JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Correlation of Glucosinolate Content to Myrosinase Activity in Horseradish (*Armoracia rusticana*)

XIAN LI AND MOSBAH M. KUSHAD*

Department of Natural Resources and Environmental Sciences, University of Illinois—Urbana/Champaign, 1201 West Gregory Drive, Urbana, Illinois 61801

Fully developed horseradish (*Armoracia rusticana* Gaertn., Mey., & Scherb.) roots from 27 accessions and leaves from a subset of 9 accessions were evaluated for glucosinolates and myrosinase enzyme activity. Eight different glucosinolates were detected (based on HPLC retention times as desulfoglucosinolates) in both root and leaf tissues. The sum of these glucosinolates, referred to as total, ranged from 2 to 296 μmol g^{-1} of dry weight (DW) in both tissues. Four glucosinolates (sinigrin, glucobrassicin, neoglucobrassicin, and gluconasturtiin) were detected in major quantities. In fully developed roots, sinigrin concentration represented ~83%, gluconasturtiin ~11%, and glucobrassicin ~1% of the total glucosinolates. Approximately the same proportions of individual glucosinolates appeared in fully developed leaves, except that glucobrassicin was substituted by neoglucobrassicin and gluconasturtiin concentration was significantly lower (<1%). At least four other glucosinolates were detected in very small quantities (<1%) in both roots and leaves. Myrosinase (β-thioglucoside glucohydrolase, EC 3.2.3.1) is the enzyme responsible for the hydrolysis of the parent glucosinolates into biologically active products. Very little is known about myrosinase activity and the correlation of its activity to total and individual glucosinolates in plant tissues. Significant differences in myrosinase activity were detected between the roots and leaves, ranging from 1.2 to 57.1 units g^{-1} of DW. Data showed no correlation between myrosinase activity and total and/or individual glucosinolates in the roots. However, in the leaves, significant correlations were found between myrosinase activity and total glucosinolates (0.78 at $P = 0.01$) and between myrosinase activity and sinigrin (0.80 at $P = 0.01$). Glucosinolates content and myrosinase activity were also correlated in young and fully developed roots and leaves and during tissue crushing. Glucobrassicin concentration in the roots and neoglucobrassicin concentration in the leaves were significantly higher in young than in fully developed tissue. Crushing of the tissue resulted in rapid hydrolysis of sinigrin and glucobrassicin, as expected, from the presence of myrosinase. Likewise, myrosinase activity declined rapidly after crushing, perhaps due to inactivation by the reaction products and/or the depletion of its substrates.

KEYWORDS: **β-Thioglucoside glucohydrolase; crushing; glucosinolates; myrosinase; sinigrin**

INTRODUCTION

Brassica vegetables are rich in sulfur-containing glucosides called glucosinolates (*1*−*3*). About 120 different glucosinolates have been identified in 15 families of dicotyledonous plants, but only about 20 have been detected in *Brassica* vegetables (*3*, *4*). The general structure of glucosinolates consists of a glucone moiety and a variable aglucone R-group (*5*). Glucosinolates are divided according to their biosynthetic precursors methionine, tryptophan, or phenylalanine into aliphatic, indolyl, or aromatic. Methionine-derived aliphatic glucosinolates are the most abundant in *Brassica* vegetables, with glucoraphanin (4-methylsulfinylbutyl glucosinolate), sinigrin (2-prophinyl glucosinolate), progoitrin (2*R*)-2-hydroxybut-3-enyl glucosinolate), glucoerucin (4-methylthiobutyl glucosinolate), and glucoiberin (3-methysulfinylpropyl glucosinolate) representing between 55 and 90% of the total, depending on tissue type, stage of development, and growing conditions (*2*, *3*, *6*, *7*). Tryptophan-derived indole glucosinolates detected in *Brassica* vegetables include glucobrassicin (3-indolylmethyl glucosinolate), neoglucobrassicin (*N*-methoxy-3-indolylmethyl glucosinolate), and 4-hydroxyglucobrassicin (4-hydroxy-3-indolylmethyl glucosinolate). Gluconasturtiin (2-phenethyl glucosinolate) is the predominant phenylalanine-derived glucosinolate in *Brassica*.

Glucosinolates provide the bitter flavor and sulfurous aroma characteristic of *Brassica* and other cruciferous crops as a result of their breakdown into isothiocyanates (*5*, *8*, *9*). Another property of glucosinolates is their allelopathic response. Glucosinolates and their breakdown products were reported to suppress soil-borne organisms such as bacteria, fungi, viruses, nematodes, and weeds (*10*−*15*).

* Author to whom correspondence should be addressed [telephone (217) 244-5691; fax (217) 333-8816; e-mail kushad@uiuc.edu].

10.1021/jf0401827 CCC: $27.50
Published on Web 10/15/2004

Glucosinolates and their breakdown products have also been linked to the neutralization of chemical carcinogens in human and animal cells by activating phase II detoxification enzymes, such as glutathione-*S*-transferase, quinone reductase, NADPH reductase, and glucouronyl transferase, and by inhibiting phase I enzymes (cytochrome P450 enzymes), which are involved in the activation of chemical carcinogens (*16*, *17*). Glucosinolates were also reported to have a direct effect on cancer development by suppressing the promotion phase of cancer cell development (*18*, *19*) and by blocking estrogen receptors of estrogen-dependent cancer (*20*, *21*).

