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Characterization of glucosinolates in 80 broccoli genotypes and different organs using UHPLC-Triple-TOF-MS method

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ABSTRACT

We aimed to characterize and quantify glucosinolate compounds and contents in broccoli, and a total of 80 genotypes and eight developmental organs were analyzed with UHPLC-Triple-TOF-MS. The method was validated in terms of performance, and the coefficients of determination (R^2) were 0.97 and 0.99 for glucoraphanin and gluconapin, respectively. In 80 genotypes, twelve glucosinolates were found in broccoli florets ranging from 0.467 to 57.156 μmol/g DW, with the highest glucosinolate content being approximately 122-fold higher than the lowest value. The principal component of glucobrassicin, neoglucobrassicin and glucoraphanin explained 60.53% of the total variance. There were positive correlations among hydroxyglucobrassicin, methoxyglucobrassicin, glucobrassicin, glucoerucin, gluconasturtiin, glucoraphanin, and glucotropaeolin ($P < 0.05$). The root contained 43% of total glucosinolates in 80 genotypes, and glucoraphanin represented 29% of the total glucosinolate content in different organs. The mutant broccoli genotypes were found by analysis of gluconapin contents in different organs.

1. Introduction

Glucosinolates (GLS) are a group of plant secondary metabolites comprising at least 120 known structures mainly found in cruciferous plants, including Chinese cabbage, broccoli, cabbage, Chinese kale and so on (Brown, Yousef, Reid, Chebrolu, Thomas, Krueger, et al., 2015; Fahey, Zalcmann, & Talalay, 2001; Halkier & Gershenzon, 2006). Glucosinolates can be divided into three groups according to their amino acid precursors: aliphatic glucosinolates are derived from methionine (Met), alanine (Ala), leucine (Leu), isoleucine (Ile) or valine (Val); benzenic glucosinolates are derived from phenylalanine (Phe) or tyrosine (Tyr); and indolic glucosinolates are derived from tryptophane (Trp) (Grubb & Abel, 2006; Sønderby, Geu-Flores, & Halkier, 2010). The glucosinolates and their degradation products have been widely helpful as anticancer agents in human health, for defense against insects and disease in plants, and for flavor regulation in cruciferous vegetables (Abdull Razis, Iori, & Ioannides, 2011; de Oliveira, Brasil, & Furstenau, 2018; Halkier & Gershenzon, 2006). Therefore, glucosinolate and its degradation products (sulforaphane, benzyl isothiocyanate, indole-3-carbinol, and 3, 3′-diindolylmethane) have become a research hotspot in food science, medicine, and botany (Alumkal, Slottke, Schwartzman, Cherala, Munar, Graff, et al., 2015; Liang, Li, Yuan, & Vriesekoop, 2008).

At present, the qualitative and quantitative analysis methods of glucosinolates focus on high-performance liquid chromatography (HPLC), ultrahigh-performance liquid chromatography (UPLC), and liquid chromatography-mass spectrometry (HPLC-MS). Since liquid chromatography-mass spectrometry can perform qualitative and quantitative analysis based on the characteristic mass spectrometry information of different glucosinolates, which is better than HPLC, liquid chromatography-mass spectrometry has become a popular tool in the analysis of glucosinolates (Alumkal, et al., 2015; Bello, Maldini, Baima, Scaccini, & Natella, 2018; Liang, Li, Yuan, & Vriesekoop, 2008). Additionally, time-of-flight high-resolution mass spectrometry (TOF-MS) has the advantages of high resolution, high sensitivity and fast analysis speed, so it is more effective in the qualitative analysis of glucosinolates than are more simple MS methods (Bell, Oruna-Concha, & Wagstaff, 2015). Mass spectrometry and secondary mass spectrometry complete the structural identification of compounds in plant samples in the absence of standards.

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The reports of glucosinolate biosynthesis have been extensively studied in *Arabidopsis*, and the regulation genes have been comprehensively described in previous research (Grubb & Abel, 2006; Sønderby, Geu-Flores, & Halkier, 2010). Recently, increasing research has focused on glucosinolates in *Brassica* plants, especially glucoraphanin and its hydrolysis product, sulforaphane, in broccoli (Z. S. Li, Liu, Li, Fang, Yang, Zhuang, et al., 2019; Liang, Li, Yuan, & Vriesekoop, 2008; Sønderby, Geu-Flores, & Halkier, 2010). Variation in glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana* has been studied over the past 40 years (P. D. Brown, Tokuhisa, Reichelt, & Gershenzon, 2003). However, there is no comprehensive report on glucosinolate variation among additional genotypes and developmental organs, though some research points out the change in total glucosinolates or several glucosinolates in broccoli florets after postharvest based on a small number of materials (Baik, Juvik, Jeffery, Wallig, Kushad, & Klein, 2003; Volker, Freeman, Banuelos, & Jeffery, 2010; Wang, Gu, Yu, Zhao, Sheng, & Zhang, 2012), representing a lack of evidence of additional genotypes and classical developmental organs. At the same time, past research in glucosinolates mostly used HPLC and lacked accurate structure calculations based on mass spectrometry information. Therefore, a comprehensive understanding of the glucosinolate profile and content during broccoli development is still needed for genetic breeding or basic research on *Brassica* plants.

