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Original article

Variation of glucosinolates on position orders of flower buds in turnip rape (*Brassica rapa*)

Mariadhas Valan Arasu [a], Na-Hyung Kim [b], Paulrayer Antonisamy [c], Yong-Han Yoon [d], Sun-Ju Kim [c,*]

[a] Department of Botany and Microbiology, Addiriyah Chair for Environmental Studies, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[b] Food and Drug Research Institute, Uiduk University, 261 Donghaedae-Ro, Gyeongju 38004, Republic of Korea
[c] Department of Bio-Environmental Chemistry, Chungnam National University, 99 Daehak-Ro, Yuseong-Gu, Daejeon 34134, Republic of Korea
[d] Department of Green Technology Convergence, College of Science and Technology, Konkuk University, Chungju 48666, Republic of Korea

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ABSTRACT

To glucosinolate (GSL) contents on flower buds depending on their position orders in turnip rape (*Brassica rapa*), three Japanese 'Nabana' cultivars such as cv. No. 21 (*Brassica rapa*, early type), cv. Husanohana (*B. rapa*, late type) and cv. Norin No. 20 (*B. napus*) were investigated using HPLC analysis. Ten GSLs including glucoraphanin, sinigrin, glucoalyssin, napoleiferin, gluconapin, 4-hydroxyglucobrassicin, glucobrassicanapin, glucobrassicin, and gluconasturtiin were detected. Differences in individual and total GSL contents were found between two plant varieties, which are also depending on various developmental stages. Among the GSLs, gluconapin (mean 23.11 μmole/g dry weight (DW) and glucobrassicanapin (mean 13.41 μmole/g DW) documented the most abundant compounds and contributed average 39 and 27% of the total GSLs, but indolyl and aromatic GSLs together accounted >10% of the total GSLs. The presence of significant quantities of gluconapin in the cultivars should be studied more extensively, since the GSL is mainly responsible for the bitter taste.

1. Introduction

Glucosinolates (GSLs) are plant secondary metabolites that have long been of toxicological and pharmacological concern. They are the chief group of phytochemicals present exclusively in 16 botanical families of the order of Capparales, and they are principally plentiful in Brassicaceae (Rosa et al., 1997; Fahey et al., 2001; Chun et al., 2016; Fu et al., 2016; Lee et al., 2017). They and/or their breakdown products are well known for their fungicidal (Pedras et al., 2006), bactericidal (Hashem and Saleh, 1999; Lin et al., 2000), nematocidal (Zasada and Ferris, 2004) and allelopathic properties (Fahey et al., 2001). In particular, sulforaphane, the isothiocyanate resultant from glucoraphanin, inhibits Phase I enzymes responsible for activation of carcinogens and induces Phase II detoxification enzyme systems established by in vitro studies, in that way rising the body's cancer resistance mechanisms (Zhang et al., 1992; Mithen et al., 2003; Pandey et al., 2017). In addition, indole-3-carbinol, the hydrolysis product derived from glucobrassicin, is thought to change the biotransformation enzyme activity and act as an anticarcinogen (Brew et al., 2009; Lee et al., 2015). However, the unhelpful features of these compounds have been a foremost spotlight of research due to their 'antinutritional' or goitrogenic possessions. Compounds with these characteristic comprise the GSLs establish in protein-rich defatted food from broadly grown oilseed crops (Fenwick et al., 1983; Chun et al., 2013, 2017; Lee et al., 2014) and in some domesticated vegetable crops (Rosa et al., 1997; Griffiths et al., 1998). Brassica vegetables hence participate in central role in the diet, and their naturally occurring GSLs should be examined.

