Korean J. Plant Res. 30(6):579-589(2017)
https://doi.org/10.7732/kjpr.2017.30.6.579

Print ISSN 1226-3591
Online ISSN 2287-8203

Original Research Article

Comparison of Glucosinolate Contents in Leaves and Roots of Radish (*Raphanus* spp.)

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Abstract - Glucosinolates (GSLs), beneficial secondary metabolites for human health are abundantly present in radish vegetable. Radish is a member of Brassicaceae family and its seed, leaf and root contain very important GSLs. The objective of this study was to determine the variation of individual and total GSL contents in leaves and roots of 44 radish (*Raphanus* spp.) germplasm (26 *R. sativus* L., 3 *R. raphanistrum*, and 15 *R. sativus* L. var. *raphanistroides* Makino), and compare the GSL contents between leaves and roots among three *Raphanus* species. Thirteen GSLs were identified, being the glucoraphasatin (GRS) and glucobrassicin (GBS) the most abundant aliphatic and indolyl GSLs in both the leaves and roots. Variation in individual and total GSL contents was found among the germplasm of three *Raphanus* species. The GRS content was higher in roots than that of leaves in all three *Raphanus* species but the GBS content was higher in leaves than roots. GRS was represented 87.0%, 92.7% and 94.7% of the total GSL in roots of *R. sativus* L., *R. raphanistrum* and *R. sativus* L. var. *raphanistroides* (Makino) germplasm, respectively. Germplasm of *R. raphanistrum* exhibited the highest (average, 79.5 μmol/g dw) total GSL with a ranged from 62.7 to 92.9 μmol/g dw. The germplasm IT119288, Joseonmu and IT119262 from *R. sativus* L., RA 504 and K046542 from *R. raphanistrum*, and Gyeongju-2003-32 (G2003-32) and IT302373 from *R. sativus* L. var. *raphanistroides* (Makino) had high total GSL contents and these could be good candidates for developing the functional compounds-rich varieties in radish breeding program.

Key words - Aliphatic, Germplasm, Glucosinolate, Indolyl, *Raphanus* species

Introduction

Radish (*Raphanus* spp.) belongs to Brassicaceae, an important annual or biennial vegetable and it is originated in the area between the Mediterranean and the Caspian Sea (Crisp, 1995). Cultivated Japanese and Chinese radish and its usages were studied by previous researchers (Crisp, 1995; Li, 1989). Small-rooted radishes are most popular in temperate regions of the world (Crisp, 1995), while larger-rooted cultivars such as Chinese radishes are predominant in East and Southeast Asia (Schippers, 2004). Radish is widely used for human consumption and consumed as raw-eaten, an appetizer vegetable in meals with high amount of medical and nutritional contents. In Korea, radish is widely used to make a fermented product '*Kimchi*' and the growing popularity of '*Kimchi*' in other countries has further demand to increase the radish production.

Radish contain GSLs, are plant secondary metabolites, and more than 120 types of GSLs have been reported in plant species (Ishida *et al*., 2012). To date, more than 30 GSLs are identified in *Brassica* species (Fahey *et al*., 2001). In general, GSLs are classified into three groups; aliphatic, indole, and aromatic GSLs, based on the precursor amino acids, i.e. methionine, tryptophan, and aromatic amino acids (tyrosine and phenylalanine) (Mithen *et al*., 2000; Rosa, 1999). GSLs are hydrolyzed with inherent myrosinase (EC 3.2.1.147) enzyme and result to several bioactive compounds; isothiocyanates (ITCs), nitriles, thiocyanates, epithionitriles and oxazolidinthiones (Antonious *et al*., 2009). GSLs and their hydrolysis products are associated with bitter, pungent and flavor components

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which are the characteristics of Brassicaceae plants (Antonious *et al*., 2009; Fahey *et al*., 2001; Ishida *et al*., 2012; Mithen *et al*., 2000). ITCs are physiological active compounds and possess anti-cancer property in human, and animals (Jakubikova *et al*., 2011; Tang *et al*., 2006), but some GSLs are harmful for human and animals (Rosa *et al*., 1997). Moreover, leaves and roots of radish (*R. sativus* L.) have been used to treat as antimicrobial and antiviral agents (Gutierrez and Perez, 2004).

