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Evaluation of Genotypic Variation of Broccoli (*Brassica oleracea* var. *italic*) in Response to Selenium Treatment

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Supporting Information

ABSTRACT: Broccoli (*Brassica oleracea* var. *italic*) fortified with selenium (Se) has been promoted as a functional food. Here, we evaluated 38 broccoli accessions for their capacity to accumulate Se and for their responses to selenate treatment in terms of nutritional qualities and sulfur gene expresion. We found that the total Se content varied with over 2-fold difference among the leaf tissues of broccoli accessions when the plants were treated with 20 μM Na_2SeO_4. Approximately half of total Se accumulated in leaves was Se-methylselenocysteine and selenomethionine. Transcriptional regulation of adenosine 5′-phosphosulfate sulfurylase and selenocysteine Se-methyltransferase gene expression might contribute to the different levels of Se accumulation in broccoli. Total glucosinolate contents were not affected by the concentration of selenate application for the majority of broccoli accessions. Essential micronutrients (i.e., Fe, Zn, Cu, and Mn) remained unchanged among half of the germplasm. Moreover, the total antioxidant capacity was greatly stimulated by selenate in over half of the accessions. The diverse genotypic variation in Se, glucosinolate, and antioxidant contents among accessions provides the opportunity to breed broccoli cultivars that simultaneously accumulate Se and other health benefit compounds.

KEYWORDS: Selenium, glucosinolate, broccoli, *Brassica oleracea* var. *italic*, germplasm, antioxidant, gene expression

INTRODUCTION

Selenium (Se) is an essential micronutrient for animals and humans and has been implicated to have important health benefits. In addition to its roles in improving immune function, in reducing viral infection, and in slowing down the aging process,[1] Se at supranutritional levels has been shown as a cancer preventative agent in reducing the incidence of prostate, colon, and lung cancer.[2] Although the SELECT trial (the Selenium and Vitamin E Cancer Preventive Trial) using selenomethionine (SeMet) shows that this particular form of Se does not prevent prostate cancer in relatively healthy men,[3] other studies have demonstrated that some methylated selenoamino acids can offer chemoprotectative effects against cancer with low body accumulation to avoid Se toxicity.[4−6] Because of large areas of soil in many parts of the world that contains low levels of Se and consequently low levels of Se supply in food, Se deficiency is a widespread problem. Thus, increasing Se intake through foods, especially cultivated Se-accumulating crops, such as Brassica vegetables, is the most effective and safe way in providing functional forms of Se and in reducing Se deficiency in the world.[4,7]

Glucosinolates (GLS) are sulfur (S)-containing secondary metabolites that are present primarily in the Brassicaceae family.[8] A substantial amount of evidence has linked ingestion of GLS-rich diets with reduced risk of cancer.[9] The cancer preventive effect of GLS is due to the breakdown products of GLS, which act as inducers of phase II detoxification enzymes in protecting against cancer.[9,10]

Broccoli (*Brassica oleracea* L. var. *italica*) contains multiple nutrients including vitamins and minerals, as well as many health beneficial secondary metabolites and antioxidants.[11] The consumption of broccoli has been steadily increasing for the past decade, due in part to its health-promoting properties.[12] Broccoli can accumulate high levels of functional forms of Se[13] and is also a rich dietary source of GLS.[11] Thus, broccoli has been promoted to serve as a functional food against cancer.[4,14] Given that Se is an analogue of S and shares S uptake and assimilation pathways,[15,16] antagonistic relationships between Se and S metabolisms are generally reported when plants are exposed to high levels of selenate or sulfate.[13,17] Thus, it is not surprising to find that high levels of Se fertilization result in low levels of GLS accumulation.[18,19] However, a number of studies have shown a synergistic instead of antagonistic relationship between Se and S metabolism under certain conditions in plants.[20,21] Indeed, Hsu et al.[22] have recently shown that it is possible to produce Se-fortified broccoli that simultaneously accumulates high levels of Se and GLS.

Only few studies have investigated the genetic variation of crop species in accumulation of Se.[20,23] A large variation in GLS content in Brassica vegetables has been shown,[24,25] and genetic variation was reported to be the most important factor in determining GLS content.[14] However, genotypic variation of broccoli accessions in accumulating Se and its effect on GLS and other nutrient contents have not been studied. Thus, the aim of this study was to evaluate the genotypic variation of broccoli germplasm in response to selenate treatment. The effects of selenate treatment on total Se accumulation and GLS synthesis,

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as well as on plant growth, total S level, Se-containing amino acid contents, macro- and micronutrient concentrations, antioxidant levels, and the expression of genes involved in Se/S uptake and assimilation were examined. Exploiting genotypic variation is likely to be effective in providing information for breeding varieties that simultaneously accumulate high levels of Se and GLS and contribute nutritional improvements in broccoli.

