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Glucosinolate Concentration in Turnip (*Brassica rapa* ssp. *rapifera* L.) Roots as Affected by Nitrogen and Sulfur Supply

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Three greenhouse pot experiments were conducted with four different nitrogen (N) treatments (80, 160, 240, and 320 kg ha^{-1}) in combination with three sulfur (S) treatments (10, 20, and 60 kg ha^{-1}) to study the effects of combined N and S supply on glucosinolate concentration and composition in turnip roots. Total glucosinolate concentration varied widely from 9.7 ($N_{320}S_{10}$) to 91.6 ($N_{160}S_{60}$) mg $(100\ g)^{-1}$ root fresh weight (FW) and individual glucosinolate concentrations were increased with increasing S supply regardless of the N treatment, whereas enhanced N supply (160 - 320 N ha^{-1}) at the high S level (60 kg ha^{-1}) did not affect total glucosinolate concentration. In contrast, assumingly attributed to the individual glucosinolate biosynthesis concentration of N-containing tryptophan-derived indole glucosinolate was highest with increased N supply, whereas S-containing methionine-derived aromatic and aliphatic glucosinolates decreased with increasing N supply combined at low S level (10–20 kg ha^{-1}).

KEYWORDS: Glucosinolate; nitrogen; sulfur; turnip; HPLC

INTRODUCTION

Glucosinolates are nitrogen (N) and sulfur (S) containing compounds that are present in all economically important species of *Brassica* vegetables. Based on the chemical structure of their side chains, glucosinolates can be subdivided into different classes such as aliphatic (alkyl, alkenyl), aromatic, and indole glucosinolates (*1*). Breakdown products of the aryl glucosinolate gluconasturtiin and indole glucosinolates can reduce the risk of cancer (*2*). For example, the hydrolysis product of gluconasturtiin, 2-phenylethyl isothiocyanate, can prevent cancer by inhibiting phase I enzymes and inducing phase II enzymes, resulting in carcinogen excretion (*3, 4*). Our previous studies showed that turnip roots contained high concentrations of health-promoting gluconasturtiin and relatively high concentrations of indole glucosinolates (*5*). Therefore, particular attention has been given to glucosinolates in *Brassica rapa*, a root vegetable turnip grown all over Europe and Asia and available all year round.

Glucosinolate concentrations are, however, known to be highly variable both in terms of levels and composition. This variability is caused by various factors, but is known to be strongly influenced by S (*6*) and N (*8, 9*) supply. The first steps of sulfate assimilation are catalyzed by ATP-sulfurylase (*10*), the activity of which is influenced by the ratio of supplied N and S (*11*). Further, plants assimilate inorganic sulfate into cysteine which is subsequently converted into methionine (*12*), and this reduction step is regulated by N content (*13*). Amino acids such as methionine, tryptophan, or phenylalanine are the precursors for glucosinolate formation (*14*). Therefore, S supply has a strong influence on the glucosinolate concentration in *Brassica* plants. This S effect varied depending on the soil's S concentration and on the glucosinolate class. S application to S-deficient soil resulted in a larger response to increase total glucosinolate concentration in vegetable tissues (*15*). A glucosinolate class-specific effect was shown for oilseed rape (*16*): aliphatic glucosinolate concentrations increased in response to S fertilizer, but exerted a negligible effect on indole glucosinolate concentration.

A correlation between glucosinolate concentration and S supply that is also partly influenced by the plant's N content has been reported for mustard, turnip leaves, kale, and broccoli (*9, 17–19*). In contrast, there is evidence that N application tends to reduce, increase, or has no effect on glucosinolate concentration in *Brassica* plants (*20, 21*), although results are partly contradictory. Glucosinolate concentration in

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Table 1. Effect of N and S Supply on N, S Concentration (g kg^{-1} DW) in Roots, N/S Ratios, Root Fresh Weight (FW), and Root Dry Weight (DW) of Turnip

