhibit activation of Nrf2 target genes mediated by myrosinase-derived glucoraphanin hydrolysis products. *Biol. Chem.* **2010**, *391*, 1281−1293.
(42) Matich, A. J.; McKenzie, M. J.; Lill, R. E.; Brummell, D. A.; McGhie, T. K.; Chen, R. K. Y.; Rowan, D. D. Selenoglucosinolates and their metabolites produced in *Brassica* spp. fertilised with sodium selenate. *Phytochemistry* **2012**, *75*, 140−152.
(43) Emmert, S. W.; Desai, D.; Amin, S.; Richie, J. P., Jr. Enhanced Nrf2-dependent induction of glutathione in mouse embryonic fibroblasts by isoselenocyanate analog of sulforaphane. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2675−2679.