MATERIALS AND METHODS

Plant Materials and Experimental Design. Thirty-eight available accessions of broccoli were obtained from the Plant Genetic Resources Unit at Geneva, NY. Seeds of each accession (Table 1) were planted and grown in a greenhouse as described previously.[20] Uniform 3 week old young seedlings were selected and transplanted into 2.2 L pots containing Hoagland nutrient solution with 40% ionic strength.[26] The seedlings from each accession were divided into two treatment groups and each with four replicates. One week after transplantation, one group of these plants was exposed to 20 μM Na_2SeO_4 (Sigma-Aldrich), and the other as control sets received no Se treatment. The nutrient solutions were changed twice each week. After 2 weeks of Se exposure, a total of 304 plants (38 × 2 × 4; accessions × treatments × repeats) were harvested individually, and the fresh weights of aerial part were weighed. The young leaf sample from each plant was either dried for elemental analysis or immediately frozen in liquid nitrogen and stored at −80 °C for RNA extraction and metabolite analyses. As the broccoli accessions develop florets at different times, we chose to do the comparative analyses on leaf samples that had the same developmental stage. To explore whether the data obtained from leaf samples would be applicable to florets, some accessions were allowed to grow until florets and floret samples were analyzed for the correlation of accumulation of metabolites between leaves and florets of broccoli.

Elemental Analysis by an Inductively Coupled Plasma (ICP) Trace Analyzer. Dried tissues (approximately 200 mg) were weighed and acid-digested in 2.0 mL of HNO_3 with 2.0 mL of $HClO_4$ at 120 °C for 1 h in tubers and then at 220 °C until $HClO_4$ fumes were observed. Total Se and other element contents were determined by ICP trace analyzer emission spectrometer (model ICAP 61E trace analyzer, Thermo Electron, San Jose, CA) as described previously.[13]

Quantification of Se-Methylselenocysteine (SeMSCys) and SeMet. Extraction, AccQTag derivatives, and analysis of SeMSCys and SeMet were performed according to the method described previously[20] with slight modifications. Leaf tissue materials (100 mg) were extracted overnight at 4 °C in 50 mM HCl (10:1, v/w) and centrifuged at 12000*g* to remove cell debris. AccQTag derivatized SeMSCys and SeMet were separated on an AccQ.Tag Ultra column (100 mm × 2.1 mm) using an Acquity ultra performance liquid chromatography (UPLC) system (Waters). SeMSCys and SeMet levels were calculated based on peak areas, in comparison with calibration curves generated from commercial standards (Sigma-Aldrich).

Analysis of Total GLS Level. Extraction and analysis of GLS were performed as described[27] with some modifications. The frozen samples (200 mg) were crushed into powder at a speed of 4.0 m s^{-1} in a FastPrep FP120 instrument (Q-BIOgene) and extracted in 1.5 mL of 80% MeOH preheated to 75−85 °C. After incubation at 80 °C for 15 min, the extracts were centrifuged at 12000*g* for 3 min, and the supernatants were added to the DEAE Sephadex A-25 columns. Sulfatase (15 U, Sigma) was added to each column and left at room temperature in the dark overnight. Desulphoglucosinolates were eluted with 80% MeOH and water, Speed-vac-dried, and resuspended in 300 μL of water. The sample (5 μL) was analyzed using an Acquity UPLC system on a HSS T3 column (1.8 μm, 100 mm × 2.1 mm) (Waters) and eluted by a mobile phase consisting of solvent A (water) and solvent B (100% acetonitrile) with a linear gradient from 0 to 90% of solvent B at a flow rate of 0.65 mL min^{-1} for a total 4 min. Quantification of the total glucosinolate contents in samples was achieved by comparison of the total peak areas with a calibration curve constructed from commercial sinigrin standard (Sigma-Aldrich).

Table 1. Broccoli (*B. oleracea* var. *italic*) Accessions Used in This Study

ID	name	GRIN[a] accession number
1	Green Sprouting Early CT Strain	G 21111
2	Purple Sprouting Late	G 28836
3	Purple Sprouting Late Improved	G 28837
4	White Sprouting Improved	G 28840
5	Cavolo Ramoso Calabrese Precoce	G 28848
6	Broccoli Neri	G 28852
7	Broccolo Natale Pied Grande Liscio	G 28853
8	Cavolo Broccolo Di Sarno	G 28855
9	Cavolo Broccolo Marzullo	G 28873
10	Cavolo Broccolo Natalino	G 28880
11	Broc 3	G 30009
12		G 30014
13	Packman F1	G 30778
14	Cavolo Broccolo Precoce	G 30928
15	Atlantic	G 30929
16	Cavolo Broc Verde Calabrese Precoce	G 30933
17	Cavolo Broccolo Di Sarno	G 30934
18	Purple Sprouting Early	G 30937
19	Persius F1	G 32206
20	Wintergarden F1	G 32209
21	Purple Sprouting Xmas	G 28832
22	Purple Sprouting Early	G 28833
23	Late Purple Sprouting	G 28835
24	White Sprouting Early	G 28838
25	Cavolo Broccolo Natalino Di Sarno	G 28854
26	Cavolo Broccolo Bronzino Di Albenga	G 28859
27	Cavolo Broccolo Verde Calbrese	G 28865
28	Cavolo Broccolo Frevarota	G 28872
29	Cavolo Cavolina Rizza	G 28878
30	Pinnacle F1	G 30414
31	Premium Crop F1	G 30415
32	Zeus F1	G 30416
33	Xmas Purple Sprouting	G 30775
34	Broccoli Grande Precoce	G 30931
35	Cavolo Broccolo Ramoso Calabrese	G 30932
36	Big Sur F1	G 32208
37	De Cicco	G 32213
38	Romano	PI 231210

[a] GRIN = Genetic Resources Information Network (http://www.ars-grin.gov/npgs/searchgrin.html).