Studies have shown that in plant tissue, glucosinolates are present in an intact form and sequestered in the vacuole (*22*). Intact glucosinolates have no allelochemical property against plant pests or biological functions against human diseases (*23*, *24*). However, when *Brassica* tissues are cut or crushed, glucosinolates (released from the vacuole) come in contact with myrosinase (β-thioglucoside glucohydrolase; EC 3.2.3.1, released from myrosine cells), causing the formation of biologically active isothiocyanates (such as sulforaphane) and indole-3-carobinol (*1*, *25*).

Horseradish (*Armoracia rusticana*) is an important *Brassica* crop in Illinois, representing nearly 60% of the total U.S. consumption (*3*). Most of the commercial crop is crushed fresh into sauces or used as a food additive for its pungent flavor. However, very little is known about the biochemical composition of horseradish, especially the distribution of glucosinolates or myrosinase enzyme activity. Also, there have been no previous studies that examined changes in both glucosinolates content and myrosinase activity in the same tissue. The objectives of this study were to evaluate the levels of glucosinolates in horseradish roots and leaves and to correlate changes in these compounds to myrosinase activity.

MATERIALS AND METHODS

Plant Material. Twenty-seven accessions of horseradish varieties were selected for this study. These accessions are part of a germplasm collection housed at the Vegetable Research Farm, University of Illinois, Urbana−Champaign, IL. The experiment was designed as a randomized complete block consisting of six plants in each of four replicates. Plants were established from root cuttings (sets) approximately 35−40 cm long and 1.0−1.5 cm in diameter. Sets from each accession were hand-planted in rows spaced at 90 cm between rows and 56 cm within each row. The proximal ends of the sets were planted facing the same direction (for ease of harvest) in furrows, then hilled-over into ridges approximately 18 cm high and 30 cm wide. Plots were maintained during the growing season using a pest control protocol as outlined in the *Midwest Vegetable Production Guide* (*26*). Water and fertilizer were applied as needed. Leaf samples were harvested when the blade had reached its full size at about the middle of July, whereas roots were harvested in November after the roots had reached their full size and the leaves had desiccated.

Tissue samples were collected from the middle four plants of each replicate, cleaned with distilled water, and dried with paper towels. About 200 g subsamples were collected from the main root or from fully developed leaf blades, quickly frozen in liquid nitrogen, lyophilized, ground into fine powder using a coffee grinder, and stored at −70 °C for further analysis.

Glucosinolates Analysis. Glucosinolates were analyzed according to previously described procedures (*3*, *27*). A 0.2 g sample of freeze-dried tissue from each accession and each replicate was placed in a sealed 15 mL glass tube and heated at 95 °C for 15 min on a heating block (Reacti-Therm III, Pierce, Rockford, IL). The heated samples were combined with 2 mL of boiling dd-H_2O and 0.5 mL of internal standard (1 μM benzylglucosinolate, VWR Scientific Products, Chicago, IL) and reheated again for an additional 5 min. The internal standard benzylglucosinolate is naturally absent in horseradish leaf and root tissues. Samples were immediately cooled and centrifuged at 12000*g* for 10 min, and the supernatant was collected and saved on ice. The pellet was re-extracted with 1 mL of boiling dd-H_2O, centrifuged as described above, and the supernatant was collected and combined with previously saved supernatant and mixed thoroughly. A 1 mL fraction of the supernatant was combined with 150 μL of 0.5 M barium acetate, vortexed for 5 s, and layered on a 1 cm DEAE-Sephadex A-25 column. Glucosinolates were desulfated on the column with 10 units of arylsulfatase type H-1 from *H. pomatia* (Sigma, St. Louis, MO) suspended in 500 μL of dd-H_2O. The columns were capped for 18 h. Desulfated glucosinolates were eluted off the column with 2 mL of dd-H_2O and filtered through a 0.2 μm Acrodisc filter (Pall Gelman Laboratory, Ann Arbor, MI). A 20 μL fraction of the water filtrate was injected into a Hitachi HPLC system (Hitachi Ltd., Tokyo, Japan) equipped with a UV detector set at a 229 nm wavelength, a refrigerated autosampler set at 5 °C, a column heater set at 32 °C, and a Lichrosphere Hibar RP-18 column (Merck, Darmstadt, Germany). A gradient of 0−20% acetonitrile at 0.8 mL/min for 53 min was used to elute the desulfoglucosinolates off the column. The type and amount of glucosinolates, except sinigrin, were estimated based on retention times and response factors developed for desulfoglucosinolates in a standardized and certified rapeseed reference material (BCR 367) by the Commission of the European Community Bureau of References (Brussels, Belgium). Sinigrin concentration was estimated by comparison of HPLC retention time with that of an authentic sinigrin standard (Sigma) after on-column sulfatase treatment as described above. Retention times of desulfated reference glucosinolates were similar to those previously reported for broccoli (*3*).

To evaluate the effect of root and leaf sizes on glucosinolate levels and myrosinase activity, two root sizes [small, 0.5−1 cm diameter; fully developed (mature), 8−10 cm diameter] and two leaf sizes [small, ~10 days after emergence, and fully developed (mature)] were collected from cultivars 1573 and 1810-A, frozen in liquid nitrogen, lyophilized, and analyzed for glucosinolates as above.

