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Research Article

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Identification and Quantification of Glucosinolates in Rapeseed (*Brassica napus* L.) Sprouts Cultivated under Dark and Light Conditions

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Abstract

BACKGROUND: This study was performed for the identification and quantification of glucosinolate (GSL) contents in seven varieties of rapeseed (*Brassica napus* L.) sprouts cultivated under dark and light conditions.

METHODS AND RESULTS: Crude glucosinolates (GSLs) were desulfated by treating with aryl sulfatase and purified using diethylaminoethyl sepharose (DEAE) anion exchange column. Individual GSLs were quantified using high-performance liquid chromatography (HPLC) with electrospray ionization-tandem mass spectrometry (ESI-MS/MS). Eleven GSLs including six aliphatic (progoitrin, sinigrin, glucoalyssin, gluconapoleiferin, gluconapin, and glucobrassicanapin), four indolyl (4-hydroxyglucobrassicin, glucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin) and one aromatic (gluconasturtiin) were identified based on the fragmentation patterns of MS spectrum. Aliphatic GSLs were noted as the predominant group with average 85.2% of the total contents. The most abundant GSLs were progoitrin which was ranged at 8.14−118.68 μmol/g dry weight (DW). The highest total GSL amounts were documented in 'Hanra' (146.02 μmol/g DW) under light condition and 'Mokpo No. 68' (86.67 μmol/g DW) in dark condition, whereas the lowest was in 'Tamra' (30.13 and 14.50 μmol/g DW) in both conditions. The sum of aliphatic GSLs attributed > 80% in all varieties, except 'Tamra' (67.7% and 64.9% in dark and light conditions, respectively) in the total GSL accumulation. Indolyl GSLs were ranged 2.41−15.73 μmol/g DW, accounted 2.78−33.6% of the total GSLs in rapeseed varieties.

CONCLUSION(S): These results provide valuable information regarding potential beneficial GSL contents individually. This study attempts to contribute to knowledge of the nutritional properties of the different varieties of rapeseed plants. These results may be useful for the evaluation of dietary information.

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Key words: *Brassica napus* L., Glucosinolates, LC-ESI-MS/MS, Light conditions

Introduction

Rapeseed (*Brassica napus* L.) is the third largest crop source of vegetable oil after palm and soybean oil on the world oilseed production. In 2012, FAOSTAT reported that the estimated cultivation and production area and the yield of rapeseed in the world were more than 34.2 million hectares and 64.8 million metric tons (MT) (FAO, 201[3]). The leading rapeseed producing countries in the world was Canada (15.4 MT), China (14.0 MT), India (6.7 MT), European countries (14.7 MT: France, Germany, UK, Poland, etc.), Australia (3.4 MT), the Russian Federation (1.0 MT), and the United States (1.1 MT). Recent report claimed that, in South Korea it was cultivated in 1,550 ha and produced 1,800 ton (FAO, 2013).