Flower buds were the vegetable products of *Brassica rapa* during winter season in China, Japan, and Korea. They are commonly consumed with/without soybean source, boiled or fried with water or cooking oil. They are distinguished by a meticulous bitter taste, which differentiate them from other vegetables in the genus Brassica, such as cabbage, broccoli, and cauliflower. However, the number of customer complaints has been recently made about the bitterness of the flower buds cultivated under inadequate environmental conditions such as drought conditions, high

* Corresponding author.
E-mail addresses: mvalanarasu@gmail.com (M.V. Arasu), kimsunju@cnu.ac.kr (S.-J. Kim).

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temperature, and insufficient fertilization (Akella et al., 1997). This has led to farmers to place emphasis on research to find the cause of the bitterness in *B. napus* in Japan in the end of the 1990s (Kim et al., 2003). It is clear that the bitter taste is attributed closely by containing some undesirable GSLs including progoitrin, sinigrin, gluconapin, glucobrassicanapin. In temperate zones of the world, oilseed rape (*B. napus*) is one of the most imperative oilseed crops even much amounts of soybean oil are imported from the United States. It accounts for up to 15% of the global oilseed production (Orlovius, 2003). The seeds contain approximately 50% oil and 25% proteins (w/w). After oil extraction, the resulting seed meal consists of more or less 40% proteins (Fenwick, 1982). While *B. napus* are mainly an oilseed crop, its meal is rich in essential amino acids and therefore attractive for animal feeds and potential human food supplements (Shahidi and Naczk, 1992; Bell, 1993). There are little information about the GSL content of flower buds of *B. rapa* and *B. napus*. Therefore the objectives of the present study were to determine individual and total GSL contents in flower buds at different stages with dissimilar positions on No. 21 (*B. rapa*, early type), Husanohana (*B. rapa*, late type) and Norin No. 20 (*B. napus*) cultivars from Japan.

2. Materials and methods

2.1. Chemicals and reagents

HPLC grade-acetonitrile and methanol were purchased from Wako Pure Chemical Industries (Osaka, Japan). Aryl sulfatase (Type H-1, EC 3.1.6.1) was purchased from Sigma Chemical Company (St Louis, MO, USA). DEAE-Sephadex A-25 was purchased from Amersham Biosciences (Uppsala, Sweden) and sinigrin (allyl-GSL) was purchased as an internal standard from Tokyo Kasei Kogyo Company (Tokyo, Japan).

2.2. Plant materials and cultural practices

Labeling started from the first flower bud produced by the plant. Every day, small labels with dates and plant identification were hanged on every newly opened flower. The flower buds were labeled when they were appeared from the branches of stems and harvested when the length was reached approximately 15 cm within 2–3 weeks later after flower bud appearance.

The seeds of No. 21 (*B. rapa*, early type), Husanohana (*B. rapa*, late type) and Norin No. 20 (*B. napus*) cultivars were sown in sand in a pot on October, 2001. Two weeks later, they were transferred to the plastic container (55.5 × 15.5 × 13.0 cm) containing 3 kg of vermiculite. Two plants were grown in each container at a distance of 20 cm apart, and each treatment was replicated four times. Plants were watered in the morning once in every two days with a nutrient solution prepared according to the Hoagland Solution (pH 6.0) as shown in Table 1. The solution was drained freely from the container. The plants were watered once a week with deionized water in order to remove salts accumulated in the media. Approximately after 70 days (DAS) of sowing, the flower buds were emerged. In addition, the flower buds were properly collected in the order of 2, 4, 6, 8 and 10 positions from the axillary end at two different development stage (stage 1 and 2) based on the Table 1. The flower buds were cut into segments about 10 cm then lyophilized, ground and stored at room temperature in a corked bottle until chemical analysis.