Generally, radishes contain high content of 4-methylthio-3-butenyl (glucoraphasatin), an aliphatic GSL and small amount of indolyl and other aliphatic compounds (Ishida *et al*., 2012; Montaut *et al*., 2010) in roots. Glucoraphasatin is a pungent compound which strongly affects the taste of fresh radish and its salad (Ishida *et al*., 2012). However, GSL types, contents and composition ratio depend on plant variety, particular species, developmental stages and tissue types (Ciska *et al*., 2000; Farnham *et al*., 2004; Kabouw *et al*., 2010; Rosa *et al*., 1997). Plant-derived products such as bioactive phytochemicals are now treated a part of the human health system (Yang and Boo, 2015). A collection of radish belonging to *R. sativs* L., *R. raphanistrum* and *R. sativus* L. var. *raphanistroides* (Makino) from different countries is maintained at the National Agro-biodiversity Center (NAC), RDA. *R. sativus* L. consists of thick root with various size, forms and color but wild radish (*R. raphanistrum*) has white, long with slender types (http://en.wikipedia.org). But wild radish (*R. sativus* L. var. *raphanistroides* Makino) is commonly available in sandy coast and the river-banks of eastern Asia which has highly variable for roots size, forms and color (Han *et al*., 2015). They are known for their phenotypic variation but no information is available on GSLs content and their variation in radish collections. With increase interest in human nutrition and health, it is necessary to investigate the profiles and level of GSLs in the collections of *Raphanus* species. This study was aimed to identify the GSL content in the germplasm of three different *Raphanus* species and recommend the potential germplasm for the development of new varieties in radish breeding program.

Materials and Methods

Plant materials

A collection of 44 population of radish (*Raphanus* spp.) consisting of *R. sativus* var. *sativus* L. (26), *R. raphanistrum* (3) and *R. sativus* L. var. *raphanistroides* Makino (15) were evaluated in this study. These germplasm were conserved in National Agrobiodiversity Center (NAC), RDA, and Korea and then, evaluated at experimental field of NAC, Suwon (37°17′27″ N 127°00′32″E). Approximately, 3-4 seeds were sown at each hill and ten hills for each accession were maintained in field at the spacing of 90 × 30 ㎝. Accession was separated from each other using label and seeds were sown in August, 2014. After seedling emergence, plants were thinned out to leave a single plant at each hill, and total 10 plants per accession were maintained for the evaluation. Cultural operations, fertilization and weed control were made according to methods recommended by RDA.

Extraction of desulfo-glucosinolates (DS-GSLs)

Plants were harvested approximately 10 to 12 weeks after sowing. Prior to experiment, leaves and roots from each accession were taken, peeled manually, and cut into pieces for leaves and small cubes for roots. Samples were then freeze-dried at -80ºC for at least 48 h, ground to a fine powder using a mortar and pestle, and, then stored in a refrigerator.

Desulfo GSLs were extracted according to procedure described by Lee *et al*. (2013). Briefly, freeze dried sample of 100 ㎎ was extracted by mixing with 10 ㎖ 70% methanol followed by heating in a water bath at 75ºC for 20 min to make myrosinase inactivation. Mixtures were centrifuged at 12,000 × g at 4ºC for 10 min and resulting supernatants were collected into 5-㎖ test tubes. The solid residue was re-extracted twice and centrifuged andextracts were loaded using 1,000 ㎕ pipette tips into a mini-column previously packed with DEAE-Sephadex A-25 (H^+ form by 0.5 M sodium acetate, approximately 40 ㎎ dry wt.). After washing with ultrapure water, GSLs were desulfated by adding an aryl sulfatase solution (75 ㎕ to the column. After overnight incubation (16-18 h) at ambient temperature, DS-GSLs were eluted with 0.5 ㎖ (× 3) of ultrapure water into 2 ㎖ micro-centrifuge tubes. Eluates were then filtered through a 0.45-㎛ hydrophilic PTFE syringe filter (Ø, 13 ㎜; Advantec, Tokyo, Japan) into brown HPLC vials and stored in a refrigerator at 4°C until the HPLC analysis.

HPLC analysis of DS-GSLs

For the qualitative and the quantitative analysis of DS-GSLs, HPLC-MS (FINNIGAN LCQ Deca XP MAX, Thermo Scientific, USA), which uses Inertsil ODS-3 (2.1×150 ㎜ I.D., 5 ㎛; GL Sciences, Japan) reversed phase column, was used. By use of photo diode array (PDA), wavelengths were detected at 265 ㎚. The oven temperature was 35°C and the velocity of the liquid was 2 ㎖/min. 1% formic acid in water (mobile phase A) and 0.1% formic acid in 20% acetonitrile (mobile phase B) were used. The pre-treatment samples described above were analyzed under these gradient conditions: the concentration of solvent B was increased from 10% to 90% consistently for 23 minutes, 90% concentration was maintained for 9 minutes, and the concentration was reduced from 90% to 10% consistently for 3 minutes. A sample, 10 ㎕ each, was injected. MS/MS analysis was conducted in the positive ionization mode using electrospray ionization (ESI) source. As MS parameter, cone voltage 3.5 kV, capillary temperature 250°C, and desolvation gas 300 L/hr were set. The range of molecular weight was 50-800 m/z in a full scan type.

Statistical analyses

Sample from each accession was replicated three times and data on individual GSL was measured three times. Descriptive statistics (mean, standard deviation and percentage) were used to analyze the data using Microsoft excel (version 10.0, Microsoft, Redmond, WA, USA). One-way analysis of variance (ANOVA) was used to test the difference between means of three *Raphanus* species using SPSS (SPSS Inc., Chicago, IL, USA) and significant means were further compared using Student-Newman-Keuls test. Principal component analysis (PCA) was done on the mean data of leaves and root GSL of each three *Raphanus* species using Multibase program (http://www.numericaldynamics.com).

