Selenium and Sulfur Increase Sulfur Uptake and Regulate Glucosinolate Metabolism in *Brassica oleracea*

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Abstract

Selenium in soils can result in increased uptake of S and a reduction in glucosinolates in Brassica species. Rapid cycling B. oleracea plants were grown hydroponically in nutrient solution with Se treatments delivered as sodium selenate in concentrations of 0.0, 0.5, 0.75, 1.0 and 1.5 ppm. Elevated S treatments of 37 ppm sulfate and 37 ppm sulfate/ 0.75 ppm selenate were incorporated to compare with Se treatments. Se concentration in the nutrient solution was positively correlated with Se and S uptake in the plants. The S concentration of plants exposed to Se was equal to or greater than the S concentration of plants exposed to elevated S in the nutrient solution. In spite of higher S concentrations, there was a decrease in production of 5 of the 7 glucosinolates analyzed in Se enriched plants. Plants in elevated S treatments had higher glucosinolate production than Se treated plants. These results suggest that Se either up-regulates or prevents the down-regulation of S uptake in B. oleracea. In addition, Se's presence within the plant seems to have a negative impact on the production of certain glucosinolates despite adequate availability of S.

INTRODUCTION

Brassicaceous plants are known for their production of the S-containing secondary plant metabolites, glucosinolates. Se, which is similar to S in both size and chemistry can substitute for S in physiological and metabolic processes (Anderson and Scarf, 1983). Charron et al. (2001), for example, found that total glucosinolate production in rapid cycling *Brassica oleracea* was decreased when grown in the presence of sodium selenate. Further, sulfate uptake has been shown to increase in *B. oleracea* with increasing selenate concentrations in nutrient solutions ranging from 0 to 9 ppm (Charron et al. 2001, Kopsell and Randle 1999, Toler and Sams 2001).

Given the importance of Se to mammalian nutrition, the beneficial anti-carcinogen inducing properties of small amounts of glucosinolate break-down products, and the negative health impacts that both glucosinolate break-down products and Se can induce in excessive quantities, it is essential to further elucidate the Se/ S relationship with regard to glucosinolate production in the widely consumed *B. oleracea*. The objectives of this study was to determine the impact of elevated sulfate in plant growing media on the uptake of S and production of glucosinolates by rapid cycling *B. oleracea*, relative to Se's impact on S uptake and glucosinolate production.

MATERIALS AND METHODS

Rapid cycling *B. oleracea* plants were grown hydroponically in half-strength, modified, Hoagland's nutrient solution under controlled light (ranging from 300 to 450 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and temperature (86.5°F ±2°F). Following one week of growth, at the appearance of the first true leaves on the plants, treatments were initiated.

The treatments consisted of the control (half-strength Hoagland's nutrient solution), four Se treatments (0.5, 0.75, 1.0 and 1.5 $mg \cdot L^{-1}$ Na_2SeO_4), an elevated S treatment (37 $mg \cdot L^{-1}$ SO_4^{2-} delivered on top of the preexisting 96 $mg \cdot L^{-1}$ SO_4^{2-} already present in the solution), and a Se/ elevated S treatment (0.75 $mg\cdot L^{-1}$ Na_2SeO_4/ 37 $mg\cdot L^{-1}$ SO_4^{2-}). Old nutrient solutions in the reservoirs were exchanged biweekly with fresh solution.

One to two days prior to anthesis, plants were harvested. At the time of harvest, plant height and stem width measurements were taken. Leaves, stems, and roots were separated, bagged, and quickly frozen in a –30°C freezer. Once frozen, all tissue was lyophilized to remove water content for dry biomass determination and to prevent glucosinolate degradation.

Glucosinolates in lyophilized leaf tissue were extracted via a method modified from the Canadian Grain Commission (McGregor, 1990) and analyzed by high performance liquid chromatography (HPLC). Se was extracted from 0.2 g of dry tissue. Se concentrations were determined using a Perkin Elmer 5000 flame atomic absorption spectrophotometer equipped with a Se hollow cathode. Total S content of leaf tissue was determined by combusting 0.2 g of leaf tissue on a LECO CNS-2000 Analyzer.

RESULTS AND DISCUSSION

Neither stem width nor tissue biomass was statistically significantly different among treatments. Plant height decreased as Se concentrations in nutrient solution increased. Plants exposed to 1.5 ppm sodium selenate exhibited a 38% decrease in height when compared to control plants. Plants exposed to the 37 ppm sulfate treatment and the 37 ppm sulfate/ 0.75 ppm selenate were taller than plants in all Se treatments.

