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Variation in Glucosinolate Contents of Cruciferous Plants

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Abstract: Glucosinolates are secondary metabolites of almost all plants of the order Brassicales, and have been known to control nematode populations. In this study, 14 glucosinolates were identified, quantified, and compared in several varieties and cultivars of cruciferous plants including *Brassica campestris* ssp. *pekinensis* (Chinese cabbage), *Brassica juncea* var. *crispifolia* L. H. Bailey (mustard), *Brassica juncea* L. Czern. var. *juncea* (leaf mustard), *Brassica oleracea* L. var. acephala (kale), *Raphanus sativus* L. (radish), and *Brassica campestris* L. ssp. *oleifera* (winter turnip rape). The most abundant glucosinolate in mustard, leaf mustard, kale, and radish was sinigrin. In leaf mustard, the sinigrin content ranged from 193.05 μmol/g to 215.52 μmol/g, and in mustard, the sinigrin contents of blue mustard and red mustard were 219.08 μmol/g and 215.73 μmol/g, respectively. Kale and radish contained 137.79 μmol/g and 120.25 μmol/g, respectively, of sinigrin. Gluconapin was the most abundant glucosinolate in winter turnip rape, at 121.17 μmol/g. Chinese cabbage contained mostly glucocochlearin (79.88 μmol/g). These results will be useful in the development of environmentally friendly plant-based pesticides by allowing for proper control of glucosinolates based on those present in the chosen plant species.

Keywords: Glucosinolate; sinigrin; gluconapin; cruciferous plant; leaf mustard.

1. Introduction

Glucosinolates are specific functional secondary metabolites that contain sulfur and nitrogen, and have been found in cruciferous crops; to date, 120 kinds have been identified in crops. The total number of glucosinolates, including those identified in recent laboratory studies, is about 200 [1]. They are classified by the characteristic functional group that is derived from one of eight different amino acids [2], based on the chemical structure of the precursor amino acid. There are aliphatic (from Met, Ala, Leu, Ile, or Val), aromatic (from Phe or Tyr), and indole (from Trp) glucosinolates [3].

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Glucosinolates are found mainly in Brassicaceae plants, and are a source of the unique bitter taste and flavor of these plants. They have been recognized as specific functional materials, and there are continual studies on systems for breeding plants to increase particular glucosinolates. The concentrations of glucosinolates can be influenced by several factors including genotype, plant tissue, and plant age [4-5]. In Brassicaceae plants, most are alkenyl-glucosinolates, the content and the composition of which vary depending on the developmental stage and tissue of the plant. The indole-glucosinolates are a minority [6].

Glucosinolates exert anti-cancer effects by inducing the activity of phase II detoxification enzymes such as quinone reductase, glutathione-S-transferase, and glucuronyl transferase [7-8]. For example, sinigrin hydrolysis catalyzed by myrosinase enzymes produces isothiocyanates (especially allyl-isothiocyanates), as bioactive material [9-13]. Pathways and mechanisms of biosynthesis of glucosinolates from the primary amino acids have been revealed through many studies [3].

Glucosinolates are also involved in clubroot disease in Arabidopsis [14] and, according to a recent report [15], the major components of glucosinolate in rapeseed are likely to be involved in reducing nematode density. Greenhouse cultivation requires a large quantity of chemical fertilizers and manure. Immature compost spreading, one of many recent environmentally friendly agriculture methods, acts as food for nematodes, causes an increase in nematode density, and inhibits crop growth and development [16]. In greenhouses, repeated cultivation causes damage to many crops such as cucumbers, melons, and tomatoes. Root-knot nematode infections result in an approximately 30%–40% reduction in the annual harvest quantity [17].

Current methods for reducing nematode damage to crops include crop rotation, soil excavation, solar disinfection, desalination treatment, soil fumigation, and nematocide treatment. Traditional nematocide treatment causes side effects in crops in a limited space and exerts an adverse effect on the health of farmers. In addition, continuous use of pesticides allows nematodes to adapt to a wider range of habitats, so in time pesticides became less effective [18]. Therefore, development of sustainable and environmentally friendly nematocides was needed.