Results and Discussion

GSL profiles in *Raphanus* species

This study analyzed the GSL in both the leaves and roots of *Raphanus* species using HPLC protocol. A total of 13 GSLs were identified in both the leaves and root tissues of radish. Identification of GSLs by chromatography were based on the known concentration of standard i.e. sinigrin and retention time. A typical HPLC chromatogram of identified GSL in the roots of *Raphanus* species is shown in Fig. 1. Likewise, GSL in leaves was also detected in the same pattern. Thirteen GSLs detected in both the leaves and roots of each *R. sativus* L., *R. raphanistrum* and *R. sativus* L. var. *raphanistroides* (Makino) germplasm were belonged to three chemical classes; aliphatic, indolyl and aromatic which are given in Table 1.

Twelve GSLs were detected in the leaves of *R. sativus*, while ten GSLs were present in roots. In *R. raphanistrum*, ten GSLs were present in leaves and nine GSLs were detected in roots. Ten GSLs were identified in leaves and roots of *R. sativus* L. var. *raphanistroides* (Makino). Genotypic differences in GSLs types and contents in radish have been reported

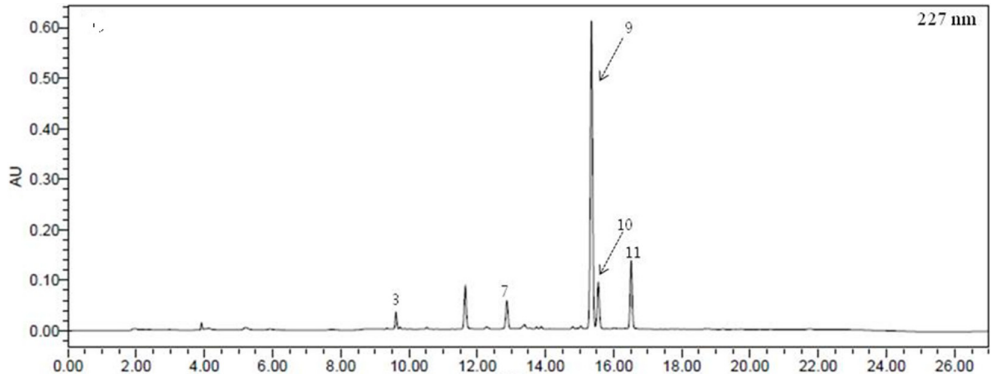

Fig. 1. HPLC chromatogram of identified GSL in the roots of *Raphanus* spp. Peak refers to following identified compounds; 1 = Progoitrin, 2 = Glucoraphanin, 3 = Sinigrin, 4 = Glucoalyssin, 5 = Gluconapoleiferin, 6 = Gluconapin, 7 = 4-Hydroxyglucobrassicin, 8 = Glucobrassicanapin, 9 = Glucoraphasatin, 10 = Glucobrassicin, 11 = Neoglucobrassicin, 12 = Glucobrassicin, and 13 = Neoglucobrassicin. The same pattern of GSL was identified in HPLC chromatogram of leaves in *Raphanus* spp.

Table 1. GSLs identified in 26 germplasm of *R. sativus* L., 3 *R. raphanistrum* germplasm and 15 *R. sativus* L. var. *raphanistroides* (Makino) germplasm grown at autumn season, their trivial name, and percentage of germplasm with GSLs

Chemical class and systematic name	Trivial name	Abbreviation	*R. sativus* L. (%)		*R. raphanistrum* (%)		*R. sativus* L. var. *raphanistroides* Makino (%)	
			Leaves	Roots	Leaves	Roots	Leaves	Roots
Aliphatic								
3-Butenyl	Gluconapin	GNA	3.8	3.8	0.0	0.0	0.0	0.0
4-Pentenyl	Glucobrassicanapin	GBN	3.8	0.0	0.0	0.0	0.0	13.3
2-(R)-2-Hydroxy-3-butenyl	Progoitrin	PRG	7.7	0.0	0.0	0.0	0.0	6.7
4-Methylsulphinylbutyl	Glucoraphanin	GRA	61.5	76.9	100.0	33.3	73.3	33.3
5-Methylsulphinylpentyl	Glucoalyssin	GAL	76.9	50.0	66.7	100.0	80.0	26.7
4-methylthio-3-butenyl	Glucoraphasatin	GRS	88.5	96.2	100.0	100.0	93.3	100.0
Allyl	Sinigrin	SIN	65.4	76.9	100.0	66.7	86.7	40.0
2-hydroxy-4-pentenyl	Gluconapoleiferin	GNL	0.0	0.0	33.3	0.0	13.3	0.0
Indolyl								
3-Indolymethyl	Glucobrassicin	GBS	100.0	100.0	100.0	100.0	100.0	93.3
1-Methox-3-indolymethyl	Neoglucobrasscin	NGBS	50.0	30.8	66.7	33.3	66.7	13.3
4-Hydrox-3-indolymethyl	4-Hydroxyglucobrassicin	4-OHGBS	42.3	76.9	33.3	100.0	33.3	93.3
4-Methoxy-3-indolymethyl	4-Methoxyglucobrassicin	4-OMGBS	96.2	88.5	100.0	100.0	100.0	93.3
Aromatic								
2-Phenylethyl	Gluconasturtiin	GST	15.4	3.8	33.3	33.3	20.0	0.0