treatment	N concentration	S concentration	N/S	root FW	root DW
$N_{80}S_{10}$	9.6 ± 0.6 a[a]	2.1 ± 0.2 c	4.6 ± 0.6	81.6 ± 8.3 ab	12.4 ± 1.9 ab
$N_{80}S_{20}$	9.2 ± 0.7 a	2.7 ± 0.2 b	3.4 ± 0.1	86.4 a ± 16.3	14.1 ± 3.1 a
$N_{80}S_{60}$	9.7 ± 0.8 a	3.5 ± 0.3 a	2.8 ± 0.2	67.3 b ± 9.9	11.0 ± 1.9 b
Average	9.5 D[b]	2.8 A		78.4 C	12.5 B
$N_{160}S_{10}$	14.2 ± 0.8 a	1.7 ± 0.2 c	8.3 ± 1.0	105.4 ± 16.7 a	14.7 ± 1.9 a
$N_{160}S_{20}$	12.8 ± 0.4 a	2.2 ± 0.1 b	5.8 ± 0.4	105.9 ± 18.0 a	15.1 ± 3.1 a
$N_{160}S_{60}$	13.5 ± 1.6 a	3.5 ± 0.3 a	3.8 ± 0.1	101.4 ± 12.3 a	14.3 ± 2.0 a
Average	13.5 C	2.5 B		104.2 B	14.7 A
$N_{240}S_{10}$	19.8 ± 0.4 a	1.8 ± 0.3 b	11.4 ± 1.7	116.7 ± 22.7 a	14.6 ± 3.3 a
$N_{240}S_{20}$	16.0 ± 0.8 b	2.3 ± 0.1 b	7.1 ± 0.1	129.4 ± 28.2 a	15.9 ± 4.1 a
$N_{240}S_{60}$	16.6 ± 2.3 b	4.0 ± 0.4 a	4.2 ± 0.4	124.4 ± 24.2 a	16.5 ± 4.3 a
Average	17.5 B	2.7 AB		123.5 A	15.6 A
$N_{320}S_{10}$	23.8 ± 0.8 a	1.6 ± 0.2 c	14.7 ± 2.4	116.5 ± 24.4 a	14.6 ± 3.5 a
$N_{320}S_{20}$	20.6 ± 1.1 b	2.0 ± 0.2 b	10.3 ± 0.3	132.1 ± 14.2 a	16.1 ± 2.8 a
$N_{320}S_{60}$	21.4 ± 2.1 ab	4.2 ± 0.2 a	5.1 ± 0.6	115.7 ± 21.4 a	14.0 ± 3.9 a
Average	22.0 A	2.6 AB		121.4 A	14.9 A

[a] Mean values of three S supply treatments with the same N treatment followed by different letters (a, b) are significantly different ($P < 0.05$). [b] Mean values of N treatment followed by different letters (A, B) are significantly different ($P < 0.05$). Values represent the mean of three experiments and three replications ($n = 3$).

oilseed rape decreased with increasing N supply in the absence of S application, but increased by additional S application (*21*). Previous studies have mostly concentrated on glucosinolates in oilseed rape and leafy or inflorescence *Brassica* vegetables, while little is known about the effects of N and S supply on the glucosinolate profile in root *Brassica* vegetables like turnip roots.

Therefore, the aims of this study were to (i) quantify the effects of various S and N supply levels and combinations on glucosinolate concentration and composition in turnip roots, and (ii) develop recommendations for designing future crop management strategies to support plant growth and optimize glucosinolate concentrations—as interesting human health-promoting compounds—by using optimal S and N fertilization.

MATERIALS AND METHODS

Plant Material and Experimental Design. Three pot experiments each with three replicates were set up in completely randomized designs and carried out successively (Expt. 1, March 18 to May 10, 2004; Expt. 2, June 1 to July 19, 2004; Expt. 3, July 30 to September 13, 2004) under greenhouse conditions. Each pot experiment was subjected to combinations of four different N and three different S supplies. N was supplied as NH_4NO_3, at rates of 80, 160, 240 and 320 kg N ha^{-1}, designated by N_{80}, N_{160}, N_{240}, and N_{320}, respectively. S supplied as K_2SO_4 at rates of 10, 20, 60 kg S ha^{-1} was designated by S_{10}, S_{20}, and S_{60}, respectively. Turnip (*Brassica rapa* ssp. *rapifera* L.) cv. Hongyuan Manjing was selected because of its high level of health-promoting glucosinolates as compared to other Brassicaceae. Turnip seeds were pregerminated in Petri dishes, and then transferred to pots containing mixed substrate of 6 kg of sand (washed, without N and S), 0.3 kg of vermiculite, 3 g of $CaCO_3$, and 1.31 g of NH_4NO_3. Each pot contained two turnip plants placed at a distance of 0.20 m.