Because glucosinolates such as progoitrin, sinigrin, and gluconapin reduce nematode density, [15,19], an investigation of glucosinolate contents in various cruciferous cultivars and native leaf mustard varieties collected in Korea was carried out to analyze the components that may be used to develop effective nematocides.

2. Materials and Methods

2.1. Plant materials

Seeds of 11 varieties of *Brassica juncea* L. Czern (leaf mustard) including local varieties, two varieties of *B. juncea* L. var *crispifolia* L. H. Bailey (mustard), *B. oleracea* L. var. *acephala* (kale), *Raphanus sativus* L. (radish), *B. campestris* L. (Chinese cabbage) and *B. campestris* L. ssp. *oleifera* (winter turnip rape) were provided by the Bio-Energy Crop Center, National Institute of Food Science (Muan, Korea) and were used to compare their glucosinolate metabolites in seed.

2.2 Analysis of glucosinolates

Glucosinolates were extracted as described in [22] and [20]. Freeze-dried and homogenized seeds (100 mg) were reacted with 1.5 mL of 70% (v/v) methanol in water in a 70°C water bath for 5 min to inactivate endo-myrosinase, then centrifuged at 12,000 rpm for 10 min (4°C). The supernatant was collected and remaining residue was re-extracted twice. Total collected supernatant was considered the crude glucosinolate content. Sinigrin (0.5 mg) was dissolved in 5 ml ultrapure water (PURELAB Option-Q, ELGA, UK) for use as an external standard. Desulfation of the crude glucosinolates was performed on a DEAE anion exchange column that was prepared by adding a slurry of Sephadex A-25 previously activated (H^+ form) with 0.5 M sodium acetate, whereas desulfation of sinigrin (external standard) was carried out separately in a DEAE anion exchange column. The crude glucosinolate extracts were loaded onto a pre-equilibrated column. After twice washing with 1 mL ultrapure water to remove cations and neutral ions, arylsulfatase (E.C.3.1.6.1) (75 μL) was loaded onto each column.

After an overnight (16 h) desulfation reaction at room temperature, the desulfated glucosinolates were eluted with 0.5 mL ultrapure water three times. The eluates were filtered through a 0.45 μm Teflon PTFE syringe filter and analyzed immediately by HPLC or stored in the refrigerator at 4°C until glucosinolate analysis.

2.3 Separation and identification of desulfo (DS)-glucosinolates using HPLC

DS-glucosinolates (DS-GLs) obtained from different lines of cabbage were analyzed with a 1200 Series HPLC system (Agilent Technologies, USA) equipped with an Inertsil ODS-3 (C18) column (150 × 3.0 mm i.d., particle size 3 μm) (GL Science, Japan). The HPLC analysis was carried out with a flow rate of 0.2 mL/min, a column oven temperature of 40°C, and a wavelength of 227 nm. The solvent system employed was ultrapure water (A) and 100% acetonitrile (B). The gradient program used was as follows: 0–2 min, 0% B; 2–7 min, 10% B; 10–16 min, 31% B; then kept constant at 31% B until 19 min, drop down to 0% B at 21 min, then kept constant at 0% B for 6 min (total 27 min). Individual glucosinolates were quantified based on the sinigrin and their HPLC areas and response factors (ISO 9167-1, 1992). In this study, all samples were designated as glucosinolates even though DS-GSLs were determined.

2.4 LC-ESI-MS/MS analysis for identification of DS-glucosinolates

An API 4000 QTRAP tandem mass spectrometer (Applied Biosystems, Foster City, USA), equipped with an Agilent 1200 Series HPLC system and an electrospray ionization tandem mass spectrometry (ESI-MS/MS) source in positive ion mode ($[M+H]^+$), was used for the identification of the individual DS-GLs. The MS operating conditions were as follows: ion spray voltage 5.5 kV; curtain gas 20 psi, nebulizing gas 50 psi, and heating gas 50 psi, high purity nitrogen (N_2); heating gas temperature 550°C; declustering potential 100 V; entrance potential 10 V; spectra scanning range *m/z* 100–1,000 (scan time 1.0 sec). Analyst® Software (SCIEX) program was used for the assessment of MS/MS peaks, the peaks were identified through a comparison of reference [23] and our results.