Myrosinase Assay. Freeze-dried tissue from each accession was suspended in dd-H_2O at 0.1 g mL^{-1} and centrifuged at 13380*g* and 4 °C for 30 min. The supernatants were collected, filtered through Whatman no. 1 filter paper, and assayed for myrosinase activity according to the method of Pessina et al. (*28*) with slight modification. The enzyme activity was determined by evaluating the rate of hydrolysis of sinigrin at 37 °C. Change in the optical density of sinigrin, as a function of time, was used to calculate myrosinase activity.

The reaction mixture consisting of 33.3 mM postassium phosphate buffer (pH 6.5) and 0.2 mM sinigrin was placed in a quartz cuvette with 1 cm path length and allowed to pre-equilibrate for 1 min at 37 °C. The reaction was initiated by adding 100 μL of crude enzyme extract to the cuvette. Decline in optical density as a result of sinigrin breakdown was plotted at 227 nm and 37 °C over a 3 min period using a Shimadzu UV160 spectrophotometer (Shimadzu Corp., Kyoto, Japan). The rate of decline in optical density during the linear phase of the graph was used to calculate the enzyme activity according to the following formula:

$$\text{units cm}^{-3} = \frac{\Delta A}{\Delta t} \times \frac{1}{\epsilon l} \times \frac{V_A}{V_E} \times 10^3$$

where the extinction coefficient (ϵ) of sinigrin at 227 nm is 6784 M^{-1} cm^{-1}, the cuvette path length (l) is 1 cm, the total volume of the assay mixture (V_A) is 3.0 cm^3, the reaction time is given in minutes (Δt), and the volume of enzyme solution (V_E) is 0.1 cm^3. One unit of activity is defined as the amount of myrosinase that catalyzes the hydrolysis of 1 μmol of substrate per minute, under the conditions described above. Specific activity was expressed in units per milligram of protein.

Protein concentration was determined by a BCA kit (Pierce, Rockford, IL). Data were subjected to analysis of variance (ANOVA) using Proc GLM of SAS (release 8.0, SAS Institute, Inc., Cary, NC). Differences among accession means were compared using Fisher's protected least significant difference (LSD) at $P = 0.05$.

Influence of Tissue Crushing on Glucosinolate Content and Myrosinase Activity in Roots. Uniform small size roots (~1 cm diameter) of accession 1573 were harvested, immediately cleaned with

Table 1. Change in Total, Individual, and Residual Glucosinolates, Myrosinase Activity, and Soluble Protein Concentrations in 27 Accessions of Horseradish Roots

accession	μmol g^{-1} of DW					myrosinase activity (units g^{-1} of DW)	protein concn (mg g^{-1} of DW)
	total GS[a]	sinigrin	glucobrassicin	gluconasturtiin	residualG[b]		
810-A	296[c]	258	2.8	20.1	15.0	4.3	94.9
753-A	233	173	0.1	48.6	11.5	21.1	80.1
113-A	191	160	1.4	18.5	11.2	10.0	46.4
239-A	175	117	0.0	46.5	10.8	22.5	71.7
813-A	162	134	0.1	14.7	13.3	3.3	97.8
Bohemian	154	130	0.9	15.2	7.3	1.2	61.9
1590	152	112	1.1	28.9	10.5	10.7	62.6
106-A	152	120	0.9	19.3	11.3	33.3	82.2
196-A	140	133	1.3	1.6	4.3	8.7	63.3
493-A	134	124	0.6	2.8	6.7	50.4	51.8
819-A	131	116	0.8	12.8	1.8	23.0	127.9
502-A	127	104	0.1	16.2	7.6	13.2	45.8
1724	107	102	0.5	1.2	3.3	26.3	72.7
104-A	78	70	0.2	2.7	5.5	25.7	70.2
125-A	75	61	0.7	4.7	3.5	57.1	74.6
1573	56	48	0.7	2.0	5.3	3.2	102.6
532-A	55	51	0.1	0.0	3.3	2.8	73.8
856-A	40	36	0.1	2.2	1.7	44.5	94.4
51-A	35	28	0.1	5.7	2.1	26.1	119.5
1069	33	32	0.1	0.0	0.8	9.5	54.9
789-A	27	21	0.8	4.5	1.3	22.7	90.6
1717	21	16	0.3	3.8	1.4	21.2	54.7
777-A	20	17	0.4	1.9	0.8	1.7	32.0
244-A	11	7	0.1	2.4	0.9	21.5	21.6
811-A	10	8	0.1	0.1	1.3	8.2	61.4
53-A	6	5	0.0	0.0	0.7	21.6	51.3
Big-Top-Western	2	2	0.0	0.1	0.1	20.7	38.5
overall mean	97	81	0.5	10.3	5.3	19.1	70.3
LSD at $P = 0.05$	64	56	1.0	9.4	4.6	10.8	30.3

[a] Total GS represent the sum of sinigrin, glucobrassicin, and gluconasturtiin plus residual glucosinolates (GS). GS were desulfated by aryl sulfatase and detected using HPLC. [b] Residual glucosinolates represent the sum of at least five additional glucosinolates. [c] Values represent the means of four replicates per accession.

distilled water, and blotted dry with paper towels. Roots were divided into 20 groups (~40 g each) of uniform size segments. Each group was place in a chilled mortar and pestle and gently crushed in dd-H_2O at 0.1 g m^{-1}. Crushing time and tissue consistency were kept as uniform as possible to represent commercial preparation of horseradish sauces. Tissue macerates were placed in beakers and incubated at 27 °C in a water bath for 0, 15, 30, and 60 min. Each group of four was assigned to each time interval as replicates. At the end of each incubation time, the four replicates per wounding time interval were removed from the water bath, immediately frozen in liquid nitrogen, and lyophilized. Samples were analyzed for glucosinolate content and myrosinase activity as described above.