2.3. Extraction and desulphation of glucosinolates

Desulfo (DS) - GSLs were extracted according to the procedure of Kim et al. (2007) and ISO 9167-1 (1992). Briefly, crude GSLs were extracted with 70% (v/v) boiling methanol (1.5 m × 3 times) from freeze-dried products (100 mg). Desulfation of the crude extracts were performed on a Sephadex A-25 DEAE (ca. 40 mg as dry matrix) column previously activated as $[H]^+$ form with 0.5 M sodium acetate. An aliquot of each methanol extract was loaded onto a pre-equilibrated column and rinsed with 1 ml (×3 times) to remove neutral and positive ions. Next, 75 µl of aryl sulfatase was loaded onto each column, and the desulphation reaction was performed overnight (16–18 h) at room temperature. The desulphated GSLs were eluted with 0.5 ml (× 3 times) of ultra-pure water and filtered through a 0.45 µm Teflon PTFE syringe filter for analysis. The eluates were analyzed immediately by HPLC or stored at −20 °C until analysis.

Table 1
Ingredients of the Hoagland Solution (pH 6.0) used for plant growth.

Nutrients	Concentration of solution	
Macronutrients		(g/l)
Solution-A		
KNO_3	5.0 mM	50.55
$MgSO_4{\cdot}7H_2O$	2.0 mM	49.296
KH_2PO_4	2.0 mM	27.218
Solution-B		
$Ca(NO_3)_2{\cdot}4H_2O$	2.0 mM	118.075
Micronutrients		(g/l)
$MnCl_2{\cdot}4H_2O$	0.50 ppm	1.801
H_3BO_3	0.50 ppm	2.86
$ZnSO_4{\cdot}7H_2O$	0.50 ppm	0.222
$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$	0.50 ppm	0.092
$CuSo_4{\cdot}5H_2O$	0.02 ppm	0.0785
Fe-EDTA	1.00 ppm	7.54

2.4. Quantification of desulpho-glucosinolate (desulpho-GSL)

For qualitative analysis, separation of desulpho-GSL was conducted on a C18 column (250 × 2.1 mm, 5 µm, Inertsil ODS-3; GL Sciences, Tokyo, Japan) using a HPLC system equipped with a diode array detector (Shimadzu, Kyoto, Japan). The elution buffers consisted of solvent A (water) and B (acetonitrile). The flow rate was 0.2 ml/min. The following elution program was applied: 0 min, 99% A/1% B; 18 min, 20% A/80% B; 30 min, 20% A/80% B; 32 min, 99% A/1% B; and 40 min, 99% A/1% B. The UV–Visible detector wavelength was set at 227 nm. For the MS analysis, the eluate was diverted to a mass spectrometer (Hitachi M-800, Tokyo, Japan) equipped with atmospheric pressure chemical ionization (APCI) in a positive mode $[M + H^+]$. The spray voltage was set to 4.5 kV and the capillary temperature was set to 250 °C. The scan of the masses ranged from *m/z* 100 to *m/z* 700. For quantitative analysis, desulpho-GSL extracts were separated on a C18 column (250 × 4.6 mm, 5 µm, Inertsil ODS-3; GL Sciences) using a HPLC system equipped with a diode array detector (LC-20A; Shimadzu). The HPLC conditions were the same as described previously, except the flow rate was 1.0 ml/min. GSL content was calculated using sinigrin as an external standard and the response factor of each compound relative to sinigrin.

2.5. Statistical analysis

Data were subjected to statistical analysis by Tukey's multiple range tests using Esumi Statistical Software version 5.0 (Esumi Incorporated, Tokyo, Japan). The ANOVA was done at a 5% level of significance.

3. Results and discussion

The weight of flower buds in cv. No. 21 (*B. rapa*, early type), cv. Husanohana (*B. rapa* late type) and cv. Norin No. 20 (*B. napus*) was

Table 2
Fresh weight and time duration of flower buds in No. 21, Husanohana and Norin No. 20 cultivars.