previously (Carlson *et al*., 1985; Ishida *et al*., 2012; Ishii *et al*., 1989). In the study of Kim *et al*. (2013), they reported thirteen GSLs in the roots of radish cultivars but our study detected only ten GSLs in the roots of *R. sativus* L. germplasm. Lee *et al*. (2013) identified eleven GSLs in turnip germplasm which is closed to our result. Eight GSLs consisting of four aliphatic (glucoraphanin, glucoalyssin, glucoraphasatin and sinigrin) and four indolyl (glucobrassicin, neoglucobrassicin, 4-hydroxyglucobrassicin and 4-methoxyglucobrassicin) were detected in both the leaves and roots of each *Raphanus* species. But in the study of Ishida *et al*. (2012), they reported three aliphatic GSLs (glucoraphanin, glucoerucin and glucoraphasatin) and three indolyl GSLs (4-hydroxyglucobrassicin, glucobrassicin and 4-methoxyglucobrassicin) in the roots of Japanese radish cultivars. Three GSLs (glucoraphasatin, glucobrassicin and 4-methoxyglucobrassicin) in leaves and roots were detected more than 85% of the germplasm of each *Raphanus* species. Previous work has shown that three GSLs (glucobrassicanapin, glucobrassicin and gluconasturtiin) were detected about 90% of the turnip varieties (Padilla *et al*., 2007). Glucoraphanin, glucoalyssin, and sinigrin were detected in roots between the 50% and 77% of the *R. sativus* L. germplasm. 4-hydroxyglucobrassicin, neoglucobrassicin and 4-methoxyglucobrassicin were detected in leaves between the 42% and 97% of the germplasm while these were detected in roots between 30% and 89% of the germplasm. In *R. raphanistrum*, glucoalyssin and sinigrin were detected in leaves between 67% and 100% of the germplasm, while glucoraphanin and sinigrin were identified in roots between 33% and 67% of the total germplasm. In *R. sativus* L. var. *raphanistrodes* (Makino), glucoraphanin, glucoalyssin and sinigrin were detected in leaves between 73% and 87% of the germplasm, while glucoalyssin, glucoraphanin and sinigrin were identified in roots between the 26% and 40% of the germplasm. Gluconapin, glucobrassicanapin and progoitrin were not present in the leaves and roots of *R. raphanistrum* germplasm while these GSLs were detected in the leaves of few *R. sativus* L. germplasm. In general, type of GSLs varies according to varietal types, species and different tissues (leaves, roots and seeds etc.) (Carlson *et al*., 1985; Rosa *et al*., 1997).

Variation of GSL contents in *Raphanus* species

Germplasm of *R. sativus* L., *R. raphanistrum* and *R. sativus* L. var. *raphanistroides* exhibited the variation for all individual GSL contents in leaves and roots (Table 2). The glucobrassicin detected the highest (4.11 μmol/g dw) in leaves followed by glucoraphasatin (3.86 μmol/g dw), whereas glucoraphasatin was the highest (24.30 μmol/g dw) in roots. In *R. sativus* L. germplasm, glucoraphasatin, a predominant aliphatic GSL, was represented 42.2% and 87.1% of the total GSL in leaves and roots, respectively. In contrast, high glucoraphanin was reported in seed (Han *et al.*, 2015) and roots of some radish cultivars (Kim *et al.*, 2013). Glucoraphasatin was identified as the dominant GSL in radish roots in the previous reports (Ishida *et al*., 2012; Kim *et al*., 2013; Li *et al*., 2010; Yi *et al*., 2016). But glucobrassicin, an indolyl GSL was represented 44.9% and 6.7% of the total GSLs in leaves and roots, respectively. Glucobrassicin was reported as a dominant GSL in radish leaves (Sang *et al*., 1984) but this is the second important GSL detected in the present study. In the germplasm of *R. raphanistrum*, the predominant GSLs in leaves were glucoraphastin (9.7 μmol/g dw) and glucobrassicin (4.07 μmol/g dw) followed by sinigrin (3.62 μmol/g dw) and represented 50.5%, 21.2% and 18.8% of the total GSL, respectively. In our study, glucoraphasatin represented 92.7% of the total GSL in roots which is close to the findings of Yi *et al*. (2016). The varied GSLs contents in roots and leaves in wild radish (*R. raphanistrum*) were reported by Malik *et al*. (2010). Quantitative and qualitative differences in GSLs

Table 2. Individual and total GSL contents (μmol/g dw) in leaves and roots of *R. sativus* L., *R. raphanistrum*, and *R. sativus* L. var. *raphanistroides* (Makino) germplasm

