Analysis of glucosinolates in ‘Nainaiqingcai’ mustard under the alpine cold climate

Peixing Lin¹, †, Fen Zhang ¹, †, Jie Ma², Wen Qu¹, Rui Wu², Chunyan Chen², Wei Ma², Yuankuan He², Ping Zhou ², and Bo Sun¹, *

¹ College of Horticulture, Sichuan Agricultural University, Chengdu 611130, China; ² Bijie Institute of Agricultural Science, Bijie 551700, China
†Peixing Lin and Fen Zhang contributed equally to this work.
*Corresponding author e-mail: 14099@sicau.edu.cn

Abstract. The composition and content of glucosinolates were investigated in ‘Nainaiqingcai’ mustard to reveal the diversity of the glucosinolates among the individual edible parts (leaves, petioles, and bolting stems). The results showed the significant differences of the composition and content of glucosinolates were found in the different edible parts of ‘Nainaiqingcai’ mustard. Eleven glucosinolates were detected in leaves of ‘Nainaiqingcai’ mustard, including six aliphatic glucosinolates, four indole glucosinolates and one aromatic glucosinolate. Only ten of the eleven glucosinolates were detected in petioles and bolting stems of ‘Nainaiqingcai’ mustard. The highest contents of glucosinolates in ‘Nainaiqingcai’ mustard was found in leaves. Aliphatic glucosinolates were predominant in the ‘Nainaiqingcai’ mustard, followed by indole and aromatic glucosinolates. Sinigrin was the predominant glucosinolate in the ‘Nainaiqingcai’ mustard. In summary, these findings provide a theoretical reference for the scientific consumption and a foundation for the further study of ‘Nainaiqingcai’ mustard in the future.

1. Introduction

Brassica juncea is an annual herb of cruciferae brassica, which originated from spontaneous hybridization of the ancestors of B.rapa (AA, n=10) and B.nigra (BB, n=8) [1]. The variety ‘Nainaiqingcai’ belongs to the leafy mustard vegetables of the cruciferous family. Its leaves, petioles, and bolting stems were usually used as the edible parts. People usually pickle it to eat kimchi, but also eat it as fresh vegetable directly. ‘Nainaiqingcai’ mustard is one of the local winter-spring vegetables in Bijie City, Guizhou Province, and has a large amount of consumption at the local. Despite being a brassica vegetable that is widely consumed in winter and spring in Southwest China, there is lack of information available on ‘Nainaiqingcai’ mustard.

Clinical studies have shown that the consumption of Brassica vegetables can significantly reduce the incidence of pancreatic cancer, prostate cancer, colon cancer and other cancers [2]. Phytochemistry and nutrition studies have shown that the anticancer activity of brassica vegetables mainly comes from secondary metabolites, glucosinolates and their degradation products [3]. Glucosinolates contain a sulfated isothiocyanate group, thioglucose, and an R-group derived from amino acids [4]. Glucosinolates can be divided into three classes based on the R-group as aliphatic, with a methionine,
isoleucine, leucine, or valine precursor; indole, with a tryptophan precursor; and aromatic, with a phenylalanine or tyrosine precursor [5].

In this experiment, to understand the contents of glucosinolates among different edible parts of ‘Nainaiqingcai’ mustard, this study used ‘Nainaiqingcai’ mustard as the material to analyze the difference of glucosinolates between different edible parts, which provided the basis and reference for the scientific consumption of ‘Nainaiqingcai’ mustard in the future.

2. Materials and methods

2.1. Plant materials

The ‘Nainaiqingcai’ mustard were sampled on December 15, 2017 at the vegetable base of Bijie Institute of Agricultural Science of Bijie City, Guizhou Province, China. The robust plants were selected at harvest stage. The samples were divided into three parts according to the leaves, petioles and bolting stems, and then all samples were frozen at −80°C, lyophilized, ground to a powder, and stored at −20°C.

2.2. Sample preparation

The samples were extracted according to the method of Sun et al. [6]. Briefly, the freeze-dried samples were boiled for 20 min in 1 ml of distilled water. The supernatant was collected after centrifugation (5 min, 7,000 g). The extract was applied to a DEAE-Sephadex A-25 (40 mg) column. The glucosinolates were converted into their desulfo analogues by overnight treatment with 100 μl of 0.1% aryl sulfatase, and the desulfoglucosinolates were eluted with water.

2.3. High performance liquid chromatography (HPLC) analysis

The HPLC analysis of the desulfoglucosinolates was carried out using a Agilent 1260 HPLC instrument equipped with a VWD absorbance detector. The samples were separated at 30°C on a C18 column using acetonitrile and water at a flow rate of 1.0 ml min⁻¹. The procedure employed isocratic elution with acetonitrile and ddwater. Absorbance was detected at 226 nm [6].