RESULTS AND DISCUSSION

Change in Glucosinolate Content and Myrosinase Activity in Roots and Leaves of Different Horseradish Accessions. There are ~130 horseradish accessions in the Univesity of Illinois germplasm collection. The geographic origins and morphological characteristics of some of these accessions have been listed in two previous studies (*3*, *29*, *30*). Most of these accessions have originated from eastern Europe and North America. However, only four of these accessions (167a, 204a, 1573, and 1590) are being grown commercially in Illinois on ~800 ha at the Mississippi River Valley in East St. Louis.

Eight different glucosinolates have been detected in variable concentrations in roots and leaves of horseradish (**Tables 1** and **2**). The sum of these glucosinolates, henceforth referred to as total, showed significant differences among the roots of the 27 accessions tested. Accessions 810-A and 753-A produced the highest total glucosinolate concentrations of 296 and 233 μmol g^{-1} of dry weight (DW), respectively (**Table 1**). Eleven accessions produced total glucosinolate concentrations between 101 and 200 μmol g^{-1} of DW, 3 accessions produced total glucosinolate concentrations between 51 and 100 μmol g^{-1} of DW, and 10 accessions produced glucosinolate concentrations between 0 and 50 μmol g^{-1} of DW (**Table 1**). Plant origin does not seem to have an effect on total glucosinolate levels. For example, 810-A and 811-A were established from seedlings originated in Switzerland, whereas 113-A and 104-A originated from Russia (*29*), yet there were differences of 30.2- and 2.5-fold, respectively, in their total root glucosinolate contents (**Table 1**).

Two of the four commercially grown cultivars, Bohemian and 1590, produced higher than average total glucosinolates (154 and 152 μmol g^{-1} of DW), 1573 produced below average concentration (56 μmol g^{-1} of DW), and Big-Top-Western produced the lowest concentration (2 μmol g^{-1} of DW). The average total glucosinolates concentration in horseradish roots is similar to previously reported values for brown and oriental mustard seeds (*31*), but significantly higher than what we had previously reported for broccoli (13 μmol g^{-1} of DW), Brussels sprouts (25 μmol g^{-1} of DW), cabbage (11 μmol g^{-1} of DW), cauliflower (15 μmol g^{-1} of DW), or kale (15 μmol g^{-1} of DW) (*3*).

Three individual glucosinolates, sinigrin, glucobrassicin, and gluconasturtiin, have been detected in the roots of all accessions, except 1069, which has no detectable level of gluconasturtiin, and 53-A, which has no glucobrassicin or gluconasturtiin (**Table 1**). Sinigrin represented on average ~83% of the total glucosinolates, followed by gluconasturtiin at ~11%, and glucobrassicin at ~1%. At least four other glucosinolates (progoitrin, gluconapin, 4-hydroxyglucobrassicin, and 4-methoxyglucobrassicin) were detected in minor concentrations (<1%) in some of the

Table 2. Changes in Total, Individual, and Residual Glucosinolates, Myrosinase Activity, and Soluble Protein Concentration in Nine Accessions of Horseradish Leaves

	μmol g^{-1} of DW					myrosinase activity	protein concn
accession	total GS[a]	sinigrin	neoglucobrassicin	gluconasturtiin	residual GS[b]	(units g^{-1} of DW)	(mg g^{-1} of DW)
493-A	201[c]	189	5.0	0.1	7.3	40.9	121.3
125-A	135	132	1.3	0.4	1.7	21.2	122.3
810-A	126	115	1.1	0.1	10.0	13.6	166.8
777-A	96	88	1.3	0.1	7.0	18.8	133.5
819-A	84	80	2.8	0.1	1.4	17.5	147.9
753-A	81	63	3.7	0.3	13.7	8.5	89.7
53-A	64	58	3.9	0.1	2.2	9.3	125.5
Bohemian	45	39	0.0	0.2	6.2	10.0	151.4
789-A	34	30	3.1	0.0	0.8	18.9	133.3
overall mean	95	88	2.5	0.2	5.6	17.6	132.4
LSD at $P = 0.05$	26	25	1.4	0.06	2.7	12.1	24.3

[a] Total GS (glucosinolates) represent the sum of sinigrin, neoglucobrassicin, and gluconasturtiin plus residual glucosinolates. GS were desulfated by aryl sulfatase and detected using HPLC. [b] Residual GS are the sum of at least four additional glucosinolates. [c] Values represent the means of four replicates per accession.

accessions. Data for these glucosinolates were combined and presented as residual glucosinolates (**Table 1**). The average sum of the residual glucosinolates in the roots was ~5% of the total.