Groups GSLs	*R. sativus* L. (N = 26)[z]		*R. raphanistrum* (N = 3)		*R. sativus* L. var. *raphanistroides* (Makino) (N = 15)	
	Leaves	Roots	Leaves	Roots	Leaves	Roots
Aliphatic						
GNA	0.01 ± 0.06[y]	0.10 ± 0.49	ND[x]	ND	ND	ND
GBN	0.04 ± 0.21	ND	ND	ND	ND	0.20 ± 0.56
PRG	0.01 ± 0.03	ND	ND	ND	ND	0.01 ± 0.06
GRA	0.06 ± 0.08	0.15 ± 0.11	0.38 ± 0.35	0.13 ± 0.23	0.21 ± 0.18	0.07 ± 0.13
GAL	0.12 ± 0.16	0.09 ± 0.14	0.35 ± 0.47	0.27 ± 0.12	0.15 ± 0.14	0.07 ± 0.13
GRS	3.86 ± 3.94	24.30 ± 11.63	9.7 ± 7.10	55.89 ± 8.98	9.2 ± 5.87	41.65 ± 16.75
SIN	0.20 ± 0.35	0.57 ± 0.60	3.62 ± 3.21	1.62 ± 2.63	0.21 ± 0.18	0.14 ± 0.37
GNL	ND	ND	0.11 ± 0.19	ND	0.02 ± 0.05	ND
Total	4.3 ± 1.34	25.21 ± 9.85	14.16 ± 3.43	57.91 ± 19.67	9.79 ± 3.22	42.14 ± 15.71
Indolyl						
GBS	4.11 ± 5.40	1.88 ± 3.64	4.07 ± 3.67	1.66 ± 0.59	3.94 ± 3.08	0.99 ± 0.87
NGBS	0.02 ± 0.04	0.01 ± 0.03	0.11 ± 0.16	0.06 ± 0.11	0.03 ± 0.04	0.01 ± 0.03
4-OHGBS	0.03 ± 0.05	0.38 ± 0.48	0.13 ± 0.23	0.17 ± 0.07	0.03 ± 0.06	0.32 ± 0.26
4-OMGBS	0.65 ± 0.68	0.42 ± 0.32	0.62 ± 0.17	0.44 ± 0.22	0.56 ± 0.36	0.53 ± 0.20
Total	4.81 ± 1.96	2.69 ± 0.83	4.93 ± 1.91	2.33 ± 0.74	4.56 ± 1.88	1.85 ± 0.41
Aromatic						
GST	0.03 ± 0.07	0.01 ± 0.03	0.04 ± 0.07	0.06 ± 0.10	0.03 ± 0.06	ND
Total GSLs	9.14 ± 8.08	27.90 ± 12.78	19.19 ± 6.91	60.30 ± 11.48	14.39 ± 6.34	44.0 ± 17.16

[z]N = Number of germplasm, GNA = Gluconapin, GBN = Glucobrassicanapin, PRG = Progoitrin, GRA = Glucoraphanin, GAL = Glucoalyssin, GRS = Glucoraphasatin, SIN = Sinigrin, GNL = Gluconapoleiferin, GBS = Glucobrassicin, NGBS = Neoglucobrasscin, 4-OHGBS = 4-Hydroxyglucobrassicin, 4-OMGBS = 4-Methoxyglucobrassicin, GST = Gluconasturtiin. [y]Mean ± SD (Standard deviation). ND[x] = not detected.

contents in different tissues of the same plant were also reported by previous researchers (Bellostas *et al*., 2004; Sang *et al*., 1984). Highest glucoraphasatin detected in leaves (9.2 μmol/g dw) and roots (41.65 μmol/g dw) in the germplasm of *R. sativus* L. var. *raphanistrodes* (Makino) were represented 63.9% and 94.7% of the total GSL, respectively. The rest of GSLs were found in lower proportions (less than 3.8% of the total GSLs). The aliphatic GSLs in leaves and roots were represented about 62.9% and 94.0% of the total GSL, respectively, while indolyl GSL represented 36.7% and 5.9% of the total GSL in leaves and roots of three *Raphanus* species, respectively (data not shown). Differences observed in aliphatic composition in this study might be due to allelic variation in few genes encoding key regulatory enzymes at key points in the GSL pathway. Cartea *et al*. (2008) reported that major aliphatic GSL represented 69.2% of the total GSLs followed by indolyl GSL (30.4%) in the leaves of Kale. In this study, we observed the differences in aliphatic, indolyl and aromatic GSLs in leaves and roots in three *Raphanus* species. Bhandari *et al.* (2015) reported the variation in aliphatic, indolyl and aromatic GSLs on different tissues of *Brassica* vegetables, while butenyl GSL was the major portion in the leaves of turnip (Kwon and Kliebenstein, 2014).