To obtain the desired N and S concentrations, fertilizers were supplied at different levels during the development of the turnips. After seeds had germinated, 1.83 g of NH_4NO_3 was applied per pot of the N_{160}, N_{240}, and N_{320} treatments, and when plants had developed six true leaves, 1.83 g of NH_4NO_3 was given to each pot of the N_{240} and N_{320} treatments. After reaching a root diameter of 0.03 m, the N_{320} treatment received another 1.83 g of NH_4NO_3 per pot. At sowing, 0.44 g of K_2SO_4 per pot was applied in the S_{10} treatment, and 0.89 g od K_2SO_4 per pot was applied in the S_{20} and S_{60} treatments. At the 6-leaf stage, 1.77 g of K_2SO_4 was applied to each pot of the S_{60} treatment. To equalize the amount of potassium, 0.96 g of KCl and 0.77 g of KCl were supplied in S_{10} and S_{20} treatment at sowing and at the 6-leaves-stage of turnip. Other nutrients were added to each pot using nutrient solution: 0.28 g of P as KH_2PO_4, 0.16 g of Mg as $Mg(NO_3)_2$, 30 mg of Mn as $MnCl_2$, 9 mg of Cu as $CuCl_2$, 3 mg of Mo as $(NH_4)_4MoO_4$, 9 mg of B, 5 mg of Fe as $FeSO_4 \cdot 7H_2O$, and 9 mg of Zn as $ZnSO_4 \cdot 7H_2O$.

Harvest and Sample Preparation. Turnip plants were harvested when the root diameter reached 0.06 m, and the root fresh mass was determined. For glucosinolate analysis, samples comprising the halves of six fresh roots were used from each replicate ($n = 3$). The mixed samples were immediately frozen (−40 °C), then freeze-dried, and finally finely ground.

Glucosinolate Analysis. The HPLC method reported by Krumbein et al. (*5*) which was modified according a method described in *Off. J. Eur. Communities* (*22*)was used for glucosinolate determination. Duplicates of freeze-dried sample material (0.5 g) were heated and incubated at 75 °C for 1 min, extracted with 4 mL of a methanol/water mixture (7:3 v/v, $T = 75$ °C), and then, after adding 1 mL of 0.4 M barium acetate, centrifuged at 4000 rpm for 10 min. Two hundred microliters of a 5 mM (2077 g/L) stock solution of sinigrin in methanol was added to one of the duplicates just before the first extraction as internal standard. The residue was extracted twice more with 3 mL of the methanol/water mixture (v/v = 7:3, $T = 75$ °C). The supernatants of the three extractions were pooled and made up to 10 mL. Five mL of the extract was applied to a 250 μL DEAE-Sephadex A-25 ion-exchange (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and rinsed with 10 mL of bidistilled water. (Preparation of DEAE-Sephadex was done in following steps: mixing 5 g of DEAE-Sephadex A-25 with 200 mL of bistilled water, subsequently activating this suspension with 5 mL of 1 mol/L CH_3COOH, and then neutralization with bidistilled water). Next, 250 μL of a purified solution of aryl sulfatase (Boehringer-Mannheim GmbH, Mannheim, Germany) was applied and left for 12 h. The desulfo-compounds were then flushed with 5 mL of bidistilled water. The analysis of the desulfo–glucosinolates was conducted by HPLC (Merck Hitachi, Darmstadt, Germany, HPLC pump 6200, DAD detector L 7455, automatic sampler model AS-2000, and HPLC Manager-Software D-6000) using a Spherisorb ODS2 column (Bischoff, Leobberg, Germany: 5 μm, 250 × 4 mm). A 0–20% gradient of acetonitrile in water was selected from 2 to 34 min, followed by 20% acetonitrile in water until 40 min, and then acetonitrile for 10 min until 50 min. The determination of desulfo–glucosinolates was conducted at a flow of 1.3 mL min^{-1} and a wavelength of 229 nm. Individual glucosinolates were identified by comparison of their retention time with that of individual glucosinolates in standard reference materials (BCR-190R and BCR-367R) of oilseed rape (*23*). The glucosinolate concentration was calculated using sinigrin as internal standard and the response factor of each compound relative to sinigrin (*22*).

Plant N and S Analysis. Plant N concentration in roots was determined using the Kjeldahl method after plant material was digested in concentrated H_2SO_4.