Ferric Reducing Antioxidant Power (FRAP) Assay. Extraction and analysis of total antioxidant activity were performed as described[28] with some modifications. The frozen samples (100 mg) were crushed and extracted in 1.0 mL of Milli Q water, followed by centrifugation at 12000*g* for 10 min. An aliquot of 5 μL of supernatant was added to 2 mL of FRAP reagent and incubated at 37 °C for 30 min. The absorbance of the reaction mixture at 593 nm was measured before and after 30 min of incubation. Quantification of the total antioxidant activity was expressed as mmol Fe^{2+} g^{-1} of fresh weight. All of the samples were measured in triplicate.

Table 2. List of Primers Used in This Study

genes	forward primer (5′-3′, top) reverse primer (5′-3′, bottom)	PCR size (bp)	GenBank accession
BoActin	CTGTGACAATGGTACCGGAATG	62	AF044573
	ACAGCCCTGGGAGCATCA		
BoSultr1;1	AAGCAGTTCATGCTCGGTCT	150	AJ311388
	AGCGAGCTTAGCGTATCCAA		
BoSultr1;2	GATTCTGCTGCAAGTGACGA	126	AJ416460
	ACGCGAATGATCAAGATTCC		
BoSultr2;1	GTTTCGCTTCTGCTTTCGTC	186	DQ091257
	AGCCATGAACCCAACAAGAG		
BoAPS1	AGACGACGAGCAAAAGGCTA	145	FN641890
	GGTTGTACCCCATGTTCTGG		
BoAPS2	CGTTGACACTCCCATCACTG	199	FN641891
	TTGATCGGAGGAAGAGGATG		
BoAPS3	TGAAACAGCACGAGAAGGTG	197	FJ626851
	ACGTTTCTCCACAGGGTGAC		
BoAPR1	TGAGGAGCTAGCGAAGAAGC	110	FN641892
	CGTCTTCGGCTCCACTAAAG		
BoAPR2	TCTTTGGTTACCCGTGCTTC	107	TC111450
	GGAGAAGCCTCTTCCAGCTT		
BoAPR3	TTCCCTTCCTCAGAGCTCAA	149	TC135211
	TCCTTTGCAACTGACTGCAC		
BoSAT1;1	ATATCCATCCAGCAGCGAAG	148	TC130359
	CTGTCTCCGCAAGCTTTACC		
BoSAT2;1	AAGAGACCCAGCTTGCGTTA	122	TC117378
	GCAAAGCGAGGATCTTTCTG		
BoSAT3;1	TCATGGAACTGGAGTGGTCA	126	TC155320
	CTTCGCCTATTTTGGGATGA		
BoSMT	AGATTCTGAAGAAGCGGCCTA	178	AY817737
	CCACCCACTCCTTCCGTTCAG		
BoHMT1	TTCAGGAATGCCTTGAAACC	169	DQ679980
	TTAGCTTTTCCGTCCCACAC		

RNA Extraction, Reverse Transcription, and Quantitative PCR Analysis. Total RNA from leaves of broccoli plants was extracted and reverse-transcribed into cDNA according to the procedure described previously.[20] A quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed using the SYBR Green Universal Master Mix (PE Applied Biosystems) with gene-specific primers as listed in Table 2. The PCR program used was 50 °C for 10 min and 95 °C for 2 min, followed by 40 cycles of denaturation for 15 s at 95 °C and annealing/extension at 60 °C for 1 min. Analysis of all gene expression was run in triplicate with two biological repeats.

Statistical Analysis. All results were analyzed using analysis of variance (ANOVA), and significantly different means between treatments were separated with the Student's *t* test at the 0.05 significance level of probability. All results were expressed as means with corresponding standard errors.

RESULTS AND DISCUSSION

Broccoli Growth. In our previous study, we have shown that broccoli plants accumulate approximately 10-fold higher levels of total and organic forms of Se in shoot when treated with selenate than selenite.[13] At the concentration of 20 μM Na_2SeO_4, broccoli accumulates substantial levels of Se without negative effects on plant growth.[13] Thus, this concentration of selenate was chosen to evaluate genotypic variation of broccoli accessions in response to selenate treatment. As shown in Figure 1, broccoli accessions exhibited different shoot fresh weights. No significant difference in growth was observed between selenate-treated and nontreated plants for all accessions (Figure 1), indicating a capacity of complete tolerance at the Se dosage used.

