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Evaluation of Glucosinolate Variation in a Collection of Turnip (*Brassica rapa*) Germplasm by the Analysis of Intact and Desulfo Glucosinolates

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Supporting Information

ABSTRACT: Glucosinolates (GLS) are secondary metabolites occurring in cruciferous species. These compounds are important for plant defense, human health, and the characteristic flavor of Brassica vegetables. In this study, the GLS in tubers from a collection of 48 turnip (*Brassica rapa*) accessions from different geographic origin were analyzed. Two different methods were used: desulfo GLS were analyzed by high-performance liquid chromatography with a photodiode array detector, and intact GLS were analyzed by accurate mass liquid chromatography−mass spectrometry. For most GLS, desulfo and intact signals correlated well, and the analytical reproducibility for individual GLS was similar for both methods. A total of 11 different GLS was monitored in the turnip tubers, through both intact and desulfo GLS analysis methods. Four clusters of accessions could be clearly distinguished based on GLS composition of the turnip tuber. Clustering based on tuber GLS differed markedly from a previously published clustering based on leaf GLS.

KEYWORDS: *glucosinolate, turnip, Brassica, mass spectrometry, HPLC*

INTRODUCTION

Glucosinolates (GLS) are nitrogen- and sulfur-containing plant secondary metabolites that are abundant in the *Brassicaceae* family. Approximately 120 different GLS are known, which share a β-D-glucopyranose residue linked via a sulfur to a (*Z*)-*N*-hydroximinosulfate ester and which differ in a variable side chain R group, derived from one of eight amino acids.[1]

The GLS in Brassicaceae play a dominant role in the consumer appreciation. The characteristic bitter taste and flavor of Brassicaceae crops are mainly due to the isothiocyanates, which are generated by hydrolysis of GLS by myrosinase.[2] A few GLS hydrolysis products have been identified as potent chemopreventive substances through inducing phase II detoxification enzymes including quinone reductase, glutathione-*S*-transferase, and glucuronosyl transferases.[3,4] The biosynthetic pathways to different GLS have been extensively studied in *Arabidopsis thaliana*.[5] Brassicaceae breeding lines with elevated content of specific health-associated GLS and improved postharvest quality have been selected.[6]

Turnip (*Brassica rapa* subsp. *rapa*) tubers were first consumed in temperate Europe around 2500−2000 BC and have been widely used for fresh vegetables or fodder. In addition to tubers, also turnip tops and greens are consumed.[7] Among the GLS detected in turnip, glucoraphanin, glucobrassicin, and gluconasturtiin are well known for their chemopreventive activity. In recent years, breeding for diversity in GLS content or elevated levels of total GLS has been initiated, for which reason collections of germplasm material have been established and are now under characterization.[7−9]

The evaluation of GLS profiles in large germplasm collections or breeding populations, in particular to characterize the diversity in GLS composition, would benefit from a reproducible and preferably simple and rapid method for comparison of content of individual GLS. Conventionally, GLS are analyzed as their desulfo compounds, after solid-phase extraction and sulfatase treatment of crude extracts, by using high-performance liquid chromatography (HPLC) with a photodiode array (PDA) detector. This method provides quantitative information on the content of individual GLS.[10−12] For the analysis of intact GLS, HPLC coupled to high-resolution mass spectrometry (MS) has been suggested, providing the relative levels of each GLS over samples.[13] MS allows the use of accurate mass information for the identification and specific detection of GLS in crude plant extracts, without preceding cleanup steps or enzymatic treatments, which can be beneficial in screening large series of plants for their variation in GLS profiles.[13−16]

The aim of this research was to investigate the variation in GLS in turnip tuber accessions, both using desulfo GLS analysis

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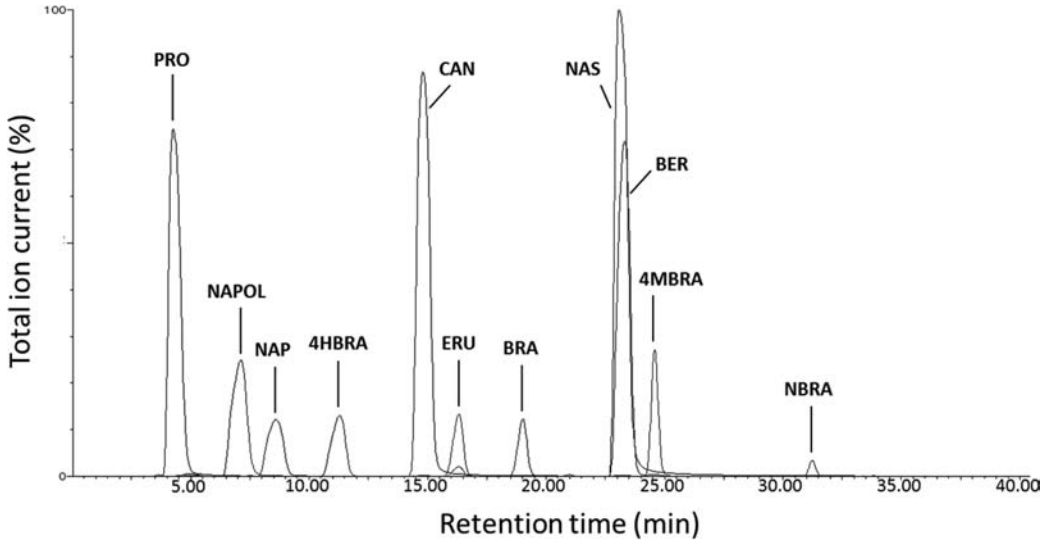

Figure 1. LC-MS chromatogram of extracts from turnip accession FT-004. Overlay of 11 mass-specific chromatograms of individual intact GLS. The information on abbreviations used, accurate mass, and retention time are shown in Table 1.

and a liquid chromatography−mass spectrometry (LC-MS) analysis for intact GLS. To this end, turnip tuber material from a collection of 48 vegetable and fodder accessions from different geographic origin was analyzed. The results of the desulfo and intact GLS analyses were compared.