Rapeseed is mainly cultivated for the production of house hold oil as nutritional oil and for the biodiesel production in the industry. The seed of rapeseed is rich in protein (36–40%) compared to stem and leaf, therefore, it is used in the cattle form as animal feed (Fenwick *et al.*, 1983). However, the use of rapeseed meal has been limited due to the presence of anti-nutrition constituents, such as phenolics and glucosinolates (GSLs), as well as its high fiber content (Bell, 1993; Brand *et al.*, 2007). The GSLs such as progoitrin, gluconapin, and glucobrassicanapin and phenolics such as sinapine, sinapic acid, and sinapate ester were abundantly noticed in rapeseed (Cai and Arntfield, 2001; Naczk *et al.*, 1998). It is reported that, rapeseed oil contained between 25–45% of erucic acid; among these phenolics, sinapine is noticed as the most abundant, accounting for 1–2% (w/w) of the whole rapeseed, whereas, GSLs detected about 60–100 μmole levels (Khattab *et al.*, 2010). In general, GSLs are categorized into three chemical classes, aliphatic, indolyl, and aromatic GSLs, according to the existence of precursor amino acid such as methionine, tryptophan or an aromatic amino acid (tyrosine or phenylalanine) (Rosa, 1997). GSLs and their break-down products were known for various biological activities such anti-cancer and insecticidal effects (Andersen and Muir, 1966; Larsen 1981; Larsen *et al.* 1992), whereas the breakdown products of *B. napus* exerts a variety of toxic and anti-nutritional effects in higher animals (adverse effects on thyroid metabolism) (Heaney and Fenwick, 1995). Among the anti-nutritional compound, goitrin derived from progoitrin is widely studied for its goitrogenic effect. Goitrin block and slowdown the thyroid hormones synthesis and metabolic pathways resulted in the absorption of iodine by the thyroid gland (Zukalova and Vasak, 2002). Therefore, removal or decreasing the contents of GSLs could potentially make rapeseed meal as valuable as the oil. To accomplish this, many researchers and plant breeders developed different inbred lines of rapeseed through the introgression of alleles containing aliphatic GSL contents from above 100 μmoles to less than 20 μmole levels (Kondra and Stefansson, 1970; Robbelen and Thies, 1980; Gland *et al.* 1981). Recently, researchers reported that applying high levels of sulfur resulted in lowering the concentrations of GSLs inbred lines of rapeseed (Hocking *et al.*, 1996; Malhi *et al.*, 2007; Egesel *et al.*, 2009). The determination of the GSL compositions of different rapeseed has become an interest of this work because of its extensive application and limited information on the individual component compositions of different rapeseeds consumed in Republic of Korea.

Individual GSLs of rapeseed has become an interest for breeders because GSLs are bioactive compounds as anti-cancer or anti-pathogen agents. In the present study, rapeseed sprouts were cultured because matured rapeseeds are relatively taken long term cultivation (6–7 months) from autumn to the following spring, and their GSLs were quantified in seven rapeseed varieties. Moreover, whether GSL contents are affected or not by light and dark conditions, seed sprouts were cultured with/without fluorescent lamp in the growth chamber. Therefore, efforts have been made to select the best variety in terms of food contents thereby to increase value in local food products which have been used for many generations.

Materials and Methods

Chemicals and reagents

HPLC-grade acetonitrile (CH_3CN), methanol (CH_3OH), and sodium acetate trihydrate ($NaC_2H_3O_2 \cdot 3H_2O$, 98.5%) were obtained from J.T Baker chemical Co. (Phillipsburg, NJ, USA) and Samchun Pure Chemical Co., Ltd. (Seoul, Korea). Sinigrin (2-propenyl GSL), aryl sulfatase (Type H-1, EC 3.1.6.1), and DEAE

Sephadex A-25 were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ultra-pure water used in this study was made by PURELAB Option-Q system (ELGA Lab Water, VWS Ltd., UK).

Plant materials

Seven varieties of *B. napus* L. seeds such as 'Naehan', 'Mokpo No. 68', 'Mokpo No. 111', 'Youngsan', 'Tammi', 'Tamra', and 'Hanra' provided from the Bio-Energy Crop Center, National Institute of Food Science (Muan, Korea) and were used to compare their GSL metabolites in seed sprouts grown under dark and light conditions.

Cultivation conditions

Seven varieties of rapeseed seeds (2 g) were soaked in tap water for 3 h, after that, the seeds were sown on sponge plastic pot (7 × 7 × 12 cm) and cultivated both in dark and light conditions for fourteen days in the growth chamber (temperature, 25℃; humidity, 70%). However, in the case of light cultivation condition, initially for three days the seeds were cultivated under dark condition after that from 4^{th} day seeds were grown under 16 h light (129 $\mu mol/m^2s^1$) by white fluorescent lamps (32 W × 8) and 8 h dark till the end of the cultivation periods for 11 days. After cultivation, the seed sprouts were lyophilized, ground with mortar and pestle, and individually stored in a plastic bottle with a lid in desiccator until GSL analysis.