Table 2. Effect of N and S Supply on Individual, Total Alkenyl, Total Alkyl, Total Indole Glucosinolate, and Total Glucosinolate Concentration (mg 100 g^{-1}) in Fresh Turnip Roots[a]

treatment	PG	GNLF	GNP	GBNP	total alkenyl	total alkyl	total indole	GNAS	total GS
$N_{80}S_{10}$	6.7 ± 2.8c [b]	2.4 ± 1.1 b	1.5 ± 0.7 c	5.4 ± 0.8 b	16.0 ± 4.6 c	1.4 ± 0.7 b	3.2 ± 1.8 b	14.8 ± 5.8 c	35.4 ± b
$N_{80}S_{20}$	12.6 ± 2.5 b	3.7 ± 0.4 ab	4.1 ± 1.6 b	11.9 ± 3.3 a	32.2 ± 6.6 b	3.7 ± 2.4 a	3.5 ± 0.7 b	25.9 ± 10.3 b	65.3 ± a
$N_{80}S_{60}$	17.8 ± 8.2 a	5.1 ± 2.3 a	6.8 ± 1.8 a	16.0 ± 6.4 a	45.7 ± 16.4 a	4.8 ± 2.4 a	6.1 ± 2.6 a	33.6 ± 5.0 a	90.2 ± a
Average	12.4 A[c]	3.7 A	4.1 A	11.1 A	32.3 A	3.3 A	4.3 A	24.8 A	63.5 A
$N_{160}S_{10}$	2.9 ± 1.4 b	1.1 ± 0.4 c	0.5 ± 0.4 b	2.9 ± 1.2 c	7.3 ± 2.8 b	0.9 ± 1.0 b	1.6 ± 0.7 b	7.9 ± 2.8 c	17.7 ± c
$N_{160}S_{20}$	7.5 ± 4.0 b	2.4 ± 1.0 b	2.2 ± 0.8 b	6.9 ± 2.0 b	19.0 ± 7.0 b	2.0 ± 1.4 b	2.9 ± 0.8 b	15.6 ± 7.6 b	39.5 ± b
$N_{160}S_{60}$	21.1 ± 11.5 a	4.2 ± 1.7 a	7.6 ± 4.0 a	13.5 ± 4.6 a	46.4 ± 20 a	5.9 ± 4.1 a	5.5 ± 2.5 a	33.8 ± 9.9 a	91.6 ± a
Average	10.5 AB	2.6 B	3.4 AB	7.81.0 B	24.2 B	2.9 A	3.3 B	19.1 B	49.6 B
$N_{240}S_{10}$	1.6 ± 0.8 b	0.8 ± 0.5 b	0.1 ± 0.1 b	2.4 ± 0.7 b	4.9 ± 1.6 b	0.4 ± 0.4 b	1.8 ± 0.6 b	4.8 ± 2.9 b	11.9 ± b
$N_{240}S_{20}$	4.5 ± 2.4 b	1.6 ± 0.9 b	0.8 ± 0.4 b	4.4 ± 2.0 b	11.3 ± 5.0 b	1.2 ± 1.3 b	2.0 ± 0.6 b	9.2 ± 1.3 b	23.7 ± b
$N_{240}S_{60}$	19.2 ± 8.7 a	4.7 ± 1.7 a	7.4 ± 3.8 a	16.7 ± 6.7 a	48.0 ± 19.7 a	4.3 ± 1.5 a	6.0 ± 1.6 a	32.0 ± 7.8 a	90.2 ± a
Average	8.3 BC	2.4 B	2.8 BC	7.8 B	21.2 BC	1.9 B	3.26 B	15.3 C	42.0 BC
$N_{320}S_{10}$	1.0 ± 0.6 b	0.6 ± 0.4 b	0.2 ± 0.3 b	2.3 ± 1.0 b	4.0 ± 1.4 b	0.3 ± 0.3 b	1.5 ± 1.0 b	3.9 ± 2.3 c	9.7 ± c
$N_{320}S_{20}$	3.6 ± 1.9 b	1.7 ± 0.8 b	0.7 ± 0.6 b	3.8 ± 1.8 b	9.7 ± 5.0 b	0.7 ± 0.4 b	2.8 ± 1.1 b	11.6 ± 5.6 b	24.9 ± b
$N_{320}S_{60}$	15.7 ± 7.5 a	5.2 ± 2.6 a	4.8 ± 2.4 a	14.7 ± 4.4 a	40.4 ± 19.7 a	4.0 ± 1.7 a	8.7 ± 3.5 a	33.6 ± 8.6 a	86.7 ± a
Average	6.8 C	2.5 B	1.9 C	6.9 B	18.0 C	1.7 B	4.3 A	16.4 BC	40.4 C