MATERIALS AND METHODS

Plant Materials and Sample Preparation. The turnip (*Brassica rapa* subsp. *rapa*) germplasm used for this study consisted of 48 accessions, mainly landraces from gene banks, including 40 vegetable and 8 fodder turnips, having different phenotypes, and from different geographic origin (31 European and 17 Asian). Leaf morphology of a subset of these accessions has been described,[17] and a clear observation is that Asian turnips have entire leaves while European turnips have serrated leaves. Forty accessions were obtained from the Laboratory of Plant Breeding, Wageningen University (WUR), The Netherlands, originally from the Dutch Crop Genetic Resources Centre (CGN), the Vavilov Research Institute of Plant Industry (VIR), and Russian State Agrarian University (RSAU). In addition, eight accessions were obtained from the National Agrobiodiversity Center, National Academy of Agricultural Science (NAAS), Rural Development Administration, Korea (Table S1, Supporting Information).

The turnip accessions were sown in February 2010 and grown for three and a half months in a temperature-controlled greenhouse at Unifarm in WUR with a complete randomized block design, to minimize seasonal and environmental differences. Four blocks were included with one plant per accession in each block. Three to four mature turnips per accession were harvested at 110 days after sowing and immediately sliced to uniform parts including the outer and inner turnip tissues, pooled per accession into a corning tube, and then immediately frozen in liquid nitrogen. As a control for variation among sets of plants from the same accession, three pools from one accession (FT-004) were analyzed (Table S2, Supporting Information). The standard error over the three pools did not exceed 15% of the mean for any of the GLS. Thus, the reported GLS profiles can be considered as representative for the accessions but do not provide information on the variation between individual plants. The plant samples were freeze-dried and milled to fine powder and then stored at −20 °C for further GLS analysis.

Chemicals. The methanol and acetonitrile used for the GLS extraction and for liquid chromatography were of HPLC grade. The DEAE Sephadex-A25, sinigrin, and aryl sulfatase from *Helix pomatia* were purchased from Sigma−Aldrich.

GLS Extraction and Desulfation. For the intact GLS analysis, 50 mg of freeze-dried turnip powder was weighed into a 10 mL glass tube with cap, extracted with 2 mL of 75% methanol and 0.1% formic acid for 15 min by sonication, and then centrifuged for 15 min at 4 °C at 1000*g*. After centrifugation of the crude extracts, the supernatants were filtered using Minisart SRP4 filters (Sartorius, Goettingen) for LC-PDA-QTOF-MS injection. A re-extraction of the insoluble material of two genotypes with 2 mL of 75% methanol and 0.1% formic acid contained less than 5% of the GLS extracted in the first extraction (not shown) and was therefore omitted from the procedure.

The desulfo GLS extraction procedure was performed according to ISO 9167-1[18] and Kliebenstein et al.[19] with slight modifications. A 50 mg amount of freeze-dried turnip powder was extracted with 1 mL of 70% boiling methanol for 20 min and immediately centrifuged at 12 000 rpm for 10 min at 4 °C. The residue was re-extracted in the same way twice with boiling methanol, and the supernatants were combined in a mini vial to give a final volume of 2.5 mL. To remove the sulfate moieties from the intact GLS, the total extract was loaded onto a Mini Biospin chromatography column (Biorad) containing 0.5 mL of DEAE-Sephadex A 25, which was preactivated with 0.1 M sodium acetate (pH 4.0). A 10 unit amount of aryl sulfatase (EC 3.1.6.1, type H-1 from *Helix pomatia*) suspended in 200 μL of distilled water was added onto each column. Columns were capped and left for 24 h at room temperature. Desulfo GLS were eluted from the column by washing with 1.5 mL of water, and the eluate was filtered over 0.45 μm before analysis.

GLS Determination using LC-PDA-QTOF-MS. Both intact and desulfo GLS extracts were analyzed for GLS composition using a LC-PDA-QTOF-MS system, essentially as described before.[13]

Intact GLS Samples were injected (5 μL) into an Alliance 2795 HT instrument (Waters) and separated on a Phenomenex Luna C18 column (2.0 mm × 150 mm, 3 mm particle size). Eluents used were water and 0.1% formic acid (A) and acetonitrile and 0.1% formic acid (B). Compounds were separated using a gradient from 5% B to 35% B in 45 min and then detected first by a PDA detector (wavelength 220−600 nm, Waters Co.) and second by a Waters Micromass QTOF Ultima mass spectrometer with negative electrospray ionization (m/z 80−1500). After the gradient, the column was washed with 75% B for 5 min and reconditioning at 5% B for 5 min, with a flow rate of 0.19 mL/min.

For desulfo GLS analysis, the LC-QTOF-PDA-MS system, column, and injection volume were the same as those for intact GLS analysis. The gradient for desulfo GLS was linear from 0.2% to 35% B for 45 min, followed by column washing at 75% B for 5 min, return to 0.2% B