Extraction and desulfation of crude GSLs

Desulfo (DS)-GSLs were extracted according to the procedure of Kim *et al.* (2007) and ISO 9167-1 (1992). Briefly, crude GSLs from freeze-dried materials (100 mg) were extracted with 1.5 ml of boiling 70% (v/v) methanol in water bath for 5 min to inactivate endo-myrosinase. After centrifugation (12,000 rpm, 4℃, 10 min), the resulting supernatant were collected, and remaining residue was re-extracted twice as above described. The combined supernatant was taken as the crude of GSLs. Separately 0.5 mg of sinigrin was dissolved in 5 ml ultrapure water which was used as an external standard. Desulfation of the crude GSL extracts was performed on DEAE anion exchange column which was prepared by adding 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 an DEAE anion exchange column. The crude GSL extracts were loaded onto a pre-equilibrated column. After washing with 1 ml (× 2 times) of ultrapure water to remove cation and neutral ions, aryl sulfatase (E.C.3.1.6.1) (75 µl) was loaded onto each column. After desulfation reaction overnight (16 h) at room temperature, the desulfated GSLs were eluted with 0.5 ml (× 3 times) of ultra-pure water (PURELAB Option-Q, ELGA). The eluates were filtered through 0.45 µm Teflon PTFE syringe filter and analyzed immediately by HPLC or stored at the 4℃ in the refrigerator until GSL analysis.

LC-ESI-MS/MS analysis for identification of DS-GSLs

An API 4000 Q TRAP tandem mass spectrometer (Applied Biosystems, Foster City, CA), equipped with an Agilent 1200 series HPLC system (Agilent Technologies) 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-GSLs. 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℃; declustering potential (100 V); entrance potential (10 V); spectra scanning range, *m/z* 100–1000 (scan time 4.8 sec).

Separation and identification of desulfo (DS)-GSLs using HPLC

DS-GSLs obtained from different lines of cabbage were analyzed by 1200 series HPLC system (Agilent Technologies, CA, USA) equipped with an Inertsil ODS-3 (C18) column 150 × 3.0 mm i.d., particle size 3 µm) (GL Science, Tokyo, Japan). The HPLC analysis was carried out with a flow rate of 0.2 ml/min at a column oven temperature of 40℃ and a wavelength of 227 nm. The solvent system employed was ultra-pure water (A) and 100% acetonitrile (B). The gradient program used as follows: 0–18 min, 7 → 24% B; 18–32 min, keep 24% B; 32.1–40 min, keep 7% B. The individual GSLs were quantified with the sinigrin with their HPLC areas and response factors (ISO 9167-1, 1992). In this study, all the samples were designated as GSLs even though DS-GSLs were determined.

Statistical analysis

Data were analyzed by application of the Tukey's

multiple range test at $P \leq 0.05$, using SPSS statistical software (version 21 for Windows, SPPS Inc., Chicago, IL, USA). The data shown in all the Tables are the means of three replicates. For comparisons of continuous parameters between groups and within a group over time, repeated measures ANOVA was used.

Results and Discussion

Identification of GSLs by LC-ESI-MS/MS analysis

The identified compounds 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. The identified GSL in seven varieties of rapeseeds were very similar, and eleven GSLs were detected in all extracts. Each GSLs were identified based on their protonated molecular ions $[M+H]^+$ and corresponding product ions such as sodium $[M+Na]^+$ and potassium $[M+K]^+$ adducts. Six aliphatic (progoitrin, sinigrin, glucoalyssin, gluconapoleiferin, gluconapin, and glucobrassicanapin), four indolyl (4-hydroxyglucobrassicin, glucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin), and one aromatic GSLs (gluconasturtiin) were identified based on fragmentation patterns of MS spectra and quantified based on the peak areas of HPLC chromatogram (Fig. 1 and Fig. 2). Peaks 1 and 5 were identified as progoitrin and gluconapin, respectively. MS data revealed that these two GSLs contained 3-butenyl group as **R**-groups backbone with variation in the hydroxyl moiety in their structure (*m/z* 310 and 294 $[M+H]^+$ as DS-GSL, respectively), peaks 6, 8, 9 and 11 differed by the presence of indolyl compounds, and peak 10 were identified as aromatic moiety in their structure ($[M+H]^+$, *m/z* 344 as DS-GSL). In general, GSLs in plants are modified by elongation of the side chain, oxidation of the parent thiol group, or formation of alkene group.