In this study, ultrahigh-performance liquid chromatography-time-of-flight mass spectrometry (UHPLC-Triple-TOF-MS) is established and used for the analysis of glucosinolates in different broccoli genotypes and developmental organs, which provides a reliable analytical technology platform for the analysis of glucosinolates in cruciferous plants in the future. Glucosinolates and their degradation products have been extensively reported but with little use of ultrahigh-performance liquid chromatography with TOF-MS scan-IDA-Product ion scan (UHPLC-Triple-TOF-MS). Our work demonstrates that glucosinolates can be simultaneously detected and quantified by using UHPLC-Triple-TOF-MS. The new method also provides necessary MS and MS/MS information from one injection based on high-resolution TOF-MS. At the same time, accurate masses of molecular ions and fragment ions were obtained and stated in this study.

2. Materials and methods

2.1. Chemical and reagents

The chemicals used for sample preparations were HPLC-grade acetonitrile (JT Baker, USA) and formic acid (Fluka, Buchs, Switzerland). Deionized water was purified through a Milli-Q system (Millipore, Bedford, MA, USA). Standards of 4-methylsulfinylbutyl glucosinolate (glucoraphanin, GRA) and 3-butenyl glucosinolate (gluconapin, NAP) were > 98% pure, purchased from LKT Laboratories, Inc. (St. Paul, Minnesota, USA).

2.2. Plant materials and pretreatment

Broccoli (*Brassica oleracea* L. var. *italic*) samples were planted and collected from the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences (IVF-CAAS). The collected samples, including different organs of roots, leaves, stalks, florets and developmental buds from 6 inbred lines (Fig. 1) and florets from the other 80 genotypes (inbred lines) genetically separated from Japan, America, the Netherlands, Switzerland and Egypt, were freeze-dried, ground into powder and stored at low temperature.

The extraction procedure of glucosinolates was performed according to previously described methods (Zheng, Zhang, Liu, Lv, Lw, Xu, et al., 2017). The dried and ground broccoli samples (0.5 g) were extracted using methanol (70% v/v), vortexed for 1 min at room temperature and extracted for 15 min in a water bath at 70 °C. Then, the mixture was extracted for 15 min in a water bath at 70 °C and sonicated for 30 min at room temperature. After centrifugation for 5 min at 13,000 × g, the supernatant was collected into a 2 mL centrifugal tube. The supernatant was dried by nitrogen flow, and the dry residue was dissolved in ultrapure water. The solution was filtered through a 0.22 μm syringe filter and stored at −20 °C until detection analysis. Meanwhile, different concentrations of 1, 10, 100 and 1000 ng/mg of 4-methylsulfinylbutyl and 3-butenyl glucosinolate (standard) were added to the dry samples, and 5 duplicates for each treatment were set to calculate the extraction recovery (n = 5).

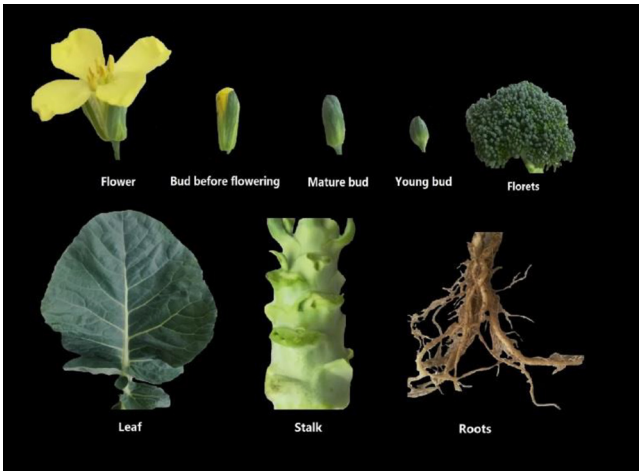

Fig. 1. Different organs of broccoli were collected in the bolting stage.

2.3. Simultaneous identification and characterization of glucosinolates

The Shimadzu LC-30A HPLC system (Japan) was equipped with an SPD-20 UV detector with a Waters ACQUITY UPLC BEH C_{18} column (2.1 × 100 mm, 1.7 μm) (Milford, MA, USA). Acidified water (0.1% formic acid, v/v) and acetonitrile (0.1% formic acid, v/v) were used as mobile phases A and B, respectively. The gradient program was carried out as follows: 0.0 min, 95% A and 5% B; 6.0 min, 60% A and 40% B; 6.5 min, 100% B; 8.0 min, 100% B; 8.1 min, 95% A and 5% B; and 10.0 min, 95% A and 5% B. The flow rate was set at 0.40 mL/min throughout the gradient. The injection volume was 1 μL, and the column temperature was maintained at 40 °C.