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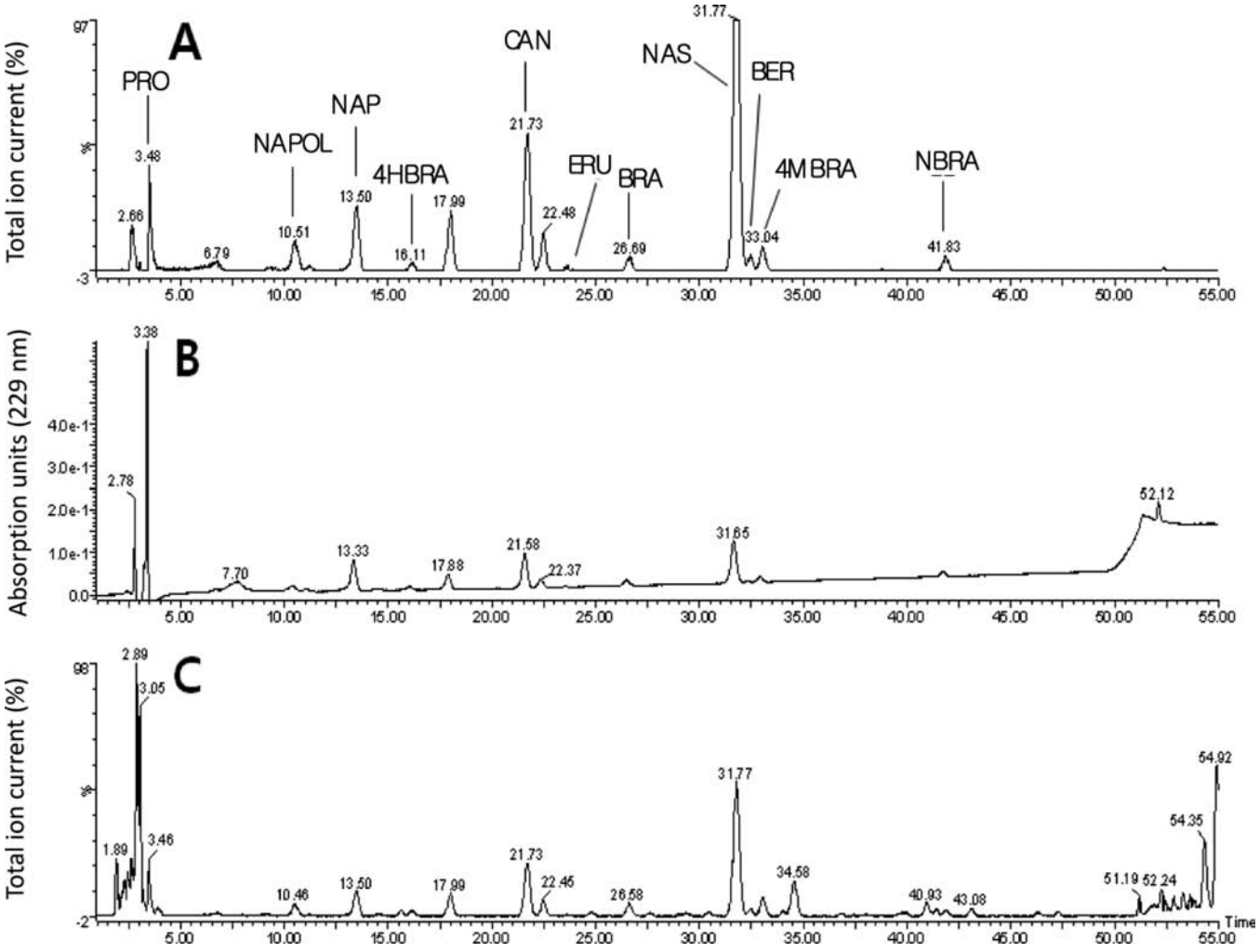

Figure 2. LC-MS and LC-PDA chromatograms of 11 desulfo GLS identified from turnip tuber tissue of accession FT-004. (A) Mass-specific chromatogram (m/z 195) representing the core structure of desulfo GLS. (B) PDA chromatogram of desulfo GLS recorded at 229 nm. (C) Ion current trace and base peak intensity of desulfo GLS extract. Abbreviations for each GLS are explained in Table 1.

Table 1. Chromatographic Information of 11 Identified Major Intact and Desulfo GLS in Turnip

peak no.	trivial name	abbreviation	side chain	group[a]	calcd mass for intact GLS $[M - H]^-$	calcd mass for desulfo GLS $[M - H]^-$	observed RT for intact GLS (min)	observed RT for desulfo GLS (min)	response factor for desulfo GLS[b]
1	progoitrin	PRO	(2*R*)-2-hydroxy-3-butenyl	aliphatic	388.0378	308.0810	4.14	3.48	1.09
2	gluconapoleiferin	NAPOL	2-hydroxy-4-pentenyl	aliphatic	402.0534	322.0966	7.10	10.51	1.00
3	gluconapin	NAP	3-butenyl	aliphatic	372.0428	292.0860	8.46	13.50	1.11
4	4-hydroxyglucobrassicin	4HBRA	4-hydroxy-3-indolylmethyl	indolyl	463.0487	383.0918	11.24	16.11	0.28
5	glucobrassicanapin	CAN	4-pentenyl	aliphatic	386.0585	306.1017	14.63	21.73	1.15
6	glucoerucin	ERU	4-methylthiobutyl	aliphatic	420.0462	340.0894	16.24	23.70	1.04
7	glucobrassicin	BRA	3-indolylmethyl	indolyl	447.0537	367.0969	18.84	26.69	0.29
8	gluconasturtiin	NAS	2-phenethyl	aromatic	422.0585	342.1017	22.93	31.77	0.95
9	glucoberteroin	BER	5-methyl-thiopentyl	aliphatic	434.0619	354.1051	23.10	32.31	unknown
10	4-methoxy-glucobrassicin	4MBRA	4-methoxy-3-indolylmethyl	indolyl	477.0643	397.1075	24.43	33.04	0.25
11	neoglucobrassicin	NBRA	*N*-methoxy-3-indolylmethyl	indolyl	477.0643	397.1075	31.06	41.83	0.20

[a]Glucosinolates are grouped by the nature of their R group. [b]Response factors as reported.[10]