Cultivars	Developed stages	Fresh weight (g) of flower buds	Time duration of flower buds[a]		
			Emergence[a]	Open	Harvested duration
No. 21	1st stage	6.16	4-Dec	26-Dec	27-Dec–13-Jan
(*B. rapa*, early type)	2nd stage[b]	5.16	10-Dec	30-Dec	31-Dec–20-Jan
Husanohana[c] (*B. rapa*, late type)	1st stage	4.83	1-Feb	15-Feb	16-Feb–8-Mar
Norin No. 20[d]	1st stage	6.40	17-Dec	2-Jan	04-Jan–24-Jan
(*B. napus*, medium type)	2nd stage	6.54	18-Dec	10-Jan	11-Jan–28-Jan

[a] The flower bud emerges at approximately 70 days after sowing (DAS) later, and the first harvesting was done at 90 DAS.
[b] The second stage means the branch of flower buds developed from the same place either after cutting the first harvest or branched (offshoot).
[c] The first flower buds of cv. Husanohana (late type) emerged at February 1 and harvested from the end of February to the beginning of March. They were two month later compared with those of No. 21 (late type) and Norin No. 20 (medium type) cultivars. Therefore, the flower buds cv. Husanohana at the second stage were not harvested because of the limitation of time schedule.
[d] The inflorescence part is harvested because it is specie for oil production.

shown in Table 2. All the cultivars exhibited significant differences in their respective mean weight when evaluated through other cultivar. Ten GSLs including progoitrin, glucoraphanin, sinigrin, glucoalyssin, napoleiferin, gluconapin, 4-hydroxy glucobrassicin, glucobrassicanapin, glucobrassicin and gluconasturtiin were identified and quantified using HPLC (Table 3). The results revealed that the total GSL contents ranged from 49.6 to 93.32 μmole/g dry weight (DW) in cv. No. 21, 5.73–19.02 μmole/g DW in cv. Husanohana, and 44.00 –67.99 μmole/g DW in cv. Norin No. 20, respectively. In the first stage total GSLs of *B. rapa* early type (No. 21) content significantly increased in flower bud all the positions except 2. The gradual increases of total GSLs were as follows 56.87 > 53.80 > 51.91 > 50.91 > 49.60 μmole/g DW. In the second stage total GSL content were significantly increased in flower bud from 8 and 10 positions when compare with flower bud harvested from other positions. Moreover, progoitrin, glucoraphanin, sinigrin, glucoalyssin, gluconapin and glucobrassicanapin levels did not differ significantly in flower bud harvested from all the positions during the first stage of *B. rapa* early type (No. 21); however GSLs such as 4-hydroxyglucobrassicin, glucobrassicin and gluconasturtiin quantity was shuffle significantly between the flower bud from a different position of cv. No. 21. In the second stage of *B. rapa* early type (No. 21), all the detected GSLs were significantly shuffled between flower bud harvested from a different position. Furthermore, the total GSL content was also increased in each and every position of flower bud resulting from the second stage of *B. rapa* (No. 21) when comparing with the respective position of the first stage flower bud.

Progoitrin and glucoraphanin level did not vary in flower buds, whereas at the second stage progoitrin level was significantly increased in flower bud harvested from 2, 8 and 10 positions of *B. rapa* early type (No. 21) when comparing with flower bud from 4 and 6 positions. Among the different GSLs, glucoraphanin is widely studied for its anticancer effect; glucoraphanin and progoitrin follow the same pathway of biosynthesis of aliphatic GSLs (Traka and Mithen, 2009). Interestingly, gluconapin accounted above 75% of the total GSLs in the cultivars, similarly, Yang and Quiros (2010) identified gluconapin as the major GSLs in 80 crops of *B. rapa* and few reports suggested that the pungent and bitter flavor was due to the presence of gluconapin in *Brassica* plants (Francisco et al., 2009).