Glucosinolate in the leaves were analyzed in a subset of nine accessions with low, medium, and high glucosinolate levels in the roots (**Table 2**). Total glucosinolate levels in the leaves were comparable to those in the roots. The highest total glucosinolate content was detected in cultivar 493-A (201 μmol g^{-1} of DW), and the lowest was detected in cultivar 789-A (34 μmol g^{-1} of DW). However, there were no correlations between total or any of the individual glucosinolates in the leaves and the roots, suggesting that the biosyntheses of glucosinolates in the leaves and roots might be independently regulated. Sang et al. (*32*) proposed that root tissue has higher total glucosinolates than leaf tissue, but they did not present quantitative data to support their conclusion. In contrast, Li et al. (*33*) found no correlation between total glucosinolates in leaves, roots, stems, or seeds in several lines of *Brassica napus*. Our results support the conclusion of Li et al. (*33*) that glucosinolate synthesis and accumulation are tissue specific.

Similar to the roots, sinigrin was the dominant glucosinolate in the leaves, representing on average ~92% of the total (**Table 2**). Leaves also contained gluconasturtiin, but at a much lower concentration (0.2 μmol g^{-1} of DW) than in the roots (10.3 μmol g^{-1} of DW). In contrast to the roots, leaf tissue contained neoglucobrassicin (2.5% of total) instead of glucobrassicin (**Table 2**). In a preliminary study, Etoh et al. (*34*) reported high concentrations of sinigrin in horseradish (78%) and wasabi (83%) roots. However, when they analyzed wasabi leaves, they found that they contained only ~24% sinigrin compared to the roots. High sinigrin concentration has also been detected in oriental mustard (*Brassica juncea*), Brussels sprouts, cabbage, cauliflower, and kale (*2*, *3*, *35*). To our knowledge, only broccoli was reported to have very low sinigrin concentration. Broccoli, instead, accumulates the aliphatic glucosinolate glucoraphanin (*3*, *6*, *7*). We did not detect any glucoraphanin in the 27 accessions of horseradish (data not shown). Magrath et al. (*5*) reported that mainly genetic factors determine the contents of aliphatic glucosinolates, such as sinigrin and glucoraphanin, whereas environmental factors have a significant influence on contents of tryptophan-derived indole glucosinolates, such as glucobrassicin and neoglucobrassicin. Pearson correlations between total glucosinolates and sinigrin in the roots and leaves were $r = 0.49$ for total and $r = 0.47$ for sinigrin, but they were not significant, suggesting that their syntheses are independently regulated.

Table 3. Changes in Total, Individual, and Residual Glucosinolates in Two Accessions of Horseradish Roots as a Function of Growth Stage

	μmol g^{-1} of DW				
accession, growth stage	total GS[a]	sinigrin	glucobrassicin	gluconasturtiin	residual GS[b]
810-A, small	74.7b[c]	58.0b	13.4b	0.2c	3.1c
810-A, mature	295.8a	258.0a	2.8c	20.1a	14.9a
1573, small	119.1b	77.3b	33.9a	0.2c	7.7ab
1573, mature	55.5c	47.5b	0.7c	2.0b	5.3bc

[a] Total GS (glucosinolates) represent the sum of sinigrin, glucobrassicin, and gluconasturtiin plus residual glucosinolates. GS were desulfated by aryl sulfatase and detected using HPLC. [b] Residual glucosinolates is the sum of at least four additional glucosinolates. [c] Values represent the means of four replicates per accession. Mean separation within each column by Duncan's multiple-range test, $P = 0.05$.

Myrosinase activity values in the root and leaf tissues are shown in **Tables 1** and **2**. Similar to glucosinolates, myrosinase activities in the root and leaf tissues were also variable, ranging from 1.2 to 57.1 units g^{-1} of DW in the roots and from 8.5 to 40.9 units g^{-1} of DW in the leaves (**Table 1**). Myrosinase activity in the roots was slightly higher than in the leaves; however, when the activity was calculated on the basis of a milligram protein basis (specific activity), roots had about double the activity in the leaves, 0.33 and 0.19 units mg^{-1} of protein, respectively. Higher myrosinase specific activity has also been reported in the roots and cotyledons than in the leaves of *Brassica napus* (*36*).

Pearson's correlation of myrosinase activity to total glucosinolates and sinigrin was not significant in the roots. However, in the leaves there were significant correlations between myrosinase activity and sinigrin (0.47, $P = 0.01$) and between myrosinase activity and total glucosinolates (0.42, $P = 0.027$). The lack of correlations between myrosinase activity, sinigrin, and total glucosinolates in the roots was observed even when we used the same subset of accessions as in the leaves.

Changes in Glucosinolates Content in Young and Fully Developed Tissue and during Tissue Crushing. In young roots total glucosinolates were statistically similar in accessions 810-A and 1573 (**Table 3**). However, in fully developed (mature) roots accession 810-A had significantly higher (>5-fold) total glucosinolates than accession 1573 (**Table 3**). The major reason for the large difference in total glucosinolates in fully developed roots was the presence of a significantly higher concentration of sinigrin in 810-A than in 1573. Young roots also contained

Table 4. Changes in Total, Individual, and Residual Glucosinolates in Two Accessions of Horseradish Leaves as a Function of Leaf Growth Stage

	μmol g^{-1} of DW				
accession, growth stage	total GS[a]	sinigrin	neogluco-brassicin	gluco-nasturtiin	residual GS[b]
810-A, small	150.2a[c]	77.6ab	66.2a	0.5a	2.3a
810-A, mature	126.0a	114.8a	1.2b	0.1a	1.1a
1573, small	129.2a	60.3b	69.7a	0.3a	2.4a
1573, mature	52.7b	48.6c	1.1b	0.4a	1.2a

[a] Total GS (glucosinolates) represent the sum of sinigrin, glucobrassicin, and gluconasturtiin plus residual glucosinolates. GS were desulfated by aryl sulfatase and detected using HPLC. [b] Residual glucosinolates is the sum of at least four additional glucosinolates. [c] Values represent the mean of four replicates per accession. Mean separation within each column by Duncan's multiple-range test, $P = 0.05$.