The HPLC system was coupled to a quadrupole-time-of-flight (AB Sciex, USA) orthogonal accelerated Q-TOF mass spectrometer equipped with an electrospray ionization source (ESI). Parameters for analysis were set using negative and positive ion modes, with spectra acquired over a mass range from *m/z* 80 to 1000. The optimal values of the ESI–MS parameters were capillary voltage, −4.5 kV; drying gas temperature, 550 °C; drying gas flow, 10.0 μL/ min; nebulizing gas pressure, 0.34 MPa; air curtain gas pressure, 0.24 MPa; auxiliary atomizing gas pressure, 0.34 MPa; collision and collision RF voltage, −35 eV and 15 eV; transfer time 70 μs; and prepulse storage, 5 μs. Moreover, automatic MS/MS experiments were performed using nitrogen as collision gas, with the collision energy values adjusted as follows: *m/z* 100, 20 eV; *m/z* 500, 30 eV; and *m/z* 1000, 35 eV.

The MS data acquisition was processed with Analyst® TF 1.7.1 software (AB Sciex Pte. Ltd., Singapore), and PeakView® 2.1 (AB Sciex Pte. Ltd., Singapore) and MasterView™ 1.0 (AB Sciex Pte. Ltd., Singapore) software was used to identify and analyze the glucosinolates. The MS/MS fragments were inferred through the FormularFinder model in PeakView®, as well as based on the first mass spectrographs, isotope-ratio mass spectrometry, molecular unsaturation, etc. In addition, the model of the Fragment Pane in MasterView® was used for structural analysis to further verify the molecular structure of the glucosinolates.

2.4. Statistical analysis

All assays were conducted in triplicate, and the results are presented as the mean ± standard deviation (SD). The data were processed using the IBM SPSS® ver. 19.0 (SPSS, Chicago, IL, USA). One-way ANOVA and Tukey's multiple-range test were used to evaluate the significant differences ($P < 0.05$). Principal component analysis (PCA), factor analysis (FA), correlation analysis, cluster analysis and regression analysis were performed to evaluate the difference and relevance of glucosinolates among genotypes and organs in broccoli ($P < 0.05$).

3. Results and discussion

3.1. Method validation and extraction development

Prevention of myrosinase activity is necessary during the glucosinolate extraction procedure in vegetative tissues. For this reason, several extraction methods have been used specifically to prevent the activation of myrosinase. Generally, extractions are conducted at temperatures of 65–100 °C, close to the water solvent or 70% aqueous methanol boiling point. Currently, a well-known analytical method used for glucosinolates is desulfonation of glucosinolates with sulfatase with subsequent analysis using a RP-HPLC gradient system (Baik, Juvik, Jeffery, Wallig, Kushad, & Klein, 2003; Wang, Gu, Yu, Zhao, Sheng, & Zhang, 2012). These methods provide reliable quantitative data and information related to glucosinolate variations, but they require much time and labor for analyses and require special equipment. Therefore, a simple method for the quantitative analysis of glucosinolates is necessary for the fields of plant breeding and food processing.

In this study, Table S1 presents the validation results, and the coefficients of determination (R^2) were 0.97 and 0.99 for glucoraphanin and gluconapin, respectively. Linear fitting was preferred to avoid possible overfitting by higher order curves. The limits of detection (LODs) and limits of quantification (LOQs) were indicative of the practical suitability for determining the two compounds. In terms of accuracy, most points appear to fit between the 75.5–83.2% ranges and 80.1–89.1% ranges for glucoraphanin and gluconapin, respectively. Finally, the RSD (%) value for stability was lower than 5% in the determination. According to the determination of florets in broccoli from 80 genotypes, twelve glucosinolate compounds were detected by UHPLC-MS/MS (Table S2).

In previous studies, the determination methods of glucosinolates include HPLC, HPLC-MS or LC-MS based on analysis of thiosine (desulfo-GSLs) compounds after desulfurization (B. G. Hansen, Kliebenstein, & Halkier, 2007; Stewart, Nho, & Jeffery, 2004; Tian, Yang, Avila, Fish, Yuan, Hui, et al., 2018). In the pretreatment, glucosinolates need to be desulfurized by a DEAE ion exchange column and sulfatase, so the whole process is complicated and time-consuming. In this study, a high-temperature water bath and ultrasonic methods were used to directly extract intact glucosinolates from plant samples (Vo, Trenerry, Rochfort, White, & Hughes, 2014), greatly simplifying the steps and time of sample preparation, and the extraction method is time-saving with good recovery and linearity (Table S1).