Table 1. Glucosinolates (GSLs) identified by LC-ESI/MS in rapeseed sprouts

No.[a]	RT[b]	Trivial names	Semisystematic names of GSLs	Compound groups	$[M+H]^+$(*m/z*)[c]	Response factor[d]
1	6.11	Progoitrin	(*2R*)-2-Hydroxy-3-butenyl GSL	Aliphatic	310	1.09
2	7.30	Sinigrin	2-Propenyl GSL	Aliphatic	280	1.00
3	8.42	Glucoalyssin	5-Methylsufinylpentyl GSL	Aliphatic	372	1.07
4	9.02	Gluconapoleiferin	2-Hydroxy-pent-4-enyl GSL	Aliphatic	324	1.00
5	13.22	Gluconapin	3-Butenyl GSL	Aliphatic	294	1.11
6	15.58	4-Hydroxyglucobrassicin	4-Hydroxy-3-indolylmethyl GSL	Indolyl	385	0.28
7	19.15	Glucobrassicanapin	Pent-4-enyl GSL	Aliphatic	308	1.15
8	21.86	Glucobrassicin	3-Indolymethyl GSL	Indolyl	369	0.29
9	24.51	4-Methoxyglucobrassicin	4-Methoxy-3-indolylmethyl GSL	Indolyl	399	0.25
10	25.26	Gluconasturtiin	2-Phenethyl GSL	Aromatic	344	0.95
11	29.96	Neoglucobrassicin	*N*-Methoxy-3-indolylmethyl GSL	Indolyl	399	0.20

[a]No., the elution order of glucosinolates from HPLC chromatograms. [b]RT, retention time (min). [c]As a desulfo (DS)-GSLs.

[d]International Organization for Stanardization (ISO 9167-1, 1992).

*4-Hydroxyglucobrassicin was not detected in LC-ESI-MS; therefore, it was identified based on our database.

Table 2. Glucosinolate contents (μmol/g DW) in rapeseed sprouts cultivated under dark condition (*n=3*)

No.	Trivial names	'Naehan'	'Mokpo No. 68'	'Mokpo No. 111'	'Youngsan'	'Tammi'	'Tamra'	Hanra
1	Progoitrin	46.31±0.70c	78.06±0.07a	50.24±0.61b	34.51±1.67d	44.61±0.52c	8.14±0.06e	45.91±0.10c
2	Sinigrin	1.28±0.53ab	2.12±0.03a	1.73±0.11ab	2.04±0.85ab	0.95±0.32bc	0.16±0.02c	1.70±0.02ab
3	Glucoalyssin	0.48[1)]a	ND[2)]	0.41±0.08a	0.53±0.51a	0.25±0.07a	0.18±0.01a	0.23±0.01a
4	Gluconapoleiferin	0.89±0.20bc	1.46±0.02abc	1.61±0.21abc	2.85±1.81a	1.47±0.04abc	0.24±0.01c	2.76±0.01ab
5	Gluconapin	3.70±0.03a	1.40±0.02c	2.37±0.52b	3.49±0.14a	2.36±0.25b	0.53±0.05d	0.78±0.04cd
6	4-Hydroxyglucobrassicin	0.41±0.01bc	0.12±0.02d	0.46±0.15ab	0.63±0.00a	0.19±0.04d	0.18±0.05d	0.25±0.03cd
7	Glucobrassicanapin	1.18±0.12b	0.63±0.07c	1.25±0.08b	2.43±0.03a	1.52±0.31b	0.56±0.16c	1.23±0.12b
8	Glucobrassicin	2.88±0.06bc	1.26±0.02e	2.54±0.04c	3.01±0.04b	1.87±0.06d	1.10±0.01e	3.68±0.32a
9	4-Methoxyglucobrassicin	4.04±0.10a	0.59±0.02e	1.80±0.04d	3.59±0.03b	2.06±0.06cd	2.38±0.14c	3.43±0.31b
10	Gluconasturtiin	ND	0.58±0.08c	0.66±0.10c	1.15±0.07b	1.02±0.02b	0.56±0.07c	1.67±0.02a
11	Neoglucobrasscin	0.54±0.03d	0.44±0.06d	0.78±0.01c	1.85±0.05b	2.91±0.04a	0.46±0.03d	0.82±0.05c
Total		**61.72±1.48b**	**86.67±0.39a**	**63.85±0.37b**	**56.08±5.06**	**59.20±0.79b**	**14.50±0.30c**	**62.46±0.62b**

[1)]*n*=1. [2)]ND, not detected.