Table 5. Change in Total Glucosinolates, Sinigrin, and Glucobrassicin Following Crushing of Small Root Tissue of Cultivar 1573

	μmol g^{-1} of DW			
time after crushing (min)	total GS[a]	sinigrin	glucobrassicin	myrosinase activity (units g^{-1} of DW)
0[b]	120.9a	81.0a	32.1a	5.3
15	1.9b	1.3b	0.4bc	1.7
30	0.7c	0.5c	0.2c	0.4
60	0.5c	0.3c	0.1c	0.1

[a] Total GS (glucosinolates) represent the sum of sinigrin, glucobrassicin, and gluconasturtiin plus residual glucosinolates. GS were desulfated by aryl sulfatase and detected using HPLC. Values represent the mean of four replicates per treatment (time). Mean separation within each column by Duncan's multiple-range test, $P = 0.05$. Crushing involved uniform grinding of the tissue using a mortar and pestle. [b] Intact tissue.

significantly higher concentrations of glucobrassicin than fully developed roots (**Table 3**).

In leaves, there were no major differences in total glucosinolates, sinigrin, gluconasturtiin, or residual glucosinolates between young and fully developed (mature) leaves, except 1573, which contained lower sinigrin in fully developed leaves (**Table 4**). The only major difference was observed in neoglucobrassicin, a derivative of glucobrassicin. Young leaves contained >60-fold higher neoglucobrassicin than fully developed leaves.

Several studies have examined changes in total and individual glucosinolates as a function of tissue age. In a previous study on *Arabidopsis thaliana*, younger leaves were reported to contain ~10-fold higher glucosinolates than older leaves and older leaves tended to accumulate more indole than aliphatic glucosinolates (*36*). In this study, however, we found no major shift in aliphatic glucosinolates in both leaves and roots as a function of tissue age except in 1810-A, which accumulated higher sinigrin in older than in younger tissue. The only shift we have noticed is that younger tissue of horseradish roots and leaves tended to accumulate more indole glucosinolates (glucobrassicin and neoglucobrassicin) than fully developed leaves. Our study is in agreement with previous studies, which showed higher levels of gluconasturtiin in roots and in leaves of *Brassica oleracea* and *A. thaliana* (*37*, *38*). Root and leaf size had no influence on myrosinase activity. Also, young and fully developed roots and leaves contained statistically similar myrosinase activities regardless of their size (data not shown).

The bulk of the commercial production of horseradish roots is crushed into relishes and sauces that are used as condiments in ethnic cooking. In crushed tissue of cultivar 1573, total glucosinolates declined to <2% of their level in the control within 15 min after tissue crushing, and by 30 min nearly all total glucosinolates were hydrolyzed (**Table 5**). The rapid breakdown of sinigrin and glucobrassicin after tissue crushing suggests that the myrosinase enzyme is very active against both aliphatic (sinigrin) and indole (glucobrassicin) glucosinolates. Myrosinase activity declined by ~67% within 15 min after crushing, possibly in response to the depletion of glucosinolates (**Table 5**) or due to inactivation by the hydrolysis products sulfate and glucose (*39*). However, allyl isothiocyanate was reported to have no significant influence on myrosinase activity (*39*).

Although it is difficult to pinpoint a specific physiological function for glucosinolates and/or their hydrolysis products in cruciferous plants, the most widely accepted hypothesis is that they are involved in a defense mechanism against several pests (*1*, *15*). For example, evaluation of the feeding behavior of herbivores on 14 homozygous lines of *B. juncea* showed less feeding of the generalist insect *Spodoptera eridania* on lines with higher glucosinolate content and less feeding of the cruciferous specialist insect *Putella xylstella* on lines with higher myrosinase activity than lower lines (*40*). Similarly, *B. napus* lines with high sinigrin content were less preferred to Bertha armyworm (*Mamestra configurata*) than lines with low sinigrin content (*41*). *Brassica* tissue with high glucosinolate levels also suppressed diseases, such as *Pythium*, when applied as green manure (*42*). On the basis of these studies and others, it is possible that the high sinigrin content and myrosinase activity in some of the horseradish accessions identified in this study may be transferred through genetic engineering or conventional breeding programs to commercial lines such as Big-Top-Western to increase their pest resistance and potentially their human health benefits.

In summary, glucosinolates and myrosinase activity were detected in varying levels in the roots and leaves of all 27 horseradish accessions. Sinigrin represented ~83% and gluconasturtiin ~10% of the total glucosinolates in the roots. In the leaves sinigrin represented ~91% and neoglucobrassicin ~2% of the total glucosinolates. However, there were no correlations between total glucosinolates or myrosinase activity in the roots and leaves, which suggested that the two are independently regulated and are site specific. The high levels of glucosinolates and myrosinase activity in some of the accessions may provide useful breeding tools for enhancing the pest resistance and human health benefits of commercial horseradish cultivars.

ACKNOWLEDGMENT

We thank Ken Horn for help with maintaining the horseradish germplasm collection.