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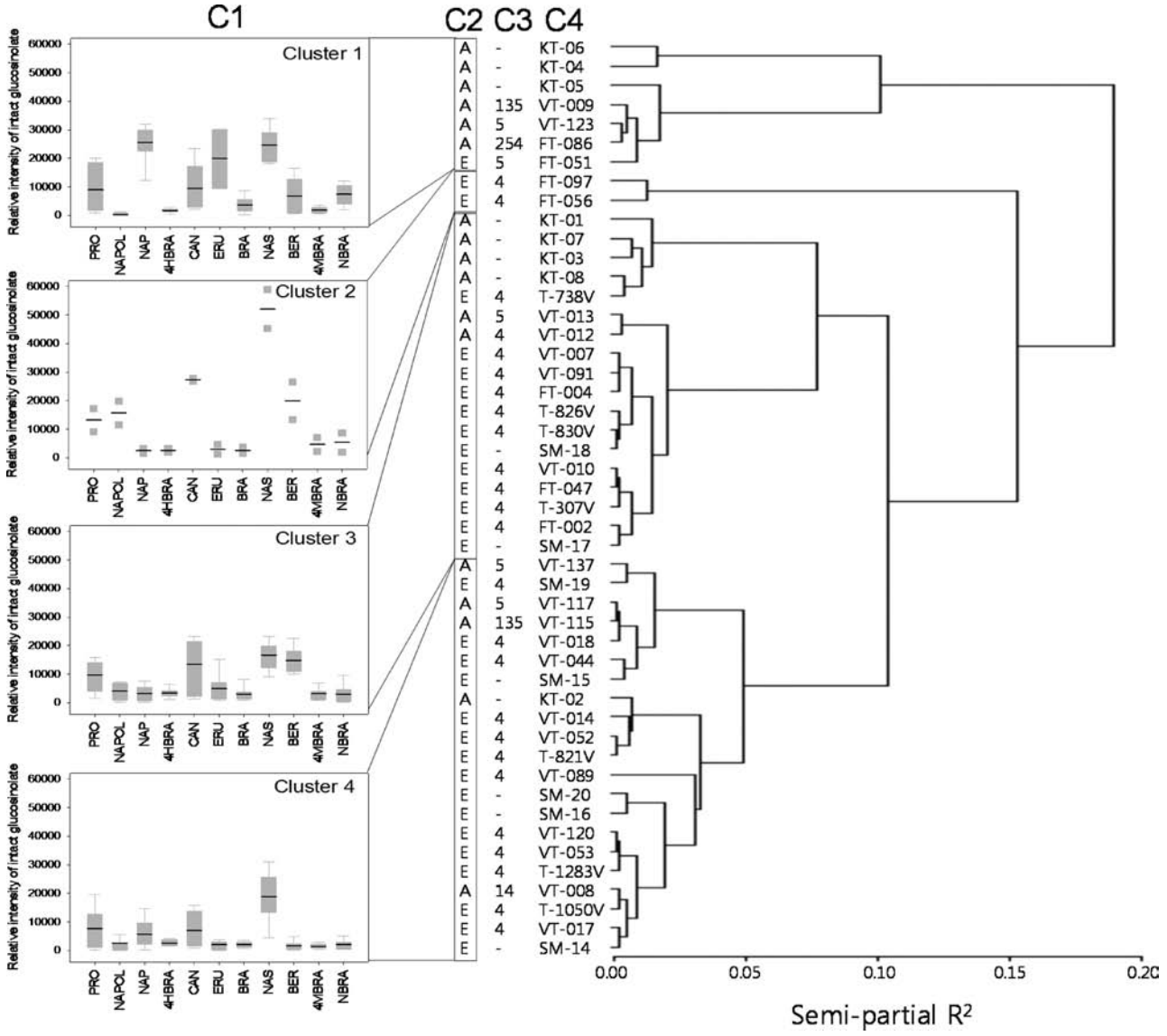

Figure 3. Nonrooted tree representing a clustering of turnip samples according to intact GLS composition. First column (C1): intact GLS in clusters 1, 2, and 3 (see Figure 4 for explanation of diagrams). Second column (C2): geographic origin; A, Asian; E, European. Third column (C3): information on population substructure as determined by the program structure based on allelic variation in 11 genetic markers profiled over the *B. rapa* core collection presented in Zhao et al.[21] For accessions with KT and SM numbers, no genotypic information was available. Fourth column (C4): accession.

in 2 min, and reconditioning at 0.2% B for 6 min, with a mobile phase flow rate of 0.19 mL/min.

The PDA signal at 229 nm was used to quantify the desulfo GLS. The type and amount of desulfo GLS in turnip were calculated in comparison to externally applied, known concentrations of standard sinigrin using recommended relative response factors.[10] In the 48 turnip accessions, a total of 11 different GLS peaks was identified based on their specific accurate masses (both as desulfo and as intact GLS) and retention times. The LC-PDA and LC-QTOF-MS signals of these major GLS compounds were quantified by integration of the peak areas using the QuantLynx module of MassLynx software (Waters Co.). One mixed sample, consisting of contrasting turnip genotypes, was extracted and analyzed in nine technical replications for the comparison of experimental error between the different GLS analysis protocols.

Statistics. To cluster turnip accessions based on intact GLS abundance, Ward's minimum variance cluster analysis was performed using SAS 9.2, based on Euclidean distances. The linear regression analysis between the mass signal abundances from intact GLS from the LC-MS data and corresponding quantified desulfo GLS from the LC-PDA data was performed using Sigmaplot 11 (Systat Software).

RESULTS

Turnip Collection. The turnip material used for this study included 48 accessions originating from 15 different European and Asian countries, which were grown simultaneously in a greenhouse. The turnip accessions are part of the Wageningen UR core collection, and their genetic diversity was previously determined.[17,20,21] The harvested mature turnip tubers had broad phenotypic characteristics, tuber shape, and color (Figure 1, Supporting Information).

Identification of GLS in Turnip. The GLS present in turnip tubers were extracted by two independent methods. The first method comprised the classical dedicated GLS extraction and pretreatment method, including repeated extraction in boiling methanol, anion-exchange chromatography, and sulfatase treatment, leading to desulfo GLS.[18] The second

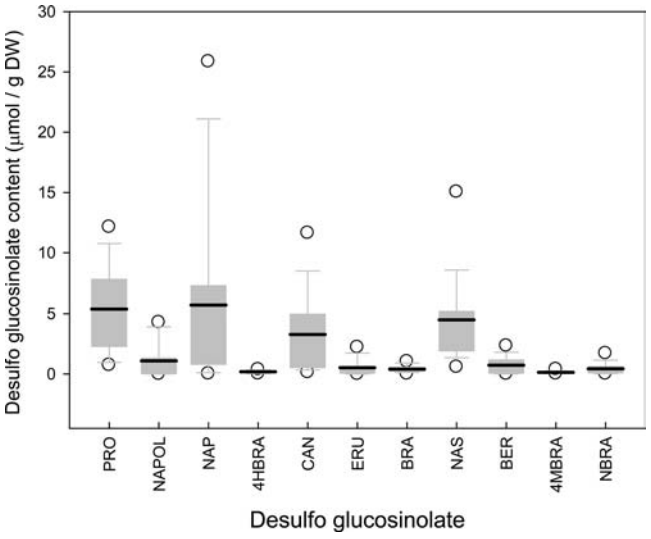

Figure 4. Variation in GLS content measured as desulfo GLS, in 48 turnip accessions. The lower and upper circles represent 5% and 95% ranges, the bidirectional vertical error bars represent 10% and 90% ranges, while the lower and upper borders of each box represent 25% and 75% ranges. The horizontal solid lines in the box plots represent the mean values of the corresponding GLS content for the 48 accessions.