3.2. Spectrometry cracking and characteristics of glucosinolates based on Triple-TOF

The UHPLC-MS/MS technique enabled the precise and concurrent identification of glucosinolates in broccoli byproducts that previously needed separate sample treatments and chromatographic conditions (Fig. 2). The structure of glucosinolates is composed of β-D-glucose (Glc) linked to a sulfonate aldoxime group and a side-chain R derived from an amino acid. In view of the hydrophilic anionic nature of glucosinolates, the compounds have a good mass spectrometric response in the negative ion mode ESI. Under CID collision energy, the R group of the amino acid side chain is broken, and it will be further broken to produce characteristic product ions and characteristic neutral missing fragment ions. The bond between the sulfur atom in the sulfonate aldehyde group and adjacent carbon atom is broken, and the hydrogen atom is rearranged to produce the characteristic product ion of glucosinolate. The *m/z* values are 290.9844, 274.9895, 259.0124, 241.0018, 195.0327, 96.9596 and 79.9568; in addition, the characteristic neutral loss of glucosinolates can also occur, and the neutral loss groups are SO (3 79.9568 u), Glc (162.0528 u), SGlc (195.0327 u), 'SGlc-OH' (178.03 u and 'Glc + SO3′ (242.0096 u). Using these characteristic product ions and neutral lost groups, we can not only analyze the molecular structure of glucosinolate compounds in the MS/MS spectra but also filter fragments and neutral losses by using the Fragment and Neutral Loss Filter function in PeakView software. Glucosinolates are screened and extracted accurately and quickly in each information association-ion scanning channel.

In addition to the above-described characteristic product ions and neutral loss fragments from the glucosinolate consensus group, the side-chain groups also produce important fragment ions. Methyl sulfinyl glucosinolate, which has a neutral loss of a side-chain methylsulfonyl (CH_3SO) under CID collision energy, produces high-intensity fragment ions. For example, 4-methylsulfinyl butyl glucosinolate, 3-methylsulfinylpropyl glucosinolate, and 5-methylsulfinyl pentyl glucosylate all have a neutral loss of the side-chain methylsulfinyl group. The corresponding fragment ions *m/z* 372.0431, *m/z* 358.0275 and *m/z* 386.0581 were generated, respectively.

Two isomers of guanidine glucosinolates were found in this study: 1-methoxy-3-mercaptomethyl glucosinolate and 4-methoxy-3-mercaptomethyl glucosinolate. These compounds have the same molecular formula ($C_{17}H_{22}N_2O_{10}S_2$) and molecular ion peaks ($[MH]^-$, *m/z* 477.0643) but have different molecular structures in which the methoxy group has different substituent positions in the anthracene ring. The extracted ion chromatograms of the two isomers are shown in Fig. 2, and it is seen that complete separation of the chromatogram is achieved with this method. Due to the difference in the molecular structure of the two isomers, the mass spectrometry cleavage pathways of the two isomers are different, resulting in different fragment ions. 1-Methoxy-3′-mercaptomethyl sulfate is cleavable at the *N*-atom of the methoxy group and indole ring; the NO bond is broken by CID collision energy, resulting in the loss of methoxy group, and the fragment ion *m/z* 446.0479 is obtained. The main fragment ion of glycoside represents the common group of glucosinolates and their fragment ions, which are characteristic of the glucosides, with values of *m/z* 274.9920, 259.0145, 195.0333, 96.9603, and 74.9915 and a retention time of 3.8 min. The distinction between two steroidal glucosinolate isomers is accomplished by observing the TOF-MS/MS fragment ions (Zheng, et al., 2017).

3.3. Evaluation of glucosinolate compounds in florets based on 80 broccoli genotypes

Glucosinolates and their secondary products are popularly recommended as anticancer agents in humans, flavor control in cruciferous plants, and chemical barriers in pest control (Gorissen, Kraut, de Visser, de Vries, Roelofsen, & Vonk, 2011; Grubb & Abel, 2006; Mithen, Bennett, & Marquez, 2010). In this study, the florets of a total of 80 broccoli genotypes from around the world were characterized, and twelve glucosinolate compounds were detected in the mature broccoli florets (Table 1). Of these twelve glucosinolates, six belonged to aliphatic glucosinolates, namely, glucoraphanin, glucoerucin, glucoiberin, gluconapin, progoitrin and sinigrin; four belonged to indole glucosinolates, namely, 4-methoxyglucobrassicin, glucobrassicin, neoglucobrassicin and 4-hydroxyglucobrassicin; and only two were phenyl glucosinolates, namely, gluconasturtiin and glucotropaeolin (Table 1). Of the twelve glucosinolates detected in florets of broccoli, some compounds were consistent with previous reports, and a unique glucosinolate, glucotropaeolin, was also detected in this work (L. P. Guo, Yang,