LITERATURE CITED

(1) Fenwick, G. R.; Heaney, R. K.; Mullin, W. J. Glucosinolates and their breakdown products in food and food plants. *CRC Crit. Rev. Food Sci. Nutr.* **1983**, *18*, 123−201.

(2) Carlson, D. G.; Daxenbichler, M. E.; VanEtten, C. H.; Kwolek, W. F.; William, P. H. Glucosinolates in crucifer vegetables: broccoli, Brussels sprouts, cauliflower, collard, kale, mustard greens and kohlrabi. *J. Am. Soc. Hortic. Sci.* **1987**, *112*, 173−178.

(3) Kushad, M. M.; Brown, A. F.; Kurilich, A. C.; Juvik, J. A.; Klein, P.; Wallig, M. A.; Jeffery, E. H. Variation of glucosinolates in vegetable crops of *Brassica oleracea*. *J. Agric. Food Chem.* **1999**, *47*, 1541−1548.

(4) Fahey, J. W.; Zalcmann, A. T.; Talalay, P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* **2001**, *56*, 5−51.
(5) Magrath, R.; Herron, C.; Giamoustaris, A.; Mithen, R. The inheritance of aliphatic glucosinolates in *Brassica napus*. *Plant Breeding* **1993**, *111*, 55−72.
(6) Rosa, E. A.; Rodrigues, A. S. Total and individual glucosinolate content in 11 broccoli cultivars grown in early and late season. *HortScience* **2001**, *36*, 56−59.
(7) Pereira, F. M.; Rosa, E.; Fahey, J. W.; Stephenson, K. K.; Carvalho, R.; Airs, A. Influence of temperature and ontogeny on the levels of glucosinolates in broccoli (*Brassica oleracea* var. italica) sprouts and their effect on the induction of mammalian phase 2 enzymes. *J. Agric. Food Chem.* **2002**, *50*, 6239−6244.
(8) Bennett, R. N.; Mellon, F. A.; Botting, N. P.; Eagles, J.; Rosa, E. A.; Williamson, G. Identification of the major glucosinolate (4-mercaptobutyl glucosinolates) in leaves of *Eruca sativa* L. (salad rocket). *Phytochemistry* **2002**, *61*, 25−30.
(9) Engel, E.; Baty, D.; Le Corre, I.; Souchon, N.; Martin, N. Flavor-active compounds potentially implicated in cooked cauliflower acceptance. *J. Agric. Food Chem.* **2002**, *50*, 6459−6467.
(10) Brabban, A. D.; Edwards, C. The effects of glucosinolates and their hydrolysis products on microbial growth. *J. Appl. Bacteriol.* **1995**, *79*, 171−177.
(11) Brown, P.; Morra, M. Glucosinolates containing plant tissue as bioherbicides. *J. Agric. Food Chem.* **1995**, *43*, 3070−3074.
(12) Glenn, M. G.; Chew, F. S.; Williams, P. H. Influence of glucosinolate content of *Brassica* (*cruciferae*) roots on growth of *vesicular-arbuscular* mycorrhizal fungi. *New Phytol.* **1988**, *110*, 217−225.
(13) Mayton, H. S.; Olivier, C.; Vaughn, S. F.; Loria, R. Correlation of fungicidal activity of *Brassica* species with allyl isothiocyanate production in macerated leaf tissue. *Phytopathology* **1996**, *86*, 267−271.
(14) Potter, M. J.; Davies, K.; Rathjen, A. J. Suppressive impact of glucosinolates in *Brassica* vegetative tissues on root lesion nematodes *Pratylenchus neglectus*. *J. Chem. Ecol.* **1998**, *24*, 6780.
(15) Spak, J.; Kolarvoa, J.; Fenwick, G. R. The effect of glucosinolates (mustard oil glucosides) and products of their enzymatic degradation on the infectivity of turnip mosaic virus. *Biol. Plant.* **1993**, *35*, 73−80.
(16) Talalay, P.; Zhang, Y. Chemoprotection against cancer by isothiocyanates and glucosinolates. *Biochem Soc. Trans.* **1996**, *4*, 806−810.
(17) Wang, H.; Griffiths, S.; Williamson, G. Effect of glucosinolate breakdown products on β-naphthoflavone-induced expression of human cytochrome P450 1A1 via the Ah receptor in Hep G2 cells. *Cancer Lett.* **1997**, *114*, 121−125.
(18) Wattenberg, L. W. Inhibition of carcinogenesis by minor dietary constituents. *Cancer Res.* **1992**, *52*, 2085S−2091S.
(19) Thorling, E. B. Dietary non-nutritive cancer protective factors. *Eur. J. Cancer Prev.* **1993**, *2*, 95−103.
(20) Chang, Y. C.; Riby, J.; Chang, G. H.; Peng, B. C.; Firestone, G.; Bjeldanes, L. F. Cytostatic and antiestrogenic effects of 2-(indole-3-ylmethyl)-3,3′-diindolylmethane, a major in vivo product of dietary indole-3-carbinol. *Biochem. Pharmacol.* **1999**, *58*, 825−834.
(21) Meng, Q.; Yuan, F.; Goldberg, I.; Rosen, E.; Auborn, K.; Fan, S. Indole-3-carbinol is a negative regulator of estrogen receptor-α signaling in human tumor cells. *J. Nutr.* **2001**, *130*, 2927−2931.
(22) Yiu, S. H.; Collins, F. W.; Fulcher, R. G.; Altosaar, I. Chromatographic and microscopic detection of glucosinolates in rapeseed using *N*,2,6-trichloro-*p*-benzoquinoneimine. *Can. J. Plant Sci.* **1984**, *64*, 869−878.