Total Se and S Contents. Broccoli as well as some other Brassica vegetables belongs to Se secondary accumulator, which can accumulate substantial levels of Se when grown on media containing low to moderate levels of Se. When broccoli accessions grew in control nutrient solution without selenate supplement, Se was undetectable. When plants were treated with 20 μM Na_2SeO_4, different levels of Se were accumulated in the broccoli germplasm (Figure 2A). The range of Se accumulation varied from 801.2 to 1798.4 μg g^{-1} dry weight, showing an over 2-fold difference in total Se levels between broccoli accessions. While wheat (*Triticum* spp.) germplasm exhibits no significant difference in accumulating Se,[23,29] lettuce (*Lactuca sativa*) accessions show over 2-fold difference.[20] In comparison with those Se nonaccumulating crops, broccoli accumulated hundreds fold more Se, which makes it an excellent supplemental food source in areas of low Se intake. In our previous study, we examined Se accumulation in leaves and florets following different concentrations of Se treatment. We have shown that the levels of total Se

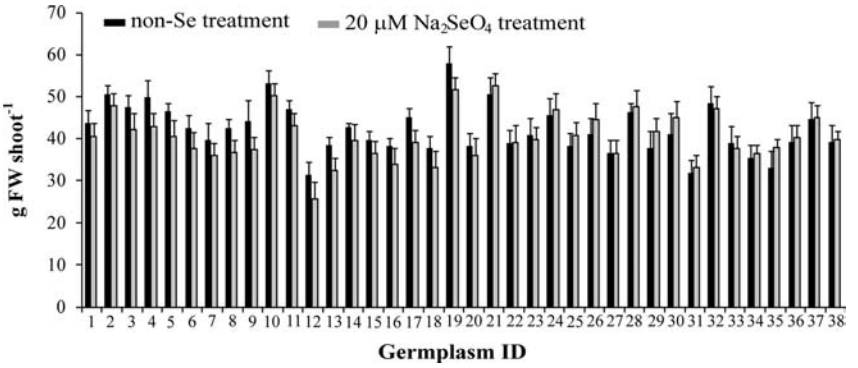

Figure 1. Shoot biomass of 6 week old broccoli (*B. oleracea* var. *italic*) accessions without and with 20 μM Na_2SeO_4 treatment for 2 weeks. The germplasm numbers correspond to the ID numbers in Table 1. Error bars indicate standard error of the mean (n = 4).

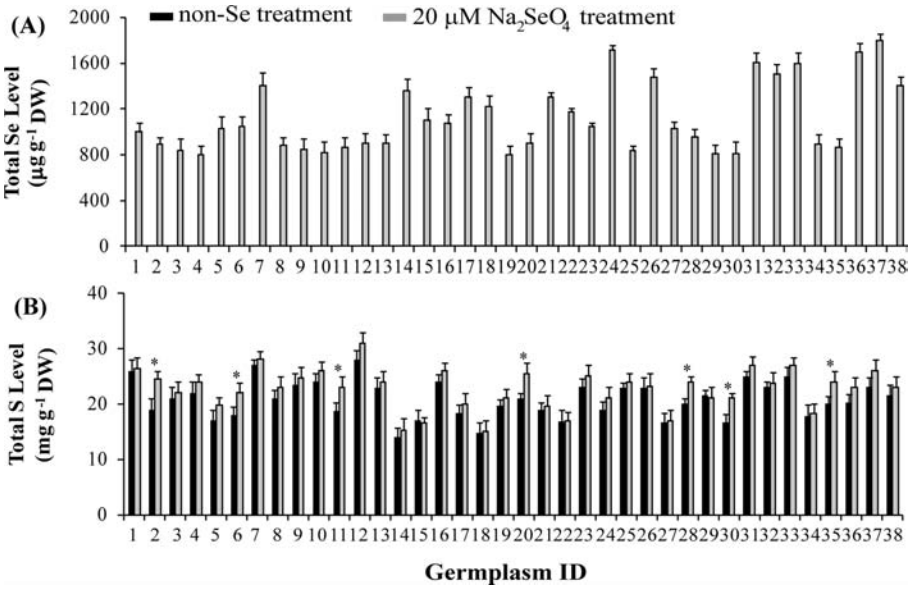

Figure 2. Effect of selenate treatment on total Se (A) and S (B) accumulation in shoot tissues of broccoli (*B. oleracea* var. *italic*) accessions. Values marked by asterisks indicate significant difference between non-Se-treated and Se-treated samples ($p < 0.05$). Error bars indicate the standard error of the mean (n = 4).

accumulation in leaves correlate well with those in florets of broccoli.[13] Thus, variation of Se levels in leaves should be a good indication of levels in florets of broccoli accessions, and a 2-fold change of Se levels in broccoli should exert a different impact in providing dietary Se.