Total GSL contents in leaves and roots of the three *Raphanus* species germplasm and their statistics are illustrated in Table 3. Total GSL contents of leaves showed the significantly ($p \leq 0.05$) different among *Raphanus* species. The highest total GSL contents (19.19 μmol/g dw) was detected in leaves of *R. raphanistrum* with a range from 11.96 to 25.74 μmol/g dw followed by *R. sativus* L. var. *raphanistroides* Makino (14.39 μmol/g dw) and represented 24.14% and 24.6% of the total GSL, respectively. Similarly, *Raphanus* species showed significant ($p \leq 0.05$) differences in total GSL contents in roots. Total GSL contents in roots was varied from 50.70 to 73.02 μmol/g dw with an average of 60.30 μmol/g dw in *R. raphanistrum* which represented 75.85% of the total GSL. Total GSL contents in roots in all three species was approximately 3-fold higher than that of leaves. The GSL contents found in higher amount in the root may act as defensive compounds to deter invasion by these soil organisms (Van Dam *et al*., 2009). Total GSL contents was significantly ($p \leq 0.05$) different among the *Raphanus* species. Total GSL contents was ranged from 62.66 to 92.90 μmol/g dw with an average of 79.49 μmol/g dw in *R. raphanistrum* which was not statistically different with total GSL contents (58.39 μmol/g dw) of *R. sativus* L.var. *raphanistroides* (Makino). Previous researchers (Bhandari *et al*., 2015; Castro *et al.* 2004; Zhu *et al*., 2013) were also reported higher GSL contents in roots than shoots of *Brassica* crops. Previous work has also shown that wild radish showed a higher total GSL contents than landrace radish (*R. sativus* L.) in roots (Choi *et al*., 2009).

Variation in total GSL contents in leaves and roots in the germplasm of three *Raphanus* species are given in Fig. 2. In *R. sativus* L., germplasm with high total GSL contents in both the leaves and roots were IT119288 (74.19 μmol/g dw), Joseonmu (68.15 μmol/g dw), and IT119262 (63.10 μmol/g dw). In *R. raphanistrum*, total GSL contents both in the leaves

Table 3. Total GSL contents (μmol/g dw) in leaves and roots of *R. sativus* L., *R. raphanistrum* and *R. sativus* L.var. *raphanistroides* (Makino) germplasm

Raphanus spp.	Statistics	Total GSL (μmol/g dw)		Total GSL (μmol/g dw)
		Leaves	Roots	
R. sativus L. (N = 26)[y]	Mean	9.14 ± 8.08 c[z]	27.9 ± 12.78 c	37.04 ± 17.28 b
	Range	(1.35 - 29.86)	(2.87 - 48.89)	(13.06 - 74.17)
R. raphanistrum (N = 3)	Mean	19.19 ± 6.91 a	60.30 ± 11.48 a	79.49 ± 15.41 a
	Range	(11.96 - 25.74)	(50.70 - 73.02)	(62.66 - 92.90)
R. sativus L. var. *raphanistroides* (Makino) (N = 15)	Mean	14.39 ± 6.34 b	44.0 ± 17.16 b	58.39 ± 20.09 a
	Range	(5.02 - 26.80)	(14.54 - 65.48)	(24.84 - 90.59)

[z]Means followed by different letter in column are significantly different at $p \leq 0.05$.
[y]N = Number of germplasm. Mean ± SD (Standard deviation).

and roots were the highest in RA 504 (92.90 μmol/g dw) and K046542 (82.92 μmol/g dw), respectively. In *R. sativus* L. var. *raphanistroides*, G2003-32 had the highest total GSL (90.59 μmol/g dw) followed by IT302373 (80.93 μmol/g dw), and K036805 (77.91 μmol/g dw) (data not shown). The differences in GSL contents in germplasm of *Raphanus* species might be influenced by genotype with varied plant species which was also reported by previous researchers (Cartea *et al*., 2012; Farnham *et al*., 2004). Regarding the total GSL contents, the germplasm IT119288, Joseonmu, IT119262,