3. Results and Discussion

The compounds identified by LC-ESI-MS/MS analysis in positive ion mode $[M+H]^+$, including systematic and common names and the principal ions, are listed in Table 1. Fourteen glucosinolates were detected in extracts; the identified glucosinolates were similar within and across species. Specifically, nine aliphatic (progoitrin, glucoraphanin, sinigrin, glucoalyssin, gluconapoleiferin, glucocochlearin, gluconapin, glucobrassicanapin, and glucoerucin), four indolyl (4-hydroxyglucobrassicin, glucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin), and one aromatic glucosinolate (gluconasturtiin) were identified based on fragmentation patterns of MS spectra and quantified based on the peak areas of HPLC chromatogram (Figure 1).

Based on previous findings that the major components of glucosinolates in rapeseed were likely to be involved in reducing nematode density [20], we investigated glucosinolate contents in various cruciferous cultivars to gain information for the most effective agents used to control nematodes.

The identified glucosinolates in varieties of leaf mustard, mustard, kale, and radish showed a similar pattern of glucosinolate composition; sinigrin was the most abundant glucosinolate. In leaf mustard, sinigrin contents were significantly different between varieties, and ‘Blue’ (215.52 μmol/g) > ‘Ulchung’ (204.45 μmol/g) > ‘Purple’ (195.94 μmol/g) > ‘Dolsan’ (193.05 μmol/g). In mustard, sinigrin contents were very similar between ‘Blue mustard’ (219.08 μmol/g) and ‘Red mustard’ (215.73 μmol/g) (Table 2).

In addition, kale and radish contained 137.79 μmol/g and 120.25 μmol/g sinigrin, respectively. Winter turnip rape and Chinese cabbage contained mostly gluconapin (121.17 μmol/g) and glucocochlearin (79.88 μmol/g), respectively (Table 3). These results were different from previous reports that progoitrin was the most abundant glucosinolate in rapeseed sprouts [20]. According to previous reports [15], the contents of progoitrin and gluconapin in two different rapeseed meals ('Jeju' Korean local rape varieties and 'Sunmang' variety) were 69.79–99.81 μmol/g and 37.17–76.81 μmol/g, respectively, accounting for 86–88% of the total glucosinolate contents in these varieties. These conflicting results suggest that the gluconsinolate contents of these and other varieties must be re-evaluated before using these data to create nematocides.

Table 1. Glucosinolates identified by LC-ESI-MS/MS in seeds of cruciferous plants.

No[a]	RT[b]	Glucosinolates	Semisystematic names	Compound groups	[M+H]+(*m/z*)[c]	Response factor[d]
1	9.10	Progoitrin	(*2R*)-2-Hydroxy-3-butenyl GSL	Aliphatic	310	1.09
2	9.68	Glucoraphanin	4-Methylsulfinylbutyl	Aliphatic	358	1.07
3	9.97	Sinigrin	2-Propenyl GSL	Aliphatic	280	1.00
4	10.95	Glucoalyssin	5-Methylsufinylpentyl GSL	Aliphatic	372	1.07
5	11.24	Gluconapoleiferin	2-Hydroxy-pent-4-enyl GSL	Aliphatic	324	1.00
6	12.56	Gluconapin	3-Butenyl GSL	Aliphatic	294	1.11
7	13.39	Glucocochlearin[e]	1-Methylpropyl	Aliphatic	296	1.00
8	13.89	4-Hydroxyglucobrassicin*	4-Hydroxy-3-indolylmethyl GSL	Indolyl	385	0.28
9	14.89	Glucobrassicanapin	Pent-4-enyl GSL	Aliphatic	308	1.15
10	15.29	Glucoerucin	4-Methylthiobutyl	Aliphatic	342	1.04[f]
11	16.12	Glucobrassicin	3-Indolymethyl GSL	Indolyl	369	0.29
12	17.13	4-Methoxyglucobrassicin	4-Methoxy-3-indolylmethyl GSL	Indolyl	399	0.25
13	17.49	Gluconasturtiin	2-Phenethyl GSL	Aliphatic	344	0.95
14	19.13	Neoglucobrassicin	*N*-Methoxy-3-indolylmethyl GSL	Indolyl	399	0.20