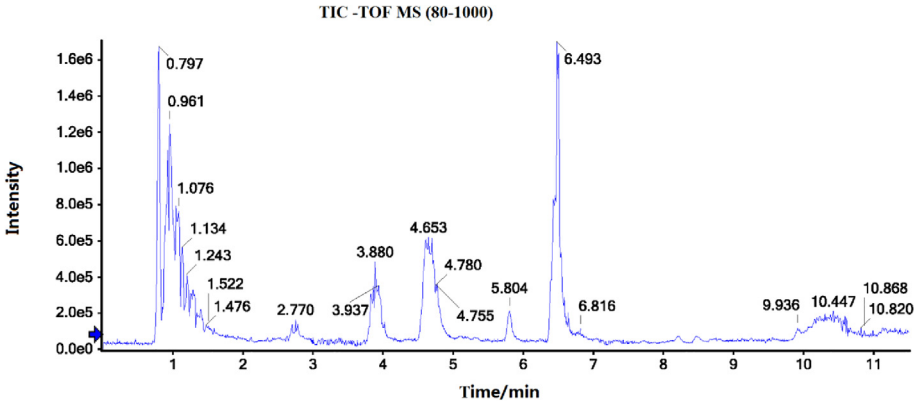

Fig. 2. The total glucosinolate chromatogram (TIC) corresponding to the analysis of broccoli samples with glucosinolates by UHPLC/TOF-MS/MS and separate MS/MS spectra of 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin, glucobrassicin, glucoraphanin, glucotropaeolin, neoglucobrassicin, glucoerucin, glucoiberin, gluconapin, gluconasturtiin, progoitrin, and sinigrin. The retention time of individual glucosinolate was shown in Table S2.

& Gu, 2016; R. F. Guo, Yuan, & Wang, 2011; Wang, Gu, Yu, Zhao, Sheng, & Zhang, 2012).

In *Brassica* plants, glucosinolate varieties and contents are closely related to A, B and C genomes (Farnham, Wilson, Stephenson, & Fahey, 2004; Liu, Liu, Yang, Tong, Edwards, Parkin, et al., 2014). It has been reported that *Brassica* plants commonly contain 18 to 27 glucosinolates (L. P. Guo, Yang, & Gu, 2016; R. F. Guo, Yuan, & Wang, 2011; M. Hansen, Moller, Sorensen, & Detrejo, 1995; Wang, Gu, Yu, Zhao, Sheng, & Zhang, 2012), with approximately twelve aliphatic glucosinolates (Cartea, Velasco, Obregon, Padilla, & de Haro, 2008). Glucoiberin, sinigrin, and glucoerucin are usually present in *B. oleracea* vegetables such as cabbage, broccoli, kohlrabi and cauliflower, and sinigrin is also produced in mustard green (*B. juncea*). Glucoraphanin, a functional component, is found in *B. oleracea* vegetables but is abundant in broccoli. Gluconapin and progoitrin are commonly present in *B. rapa* vegetables such as Chinese cabbage, mustard and turnip, *B. oleracea* vegetables such as cabbage, broccoli and cauliflower, *B. juncea* vegetables, mainly in mustard green, and *B. napus* (rapeseed). Dehydroerucin is the dominant aliphatic glucosinolate in radish, accounting for over 80% of the total glucosinolates, and glucobrassicanapin is the main glucosinolate in *B. rapa* vegetables (L. P. Guo, Yang, & Gu, 2016; Ishida, Hara, Fukino, Kakizaki, & Morimitsu, 2014; Ishida, Nagata, Ohara, Kakizaki, Hatakeyama, & Nishio, 2012). However, some glucosinolates, such as gluconasturtiin and glucotropaeolin, are rarely detected in *B. oleracea* vegetables, but we have detected both of those glucosinolate compounds, although they were in a relatively low content in broccoli florets shown in Table 1.

The glucosinolate concentrations in *Brassicaceae* are influenced by genotypes, plant tissues, growth and harvest time, environmental factors such as climate, and cultivation conditions including fertilization and soil, and genetic variations in the composition and contents of glucosinolates have been reported (Farnham, Wilson, Stephenson, & Fahey, 2004; Ishida, Nagata, Ohara, Kakizaki, Hatakeyama, & Nishio, 2012; G. Li & Quiros, 2003). However, the main factor influencing the amount of glucosinolates is usually dependent on genotype and assessed by investigating glucosinolate changes in different species, environments, or genotype-environment interactions (Fahey, Zalcmann, & Talalay, 2001; Farnham, Wilson, Stephenson, & Fahey, 2004; L. P. Guo, Yang, & Gu, 2016). In our work, twelve glucosinolate compounds were detected in the florets of broccoli, and seven glucosinolates, glucoraphanin, glucoerucin, 4-methoxyglucobrassicin, glucobrassicin, neoglucobrassicin, 4-hydroxyglucobrassicin and gluconasturtiin, were all found in mature florets. Two glucosinolates, glucoiberin and glucotropaeolin, were found in most materials, and three glucosinolates, gluconapin and progoitrin and sinigrin, were just found in a few broccoli genotypes. In contrast to previous studies, gluconasturtiin and especially glucotropaeolin were detected in florets, and both glucosinolate compounds were present at relatively low levels, which might be the reason that they were not detected in broccoli (Farnham, Wilson, Stephenson, & Fahey, 2004; L. P. Guo, Yang, & Gu, 2016; Wang, Gu, Yu, Zhao, Sheng, & Zhang, 2012).