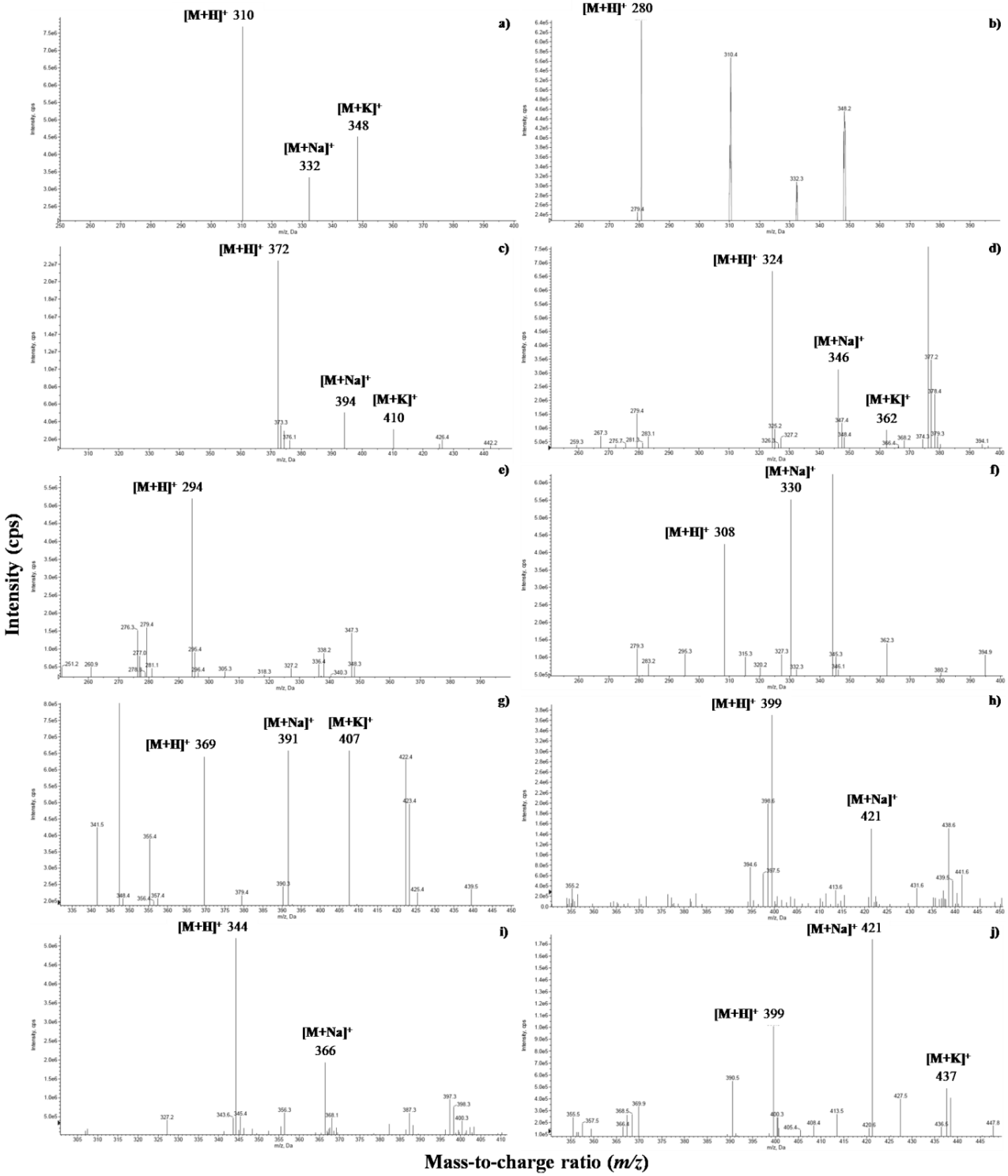

Fig. 1. Mass spectra of desulfo-glucosinolates in rapeseed sprouts ('Mokpo No. 68'). 1, progoitrin (*m/z* 310 $[M+H]^+$); 2, sinigrin (280); 3, glucoalyssin (372); 4, gluconapoleiferin (324); 5, gluconapin (294); 7, glucobrassicanapin (308); 8, glucobrassicin (369); 9, 4-methoxyglucobrassicin (399); 10, gluconasturtiin (344); 11, neoglucobrassicin (399). Peak 6, 4-hydroxyglucobrassicin was identified based on our data base and HPLC retention time.