extraction method deployed a single acidified aqueous-methanol extraction followed by centrifugation, producing a crude extract in which intact GLS are present.[13] The identification and relative quantification of both intact and desulfo GLS were performed using the same LC-PDA-QTOF-MS system.

As a first step in the identification, a list of calculated accurate masses ($[M - H]^-$) of 120 known GLS[1] was made, based on their elemental composition, both for intact and desulfo forms. Subsequently, the LC-MS chromatograms of the intact and desulfo GLS extracts of the 48 accessions were investigated for the presence of these exact masses (Figure 1). To further confirm the identity of accurate mass peaks as a GLS, desulfo GLS mass chromatograms were inspected for the presence of m/z 195, representing a thioglucose group, which is typical for desulfo GLS[14] (Figure 2A). Intact GLS were further checked for loss of a fragment of m/z 96.96, indicative of the sulfate moiety, and for a fragment of m/z 259, which is indicative of the GLS core structure.[22,23]

In the tubers of the 48 analyzed turnip accessions, a total of 11 different GLS could be identified (Table 1 and Figures 1 and 2C). These comprised six aliphatic GLS, four indole GLS, and one aromatic GLS. The aliphatic GLS contain a four carbon side chain, such as progoitrin (PRO), gluconapin (NAP), and glucoerucin (ERU), or a five carbon side chain, such as gluconapoleiferin (NAPOL), glucobrassicanapin (CAN), and glucoberteroin (BER). The four indole GLS identified were 4-hydroxyglucobrassicin (4HBRA), glucobrassicin (BRA), 4-methoxyglucobrassicin (4MBRA), and neoglucobrassicin (NBRA). Gluconasturtiin (NAS) was the only aromatic GLS found in turnip. Indeed, all identified intact GLS could also be found as desulfo GLS in the LC-MS analysis of the desulfo extracts, further confirming their identity (Table 1). In the intact GLS analysis, the LC-MS peaks of NAS and BER (Figure 1) were overlapping but were separated in the desulfo GLS analysis (Figure 2C). These results overlap with those found in previous studies, though not all studies report the presence of ERU, BRA,[24,25] 4HBRA, 4MBRA, and NBRA,[24−26] or BER.[24−27] On the other hand, glucoraphanin and glucoalyssin[27] have been described by others, while we could not detect these.

To obtain data about absolute levels of the GLS in each accession, the peak area of the desulfo GLS detected at 229 nm by LC-PDA analysis was used (Figure 2B) and converted by relative response factors as published.[10] For PRO, quantification based on the PDA signal was not straightforward, as this compound eluted very close to the injection peak in the LC-PDA chromatogram (Figure 2B). Minor compounds, such as NAPOL, 4HBRA, ERU, BRA, and BER, were difficult to detect by PDA, in comparison to the accurate mass traces (Figure 2B,C).

Variation of GLS in Tubers of 48 Turnip Accessions. The LC-MS analysis of intact GLS showed clear differences in their relative abundance among the turnip accessions. The relative abundance levels of intact GLS of the turnip tubers were used to cluster the 48 accessions (Figure 3). Geographic origin (column C2) and genotypic characteristics (column C3) were included in Figure 3.[17,20] Four clusters can be clearly distinguished with an r^2 value of greater than 0.10. Identical clustering was obtained using desulfo GLS data (not shown). The variation in GLS content based on intact GLS was plotted per cluster (column C1).

In Figure 4, the variation in the absolute content of 11 desulfo GLS among the 48 accessions is shown. The predominant desulfo GLS were PRO (on average 5.4 μmol/g DW), NAP (5.7 μmol/g DW), CAN (3.3 μmol/g DW), and NAS (4.5 μmol/g DW), while the other GLS were evaluated as minor constituents after desulfation (<1.1 μmol/g DW). NAP showed the highest and most broad variation (0.06−29.2 μmol/g DW).

Correlation between Intact and Desulfo GLS Quantification. The correlation between intact GLS relative abundance and desulfo GLS content data were compared by a linear regression analysis. The scatter plots are shown for NAP, CAN, and NAS in Figure 5, and all the linear regression fits for the 11 compounds are given in Table 2. Most GLS showed linear correlations with r^2 values between 0.77 and 0.97, indicating a good agreement between the two methods. However, correlation is less in the lower detection range: when desulfo GLS LC-PDA signals for NAS are around zero, the intact GLS LC-MS method is still able to clearly detect this GLS and to determine the variation between accessions (Figure 5C). At the highest concentrations, correlations between PDA signals and intact LC-MS signals are somewhat lower, possibly due to the onset of saturation of the MS signals. For the polar GLS PRO, the correlation between desulfo and intact GLS was very low (r^2 = 0.39; Table 2), which was mainly caused by the fact that, for many samples, the peak area of desulfo PRO at 229 nm was very difficult to integrate correctly, due to its low retention on the C18 column under the applied LC conditions. In contrast to its PDA signal, the accurate mass of PRO (either desulfo or intact) is unique and can be used for sample comparison. Also for 4HBRA, we observed a low correlation coefficient (r^2 = 0.43). This compound was present at low peak intensities in all analyzed turnip tuber samples, which may have hampered its quantification by LC-MS and/or LC-PDA. However, for ERU, another compound occurring at low concentration, the correlation was higher than 0.9. The correlation value did not improve for 4HBRA when LC-MS data from the intact GLS extracts were correlated to LC-MS data of the desulfo extracts, while the LC-PDA and LC-MS data