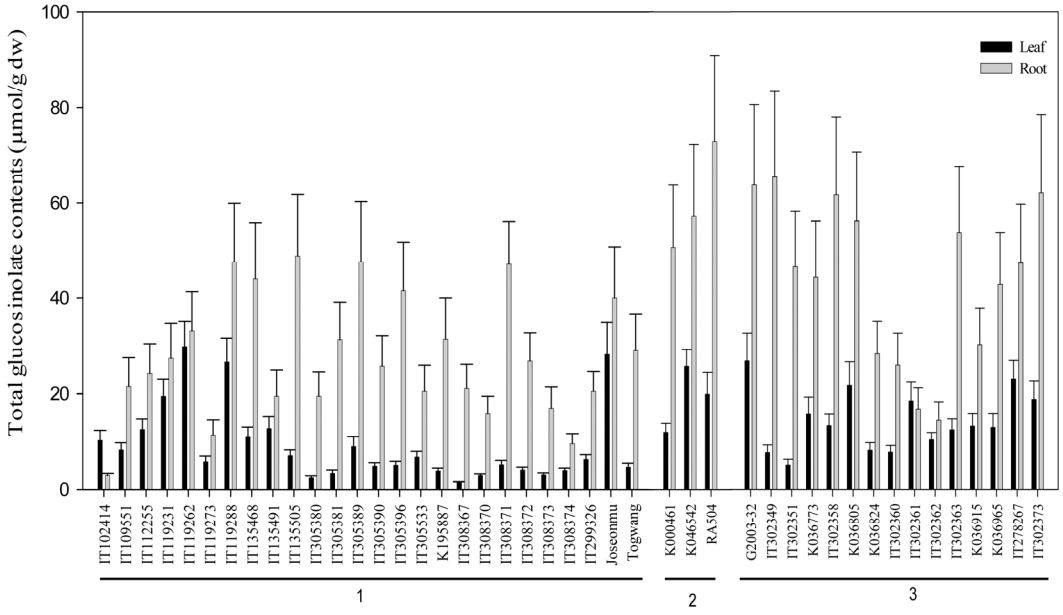

Fig. 2. Variation in total GSL contents (μmol/g dw) in the leaf and root of 44 germplasm of radish *(Raphanus* spp.). 1 = *R. sativus* L. germplasm (44); 2 = *R. raphanistrum* germplasm (3); and 3 = *R. sativus* L. var. *raphanistroides* (Makino) germplasm (15). Each vertical bar represents mean ± of three replications.

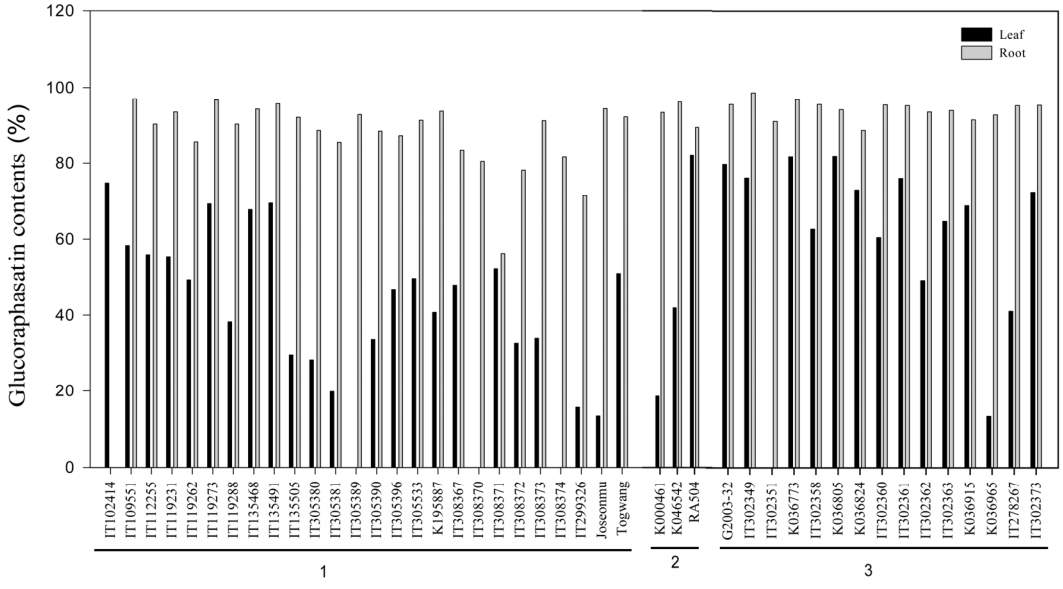

Fig. 3. Glucoraphasatin relative content (%) in the leaf and root tissue of 44 radish (*Raphanus* spp.) germplasm. 1= *R. sativus* L. germplasm (44); 2 = *R. raphanistrum* germplasm (3) and 3 = *R. sativus* L. var. *raphanistroides* (Makino) germplasm (15).

RA 504, K046542, G2003-32, IT302373 and K036805 could have potential health benefit as well as promising source for future breeding purpose.

The relative content of glucoraphasatin with the total GSL in leaves and roots in three *Raphanus* species is shown in Fig. 3. In *R. sativus* L., germplasm with high glucoraphasatin content in both the leaves and roots were IT119273 (83.0%), IT135491 (82.6%) and IT135468 (81.0%). In *R. raphanistrum*, RA 504 had the highest GRS content (85.7%) in both the leaves and roots whereas in *R. sativus* L. var. *raphanistroides* (Makino), it had the highest in K036773 (89.4%) followed by K036805 (88.0%) in both the leaves and roots (data not shown). In contrast, Bhandari *et al*. (2015) reported glucoraphanin as the predominant GSL in roots of radish and comprised 64.9% of the total GSL. But GRS was detected as the major GSL in this study and similar results were reported in previous studies (Barillari *et al*., 2005; Montaut *et al*., 2010). Glucoraphasatin, one of the important isothiocyanate components, are responsible for pungency in roots and it is known to have antimutagenic, antifungal, and antibacterial and direct antioxidant activity (Montaut *et al*., 2010). Germplasm IT119273, IT135491, IT135468, RA 504, K036773 and K036805 had high GRS levels and that could have potential sources to develop genotypes for high GSL content.