The glucosinolates that were extracted from the rapid cycling *B. oleracea* plants were glucoiberin, progoitrin, glucoraphanin, sinigrin, gluconapin, glucobrassicin, and neoglucobrassicin. All glucosinolates identified in rapid cycling *B. oleracea* have been identified in *B. oleracea* plants: white cabbage, red cabbage, Savoy cabbage, Brussels sprouts, kale, cauliflower and kohlrabi (Ciska et al., 2000).

While qualitative glucosinolate make-up of *B. oleracea* is consistent from one variety to the next, individual glucosinolate concentrations differ quantitatively among varieties. With the exception of neoglucobrassicin, the glucosinolate concentrations of rapid cycling *B. oleracea* plants tended to be an average of the concentrations examined in other varieties. Neoglucobrassicin concentrations in rapid cycling plants were nearly double that of other varieties analyzed by Ciska et al. (2000) and Kushad et al. (1999).

Total glucosinolate concentrations in rapid cycling *B. oleracea* were higher in the elevated sulfate treatment. This is consistent with increases in total glucosinolate concentrations observed in *B. napus* and *B. rapa* grown under increasingly elevated S (Mailer 1989). Individually, the production of glucoiberin and glucoraphanin was negatively impacted with increasing Se concentrations in nutrient solutions. Plants in the 1.5 ppm selenate treatment exhibited a 58% and 68% reduction in the production of glucoiberin and glucoraphanin, respectively, when compared to plants in the control treatment. Further, plants exposed to elevated sulfate levels in nutrient solution exhibited a significant increase in both glucoiberin and glucoraphanin production, with concentrations 11% and 16% higher than the control, respectively. Plants in the 0.75 ppm selenate/ 37 ppm elevated sulfate treatment had glucoiberin and glucoraphanin production equal to plants in the 0.5 ppm selenate treatment.

Progoitrin production was not significantly impacted by any of the treatments. Like glucoiberin and glucoraphanin, the production of sinigrin and gluconapin was significantly hindered by the presence of Se. Unlike glucoiberin and glucoraphanin, however, the decrease in sinigrin and gluconapin concentrations was a drop in production at any Se exposure (approximately 34% to 38% less than control plants) rather than a gradual decrease with increasing treatment concentrations. There were no notable differences in glucosinolate concentrations among the Se treatments themselves. The 37 ppm sulfate treatment had higher sinigrin and gluconapin concentrations than control plants, with concentrations being 4% and 16% higher, respectively. Plants in the 0.75 ppm selenate/ 37 ppm sulfate treatment had sinigrin concentrations falling between controls and Se treatment plants. Interestingly, gluconapin concentrations in plants in the 0.75 ppm selenate/ 37 ppm sulfate treatment was significantly higher than any other treatment, including 9.5% higher than the 37 ppm sulfate treatment.

Glucobrassicin concentrations in leaf tissue were negatively impacted by the presence of Se. Plants in the 1.5 ppm selenate treatment had a 34% reduction in glucobrassicin production when compared to control plants. Plants in the 37 ppm elevated sulfate treatment exhibited a 17.5% increase in glucobrassicin production when compared to control. Plants in the 0.75 ppm selenate/ 37 ppm elevated sulfate treatment had the lowest concentrations of glucobrassicin, including 22% lower than the 1.5 ppm selenate treatment. Unlike glucobrassicin, neoglucobrassicin concentrations were not significantly impacted by any of the treatments.

With increasing Se concentrations, Se content of leaf tissue increased from 0 to 373 $\mu g \cdot g^{-1}$ in the 0 to 1.5 ppm selenate treatments, respectively. Se concentrations associated with a decreased risk of cancer in humans occur at intakes of 200 $\mu g \cdot day^{-1}$. In the current study, equating Se concentrations in *Brassica* tissues occurred at the Se treatment concentrations of 0.5 ppm and 0.75 ppm (concentrations of 178 and 218 $\mu gSe \cdot g^{-1}$, respectively). Plants exposed to both 0.75 ppm selenate, in addition to elevated sulfate concentrations of 37 ppm, had a mean Se concentration in their leaf tissue of 245 $\mu g \cdot g^{-1}$. These Se concentrations in leaf tissue (in the 0 to 1.5 ppm treatments) are consistent with concentrations found by Charron et al. (2001), Kopsell and Randle (1999) and Kopsell et al. (2000).