The results revealed that the level of sinigrin at the second stage was significantly augmented in flower bud harvested from 4, 6 and 8 positions of *B. rapa* early type (No. 21), similarly, Rangkalidok et al. (2002) reported that the amount of sinigrin was higher in buds in three *Brassica* species and the derivative product of sinigrin used for the reduction of cholesterol levels in mice (Balasinska et al., 2005). Glucobrassicanapin level did not unrelated in flower buds yield from diverse positions of *B. rapa* early type (No. 21) by the first stage. At the second stage glucobrassicanapin level was significantly greater in flower bud harvested from 2, 6, 8 and 10 positions of *B. rapa* early type (No. 21) when comparing with flower bud from 4 positions. Glucobrassicin mass was significantly increased in flower bud from 2, 6, 8 and 10 positions by first stage and only 8 positions by the second stage of *B. rapa* early type (No. 21) when comparing with flower bud harvested from other positions.

Progoitrin, sinigrin, glucoalyssin and glucoraphanin level were significantly increased in flower bud harvested from 2 and 4 positions of cv. Husanohana (*B. rapa*) when compare with flower bud from 6, 8 and 10 positions, whereas progoitrin level did not varied in flower buds yield from different position of *B. napus* (Norin No. 20) by the first stage. At the second stage progoitrin level was significantly increased in flower bud harvested from 8 positions of *B. napus* (Norin No. 20) when compare with flower bud from 2, 4, 6 and 10 positions. Glucoraphanin, sinigrin and 4-hydroxyglucobrassicin were not detected in the flower bud harvested from a different position of *B. napus* (Norin No. 20) by the different stage. Glucobrassicin mass was significantly increased in flower bud from 6 positions through the first stage and 8 position by the second stage of *B. napus* (Norin No. 20) when compare with flower bud harvested from other positions. Glucobrassicin together with the degraded compound sulforaphane acts as anticancer agents (Kushad et al., 1999).

4. Conclusion

In conclusion, the content of GSLs in the flower buds position was varied depending upon the stages. In the first and second stages, the total GSLs were higher in 10 flower bud position (56.87 and 56.23 μmole/g DW) and 8 flower bud position (93.32 and 67.99 μmole/g DW) in No. 21 and Norin No. 20 cultivars. Among the three cultivars, cv. No. 21 produced the highest amount of total GSL (93.32 μmole/g DW). Remarkable differences in the total and individual GSL contents were observed in the flower bud positions. The variation in GSL levels in the bud position reported in this study suggests that the potential health benefits of turnip rape are greatly dependent on the particular position of the flower bud which has been selected. Ten glucosinolates molecules including progoitrin, glucoraphanin, sinigrin, glucoalyssin, napoleiferin, gluconapin, 4-hydroxyglucobrassicin, glucobrassicanapin, glucobrassicin and gluconasturtiin were identified and quantified in the different positions of *Brassica rapa*. The identification quantification of these bioactive compounds in *Brassica rapa* will guide the researchers to find the better method to select the plants for further breeding.

Table 3
Individual and total glucosinolate contents identified from No. 21, Husanohana and Norin No. 20 cultivars at different development stages.