Because Se and S share the same uptake and assimilation pathways in plants,[16] the S levels were also examined in these broccoli accessions following selenate treatment. The total S content varied among accessions, which appears to have no correlation with plant growth. In all accessions, S levels were not decreased when plants were exposed to 20 μM Na_2SeO_4, indicating no antagonistic relationship between Se and S accumulation at the dosage used. Among the accessions, seven lines (i.e., 2, 6, 11, 20, 28, 30, and 35) instead showed enhanced S levels with an average of 19.6% increase when Se was applied in nutrient solution (Figure 2B). This result implies a synergism relationship between Se and S metabolism at the dosage in these accessions, suggesting a different capacity of them in uptaking and metabolizing S in the presence of Se. The data also agree with other studies showing that selenate application at suitable levels could promote S accumulation in some plants.[17,20,22]

Accumulation of Organic Forms of Se. SeMSCys has been reported to be an effective form of Se in serving as a chemopreventive agent.[5,30,31] Among various crop species, broccoli accumulates a high level of Se with SeMSCys and SeMet that account for the majority of organic forms of Se.[13,31] To examine the accumulation of organic Se species in these broccoli accessions, we extracted and analyzed SeMSCys and SeMet contents. While SeMSCys and SeMet were absent in plants without Se treatment, they are readily detectable when plants were grown in Se-containing nutrient solution. The levels of SeMSCys and SeMet varied among accessions (Figure 3). A general correlation of SeMSCys with total Se accumulation was observed. Analysis of the amounts of organic Se species accumulated in these broccoli accessions revealed that approximately 43% of the total Se accumulation was SeMSCys and SeMet, which represented an average of 26 and 17%, respectively, of the total Se levels following 20 μM Na_2SeO_4 supplement for 2 weeks. Our previous

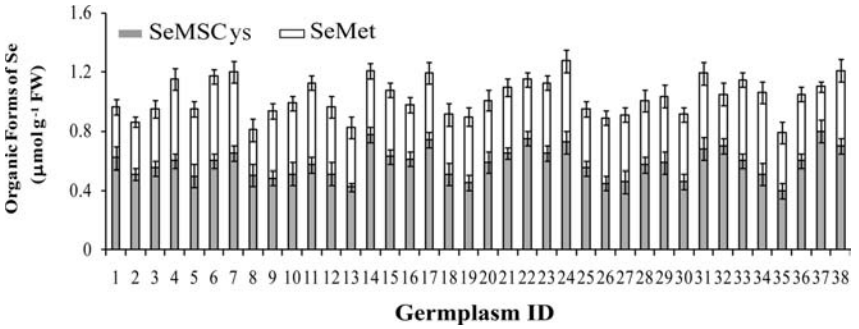

Figure 3. SeMSCys and SeMet accumulation in shoot tissues of broccoli (*B. oleracea* var. *italic*) accessions treated with 20 μM Na_2SeO_4 for 2 weeks. Error bars indicate the standard error of the mean (n = 4).

study has shown that the SeMSCys level is highly correlated between leaves and florets in broccoli, and its accumulation increases with increased levels of selenate supply.[13] We also observed that SeMSCys content increased with increasing length of selenate exposure (unpublished data). Thus, a high percentage of conversion into organic Se species may be obtained by extending the period of Se treatment. Higher percentages of SeMSCys and SeMet accumulation also have been reported in a few Se-accumulating crop species, such as radish (*Raphanus sativus*), garlic (*Allium sativum*), onions (*Allium cepa*), and leeks (*Allium ampeloprasum* var. *porrum*).[31,32]

Other Essential Nutrient Accumulation. It is well established that Se affects S uptake and assimilation. To investigate whether exposure of broccoli accessions to selenate influenced nutrient balance and whether there was genotypic variation in their responses, we evaluated the levels of macronutrients (Ca, Mg, and P) and micronutrients (Fe, Zn, Cu, and Mn) in plants without and with selenate treatment. No significant differences in these macronutrient contents were observed between Se-treated and nontreated plants at the dosage applied (data not shown). In contrast, micronutrient accumulation in leaf samples of broccoli accessions responded differently to selenate treatment. Approximately half of accessions remained unchanged, and the other half showed decreased levels of micronutrients when exposed to 20 μM Na_2SeO_4 ($P < 0.05$; Figure 4). The decreased levels of micronutrient accumulation upon Se treatment have been reported for Mn, Fe, and Zn in kale (*B. oleracea* Acephala Group)[33] and for Fe and Mn in leaf tissue of rapid-cycling *B. oleracea*.[34] The uptake of Mn, Cu, Fe, and Zn is reported to be inhibited by an increasing level of Se treatment in *Sinapis alba* seedlings.[35] It is clear that by examining genotypic variation of broccoli accessions, different capacity in uptake and accumulation of these essential micronutrients were found among germplasm when treated with selenate.

Glucosinolate Levels. Broccoli along with other Brassica vegetables contains specific phytochemicals of GLS, which have been proposed to confer protection against certain degenerative diseases such as cancer.[9,36] Glucosinolates are S-containing metabolites; thus, their levels of accumulation in plants are influenced by Se status. A high concentration of Se can suppress GLS accumulation in plants.[18,19] For simultaneous enrichment of GLS and Se level, we evaluated GLS levels in broccoli accessions and assessed genotypic variation of GLS levels in response to selenate treatment. As shown in Figure 5, an approximately 3-fold variation in GLS levels was observed between leaf samples of broccoli accessions. Similarly, much wide variation in GLS content in broccoli florets has been reported.[25] While 13 accessions had reduced levels of total GLS content when plants were exposed to 20 μM Na_2SeO_4, the majority of broccoli accessions contained similar levels of total GLS following Se treatment. A recent study shows that selenate application even at 40 μM does not affect total GLS level in a broccoli variety.[22] We examined total GLS in florets of eight accessions that had similar stages of floret development. As the case for Se accumulation, a high degree of correlation (Pearson R = 0.88, $p < 0.05$) in total GLS contents between leaves and florets was observed (Supporting Information, Figure S1). Clearly, genotypic variation in GLS contents among broccoli accessions in response to Se treatments was present. Thus, it is possible to select and breed cultivars with high levels of Se accumulation without a negative effect on GLS contents.