[a] PG = Progoitrin, GNLF = Gluconapoleiferin, GNP = Gluconapin, GBNP = Glucobrassicanapin, GNAS = Gluconasturtiin. [b] Mean values of three S supply treatments with the same N treatment followed by different letters (a, b) are significantly different ($P < 0.05$). [c] Mean values of N treatment followed by different letters (A, B) are significantly different ($P < 0.05$). Values represent the mean of three experiments and three replications ($n = 3$).

An NDIR detector (employing nondispersive infrared gas analysis) was used to determine the S concentration in roots and leaves. Fine ground samples were weighted into quartz boats and delivered into the hot zone of multi EA 2000 CS (Analytic Jena AG, Jena, Germany). Then, the sample were pyrolyzed and oxidized at 1300 °C in a stream of oxygen (99.5%). The measurements were performed in duplicate.

Statistical Analysis. Statistical significance of differences between treatments were determined by least significant differences (LSD) for multiple comparisons after analysis of variance (ANOVA), using SAS (SAS Institute, 1985). All tests were performed at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

The individual aromatic (gluconasturtiin), aliphatic (alkyl glucosinolates: glucoraphanin, glucoalyssin, and glucoerucin; alkenyl glucosinolates: progoitrin, gluconapoleiferin, gluconapin, and glucobrassicanapin) and indole glucosinolates (glucobrassicin, neoglucobrassicin, and 4-methoxy-glucobrassicin) were quantitatively determined in turnip roots. The total GS concentration was calculated by the sum of the individual GS.

Total Glucosinolate Concentration. The total glucosinolate concentration in turnip roots was strongly influenced by N and S supply. Increasing S supply enhanced total glucosinolate concentration at all levels of N supply. The highest total glucosinolate concentrations resulted from the highest S supply, irrespective of N fertilization (**Table 2**). They were about 9 times higher than the lowest total glucosinolate concentration at $N_{320}S_{10}$.

Our present study shows that glucosinolate concentration in turnip roots as a response to S supply is dependent on N supply. With low S supply (10 and 20 kg ha^{-1}), total glucosinolate concentrations were 0.8–2.3 times higher at 80 kg N ha^{-1} compared to treatments with 160 and 240 kg N ha^{-1}. However, at the high S level (60 kg ha^{-1}), increasing N supply (160, 240, and 320 N ha^{-1}) did neither affect total glucosinolate concentration (**Table 2**) nor root fresh weight (**Table 1**) as it also was found in broccoli sprouts (*24*). Enhancing S supply also increases glucosinolate concentration in broccoli (*7*, *8*) and oilseed rape (*25*, *26*). Averaged over all S supply levels, total glucosinolate concentration in the N_{80} treatment was significantly higher than that in the N_{160}, N_{240}, and N_{320} treatments (**Figure 1**).

Glucosinolates are S- and N-containing compounds (*1*) suggesting that S and N concentrations in the plant tissue strongly affect glucosinolate concentration. S concentration in turnip roots increased by about 2-fold with increasing S supply (**Table 1**). In contrast to S concentration, there was no difference in N concentrations of turnip roots between the three S supply treatments for N_{80} and N_{160}. However, when N supply was >160 kg ha^{-1}, N concentration in roots for the S_{20} and S_{60} treatments

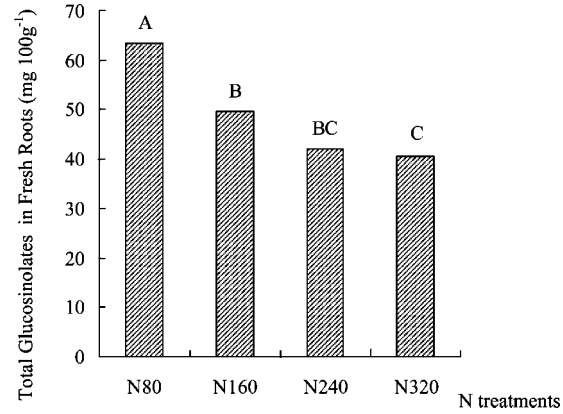

Figure 1. Average total glucosinolate concentration of three S supply in turnip roots in each N supply level. Bars with different capital letters (A, B) mean significantly different between N treatments ($p < 0.05$).