Cultivars	Development stage	Flower bud	Glucosinolates										Total
		Positions from ground	Progoitrin	Glucoraphanin	Sinigrin	Glucoalyssin	Napoleiferin	Gluconapin	4-OH-Glucobrassicin	Glucobrassicanapin	Glucobrassicin	Gluconasturtiin	
No. 21 (*B. rapa*, early type)	1st	2[a]	0.62 a	0.62 a	0.47 a	0.80 a	ND	35.85 a	0.05 b	10.11 a	0.48 a	0.60 b	49.60 b
		4	0.60 a	0.64 a	0.43 a	0.73 a	ND	36.24 a	0.05 b	11.05 a	0.40 b	0.77 a	50.91 ab
		6	0.64 a	0.63 a	0.47 a	0.85 a	ND	36.65 a	0.06 a	11.53 a	0.48 a	0.62 b	51.91 ab
		8	0.62 a	0.68 a	0.42 a	0.80 a	ND	38.11 a	0.05 b	12.00 a	0.47 ab	0.64 b	53.80 ab
		10	0.62 a	0.71 a	0.46 a	0.83 a	ND	41.67 a	0.05 b	11.43 a	0.45 ab	0.66 ab	56.87 a
	2nd	2	1.08 ab	0.77 b	0.58 b	1.29 a	ND	54.45 b	0.02 d	17.48 a	0.45 c	0.59 a	76.70 b
		4	0.98 b	0.77 b	0.73 a	0.85 b	ND	40.57 c	0.03 c	11.64 b	0.50 bc	0.32 c	56.38 c
		6	1.00 b	0.85 ab	0.63 ab	1.31 a	ND	58.49 ab	0.04 b	17.22 a	0.59 b	0.34 bc	80.47 b
		8	1.23 a	0.97 a	0.69 ab	1.20 a	ND	69.03 a	0.05 a	18.79 a	0.95 a	0.42 b	93.32 a
		10	1.03 ab	0.98 a	0.42 c	1.33 a	ND	61.42 ab	0.05 a	16.12 a	0.60 b	0.66 a	82.61 ab
Husanohana (*B. rapa*, late type)	1st	2	5.03 a	0.28 d	1.27 a	1.04 a	0.49 c	4.18 a	0.01 b	5.79 a	0.30 a	0.62 b	19.02 a
		4	4.56 a	0.45 b	1.07 b	1.01 a	0.60 b	1.68 b	0.08 a	2.52 b	0.18 b	2.11 a	14.26 b
		6	2.85 c	0.37 bc	0.87 bc	0.58 b	0.38 d	0.99 c	tr	1.57 c	0.18 b	0.31 c	8.09 d
		8	3.81 b	0.63 a	0.85 a	0.97 a	0.70 a	1.81 b	0.01 bc	1.95 c	0.18 b	0.21 c	11.11 c
		10	1.99 d	0.35 cd	0.57 cd	0.44 b	0.31 d	0.73 c	tr	0.92 d	0.11 c	0.31 c	5.73 e
Norin No. 20 (*B napu*, medium type)	1st	2	17.10 a	ND	ND	1.37 c	2.10 ab	7.15 c	ND	16.20 b	0.28 b	1.65 ab	45.87 b
		4	16.90 a	ND	ND	1.95 a	2.28 a	7.35 c	ND	18.94 a	0.31 b	1.49 b	49.22 b
		6	18.08 a	ND	ND	1.66 b	2.18 ab	9.61 b	ND	18.87 ab	0.40 a	1.88 a	52.70 ab
		8	16.65 a	ND	ND	1.58 bc	1.92 b	10.71 ab	ND	17.51 ab	0.30 b	1.95 a	50.62 b
		10	19.66 a	ND	ND	1.50 bc	1.92 b	12.12 a	ND	18.96 a	0.34 b	1.74 ab	56.23 a
	2nd	2	18.14 b	ND	ND	1.29 b	1.96 b	11.35 a	ND	17.50 b	0.30 b	1.62 b	52.15 b
		4	17.11 b	ND	ND	1.49 b	1.97 b	11.06 ab	ND	18.21 b	0.32 b	2.57 a	52.72 b
		6	15.44 b	ND	ND	1.49 b	2.01 b	8.96 bc	ND	16.52 b	0.34 b	1.74 b	46.50 b
		8	22.73 a	ND	ND	2.81 a	3.28 a	10.47 ab	ND	26.67 a	0.43 a	1.60 b	67.99 a
		10	16.03 b	ND	ND	1.27 b	1.99 b	7.17 c	ND	15.77 b	0.28 b	1.47 b	44.00 b

[a] The flower buds were harvested at 2, 4, 6, 8 and 10 order positions from ground because of too many flower buds (around 12–20 positions) in one plants. Cultivar No. 21, 15–21 (1st) and 16–19 (2nd) positions; cv. Husanohana, 12–19 (1st) position; cv. Norin No. 20, 14–15 (1st) and 13–15 (2nd) positions.

Conflict of interest statement

The corresponding author and the contributing authors declare that they have no conflicts of interest in this research work.

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