[a] No., number, the elution order of glucosinolates from HPLC chromatograms.
[b] RT, retention time (min).
[c] As desulfo (DS)-glucosinolates.
[d] International Organization for Standardization (ISO 9167-1, 1992).
[e] Confirmed by [24].
[f] According to [1].
* 4-Hydroxyglucobrassicin was not detected in LC-ESI-MS; therefore, it was identified based on our database.

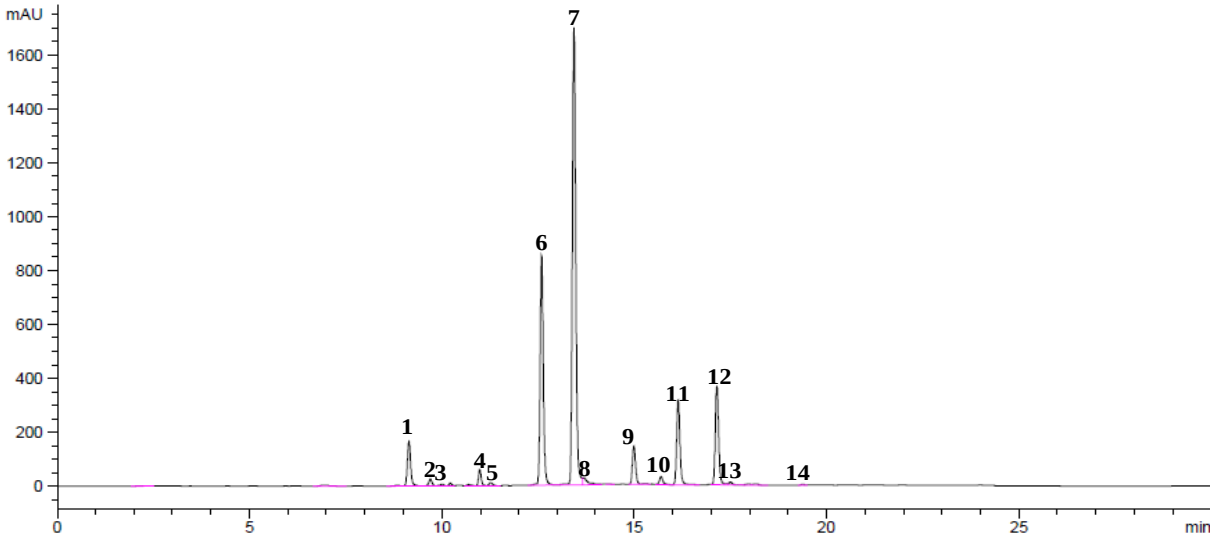

Figure 1. HPLC chromatogram of glucosinolates in seeds of cruciferous plants. 1, progoitrin; 2, glucoraphanin; 3,sinigrin; 4, glucoalyssin; 5, gluconapoleiferin; 6, glucinapin; 7, glucococochlearin; 8, 4-hydroxyglucobrassicin; 9, glucobrassicanapin; 10, glucoerucin; 11, glucobrassicin; 12, 4-methoxyglucobrassicin; 13, gluconasturtiin; 14, neoglucobrasscin. Peaks were detected using the following integration parameters: peak width 0.01, area reject 3 and height reject 1.

Table 2. Glucosinolate contents in seeds of leaf mustard and mustard plants.