PCA on GSL contents in *Raphanus* species

The data obtained for the thirteen GSLs content in leaves and roots of the three *Raphanus* species were subjected to PCA to outline GSL profile differences among the radish germplasm. PCA revealed that the two highest ranking principal components accounted for 99.37% of the total variance (Fig. 4). The GSLs in the principal component were identified on the basis of eigenvectors. The first principal component (PC1) accounting for 92.85% of the total variance and glucoraphasatin was the major contributor in PC1, while in second principal component (PC2) explained 6.52% of the total variance and it was mostly associated with glucobrassicin. In particular, IT119288 from *R. sativus* L., K046547 from *R. raphanistrum* and G2003-32 from *R. sativus* var. *raphanistroides* (Makino) stood out from other radish germplasm (data not shown). The scatter diagram showed that most of radish germplasm belonging to *R. sativus* grouped together but only a few accessions were closed to *R. sativus* L. var. *raphanistrodes* (Makino). Three accessions from *R. raphanistrum* were closed with *R. sativus* L. var. *raphanistroides* (Makino) accessions. Likewise, germplasm of *R. sativus* L. var. *raphanistroides* (Makino) were grouped together except few accessions. In this study, PCA could not distinguish clearly among the three *Raphanus* species. GSL contents and their types vary on among *Brassica* species and between cultivars of the same species (Bradshaw *et al*., 1984; Rosa *et al*., 1997). GSL contents of few accession of wild radish might be similar with the accessions of some domesticated radish (*R. sativus* L.) accessions. PCA is a widely used multivariate technique which exhibits the greatest variance within a population and determines closely related compounds (Kim *et al*., 2007). Our result also identified glucoraphasatin and glucobrassicin as the predominant GSLs within data and PCA was used to analyze the variation among different crop varieties at the metabolome level (Hong and Kim, 2014; Jo *et al*., 2016; Kim *et al.*, 2010).

This study analyzed the individual and total GSL contents in leaves and roots of *R. sativus*, *R. raphanistrum*, and *R. sativus* L. var. *raphanistroides* (Makino) germplasm. Our study provided the valuable information regarding on GSL contents in radish germplasm. Despite the thirteen GSLs were

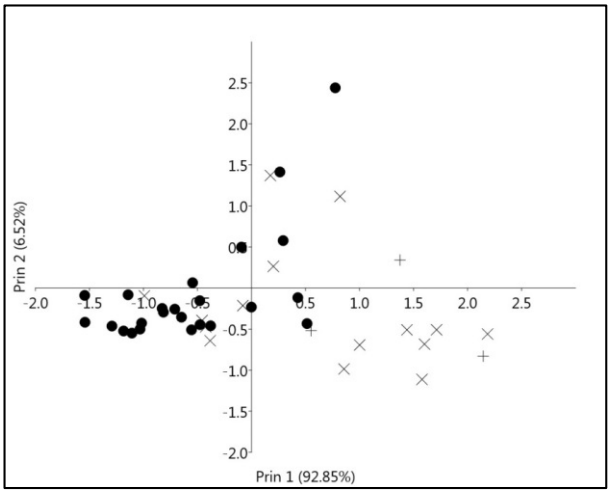

Fig. 4. Scatter diagram of 44 radish (*Raphanus* spp.) accessions for the first (PC1) and the second (PC2) principal component produced by analysis from mean data of leaves and root GSL content. • = *R. sativus* L. (26), + = *R. raphanistrum* (3), and × = *R. sativus* L. var. *raphanistroides* Makino (15).

detected in leaves and roots of each three *Raphanus* species, mainly eight GSLs consisting of four aliphatic (glucoraphanin, glucoalyssin, glucoraphasatin and sinigrin) and four indolyl (glucobrassicin, neoglucobrassicin, 4-hydroxyglucobrassicin and 4-methoxyglucobrassicin) were detected in both the leaves and roots.

Glucoraphasatin was the predominant GSL in all the *Raphanus* species followed by glucobrassicin, while sinigrin identified as the third GSL. Three accessions (IT119273, IT135491 and IT135468) from *R. sativus* L., two accessions (RA 504 and K046542) from *R. raphanistrum* and three (K036773, K036805 and G2003-32) from *R. sativus* L. var. *raphanistroides* (Makino) were identified as high glucoraphasatin content. The germplasm having high glucoraphasatin content will enhance the possibility of studying genes involved in the GSL regulation and explore the feasibility of modifying in specific plant genotypes in future. Glucoraphasatin is one of the potent compounds of isothiocyanates which have a chemoprotective effect in human. PCA also confirmed that glucoraphasatin is a major GSL followed by glucobrassicin.

Thus, IT119288, Joseonmu, IT119262, RA 504, K046542, G2003-32, IT302373 and K036805 had high GSL levels and these germplasm can be good candidates for developing GSL-rich varieties in radish breeding program.

Acknowledgement

This study was carried out with the support of "Research Program for Agricultural Science & Technology Development (Project No. PJ01192202)" National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea.

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(Received 7 March 2017 ; Revised 4 July 2017 ; Accepted 10 July 2017)