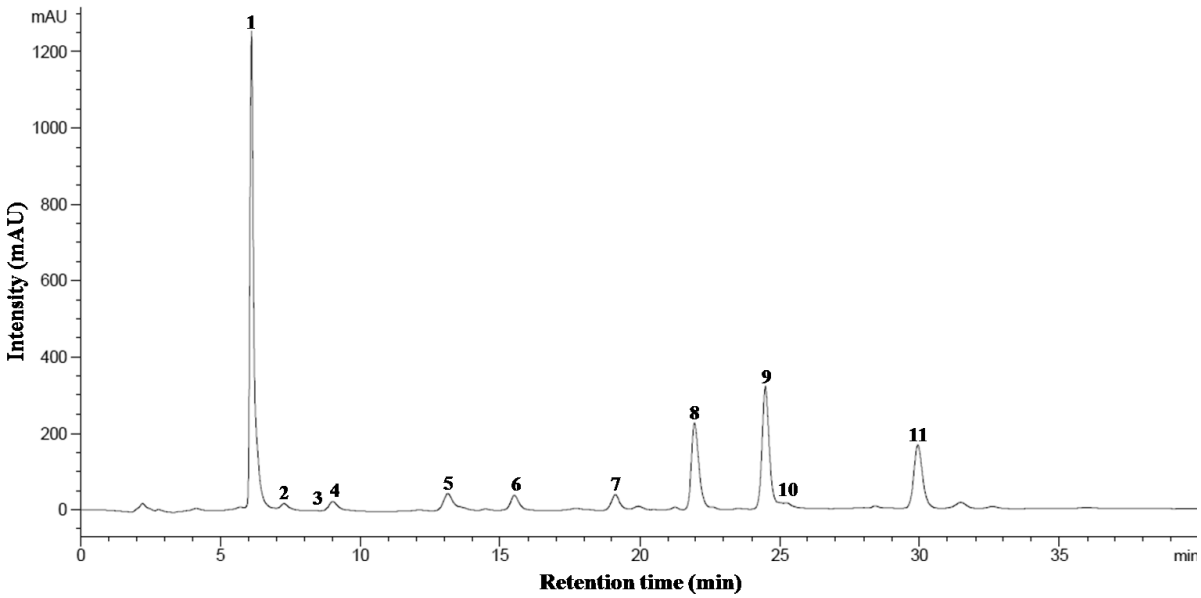

Fig. 2. HPLC chromatogram of glucosinolates in rapeseed sprouts ('Youngsan') cultivated under dark condition. 1, progoitrin; 2, sinigrin; 3, glucoalyssin; 4, gluconapoleiferin; 5, gluconapin; 6, 4-hydroxyglucobrassicin; 7, glucobrassicanapin; 8, glucobrassicin; 9, 4-methoxyglucobrassicin; 10, gluconasturtiin; 11, neoglucobrasscin.