2.4. Data analysis

Results are shown as mean ± standard deviation of three replicates. Microsoft Excel 2013 was adopted for data processing. Correlation analysis was performed using the SPSS 18.0 software.

3. Results

3.1. Glucosinolate composition in ‘Nainaiqingcai’ mustard

Eleven glucosinolates were detected in leaves of ‘Nainaiqingcai’ mustard, including six aliphatic glucosinolates: glucoiberin (GIB), progoitrin (PRO), glucoraphanin (GRA), sinigrin (SIN), glucoalyssin (GAL) and gluconapin (GNA); four indole glucosinolates: 4-hydroxyglucobrassicin (OHGB), glucobrassicin (GBS), 4-methoxyglucobrassicin (OMGB), and neoglucobrassicin (NGBS), and one aromatic glucosinolate: gluconasturtiin (GST). Only ten of the eleven glucosinolates were detected in petioles and bolting stems of ‘Nainaiqingcai’ mustard, whereas GST were not detected. SIN was the predominant glucosinolates in the three edible parts of ‘Nainaiqingcai’ mustard, whose content ranging from 86.54% in petioles of ‘Nainaiqingcai’ mustard to 96.27% that in leaves.

3.2. Total glucosinolates

The total glucosinolates (TG) distribution in the three edible parts was absolutely different (Figure 1). The TG content in the leaves was remarkably higher than those in the bolting stems and petioles. However, no significant differences were observed between the petioles and bolting stems. The TG contents ranged from 10.33 μmol g⁻¹ in petioles to 42.33 μmol g⁻¹ that in leaves.
Figure 1. The content of total glucosinolates in ‘Nainaiqingcai’ mustard

3.3. Aliphatic glucosinolates

The aliphatic glucosinolate contents and distribution in different edible parts are presented in Table 1. The results show that all six aliphatic glucosinolates were detected in three edible parts. SIN was the major glucosinolate in all edible parts. The total aliphatic glucosinolates (TALG) contents in the petioles and bolting stems were remarkably lower than that in the leaves. TALG contents ranged from 10.01 μmol g⁻¹ in the petioles to 42.06 μmol g⁻¹ that in the leaves. The contents of GRA, SIN and GNA in leaves were all significantly higher than those in the petioles and bolting stems, whereas the contents of GIB and PRO in bolting stems were all significantly higher than those in the leaves and petioles.

Table 1. The contents of aliphatic glucosinolates in ‘Nainaiqingcai’ mustard (μmol g⁻¹ DW)

| Edible parts  | GIB     | PRO     | GRA     | SIN     | GAL     | GNA     | TALG     |
|---------------|---------|---------|---------|---------|---------|---------|----------|
| Leaves        | 0.05±0.01 b | 0.34±0.08 b | 0.16±0.04 a | 40.75±9.75 a | 0.39±0.09 a | 0.36±0.07 a | 42.06±10.01 a |
| Petioles      | 0.05±0.00 b | 0.30±0.02 b | 0.07±0.01 b | 8.94±0.54 b  | 0.15±0.02 b | 0.18±0.01 b | 10.01±0.35 b  |
| Bolting stems | 0.10±0.02 a | 0.55±0.04 a | 0.10±0.01 b | 11.52±0.47  | 0.30±0.03 a | 0.18±0.04 b | 12.76±0.47 b  |

Note: Each value represents the mean (n = 3). Different letters indicate significant difference at 0.05 level.

3.4. Indole glucosinolates

The content of total indole glucosinolates (TING) in bolting stems was significantly higher than that in leaves and petioles (Table 2). The predominant indole glucosinolate was OMGB. The TING content of bolting stems was the highest (0.25 μmol g⁻¹), and that of leaves was the lowest (0.11 μmol g⁻¹).

Table 2. The contents of indole glucosinolates in ‘Nainaiqingcai’ mustard (μmol g⁻¹ DW)

| Edible parts  | OHGB     | GBS     | OMGB    | NGBS    | TING     |
|---------------|----------|---------|---------|---------|----------|
| Leaves        | 0.03±0.01 c | 0.03±0.01 a | 0.11±0.03 b | 0.01±0.00 c | 0.25±0.10 b |
| Petioles      | 0.10±0.02 b | 0.02±0.00 b | 0.14±0.01 b | 0.06±0.01 a | 0.32±0.04 b |
| Bolting stems | 0.23±0.01 a | 0.02±0.00 b | 0.25±0.01 a | 0.04±0.00 b | 0.54±0.02 a |

Note: Each value represents the mean (n = 3). Different letters indicate significant difference at 0.05 level.
3.5. Aromatic glucosinolate

Only one type of aromatic glucosinolate, GST, was detected in the leaves, and aromatic glucosinolate were not detected in the petioles and bolting stems (Table 3). The total aromatic glucosinolate (TARG) in the leaves is 0.02 μmol g⁻¹.

| Edible parts | GST | TARG |
|--------------|-----|------|
| Leaves       | 0.02±0.00 | 0.02±0.00 |
| Petioles     | ND  | ND   |
| Bolting stems| ND  | ND   |

Note: Each value represents the mean (n = 3). Different letters indicate significant difference at 0.05 level. ND: Not detected.