As shown in Table 1, we found that the total content of glucosinolates in florets ranged from 0.467 to 57.156 μmol/g DW in the 80 genotypes, with the highest content being approximately 122-fold higher than the lowest: glucoraphanin ranging from 0.136 to 14.973 μmol/g DW, glucoerucin ranging from 0.008 to 6.273 μmol/g DW, glucoiberin ranging from 0 to 0.878 μmol/g DW, gluconapin ranging from 0 to 2.728 μmol/g DW, progoitrin ranging from 0 to 4.537 μmol/g DW, sinigrin ranging from 0 to 3.161 μmol/g DW, 4-methoxyglucobrassicin ranging from 0.014 to 3.915 μmol/g DW, glucobrassicin ranging from 0.103 to 27.690 μmol/g DW, neoglucobrassicin ranging from 0.018 to 45.954 μmol/g DW, 4-hydroxyglucobrassicin ranging from 0.014 to 3.289 μmol/g DW, gluconasturtiin ranging from 0.004 to 0.441 μmol/g DW, and glucotropaeolin ranging from 0 to 0.040 μmol/g DW. According to the results, < 22% of the genotypes contained the compounds gluconapin, progoitrin or sinigrin, and the corresponding contents of the three glucosinolates were all at low levels (< 4.537 μmol/g DW), which coincides with some reports but differs from several others (high content in broccoli) (Gorissen, Kraut, de Visser, de Vries, Roelofsen, & Vonk, 2011; L. P. Guo, Yang, & Gu, 2016; Mithen, Bennett, & Marquez, 2010; Wang, Gu, Yu, Zhao, Sheng, & Zhang, 2012), suggesting that some mutant plants exist in these materials.

Table 1 shows the presence of neoglucobrassicin and glucotropaeolin in some genotypes of broccoli, which not only provides new information but also validates previous research in *B. oleracea* broccoli (Wang, Gu, Yu, Zhao, Sheng, & Zhang, 2012). Few reports state that gluconapoleiferin might be detected in broccoli. However, the compound is widely and commonly detected in *B. rapa* Chinese cabbage (Wiesner, Zrenner, Krumbein, Glatt, & Schreiner, 2013). Meanwhile, we found a large variation in glucosinolate amounts in different genotypes of broccoli, enriching previous research.

A dendrogram using ward linkage was established based on a re-scaled distance cluster combined with SPSS 19.0 software (Fig. S1). From Figure S1, the cluster analysis presented 80 genotypes that can be divided into three subgroups; bred lines B100 and B125 were clustered together and contained all twelve glucosinolates, which was different from the other two large clusters containing only some glucosinolates. Therefore, the results suggested that *Brassica* plants with different compounds and contents of glucosinolates, including broccoli, cabbage, cauliflower, Chinese cabbage and so on, should be cultivated and chosen by humans. Nilsson et al. (2006) detected glucobrassicin and glucoiberin as the main glucosinolates in kale, while sinigrin was found as the major glucosinolate in white cabbage (Nilsson, Olsson, Engqvist, Ekvall, Olsson, Nyman, et al., 2006). Glucoiberin was reported as the only important glucosinolate in kales, while glucoraphanin, sinigrin, glucobrassicin, and glucoiberin were found to be the most abundant in cabbage (Kushad, Brown, Kurilich, Juvik, Klein, Wallig, et al., 1999).

Table 1
Glucosinolate contents in florets based on different genotypes of broccoli (umol/g DW).