Variation of GSLs under dark condition

The total GSL contents of the rapeseed sprouts cultivated under dark condition were ranged from 14.50–86.67 μmol/g DW (Table 2). Based on various reports, the major GSLs in rapeseeds are progoitrin, gluconapin, glucobrassicanapin, and glucobrassicin (Font *et al.*, 2005). Among them, progoitrin is the most abundant species accounting for about 50% in rapeseed. However, the HPLC quantification method revealed that the major GSLs found in the sprouts of rapeseeds were progoitrin, gluconapin, glucobrassicanapin, glucobrassicin, and 4-methoxyglucobrassicin. Other minor GSLs that were present in the sprouts considered herein were gluconapoleiferin, glucoalyssin, gluconasturtiin, and neoglucobrassicin. Data show significant differences in content of individual GSL levels among the varieties cultivated under dark condition. The predominant GSLs were progoitrin (ranged 8.14–78.06 μmol/g DW) and gluconapin (ranged 0.53–3.70 μmol/g DW). Among the samples; 'Mokpo No. 68' contained comparatively higher progoitrin levels (78.06 μmol/g DW) and contributed 90% of the total GSLs concentration, whereas, 'Tamra' detected the lowest amount of progoitrin (8.14 μmol/g DW). The cumulative amount of aliphatic and indolyl GSLs detected in 'Mokpo No. 68' were (83.63 and 2.40 μmol/g DW) which clearly indicated that the concentration of aliphatic GSLs were predominant (96.6%) in total amount of GSLs. Results indicated that total GSL, aliphatic GSL, and indolyl GSL contents of the sprouts of 'Naehan' (61.72, 53.85, and 7.87 μmol/g DW, respectively), 'Mokpo No. 111' (63.85, 57.60, and 5.59 μmol/g DW, respectively), and 'Hanra' (62.46, 52.62, and 8.18 μmol/g DW, respectively) under dark condition. Also, 'Mokpo No. 111' and 'Hanra' contain a small quantity of gluconasturtiin (0.66 and 1.67 μmol/g DW, respectively), and the content of gluconasturtiin in the two varieties did not exceed 4% of the total GSLs. Sinigrin is the most abundant GSLs in *Brassicaceae* family, and its breakdown product, allyl-isothiocyanate, is mainly involved in the prevention of the proliferation of human colorectal carcinoma cell line (Smith *et al.*, 2004). In contrary to Rosa (1997), our result detected sinigrin in all the varieties in the range of 0.16–2.12 μmol/g DW.

Variation of GSLs under light condition

The total GSL contents of the rapeseed sprouts cultivated under light condition were ranged from 30.13–146.02 μmol/g DW (Table 3). Under light condition the total GSL contents of the sprouts of 'Hanra' were higher (146.02 μmol/g DW) followed by 'Mokpo No. 111', (137.13 μmol/g DW). It is noted that rapeseed cultivated under light condition exhibited comparatively 1.83 fold higher GSL than

Table 3. Glucosinolate contents (μmol/g DW) in rapeseed sprouts cultivated under light condition (*n*=3)

No.	Trivial names	'Naehan'	'Mokpo No. 68'	'Mokpo No. 111'	'Youngsan'	'Tammi'	'Tamra'	Hanra
1	Progoitrin	71.96±1.65e	118.68±0.47a	90.26±0.06c	76.12±0.08d	70.40±0.21e	16.92±0.03f	112.80±0.15b
2	Sinigrin	3.24±3.03ab	2.86±0.01ab	2.51±0.01ab	2.25±0.02ab	1.66±0.02ab	0.44±0.01b	4.12±0.01a
3	Glucoalyssin	0.29±0.02e	0.57±0.00a	0.52±0.00b	0.41±0.01c	0.37±0.00d	0.27±0.01f	0.56±0.01a
4	Gluconapoleiferin	2.84±3.23b	1.90±0.00b	2.29±0.00b	3.03±0.01b	2.27±0.01b	0.75±0.01b	7.24±0.02a
5	Gluconapin	6.60±1.09a	1.53±0.01c	4.46±0.01bc	5.77±0.18ab	4.04±0.09c	0.58±0.01c	0.79±0.02c
6	4-Hydroxyglucobrassicin	0.23±0.14b	0.18±0.00b	0.15±0.00b	0.48±0.02a	0.32±0.01ab	0.27±0.10b	0.21±0.01b
7	Glucobrassicanapin	2.19±0.04e	0.73±0.02f	3.11±0.03b	5.06±0.01a	2.30±0.01d	0.60±0.07g	2.62±0.05c
8	Glucobrassicin	5.09±0.23b	5.13±0.02b	4.12±0.03d	4.55±0.01c	4.49±0.00c	4.48±0.06c	7.24±0.02a
9	4-Methoxyglucobrassicin	4.70±0.29b	2.76±0.02e	1.90±0.02f	4.06±0.01c	3.42±0.02d	4.74±0.09b	6.75±0.02a
10	Gluconasturtiin	0.60±0.03e	0.98±0.09d	1.34±0.08c	1.82±0.06b	0.85±0.07d	0.45[1)]e	2.16±0.02a
11	Neoglucobrasscin	1.08±0.02f	1.80±0.04d	3.76±0.04a	2.26±0.03b	1.90±0.03c	0.64±0.02g	1.54±0.03e
Total		**98.82±9.13cd**	**137.13±0.63a**	**114.61±0.18b**	**105.80±0.11bc**	**92.01±0.34d**	**30.13±0.36e**	**146.02±0.23a**