3.6. Correlation analysis

A correlation analysis was performed to investigate the correlations among the different glucosinolates (Table 4). There were significantly positive correlation between GRA, SIN, GAL and GNA. The four above significantly and positively correlated with GBS, TALG and TG, whereas significantly and negatively correlated with NGBS. There was a significantly positive correlation between GIB and PRO, and both of them were significantly positive correlated with OHGB, OMGB and TING. In addition, OHGB is extremely significantly positively correlated with OMGB, and both of them are significantly positively correlated with TING. And GBS is extremely significantly positively correlated with TING and TG.

|            | PRO  | PRO  | GRA  | SIN  | GAL  | GNA  | OHGB | GBS  | OMGB | NGBS | GST  | TALG | TING | TARG |
|------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| PRO        | 0.753* |      |      |      |      |      |      |      |      |      |      |      |      |      |
| GRA        | -0.231 | 0.102|      |      |      |      |      |      |      |      |      |      |      |      |
| SIN        | -0.402 | -0.176| 0.925** |      |      |      |      |      |      |      |      |      |      |      |
| GAL        | 0.079 | 0.376| 0.907** | 0.824** |      |      |      |      |      |      |      |      |      |      |
| GNA        | -0.464 | -0.174| 0.909** | 0.970** | 0.771* |      |      |      |      |      |      |      |      |      |
| OHGB       | 0.823** | 0.828** | -0.455 | -0.665 | -0.169 | -0.486* |      |      |      |      |      |      |      |      |
| GBS        | -0.494 | -0.214| 0.857** | 0.922** | 0.731* | 0.929** | -0.687** |      |      |      |      |      |      |      |
| OMGB       | 0.829** | 0.919** | -0.268 | -0.468 | 0.041 | -0.904 | 0.972** | -0.903 |      |      |      |      |      |      |
| NGBS       | -0.009 | -0.068 | -0.792* | -0.822** | -0.867** | -0.785* | 0.181 | -0.635 | 0.227 |      |      |      |      |      |
| GST        | 0.999 | -0.999* | -0.946 | -0.971 | -0.988* | -0.918 | -0.711 | -0.980 | -0.810 | -0.866 |      |      |      |      |
| TALG       | 0.855 | -0.170 | 0.927** | 1.000** | 0.828** | 0.970** | -0.662 | 0.926** | -0.483 | -0.823** | -0.955 |      |      |      |
| TING       | 0.752* | 0.922** | -0.163 | -0.440 | 0.087 | -0.454 | 0.928** | -0.411 | 0.968** | -0.526 | -0.980 | -0.435 |      |      |
| TARG       | 0.899 | -0.999* | -0.946 | -0.971 | -0.989* | -0.918 | -0.711 | -0.950 | -0.810 | -0.866 | 1.000** | -0.955 | -0.980 |      |
| TG         | -0.394 | -0.163 | 0.922** | 1.000** | 0.832** | 0.975** | -0.658 | 0.926** | -0.477 | -0.826** | -0.957 | 1.000** | -0.928 | -0.957 |

4. Discussion

As reported by many studies, there were obvious differences in the contents of metabolites among different tissues [7-9]. Our study also shows the diversity of the glucosinolate contents in the different edible parts of ‘Nainaiqingcai’ mustard. Regulation of glucosinolates biosynthesis is a complex process. Glucosinolate biosynthesis is influenced by internal factors, such as organs and tissues [10], which is consistent with our results. The TG content was highest in leaves, followed by bolting stems and petioles of ‘Nainaiqingcai’ mustard, while aliphatic glucosinolate contents accounted for 98.29% of the TG content. And in the synthetic pathway of aliphatic glucosinolate, including the extension of precursor amino acids which require the participation of the chloroplast [10]. However, the number of chloroplasts in the leaves is obviously greater than that in the bolting stems and petioles. Therefore, it is speculated that the high content of glucosinolates in leaves may be related to the large number of chloroplasts in leaves. This is consistent with the experimental results of Hu [11]. However, the results
are inconsistent with experimental results of Maldini et al. [12]. Therefore, there are differences in the content of glucosinolates in different tissues and organs, which need to be further studied.

The predominant glucosinolate in the three edible parts of ‘Nai naiqingcai’ mustard was SIN. However, the predominant glucos inolate it is GNA in Chinese kale [13] and most varieties of B. rapa [14]. That maybe they have different genetic backgrounds [15]. Most of the edible parts of cruciferous Brassica vegetables have a high content of aliphatic glucosinolates followed by indole and aromatic glucosinolates [7], which is consistent with our results.