Total Antioxidant Activity. Increasing antioxidant levels in food crops can reduce the risk of a number of chronic diseases.[37] Broccoli is a rich source of antioxidants comprising ascorbic acid, tocopherol, phenolics, and carotenoids. Se often acts as an antioxidant in plants.[38] To examine the response of broccoli germplasm to selenate treatment in inducing antioxidant production, the total antioxidant activity was measured. Various levels of antioxidants were observed in broccoli accessions as shown in other studies.[39] Selenate treatment increased the total antioxidant capacity in over half of the germplasm (Figure 6). Some of the accessions, that is, 8, 11, 20, 23, 25, 27, 32, and 34, exhibited an approximately 2-fold enhancement. A high degree of correlation (Pearson R = 0.95, $p < 0.05$) in total antioxidant capacity, and its response to Se stimulation was also obtained between leaves and florets of the selected accessions tested (Supporting Information, Figure S2). The increased total antioxidant activity in response to Se treatment has been reported in a number of Se-enriched plants, such as in garden cress (*Lepidium sativum*)[40] and lettuce.[41] The diverse response of broccoli germplasm in promoting antioxidant production along with varied Se levels offers the opportunity for enhancing their health-promoting properties.

Expression of Genes Involved in Se Metabolism. Plants take up selenate, the major soluble form of Se in soil, via sulfate transporters and metabolize it through the S assimilation pathway.[15,16] To gain a better understanding of Se assimilation in broccoli, we investigated the transcript levels of a number of key genes in S/Se uptake and assimilation pathways. As shown in Figure 7, no significant difference in expression was observed for all genes examined between the eight selected accessions that accumulated high and low levels of Se. While Se treatment did not dramatically alter the transcript levels for most of the genes examined, significantly high expression of *ATP sulfurylase 1*