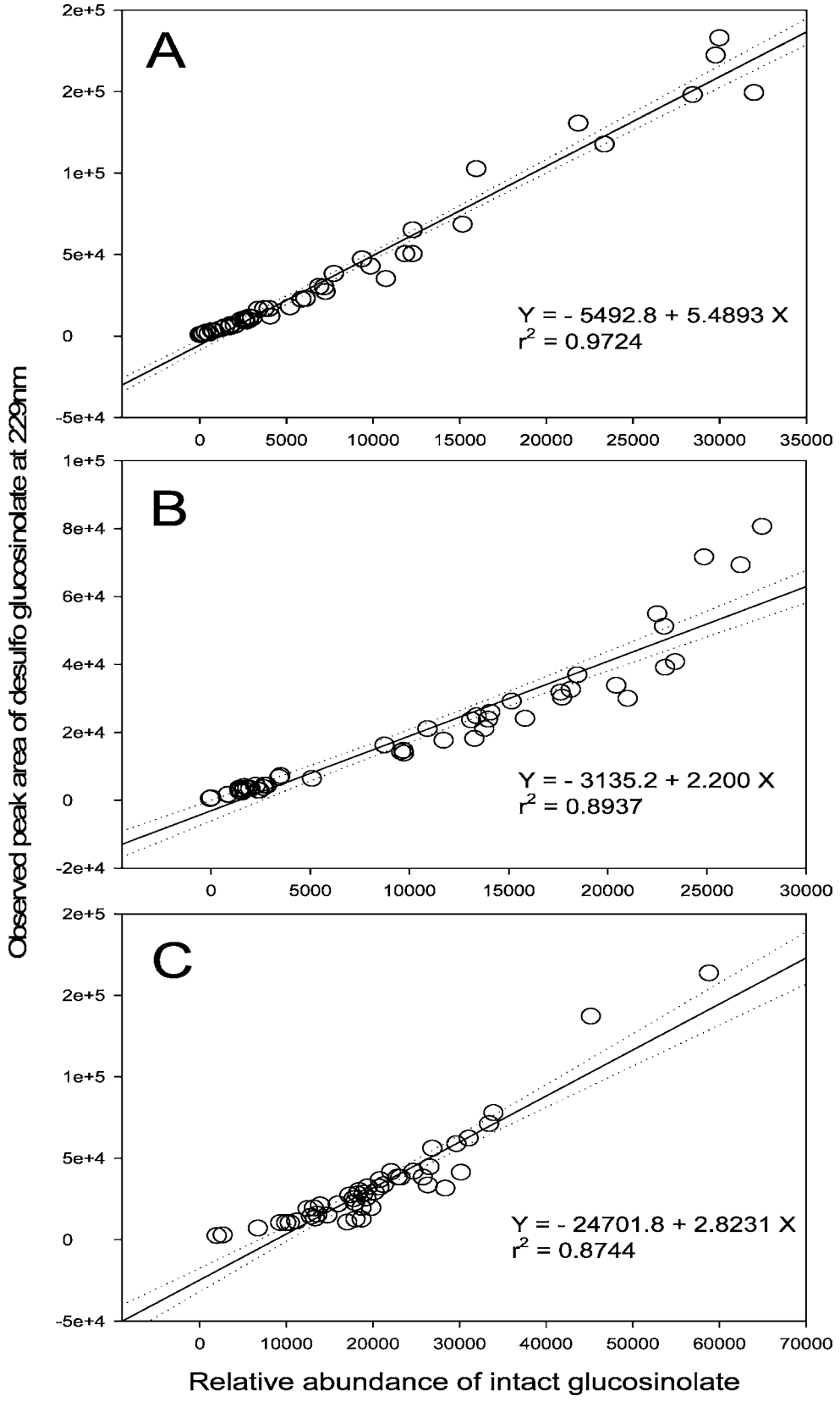

Figure 5. Linear regression analysis for gluconapin (A; NAP), glucobrassicanapin (B; CAN), and gluconasturtiin (C; NAS) between the relative abundance of intact and desulfo GLS over 48 turnip accessions. The linear regressions and its 95% confidence intervals are shown with solid and dotted lines, respectively.

both from the desulfo samples showed high correlation (R^2 = 0.85). This suggests that differences in the extraction methods between intact and desulfo GLS, rather than in the detection methods, caused a poor correlation for this compound.

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Table 2. Linear Regression Coefficients between Intact and Desulfo GLS Abundances in 48 Turnip Accessions for 11 Identified Compounds

GLS	Y (desulfo GLS peak area) = $b + a \times X$ (intact GLS mass)	r^2
PRO	$Y = -11\,346.4 + 2.3011 \times X$	0.3915
NAPOL	$Y = -733.6 + 2.1119 \times X$	0.7713
NAP	$Y = -5492.8 + 5.4893 \times X$	0.9724
4HBRA	$Y = -1684.7 + 1.2534 \times X$	0.4373
CAN	$Y = -3135.2 + 2.2000 \times X$	0.8937
ERU	$Y = -322.1 + 1.3876 \times X$	0.9576
BRA	$Y = -2093.5 + 2.8361 \times X$	0.8858
NAS	$Y = -24\,701.8 + 2.8231 \times X$	0.8744
BER	$Y = -522.0 + 0.7447 \times X$	0.8807
4MBRA	$Y = 1127.9 + 1.2958 \times X$	0.7708
NBRA	$Y = -995.0 + 4.4764 \times X$	0.8706

The reproducibility of GLS quantification using both intact and desulfo GLS methods was compared using nine technical replications from a mix of four turnip samples (Table 3). The coefficients of variation (CV) in intact GLS mass abundance, desulfo GLS mass abundance, and desulfo GLS quantification at 229 nm were partly dependent on both GLS species and analysis protocol. CV values for intact GLS detection by LCMS (6.2−18.8%) did not differ strongly from values for the desulfo GLS by the LC-PDA method (2.9−13.5%).