No	Aliphaticsglucosinolates						Indolesglucosinolates				Phenylglucosinolates	
	GRA[a]	GER	GIB	NAP	PRO	SIN	4MGBS	GBS	NGBS	4HGBS	GNS	GTL
B57	10.401 ± 0.946[b]	0.070 ± 0.007	0.014 ± 0.002	ND	ND	ND	0.232 ± 0.025	6.007 ± 0.646	3.366 ± 0.370	0.853 ± 0.078	0.123 ± 0.015	ND
B58	6.380 ± 0.580	0.117 ± 0.012	0.215 ± 0.027	ND	ND	ND	0.960 ± 0.104	19.816 ± 2.131	13.059 ± 1.435	0.383 ± 0.035	0.441 ± 0.054	0.013 ± 0.002
B59	3.118 ± 0.283	0.485 ± 0.051	0.009 ± 0.001	ND	ND	ND	1.701 ± 0.185	7.386 ± 0.794	10.783 ± 1.185	1.176 ± 0.107	0.117 ± 0.014	0.011 ± 0.001
B60	0.602 ± 0.055	0.123 ± 0.013	ND	0.038 ± 0.004	0.467 ± 0.055	ND	0.174 ± 0.019	1.577 ± 0.170	1.204 ± 0.132	0.090 ± 0.008	0.033 ± 0.004	ND
B61	2.442 ± 0.222	0.773 ± 0.081	0.295 ± 0.037	ND	ND	ND	0.743 ± 0.081	13.552 ± 1.457	3.094 ± 0.340	1.629 ± 0.148	0.069 ± 0.009	0.009 ± 0.001
B62	0.984 ± 0.089	0.080 ± 0.008	ND	0.390 ± 0.044	0.446 ± 0.052	ND	0.258 ± 0.028	5.324 ± 0.573	0.934 ± 0.103	0.173 ± 0.016	0.042 ± 0.005	0.011 ± 0.001
B63	1.425 ± 0.130	1.300 ± 0.137	ND	ND	ND	ND	0.076 ± 0.008	3.072 ± 0.330	0.429 ± 0.047	0.274 ± 0.025	0.097 ± 0.012	ND
B64	0.529 ± 0.048	0.308 ± 0.032	0.076 ± 0.010	ND	ND	ND	0.843 ± 0.092	5.666 ± 0.609	3.129 ± 0.344	1.672 ± 0.152	0.028 ± 0.003	0.016 ± 0.002
B65	2.662 ± 0.242	6.273 ± 0.660	0.164 ± 0.020	0.438 ± 0.049	0.889 ± 0.105	0.298 ± 0.038	1.053 ± 0.114	11.514 ± 1.238	13.740 ± 1.510	0.999 ± 0.091	0.174 ± 0.021	0.011 ± 0.001
B66	0.351 ± 0.032	0.013 ± 0.001	ND	ND	ND	ND	0.027 ± 0.003	0.693 ± 0.074	0.018 ± 0.002	0.043 ± 0.004	0.009 ± 0.001	ND
B67	1.077 ± 0.098	0.052 ± 0.005	ND	ND	ND	ND	0.029 ± 0.003	0.201 ± 0.022	0.129 ± 0.014	0.046 ± 0.004	0.017 ± 0.002	ND
B68	0.136 ± 0.012	0.008 ± 0.001	ND	0.008 ± 0.001	ND	ND	0.017 ± 0.002	0.343 ± 0.037	0.108 ± 0.012	0.014 ± 0.001	0.010 ± 0.001	ND
B69	1.257 ± 0.114	0.311 ± 0.033	0.008 ± 0.001	ND	ND	ND	0.733 ± 0.080	0.746 ± 0.080	3.325 ± 0.365	1.235 ± 0.112	0.047 ± 0.006	0.008 ± 0.001
B70	9.290 ± 0.845	0.297 ± 0.031	0.021 ± 0.003	ND	ND	ND	0.787 ± 0.086	8.652 ± 0.930	4.505 ± 0.495	1.467 ± 0.133	0.188 ± 0.023	0.021 ± 0.003
B71	1.850 ± 0.168	0.176 ± 0.019	ND	ND	ND	ND	0.132 ± 0.014	3.751 ± 0.403	1.980 ± 0.218	0.409 ± 0.037	0.032 ± 0.004	0.009 ± 0.001
B72	1.279 ± 0.116	0.126 ± 0.013	ND	ND	ND	ND	0.354 ± 0.038	4.772 ± 0.513	3.743 ± 0.411	0.870 ± 0.079	0.038 ± 0.005	0.008 ± 0.001
B73	0.169 ± 0.015	0.008 ± 0.001	0.007 ± 0.001	ND	ND	ND	0.014 ± 0.002	0.103 ± 0.011	0.087 ± 0.010	0.070 ± 0.006	0.008 ± 0.001	ND
B74	2.155 ± 0.196	0.030 ± 0.003	0.118 ± 0.015	0.288 ± 0.032	0.921 ± 0.108	0.321 ± 0.041	0.282 ± 0.031	3.364 ± 0.362	1.609 ± 0.177	0.465 ± 0.042	0.027 ± 0.003	0.019 ± 0.002
B75	3.344 ± 0.304	0.412 ± 0.043	0.143 ± 0.018	ND	ND	ND	0.364 ± 0.040	8.132 ± 0.874	7.663 ± 0.842	2.728 ± 0.248	0.112 ± 0.014	0.024 ± 0.003
B76	0.604 ± 0.055	0.484 ± 0.051	0.013 ± 0.002	ND	ND	ND	0.326 ± 0.035	0.337 ± 0.036	0.449 ± 0.049	0.492 ± 0.045	0.025 ± 0.003	ND
B77	5.059 ± 0.460	1.353 ± 0.142	0.009 ± 0.001	ND	ND	ND	1.369 ± 0.149	12.279 ± 1.320	2.543 ± 0.279	0.903 ± 0.082	0.111 ± 0.014	0.007 ± 0.001