[1)] $n=1$.

under dark condition. Similar to dark condition, progoitrin identified as the major GSLs with higher contents in all the varieties together with gluconapin and shared 74.3% and 3.45% of total GSLs. In 'Hanra', the predominant GSLs were progoitrin (112.80 μmol/g DW) followed by gluconapoleiferin (7.24 μmol/g DW), glucobrassicin (7.24 μmol/g DW), 4-methoxyglucobrassicin (6.75 μmol/g DW), sinigirin (4.12 μmol/g DW), glucobrassicanapin (2.62 μmol/g DW), gluconasturtiin (2.16 μmol/g DW), and neoglucobrasscin (1.54 μmol/g DW), whereas, glucoalyssin, gluconapin and 4-hydroxyglucobrassicin documented less than 1.0 μmol/g DW. Under light cultivation condition the contents of indolyl GSLs varied significantly. Glucobrassicin is the most abundant indolyl GSLs, representing nearly 44.8% of the indolyls and about average 5.92% of the total GSLs (Table 3). The highest level of glucobrassicin in rapeseed sprout was found in the 'Hanra' (7.24 μmol/g DW) and 'Mokpo No. 68' (5.13 μmol/g DW), while the lowest concentration was found in 'Mokpo No. 111' (4.12 μmol/g DW). The sum of residual indolyl GSLs (4-hydroxyglucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin) was less and exhibited comparatively significant differences in their contents among the rapeseed varieties, except in 'Hanra' which contained a significant amount of 4-methoxyglucobrassicin (6.75 μmol/g DW). In contrast to indolyl GSLs, aliphatic GSLs such as progoitrin, sinigrin, glucoalyssin and gluconapoleiferin are mainly synthesized under the control of genetic materials (Magrath *et al.*, 1994), whereas the production of the indolyl GSLs namely, 4-hydroxyglucobrassicin, glucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin has been proposed to be regulated primarily by environmental and/or physiological factors (Mithen *et al.*, 1995). Rosa (1997) reported a significant difference in their individual GSL levels with regards to the cultivation of different accessions in *B. napus* and *B. oleracea*.

Conclusions

Rapeseed sprouts have similar GSL profiles to irrespective of their cultivation condition, and progoitrin is the most abundant GSLs. The total contents of GSLs in light cultivated rapeseed sprouts are 1.55–2.33 times higher than those in dark cultivated sprouts. Total GSL contents were higher in 'Hanra' and 'Mokpo No. 68' (146.02 and 137.13 μmol/g DW, respectively). The glucobrassicin contents of sprouts grown under light are much higher than those of the dark, but the total GSL contents in other indolyl groups are comparatively similar. In both dark and light cultivation conditions, the total contents of progoitrin are much higher than those of other individual GSLs. From this study it is confirmed that dark growing condition is better if our interest to reduce the content of GSLs, whereas the light grown condition is preferable if research is interested in biodiesel production. In the rape crop breeding program, continuing efforts have sought to decrease the contents of individual GSLs, for food and safety reasons. To decrease the content of anti-nutritional components in rapeseeds, a new variety has to be made through pollination and breeding methods, which attracts the rapeseed crop growers and the animal feed industries for the production of

cattle feed.

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