DISCUSSION

GLS Diversity in Turnip Tuber. A large diversity of GLS in vegetable material is essential for breeding toward crop varieties with a specific metabolite composition. In this research, we screened a population of 48 turnip accessions. Turnips are either cultivated as fodder crop, where both leaves and tubers are consumed, or as vegetables, where depending on the country, the tubers are consumed (Northern and Eastern Europe, China), or the leaves (turnip greens) and shoots (turnip tops), mainly in Southern European countries. This research was focused on the tuber. For turnip tuber material, a limited number of desulfo analyses of GLS content have previously been reported.[24−27] More extensive characterizations of turnip populations were performed on leaf material, also including intact GLS analysis.[10,20,28−31] The current work analyzes the GLS content of a collection of turnip tubers by two methods, analyzing intact and desulfo molecules. This turnip collection is part of a large core collection including diverse *B. rapa* morphotypes (Chinese cabbages, PakChois, broccoletto's, oil types, Japanese leafy types) and has been profiled with genetic markers to reveal population structure and to enable association mapping studies.[21,34] Earlier, we have already published about the intact GLS content in leaf material of a part of the collection. This turnip collection has been grown in greenhouse conditions in plots, to minimize environmental variation.

The analysis of turnip tuber GLS distinguishes four clusters. Cluster 1, in which mostly Asian accessions occur, is characterized by a relative high content of NAP (Figure 4, top): the seven accessions in this cluster represent the top seven NAP content of the collection. On a genetic level, this cluster is characterized by the absence of genetic group 4, while in the other clusters, genetic group 4 predominates. The small cluster 2, formed by FT056 and FT097, is characterized by very high levels of both NAS and CAN and lower levels of NAP. Cluster 3 is characterized by a high content of BER: all 19 accessions from cluster 3 are represented among the 20 genotypes with the highest BER content. Most accessions in this cluster have been collected in Europe, except for some accessions from Korea. Cluster 4, which has mostly been collected in Europe, does not represent a characteristic GLS profile but generally is low in aliphatic GLS (Figure 4, bottom). The same clusters were also found when desulfo GLS were considered (not shown).

Most of GLS diversity studies in *Brassica rapa* are based on green tissue. For turnip tuber, such an analysis may not necessarily yield relevant information. Previously, we[32] reported the LC-MS analysis of young leaves of 168 *B. rapa* accessions in which 26 turnips were included. The 19 GLS metabolites that were identified in this work were used for clustering the 20 genotypes that were analyzed both in turnip tuber tissue (this work) and in young leaf[32] (Figure 6 and Table S3, Supporting Information). In the analysis based on leaf GLS, again, four clusters can be distinguished that separate at $r^2 > 0.1$ (Figure 6). However, for leaf tissue, the division of the accessions across the clusters is completely different, compared to the turnip tuber. We conclude that the GLS analysis of young turnip leafs does not provide indications on the composition of the turnip tuber. It is notable that, in the GLS analysis of the tubers, vegetable turnips (VT accessions, Figure 6 and Table S1,

Table 3. Reproducibility of GLS Identification among Intact and Desulfo GLS Analysis Protocols Using Mixed Turnip Samples with Nine Technical Replications

	intact GLS mass				desulfo GLS mass				desulfo GLS PDA at 229 nm			
GLS	calcd mass for intact GLS $[M-H]^-$	relative abundance	SD	CV (%)	calcd mass for desulfo GLS $[M-H]^-$	relative abundance	SD	CV	observed retention time (min)	content (μmol/g DW)	SD	CV (%)
PRO	388.0378	8364.85	686.37	8.21	308.0810	52.04	6.12	11.76	3.2	3.94	0.35	8.98
NAPOL	402.0534	230.00	24.09	10.47	322.0966	197.26	34.61	17.55	9.8	25.96	0.75	2.90
NAP	372.0428	3352.12	479.10	14.29	292.0860	107.99	16.85	15.61	12.6	32.51	3.37	10.36
4HBRA	463.0487	709.58	126.80	17.87	383.0918	100.93	19.21	19.03	15.0	9.70	1.07	11.01
CAN	386.0585	10637.97	1018.34	9.57	306.1017	211.17	19.50	9.23	21.0	3.64	0.29	8.09
ERU	420.0462	755.99	133.20	17.62	340.0894	24.74	7.80	31.51	23.0	1.04	0.11	10.80
BRA	447.0537	664.04	121.35	18.27	367.0969	28.19	6.12	21.70	25.9	0.54	0.06	10.47
NAS	422.0585	15954.01	987.94	6.19	342.1017	561.71	39.19	6.98	31.1	37.40	3.44	9.20
BER	434.0619	2598.32	286.86	11.04	354.1051	123.13	15.56	12.63	31.8	1.09	0.11	10.18
4MBRA	477.0643	511.50	91.63	17.91	397.1075	49.83	5.80	11.64	32.4	0.79	0.07	9.19
NBRA	477.0643	352.63	66.28	18.79	397.1075	7.45	2.01	26.92	41.1	0.44	0.06	13.55