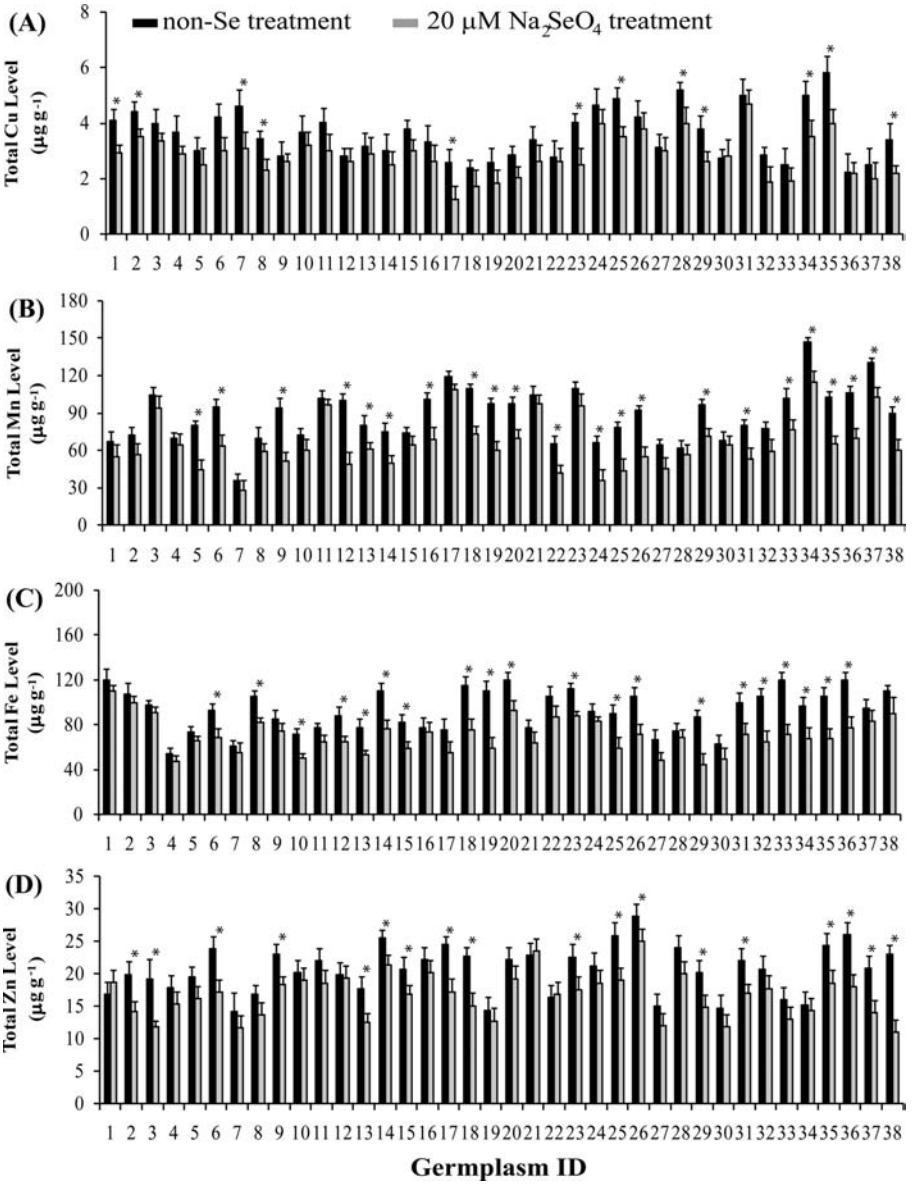

Figure 4. Micronutrient Fe, Zn, Cu, and Mn accumulation in shoot tissues of broccoli (*B. oleracea* var. *italic*) accessions treated with and without 20 μM Na_2SeO_4. Values marked by asterisks indicate significant difference between non-Se-treated and Se-treated samples ($p < 0.05$). Error bars indicate the standard error of the mean ($n = 4$).

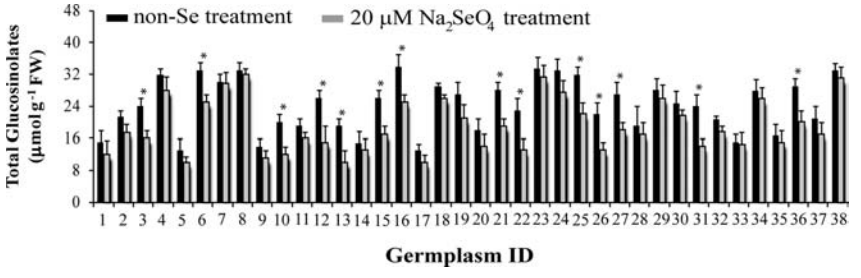

Figure 5. Total glucosinolate levels in shoot tissues of broccoli (*B. oleracea* var. *italic*) accessions without and with selenate treatment. Values marked by asterisks indicate significant difference between non-Se-treated and Se-treated samples ($p < 0.05$). Error bars indicate the standard error of the mean ($n = 4$).

(*APS1*), a plastidic isoform of *APS*, and *selenocysteine Se-methyltransferase* (*SMT*) were observed in those accessions that accumulated high levels of Se.

ATP sulfurylase is the first and rate-limiting enzyme in the S assimilation pathway.[42] Selenate application significantly increased the expression of *APS1* in those accessions that accumulated more

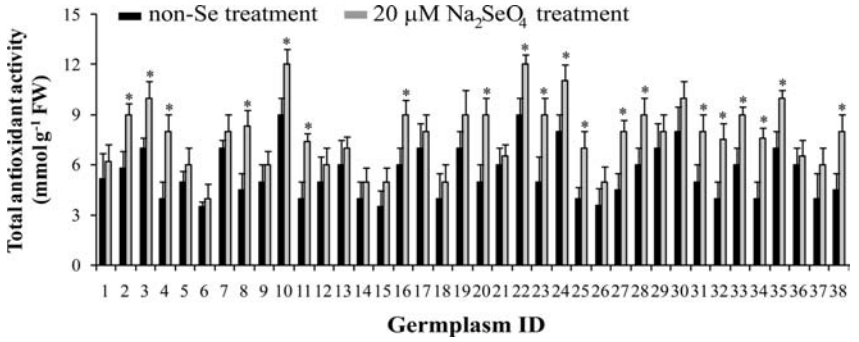

Figure 6. Total antioxidant levels in shoot tissues of broccoli (*B. oleracea* var. *italic*) accessions without and with selenate treatment. Values marked by asterisks indicate significant difference between non-Se-treated and Se-treated samples ($p < 0.05$). Error bars indicate the standard error of the mean ($n = 3$).