Glucosinolates	Blue leaf mustard	Ulchug leaf mustard	Purple leaf mustard	Dolsan leaf mustard	Blue mustard	Red mustard
Progoitrin	0.22±0.03	0.34±0.01	0.36±0.02	0.36±0.01	0.39±0.00	0.10±0.00
Glucoraphanin	0.32±0.02	ND	ND	ND	ND	0.34±0.01
Sinigrin	215.52±5.17	204.45±0.44	195.94±4.10	193.05±5.46	219.08±2.77	215.73±0.01
Glucoalyssin	2.78±0.36	2.50±0.08	2.38±0.38	2.21±0.02	2.76±0.37	2.74±0.37
Gluconapoleiferin	ND	0.11	ND	ND	ND	ND
Gluconapin	38.97±2.74	1.37±0.02	1.26±0.03	5.30±0.15	2.79±0.07	5.65±0.03
Glucococochlearin	43.21±1.25	51.17±5.62	43.56±4.15	52.02±2.23	42.77±3.04	45.03±4.75
4-Hydroxyglucobrassicin	ND	0.29	ND	0.12±0.01	ND	0.11±0.02
Glucobrassicanapin	ND	ND	ND	0.10±0.01	ND	0.18±0.00
Glucoerucin	0.28±0.04	0.31±0.04	0.31±0.01	0.36±0.01	0.25	0.24±0.00
Glucobrassicin	0.30±0.01	0.11±0.01	0.23±0.00	0.23±0.00	0.15±0.02	0.08±0.01
4-Methoxyglucobrassicin	0.63±0.04	0.03±0.01	0.30±0.06	1.03±0.06	0.08±0.00	0.06±0.01
Gluconasturtiin	1.69±0.02	0.78±0.01	0.75±0.02	0.69±0.04	0.13±0.02	0.34±0.05
Neoglucobrassicin	0.06±0.01	0.03±0.00	0.04±0.01	0.04±0.01	0.07±0.02	0.03±0.01
Total	305.70±3.92	263.94±5.32	247.41±8.70	257.72±3.48	271.08±0.24	272.56±5.17

All data are mean ± standard deviation. Units are μmol/g DW. ND = none detected.

Table 3. Glucosinolate contents in seeds of cruciferous plants.

Glucosinolates	*Brassica campestris* L. ssp. *oleifera* (Winter turnip rape)	*Brassica campestris* L. (Chinese cabbage)	*Brassica oleracea* L. var. *acephala* (kale)	*Raphanus sativus* L. (radish)
Progoitrin	11.96±0.84	8.28±0.12	17.17±0.01	0.25±0.13
Glucoraphanin	1.71±0.07	1.13±0.03	17.55±0.41	4.72±0.06
Sinigrin	0.50±0.14	0.35±0.05	137.79±0.93	120.25±8.76
Glucoalyssin	3.55±0.06	2.43±0.07	3.06±0.13	29.99±0.07
Gluconapoleiferin	2.15±0.17	0.54±0.02	ND	4.49±0.10
Gluconapin	121.17±3.09	41.54±1.53	5.25±0.18	3.19±0.01
Glucocochlearin	40.47±5.09	79.88±0.82	72.15±2.17	44.93±4.93
4-Hydroxyglucobrassicin	0.10±0.01	0.51±0.06	0.44±0.02	ND
Glucobrassicanapin	18.89±0.21	7.12±0.33	ND	ND
Glucoerucin	0.51±0.13	0.37±0.03	4.51±0.08	0.79±0.08
Glucobrassicin	0.32±0.02	4.06±0.34	0.50±0.02	4.48±0.27
4-Methoxyglucobrassicin	0.75±0.05	4.21±0.02	0.02±0.00	0.24±0.02
Gluconasturtiin	1.08±0.11	0.58±0.08	0.52±0.01	4.74±1.75
Neoglucobrassicin	0.30±0.00	0.03±0.00	0.02±0.00	0.84±0.73
Total	203.59±0.63	151.37±3.22	259.47±2.78	219.11±16.47

All data are mean ± standard deviation. Units are μmol/g DW. ND = none detected.