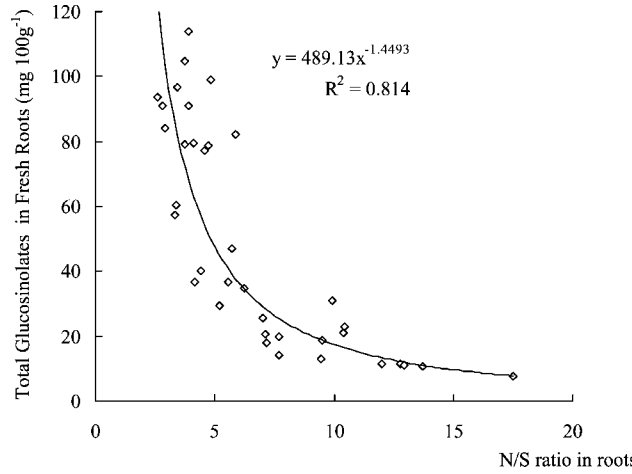

Figure 2. Correlation between the total glucosinolate concentration and the N/S ratios in roots.

Table 3. Effect of N and S Supply on Percentage Composition (%) of Individual, Total Alkenyl, Total Alkyl and Total Indole Glucosinolates in Fresh Turnip Roots[a]

treatment	PG	GNLF	GNP	GBNP	total alkenyl	total alkyl	total indole	GNAS
$N_{80}S_{10}$	19.1 ± 6.2 a[b]	6.5 ± 1.9 a	4.4 ± 1.9 b	16.1 ± 3.5 a	46.1 ± 9.4 a	3.7 ± 1.3 a	8.7 ± 2.6 a	41.4 ± 8.4 a
$N_{80}S_{20}$	19.9 ± 4.4 a	5.9 ± 1.4 a	6.1 ± 1.5 ab	18.5 ± 3.0 a	50.3 ± 7.1 a	5.4 ± 2.7 a	5.7 ± 1.6 b	38.7 ± 7.6 a
$N_{80}S_{60}$	18.8 ± 5.5 a	5.6 ± 2.2 a	7.8 ± 2.2 a	17.4 ± 4.0 a	49.5 ± 7.7 a	5.2 ± 2.5 a	6.6 ± 2.0 b	38.8 ± 8.6 a
Average	19.2 A[c]	6.0 A	6.0 A	17.2 A	48.6 A	4.7 A	7.0 C	39.6 B
$N_{160}S_{10}$	15.8 ± 4.8 b	6.0 ± 1.4 ab	2.6 ± 2.0 c	16.5 ± 5.1 a	40.9 ± 7.5 b	4.4 ± 3.7 a	9.1 ± 3.2 a	45.5 ± 8.3 a
$N_{160}S_{20}$	18.2 ± 5.8 ab	6.5 ± 2.2 a	5.7 ± 2.5 b	18.3 ± 3.3 a	48.7 ± 7.9 a	4.6 ± 2.2 a	7.9 ± 2.5 ab	38.7 ± 8.6 ab
$N_{160}S_{60}$	21.9 ± 4.6 a	4.6 ± 0.9 b	8.5 ± 3.2 a	15.1 ± 2.6 a	50.1 ± 4.8 a	6.1 ± 2.9 a	5.9 ± 1.7 b	37.9 ± 5.0 b
Average	18.7 AB	5.7 A	5.6 A	16.6 A	46.6 A	5.0 A	7.7 C	40.7 AB
$N_{240}S_{10}$	12.6 ± 4.1 b	6.5 ± 2.7 a	1.2 ± 1.1 c	22.9 ± 10.9 a	43.2 ± 10.1 b	2.8 ± 2.3 a	15.7 ± 3.3 a	38.4 ± 11.0 a
$N_{240}S_{20}$	17.6 ± 6.5 a	6.2 ± 2.4 a	3.9 ± 1.6 b	18.3 ± 5.1 a	45.8 ± 7.4 ab	4.3 ± 3.6 a	8.5 ± 2.3 b	41.4 ± 10.0 a
$N_{240}S_{60}$	20.5 ± 4.0 a	5.2 ± 1.1 a	7.8 ± 2.0 a	18.2 ± 3.9 a	51.7 ± 6.7 a	5.0 ± 1.9 a	6.9 ± 1.4 b	36.4 ± 5.0 a
Average	16.9 B	6.0 A	4.3 B	19.8 A	46.9 A	4.0 B	10.4 B	38.7 AB
$N_{320}S_{10}$	10.0 ± 4.6 b	6.9 ± 4.7 a	1.4 ± 1.7 b	26.0 ± 13 a	44.3 ± 9.9 a	2.9 ± 2.0 b	14.9 ± 4.6 a	37.9 ± 11.4 a
$N_{320}S_{20}$	14.4 ± 3.9 ab	6.7 ± 1.6 a	2.6 ± 1.6 b	15.0 ± 2.5 b	38.7 ± 5.9 a	2.8 ± 1.2 b	11.9 ± 3.8 ab	46.7 ± 8.2 a
$N_{320}S_{60}$	17.4 ± 5.2 a	5.9 ± 2.4 a	5.4 ± 2.3 a	16.9 ± 4.0 b	45.5 ± 10.3 a	4.5 ± 1.1 a	9.9 ± 2.3 b	40.1 ± 11.6 a
Average	13.9 C	6.5 A	3.1 C	19.3 A	42.8 B	3.4 B	12.2 A	41.5 A