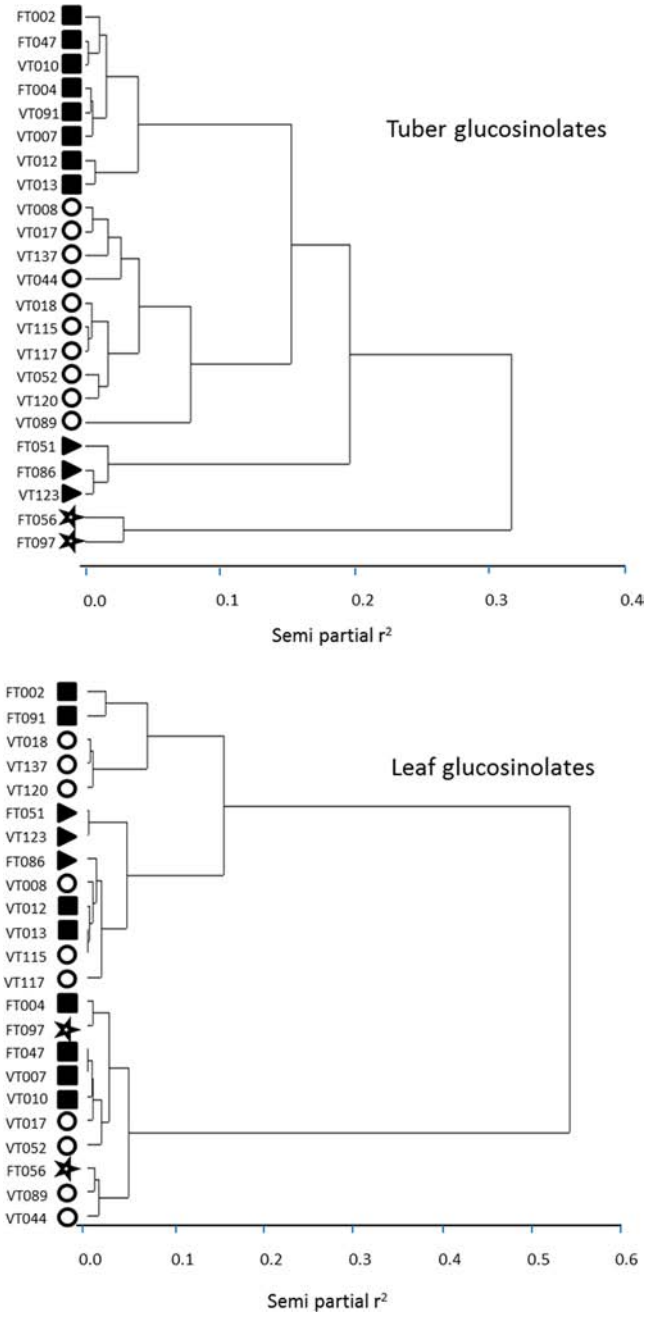

Figure 6. Comparison of turnip accessions clustering based on turnip tuber GLS (top) and leaf GLS (bottom). Leaf GLS data were taken from an earlier publication and are shown in Supporting Information S3.[19] Symbols identify clusters based on turnip tuber GLS.

Supporting Information) separate more from the fodder turnips (FT) into separate clusters, compared to leaves.

Large-Scale Analysis of GLS in Brassica Collections and Populations. The standardized method for quantitative GLS analysis[18] involves complex sample treatment, including an SPE step and an overnight sulfatase incubation in order to enable HPLC with UV/vis detection. This method is well established but laborious. Moreover, the sulfatase treatment can be prone to experimental error, mainly due to variation in the quality of the sulfatase enzyme preparation.[11] Alternative methods have been developed, focusing on LC-MS-based analysis of intact GLS present in crude aqueous-methanol extracts. The sample preparation for intact GLS is much less laborious and does not deploy the sulfatase treatment. In this research on variation in GLS, we compared both methods in terms of the analytical efficiency and reproducibility, using samples from the 48 turnip accessions. The correlations between data from both methods were generally strong (r^2 > 0.7; Table 2), especially for dominant GLS such as NAP and NAS. However, poor correlations (r^2 < 0.5) were observed for both PRO and 4HBRA. In the case of PRO, this was likely due to the poor chromatography of the polar desulfo PRO, while in the case of 4HBRA, which is only a minor GLS in turnip, variation in the extraction and pretreatment likely accounted for the lack of correlation. Both methods show high technical reproducibility, based on nine extractions using the same material (Table 3).

An advantage of accurate mass-based detection over UV/vis detection is the ability to distinguish GLS masses that partly coelute in their chromatography. The 11 GLS identified in this study are consistent with those reported in previous studies, while BER was not commonly found in other reports.[24−27] BER, both as an intact GLS and in its desulfo form, elutes at a retention time very close to that of NAS, the predominant GLS in many turnip accessions (Table 1; Figure 1). In the case of LC-PDA analysis only, the (partial) coelution of BER with the abundant NAS peak may easily obscure the presence of BER.[25]

One of the major draw-backs of quantification of GLS by MS techniques is that each GLS species has its own specific ionization characteristics, so that standard calibration curves for each GLS would be required to accurately quantify the levels of individual GLS.[34] Most turnip GLS standards are not commercially available or very expensive. Nevertheless, signals from the same GLS can well be compared over samples and can provide information on the relative abundance of that GLS over the samples. In contrast, for the detection by PDA, stable conversion factors relative to sinigrin have been documented for many GLS, facilitating their quantification in an extract,[10] provided that they are chromatographically well separated, their peaks can be integrated accurately, and their identities are unambiguously known. However, these provisions are not met at all times for all compounds detectable in desulfo extracts of Brassicas, and without confirmation of the identity and purity of an assumed GLS peak by accurate mass MS, some care should be taken when comparing GLS profiles between different species, accessions or tissues.

The linear correlations between GLS data from intact GLS (by LC-MS) and desulfo GLS (by LC-PDA) were found to be high for most of GLS species. Thus, accurate mass LC-MS analysis of intact GLS is suitable for comparative research, for example, for screening of plant populations, investigating the relative abundance in tissues, the behavior of GLS during plant development, the effect of treatments, and so forth. The desulfo method is indispensable for determining absolute GLS concentrations. For large populations, these could be calculated afterward, by UV-based quantification of desulfo GLS on a selected sample set covering the range of relative levels observed within the entire sample series.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Morphology and color variation among tubers from 40 turnip accessions. The white arrow in each picture is of constant size (5 cm in length); list of *B. rapa* ssp. *rapa* accessions used for this study; variation in turnip tuber GLS content within one accession; variation in turnip leaf GLS content in a subset of the

accessions, analyzed for intact GLS by LC-MS. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

GLS, glucosinolate; PRO, progoitrin; NAPOL, gluconapoleiferin; NAP, gluconapin; 4HBRA, 4-hydroxyglucobrassicin; CAN, glucobrassicanapin; ERU, glucoerucin; BRA, glucobrassicin; NAS, gluconasturtiin; BER, glucoberteroin; 4MBRA, 4-methoxyglucobrassicin; NBRA, neoglucobrassicin; DW, dry weight; SD, standard deviation; LC, liquid chromatography; MS, mass spectrometry; PDA, photodiode array

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