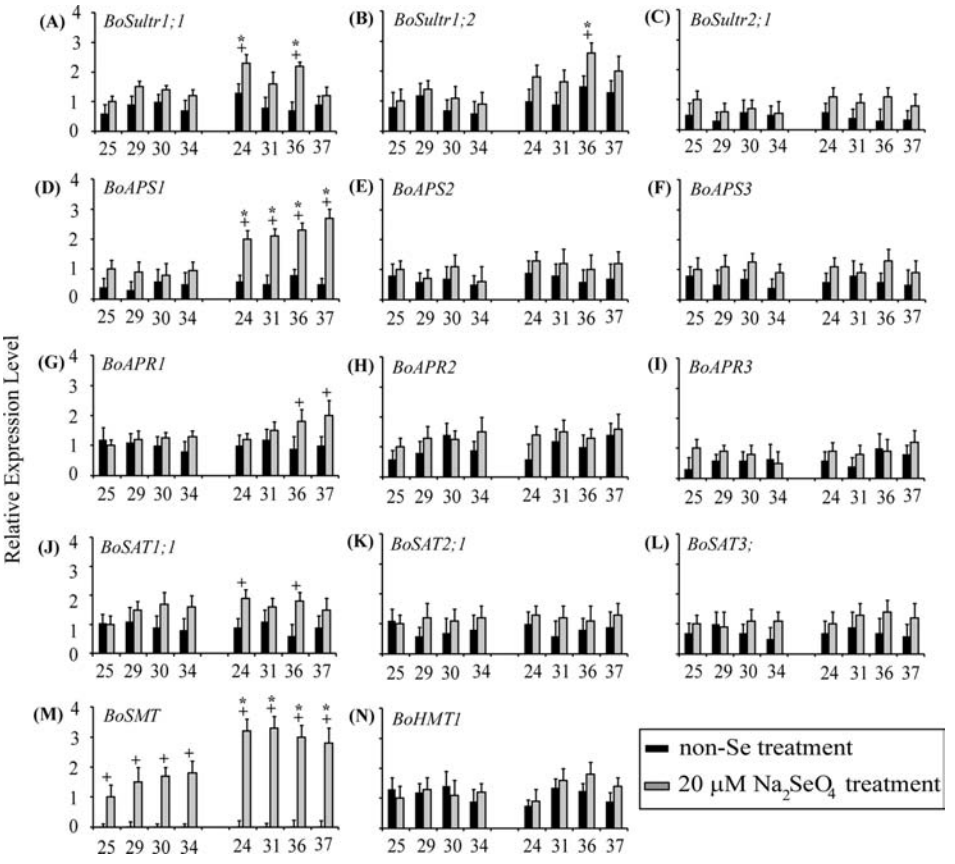

Figure 7. Relative expression of genes involved in Se/S transport and assimilation in shoot tissues of broccoli (*B. oleracea* var. *italic*) accessions. Transcript levels of genes in broccoli accessions with low (25, 29, 30, and 34) and high (24, 31, 36, and 37) Se contents upon 20 μM Na_2SeO_4 treatment were measured by qRT-PCR. The expression of accession ID 25 in Se-treated sample was set to 1. Data are means of three technical trials with two biological repeats. Values marked by asterisks and plus indicate significant differences between plants with high and low Se levels and between Se-treated and non-Se-treated samples, respectively ($P < 0.05$). Error bars indicate the standard error of the mean ($n = 3$).

Se (Figure 7D), indicating the important role of APS1 in controlling Se accumulation in Broccoli. Indeed, previous reports show that alteration of *APS* expression dramatically enhances Se metabolism in plants.[43] Selenocysteine Se-methyltransferase represents a key enzyme responsible for SeMSCys production. The expression of *SMT* in broccoli can be dramatically induced upon selenate treatment.[13] In comparison with non-Se-treated plants, high levels of expression of *SMT* were observed when exposed to selenate (Figure 7M). Significantly high expression was seen in accessions accumulating more Se, implying a greater potential for SeMSCys synthesis. Overexpression of *SMT* can increase organic Se species accumulation in both *Arabidopsis* and Indian mustard.[44,45] The results suggest that *APS1* and *SMT* expression may be important for high levels of Se accumulation in leaves of broccoli.

As Se secondary accumulators, all broccoli accessions accumulate high levels of Se, but genotypic variation among broccoli

germplasm was observed. Se application at proper concentrations did not exert a negative effect on plant growth and total S level. Broccoli germplasm exhibited various responses in organic Se species production, micronutrient accumulation, GLS synthesis, and total antioxidant contents following Se treatment. The diversity in broccoli germplasm offers the opportunity to develop varieties with high levels of Se and GLS production, as well as other nutritional qualities. A general correlation between total Se levels and organic forms of Se was observed in broccoli accessions. Some accessions accumulate high levels of both Se and GLS (e.g, accession ID 7, 16, 18, 21, 23, 24, 36, and 38). A majority of broccoli accessions have the capacity to simultaneously accumulate Se and GLS without antagonistic effect (e.g, accession ID 4, 7, 8, 18, 19, 23, 24, 29, 30, 34, and 38). Among them, many also produced enhanced levels of total antioxidants in response to Se treatment. Thus, this study provides important information for breeding of varieties with enhanced health benefits and as a Se supplemental food source in areas of low Se intake.

■ ASSOCIATED CONTENT

Supporting Information. Materials and methods and figures of total glucosinolate contents in florets of selected broccoli accessions without selenate treatment and total antioxidant levels in florets of selected broccoli accessions without and with selenate treatment. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

Se, selenium; S, sulfur; Ca, calcium; Mg, magnesium; P, phosphorus; Cu, copper; Fe, iron; Mn, manganese; Zn, zinc; GLS, glucosinolate; Sultr, sulfate transporter; APS, adenosine 5′-phosphosulfate sulfurylase; APR, adenosine phosphosulfate reductase; SAT, serine acetyl transferase; SMT, selenocysteine Se-methyltransferase; HTM, homocysteine S-methyltransferases; SeMSCys, Se-methylselenocysteine; SeMet, selenomethionine; qRT-PCR, quantitative reverse transcription polymerase chain reaction; ICP, inductively coupled plasma; UPLC, ultra performance liquid chromatography.

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