[a] PG = Progoitrin, GNLF = Gluconapoleiferin, GNP = Gluconapin, GBNP = Glucobrassicanapin, GNAS = Gluconasturtiin. [b] Mean values of three S supply treatments with the same N treatment followed by different letters (a, b) are significantly different ($P < 0.05$). [c] Mean values of N treatment followed by different letters (A, B) are significantly different ($P < 0.05$). Values represent the mean of three experiments and three replications ($n = 3$).

was decreased compared to that of the S_{10} treatment. This effect could be explained by increasing root fresh matter (**Table 1**).

When S supply is limited, most S is incorporated into proteins, and therefore less S is available for glucosinolate synthesis (*26*). Also, high N supply can suppress S uptake when S is limited (*11*). Therefore, the observed decrease of total glucosinolate concentration in turnip roots with increasing N supply in low S treatments (S_{10} and S_{20}) could be attributed to low S concentration in roots. This result is consistent with reports by Zhao et al. (*25*) and Kim et al. (*19*).

In addition to S uptake, N nutrition seems also to regulate S assimilation, since, in *Arabidopsis thaliana*, a decrease of adenosine 5′-phosphosulfate reductase activity occurred in N-deficient plants (*13*). Therefore, S concentration in plants could be regulated by N supply. *O*-acetylserine (OAS) plays a major role in this S–N interaction (*27*) as OAS is derived from S and N assimilation pathways. When S was limited, OAS was seen to accumulate, whereas when N was limited OAS formation was reduced (*28*). OAS is a precursor of the N- and S-containing amino acids cysteine and methionine which are basic metabolites of glucosinolate synthesis. Therefore, high N supply (160–320 kg ha^{-1}) combined with insufficient S supply (10–20 kg ha^{-1}) could lead to an accumulation of OAS and reduced cysteine and methionine synthesis, resulting in a lack of precursors for glucosinolate synthesis and in a reduced glucosinolate concentration.

The interactive S and N effect on glucosinolate concentration is supported by the N/S ratio in turnip roots, as low glucosinolate concentrations occurred when N/S ratio < 5 (**Figure 2**). Also in cabbage (*29*) and in broccoli (*9*) glucosinolate concentrations were negatively correlated with N/S ratios.

Individual Glucosinolate Concentration. Eleven individual glucosinolates were identified in turnip roots of cv. Hongyuan Manjing. Major glucosinolates in turnip roots were gluconasturtiin, progoitrin, glucobrassicanapin, and neoglucobrassicin, whereas in cv. Goldball the predominant glucosinaolates were progoitrin, gluconasturtiin, and 4-hydroxyglucobrassicin (*30*). These different findings indicate genotypic effects on glucosinolate profile and concentrations. Our results showed that all individual glucosinolates exhibited trends similar to the total glucosinolate concentration. Increasing S supply increased the predominate aromatic gluconasturtiin, the alkyl and alkenyl glucosinolate concentrations dramatically regardless of N treatments, whereas increasing N supply decreased the individual glucosinolate concentrations (**Table 2**). This is in agreement with results obtained in rape seed (*31*), whereas alkyl glucosinolates in broccoli were high at insufficient N supply independent of the S level and low at insufficient S supply in combination with an optimal N supply (*9*). The alkyl glucosinolate concentration in turnip decreased with insufficient S supply. S deficiency affects the amino acid profile in plants with a decrease in the share of amino acids rich in S such as cysteine and methionine (*32, 33*). Due to reduced S assimilation, cysteine synthesis and the production of derived compounds such as methionine are therefore reduced (*28*). Nikiforova et al. (*12*) found that genes involved in the direct methionine biosynthetic pathway were not induced by insufficient S supply. Aliphatic glucosinolate biosynthesis requires methionine, which can therefore be expected to respond sensitively to the plant's S status (*20*).