Table 4. Glucosinolate contents in seeds of Korean leaf mustard varieties.

Glucosinolates	'Muan'	'Haman'	'Suncheon'	'Janghueng'	'Bosung'	'Gangjin'	'Haenam'
Progoitrin	0.11±0.01	0.34±0.01	0.33±0.00	0.31±0.01	0.35±0.00	0.30±0.05	0.42±0.01
Glucoraphanin	0.42±0.01	1.00±0.03	1.10±0.09	1.10±0.02	1.10±0.00	1.19±0.14	0.87±0.16
Sinigrin	224.98±4.00	201.36±3.18	212.13±3.65	198.81±3.32	207.61±0.32	219.91±0.68	215.78±0.96
Glucoalyssin	2.53±0.15	2.58±0.21	3.05±0.05	2.88±0.20	3.18±0.21	3.54±0.13	2.81±0.16
Gluconapoleiferin	0.27±0.01	ND	ND	ND	ND	ND	ND
Gluconapin	0.81±0.98	1.10±0.04	1.48±0.02	1.39±0.03	1.39±0.03	1.92±0.02	1.20±0.09
Glucocochlearin	40.06±5.15	34.29±2.12	45.16±1.16	34.86±2.69	40.19±2.35	41.63±0.04	51.46±2.92
4-Hydroxyglucobrassicin	0.10±0.00	ND	ND	ND	ND	ND	ND
Glucobrassicanapin	ND	ND	ND	ND	ND	ND	ND
Glucoerucin	0.08±0.01	0.16±0.00	0.23±0.02	0.16±0.01	0.19±0.01	0.20±0.00	0.16±0.00
Glucobrassicin	0.15±0.03	0.34±0.02	0.13±0.01	0.14±0.01	0.09±0.00	0.12±0.01	0.20±0.00
4-Methoxyglucobrassicin	0.16±0.03	0.11±0.00	0.10±0.01	0.32±0.05	0.03±0.00	0.07±0.01	0.10±0.00
Gluconasturtiin	1.27±0.19	1.00±0.05	1.14±0.00	1.14±0.07	1.04±0.01	1.45±0.10	1.38±0.03
Neoglucobrassicin	0.03±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.03±0.00	0.04±0.00	0.03±0.00
Total	273.13±8.46	244.02±5.68	267.68±5.24	243.23±6.27	257.71±2.87	272.61±0.92	276.45±4.29

All data are mean ± standard deviation. Units are μmol/g DW. ND = none detected.

There were differences in sinigrin content among several Korean leaf mustard varieties. Results indicated that sinigrin content of 'Muan' was highest (224.98 μmol/g). This level is 26.17 μmol/g higher than the lowest, 'Jangheung' (198.81 μmol/g) (Table 4). According to previous studies [15,21], rapeseed meal containing mainly progoitrin showed a visible effect of nematode control, and *Brassica juncea* 'Pacific Gold' containing mainly sinigrin reduced nematode populations significantly.

3. Conclusion

In this study, the content and composition of glucosinolates extracted from various cruciferous plants seeds were analyzed. Our results show that the identified glucosinolates in varieties of leaf mustard, mustard, kale, and radish presented a similar pattern of glucosinolate composition; sinigrin was the most abundant glucosinolate of all. However, there were differences in sinigrin content among the several varieties. Winter turnip rape and Chinese cabbage contained mostly gluconapin and glucocochlearin, respectively. Based on these information, we suggest that using a mixture of rapeseed meal and leaf mustard meal as nematocide might increase the effectiveness of soil nematode control by compensating for relatively low sinigrin content of rapeseed meal. Our results, detailing the variation in glucosinolate content and composition among cruciferous plants, will be valuable for the development of effective plant-based nematocides by combining specific glucosinolate-containing plants.

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