(23) Loft, S.; Otte, J.; Paulson, H.; Sorensen, H. Influence of intact and myrosinase-treated indolyl glucosinolates on the metabolism in vivo of metronidazole and antipyrine in the rat. *Food Chem. Toxicol.* **1992**, *30*, 927−935.
(24) Raybould, A. F.; Moyes, C. L. The ecological genetics of aliphatic glucosinolates. **2001**, *87*, 383−391.
(25) Johnson, I. T. Glucosinolates: bioavailability and importance to health. *Int. J. Vitam. Nutr. Res.* **2002**, *72*, 26−31.
(26) Foster, R.; Maynard, L. *Midwest Vegetable Production Guide for Commercial Growers*; University of Illinois Extension Publication C1364; Urbana−Champaign, IL, 2003; pp 47−55.
(27) Wathelet, J. P.; Wagstaffe, P. J.; Boenke, A.; Marlier, M.; Severin, M. The certification of the total glucosinolate contents in 3 rapeseeds (CRM-190, CRM-366 and CRM-367). *Fresenius' J. Anal. Chem.* **1993**, *347*, 396−399.
(28) Pessina, A.; Thomas, R. M.; Palmieri, S.; Luisi, R. An improved method for the purification of myrosinase and its physiochemical characterization. *Arch. Biochem. Biophys.* **1990**, *280*, 383−389.
(29) Rhodes, A. M.; Courter, J. W.; Churtleff, M. C. Identification of horseradish types. *Illinois State Acad. Sci.* **1965**, *58*, 115−122.
(30) Rhodes, A. M.; Cramer, S. G.; Courter, J. W. Measurement and classification of genetic variability of horseradish. *J. Am. Soc. Hortic. Sci.* **1969**, *94*, 98−102.
(31) Szmigielska, A. M.; Schoenau, J. J. Use of anion-exchange membrane extraction for the high-performance liquid chromatographic analysis of mustard seed glucosinolates. *J. Agric. Food Chem.* **2000**, *48*, 5190−5194.
(32) Sang, J. P.; Minchinton, I. R.; Johnstone, P. K.; Truscott, R. J. Glucosinolate profiles in the seed, root, and leaf tissue of cabbage, mustard, rapeseed, radish and Swede. *Can. J. Plant Sci.* **1984**, *64*, 77−93.
(33) Li, Y.; Kiddle, G.; Bennett, R.; Doughty, K.; Wallsgrove, K. Variation in the glucosinolate content of vegetable tissues of Chinese lines of *Brassica napus* L. *Ann. Appl. Biol.* **1999**, *134*, 131−136.
(34) Etoh, H.; Nishimura, A.; Takasawa, R.; Yagi, A.; Saito, K.; Sakata, K.; Kishima, I.; Ina, K. ω-Methylsulfinylalkyl isothiocyanates in Wasabi, *Wasabia japonica* Matsum. *Agric. Biol. Chem.* **1990**, *54*, 1587−1589.
(35) Tsao, R.; Yu, Q.; Friesen, I.; Potter, J.; Chiba, M. Factors affecting the dissolution and degradation of oriental mustard-derived sinigrin and allyl isothiocyanate in aqueous media. *J. Agric. Food Chem.* **2000**, *48*, 1898−1902.
(36) Bones, A. M. Distribution of β-thioglucosidase activity in intact plants, cell and tissue cultures and regenerant plants of *Brassica napus* L. *J. Exp. Bot.* **1990**, *41*, 737−744.
(37) Rosa, E. Daily variation of glucosinolates concentrations in the leaves and roots of cabbage seedlings in two constant temperature regimes. *J. Sci. Food Agric.* **1997**, 364−368.
(38) Brown, P. D.; Tokuhisa, J. G.; Reichelt, M.; Gershenzon, J. Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. *Phytochemistry* **2003**, *62*, 472−482.
(39) Shikita, M.; Fahey, J.; Golden, T. R.; Holtzclaw, D.; Talalay, P. An unusual case of 'uncompetitive activation' by ascorbate: purification and kinetic properties of myrosinase from *Raphanus sativus* seedlings. *Biochem. J.* **1999**, 725−732.
(40) Li, Q.; Eigenbrode, S.; Stringham, G.; Thiagarajah, M. Feeding growth of *Putella xylostella* and *Spodoptera eridania* on *Brassica juncea* with varying glucosinolate concentrations and myrosinase activities. *J. Chem. Ecol.* **2000**, *26*, 2401−2419.
(41) Ulmer, B.; Gillott, C.; Erlandson, M. Feeding preference, growth, and development of *Mamestra configurata* (Lepidoptera: Noctuidae) on *Brassicaceae*. *Can. Entomol.* **2001**, *133*, 509−519.
(42) Lazzeri, L.; Manici, M. Allelopathic effect of glucosinolate containing plant green manure on *Pythium* sp. and total fungal population in soil. *HortScience* **2001**, *37*, 1283−1289.

Received for review April 14, 2004. Revised manuscript received August 6, 2004. Accepted September 10, 2004. This study was support by grants from the University of Illinois Functional Food for Health Program and the Illinois Horseradish Growers Association.

JF0401827