In this experiment, the distribution of glucosinolates in leaves, petioles and bolting stems of ‘Nainaiqingcai’ mustard was analyzed. These findings provide a theoretical reference for human dietary nutrition and a foundation for the further study of ‘Nainaiqingcai’ mustard.

Acknowledgments
This work was supported by Agricultural Support Project of Guizhou Province (QianKeHeZhiCheng[2018]2372-1; QianKeHeZhiCheng[2018]2372-2), and Science and Technology Special Fund Project of Central Subsidized Place (QianKeHeTiaoZhongBuDi[2015]4003).

References
[1] J. L. Wang, Y. He, Y. F Luan, Dacizhuoga, Y. Q. Zhang, A Study on origin, evolution and spread of Brassica in China, Chinese Agricultural Science Bulletin. 22 (2006) 489-494.
[2] M. Y. Wang, W. X. Yuan, H. Y. Miao, B. L. Wang, Q. M. Wang, Effects of different postharvest treatments on glucosinolate metabolism and nutritional quality in Brassica vegetables: A review, Journal of Zhejiang University. 44 (2018) 269-274.
[3] E. Capuano, M. Dekker, R. Verkerk, T. Oliviero, Food as Pharma? The Case of Glucosinolates, Current pharmaceutical design. 23 (2017) 2097-2721.
[4] H. W. Kim, H. C. Ko, H. J. Baek, S. M. Cho, H. H. Jang, Y. M. Lee, J. B. Kim, Identification and quantification of glucosinolates in Korean leaf mustard germplasm (Brassica juncea var. integrifolia) by liquid chromatography–electrospray ionization/tandem mass spectrometry, European Food Research and Technology. 242 (2016) 1479-1484.
[5] R. Agneta, F. Lelario, S. De Maria, C. Möllers, S. A. Bufo, A. R. Rivelli, Glucosinolate profile and distribution among plant tissues and phenological stages of field-grown horseradish. Phytochemistry. 106 (2014) 178-187.
[6] B Sun, Y. X. Tian, Q. Chen, Y. Zhang, Y. Luo, Y. Wang, M. Y. Li, R. G. Gong, X. R. Wang, F. Zhang, H. R. Tang, Variations in the glucosinolates of the individual edible parts of three stem mustards (Brassica juncea), Royal Society Open Science. 6 (2019) 182054.
[7] F. G. K. Vieira, G. D. S. C. Borges, C. Copetti, P. F. D. Pietro, E. D. C. Nunes, R. Fett, Phenolic compounds and antioxidant activity of the apple flesh and peel of eleven cultivars grown in Brazil, Scientia Horticulturae. 182 (2017) 261-266.
[8] I. M. Abu-Reidah, Á. Gil-Izquierdo, S. Medina, F. Ferreres, Phenolic composition profile of different edible parts and by-products of date palm (Phoenix dactylifera L.) by using HPLC-DAD-ESI/MS, Food Research International. 100 (2017) 494-500.
[9] F. Liu, M. Wang, M. Wang, Phenolic compounds and antioxidant activities of flowers, leaves and fruits of five crabapple cultivars (Malus Mill. species), Scientia Horticulturae. 235 (2018) 460-467.
[10] H. Y. Miao, Glucose and plant hormones synergetically modulate glucosinolates biosynthesis in cruciferae plants, (2015) 167-173.
[11] X. Y. Hu, Study on change rule of total glucosinolates during harvest time of tumorous stem mustard, Chongqing Industry & Trade Polytechnic. 11 (2015) 33-36.
[12] M. Maldini, M. Foddai, F. Natella, G. L. Petretto, J. P. Rourke, M. Chessa, G. Pintore, Identification and quantification of glucosinolates in different tissues of Raphanus raphanistrum by liquid chromatography tandem-mass spectrometry, Journal of Food Composition and Analysis. 61 (2017) 20-27.
[13] B. Sun, N. Liu, Y. Zhao, H. Yan, Q. Wang, Variation of glucosinolates in three edible parts of Chinese kale (Brassica alboglabra Bailey) varieties, Food Chemistry. 124 (2011) 941-947.

[14] G. Padilla, M. E. Cartea, P. Velasco, A. D. Haro, A. Ordás, Variation of glucosinolates in vegetable crops of Brassica rapa, Phytochemistry. 68 (2007) 536-545.

[15] M. J. Kim, Y. C. Chiu, N. K. Kim, H. M. Park, C. H. Lee, J. A. Juvik, K. M. Ku. Cultivar-specific changes in primary and secondary metabolites in pak choi (Brassica rapa, Chinese group) by methyl jasmonate, International Journal of Molecular Sciences. 18 (2017) 1004.