Broccoli and cabbage grown in soils with a base Se concentration of 0.1 $\mu g \cdot g^{-1}$ and fertilized with 3 ppm K_2SeO_4 were shown to have Se concentrations of approximately 150 $\mu g \cdot g^{-1}$ dry weight. Carrots and lettuce grown under the same conditions had Se concentrations of 30 to 50 $\mu g \cdot g^{-1}$ dry weight (Hamilton and Beath 1964). The fact that the broccoli and cabbage were grown in soil may explain the lower Se concentrations in their tissue when compared to hydroponically grown rapid cycling *B. oleracea* plants exposed to lower selenate concentrations. Some of the selenate molecules administered would adhere to soil particles, making them unavailable to the plants for uptake. Further, the real-life increase of Se concentrations in economically grown *B. oleracea* varieties by application of selenate fertilizer suggests that it may be readily possible to produce niche crops of Se-enriched *B. oleracea* with Se concentrations ranging from 150 to 200 $\mu g \cdot g^{-1}$.

The S content of leaf tissue was significantly impacted by Se and S treatments. S content of leaf tissue increased from 1.2% to 1.8% as Se concentrations increased. S content of plants exposed to the elevated S treatment was similar to that of plants exposed to the 0.75 ppm selenate treatment. Plants exposed to the 0.75 ppm selenate/ 37 ppm sulfate treatment had S content in their leaf tissue equal to that of plants exposed to the 1.0 ppm selenate treatment.

Se and S significantly impacted the concentration of sulfate in rapid cycling *B. oleracea* plants. Increasing Se concentration resulted in an increase in sulfate concentrations in leaf tissue, ranging from 46.4 $mg \cdot g^{-1}$ in the control treatment to 65.9 $mg \cdot g^{-1}$ in the 1.5 ppm selenate treatment. Plants exposed to elevated sulfate in nutrient solutions had sulfate concentrations in their leaf tissue equal to that of plants in the 0.5 ppm selenate treatment. Plants in the 0.75 ppm selenate/ 37 ppm sulfate treatment had mean sulfate concentrations in their leaf tissue equal to that of plants in the 1.0 ppm selenate treatment.

These results are consistent with findings by Charron et al. (2002), Kopsell and Randle (1999) and Kopsell et al. (2000). In rapid cycling *B. oleracea*, Se appears to either up-regulate, or prevent the down-regulation of S uptake by the plant's roots. A similar influence on S concentrations as a result of Se in growing medium is seen in *Allium cepa* plants (Kopsell and Randle, 1997).

CONCLUSIONS

A primary research objective of this project was to determine Se concentrations in growing media for production of *Brassica* plants with maximum nutritional value. If maximum nutritional value can be achieved without a significant decrease in plant biomass or glucosinolate production, the potential for a super-healthy commercial niche crop of Se enriched *Brassica* vegetables is feasible. The increase of S in the tissue of plants exposed to Se in nutrient solutions, such that their S content was equal to or exceeding that of plants exposed to elevated sulfate levels, suggests Se up-regulates, or prevents the down-regulation of S uptake in *B. oleracea*.

In spite of higher S concentrations in plants exposed to the selenate treatments, when compared to the 37 ppm elevated sulfate treatment, glucosinolate production (of 5 of the 7 glucosinolates analyzed) was lower in Se-exposed plants. The availability of S, and lack of S-containing glucosinolate production, suggest that Se interferes with glucosinolate metabolism. The 0.75 ppm selenate/ 37 ppm sulfate treatment supports this conclusion in the case of glucoiberin, glucoraphanin and sinigrin.

Glucoiberin and glucoraphanin were the two glucosinolates most heavily reduced by the presence of Se in nutrient solution. Neoglucobrassicin was not significantly affected by any treatments.

Research findings by Charron et al. (2001) and Kopsell and Randle (1999), in which increasing sodium selenate concentrations in hydroponic nutrient solutions caused an increase in both Se and S uptake by rapid cycling *B. oleracea*, were corroborated in this experiment. Further, while the production of sinigrin, glucoraphanin, and glucobrassicin was negatively impacted by the presence of Se, concentrations of these glucosinolates at the 0.5 ppm selenate treatment was still relatively high. The 0.5 ppm selenate treatment that had approximately 180 $\mu g{\cdot}g^{-1}$ Se in leaf tissue. Experiments utilizing economic varieties of *B. oleracea* fertilized with selenate yielded similar Se concentrations in plant tissue (Hamilton and Beath, 1964). It may therefore be possible to produce a crop of *B. oleracea* vegetables that not only have preexisting anti-carcinogen inducing health benefits from glucosinolates, but have the added health benefit of appropriate amounts of Se as well.

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