In contrast to aromatic and aliphatic glucosinolates, the total indole glucosinolate concentration increased at the highest N supply level. These results agree with those of Kim et al. (*19*) who found in turnip leaves that indole glucosinolates increased with increasing N application, regardless of S application. In broccoli, the highest indole concentrations were found at an optimal N supply in combination with an optimal or excessive S supply compared to an insufficient N supply (*9*). The increase of indole glucosinolates concentration with increased N supply might be attributed to the biosynthesis process since indole glucosinolates are derived from tryptophan, an S-free but N-containing amino acid, suggesting an increased tryptophan formation by increased N supply.

In this study the N/S ratio clearly influenced the aromatic, aliphatic, and indole glucosinolates and hence the glucosinolate profile of vegetable turnip roots. This result is similar to the recent observation in cabbage (*29*). In turnip roots supplied with 60 kg ha^{-1} S total glucosinolate and gluconasturtiin concentrations were not influenced significantly by N supply (**Figure 1**, **Table 2**). In contrast, in Bai Cai (*Brassica campestris* ssp. *chinensis*) (*13*) and broccoli (*9*) increased N application tends to reduce individual glucosinolate concentrations indicating also *Brassica* species-specific responses on N/S supply. It seemed likely that the differing effects elicited by the N supply might arise from the different status of S in plant tissues.

Percentage Composition of Individual Glucosinolates. Alkenyl glucosinolates, mainly progoitrin and glucobrassicanapin, and the aromatic gluconasturtiin were the major components of glucosinolates, which accounted for, on average, 46.2% and 40.2%, respectively, of total glucosinolates in turnip roots (**Table 3**). However, different S rate supply had no distinct influence on total alkenyl glucosinolate and gluconasturtiin percentage composition in each level N supply. In this study, average total indole glucosinolate percentage composition for all S treatments in N_{320} treatment increased by 71.8% compared with the N_{80} treatment. Average total indole glucosinolate percentage significantly increased with increasing nitrogen supply. Lee et al. (*24*) also found that total indole glucosinolate percentage composition significantly decreased with increasing S supply and increased with increasing N supply. Resulting differences in the alkenyl and indole glucosinolate percentage compositions as a response to varying N/S ratio could be due to differences in amino acid precursor utilization. Indole glucosinolates are derived from tryptophan, whereas alkenyl glucosinolates are synthesized from methionine (*1*). Nikiforova et al. (*12*) found that under S depletion, the tryptophan level increased by 6- to 28-fold and methionine level reduced nonmeasurably compared to the control in *Arabidopsis thaliana*. In plants, tryptophan is produced from indole and serine by the tryptophan synthase β-subunit which is induced under S-deficient conditions (*34*). Moreover, S-deficient conditions decreased cysteine concentration resulting in accumulation of OAS and its precursor, serine (*35*). The surplus serine might be converted to tryptophan, which in turn might increase total indole glucosinolate concentrations under limited-S conditions. Recently, Rosen (*29*) also found that the percentage composition of indole glucosinolates in cabbage increased and aliphatic glucosinolates decreased with increasing N supply. However, glucosinolate synthesis requires the participation of two S-containing compounds: cysteine and 3′-phosphoadenosyl-5-phosphosulphate (*36*). Hence, if S supply were insufficient, glucosinolate concentration in crops would be decreased due to the absence of basic S-containing metabolites.

The present study showed that manipulating the N and S supply might be one means of altering glucosinolate concentration level and individual glucosinolate percentage composition in turnip roots, and thereby potentially increase the health benefits when consuming this vegetable. The results presented here might be used to enable practical crop management strategies to be developed in which N and S supply is controlled in order to produce turnips with the highest health-promoting glucosinolate content and composition.

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