B78	11.346 ± 1.031	0.260 ± 0.027	0.043 ± 0.005	0.581 ± 0.065	2.093 ± 0.246	0.038 ± 0.005	0.540 ± 0.059	7.128 ± 0.766	1.482 ± 0.163	1.463 ± 0.133	0.262 ± 0.032	ND
B79	3.802 ± 0.346	0.878 ± 0.092	ND	ND	ND	ND	0.665 ± 0.072	27.690 ± 2.977	16.730 ± 1.839	2.274 ± 0.207	0.155 ± 0.019	0.040 ± 0.005
B80	2.389 ± 0.217	0.046 ± 0.005	ND	ND	ND	ND	0.218 ± 0.024	0.936 ± 0.101	5.002 ± 0.550	0.182 ± 0.017	0.020 ± 0.002	0.009 ± 0.001
B81	14.973 ± 1.361	1.112 ± 0.117	0.025 ± 0.003	ND	ND	ND	0.439 ± 0.048	7.227 ± 0.777	0.632 ± 0.069	1.181 ± 0.107	0.236 ± 0.029	0.016 ± 0.002
B82	0.961 ± 0.087	0.200 ± 0.021	ND	0.059 ± 0.007	0.197 ± 0.023	ND	0.384 ± 0.042	3.803 ± 0.409	3.008 ± 0.331	0.160 ± 0.015	0.055 ± 0.007	ND
B83	0.330 ± 0.030	0.072 ± 0.008	0.034 ± 0.004	0.048 ± 0.005	0.104 ± 0.012	0.046 ± 0.006	0.793 ± 0.086	4.510 ± 0.485	7.797 ± 0.857	0.472 ± 0.043	0.022 ± 0.003	0.018 ± 0.002
B84	1.442 ± 0.131	0.139 ± 0.015	0.075 ± 0.009	ND	ND	ND	0.704 ± 0.077	7.051 ± 0.758	4.926 ± 0.541	0.252 ± 0.023	0.226 ± 0.028	0.010 ± 0.001
B85	1.371 ± 0.125	0.173 ± 0.018	ND	ND	ND	ND	0.680 ± 0.074	1.913 ± 0.206	4.410 ± 0.485	1.284 ± 0.117	0.083 ± 0.010	ND
B86	1.735 ± 0.158	0.171 ± 0.018	ND	ND	ND	ND	0.245 ± 0.027	2.424 ± 0.261	0.520 ± 0.057	1.205 ± 0.110	0.122 ± 0.015	ND
B87	4.170 ± 0.379	0.121 ± 0.013	ND	ND	ND	ND	0.489 ± 0.053	7.850 ± 0.844	2.770 ± 0.304	0.711 ± 0.065	0.049 ± 0.006	0.014 ± 0.002
B88	1.502 ± 0.137	0.110 ± 0.012	ND	ND	ND	ND	0.379 ± 0.041	13.021 ± 1.400	0.655 ± 0.072	0.801 ± 0.073	0.022 ± 0.003	0.011 ± 0.001
B89	1.953 ± 0.178	0.255 ± 0.027	0.349 ± 0.044	ND	ND	ND	0.082 ± 0.009	0.999 ± 0.107	1.395 ± 0.153	0.764 ± 0.069	0.039 ± 0.005	0.016 ± 0.002
B90	0.920 ± 0.084	0.818 ± 0.086	0.113 ± 0.014	ND	ND	ND	0.203 ± 0.022	6.461 ± 0.695	3.291 ± 0.362	0.788 ± 0.072	0.092 ± 0.011	0.020 ± 0.003
B91	2.572 ± 0.234	0.308 ± 0.032	0.249 ± 0.031	ND	ND	ND	1.045 ± 0.114	7.703 ± 0.828	16.589 ± 1.823	1.428 ± 0.130	0.109 ± 0.013	ND
B92	6.342 ± 0.577	0.345 ± 0.036	ND	2.419 ± 0.272	4.537 ± 0.534	ND	1.024 ± 0.111	9.594 ± 1.032	2.425 ± 0.266	1.531 ± 0.139	0.189 ± 0.023	ND
B93	6.737 ± 0.612	0.473 ± 0.050	0.014 ± 0.002	ND	ND	ND	0.541 ± 0.059	10.165 ± 1.093	31.315 ± 3.441	0.671 ± 0.061	0.307 ± 0.038	0.010 ± 0.001
B94	5.946 ± 0.541	0.071 ± 0.007	ND	ND	ND	ND	0.417 ± 0.045	2.853 ± 0.307	0.937 ± 0.103	0.321 ± 0.029	0.050 ± 0.006	ND
B95	0.896 ± 0.081	0.091 ± 0.010	0.109 ± 0.014	ND	ND	ND	0.059 ± 0.006	0.284 ± 0.031	0.329 ± 0.036	0.085 ± 0.008	0.056 ± 0.007	ND
B96	11.928 ± 1.084	0.281 ± 0.030	0.029 ± 0.004	ND	ND	ND	0.512 ± 0.056	8.613 ± 0.926	15.111 ± 1.660	1.200 ± 0.109	0.121 ± 0.015	ND
B97	2.348 ± 0.213	0.500 ± 0.053	0.013 ± 0.002	ND	ND	ND	1.353 ± 0.147	9.358 ± 1.006	9.159 ± 1.006	1.196 ± 0.109	0.062 ± 0.008	0.012 ± 0.001
B98	1.219 ± 0.111	0.341 ± 0.036	ND	0.151 ± 0.017	0.443 ± 0.052	ND	1.405 ± 0.153	6.867 ± 0.738	8.799 ± 0.967	1.125 ± 0.102	0.034 ± 0.004	0.012 ± 0.001
B99	11.864 ± 1.079	1.432 ± 0.151	0.013 ± 0.002	ND	ND	ND	1.453 ± 0.158	9.672 ± 1.040	8.389 ± 0.922	1.754 ± 0.159	0.051 ± 0.006	0.009 ± 0.001
B100	8.548 ± 0.777	1.079 ± 0.114	0.557 ± 0.070	2.728 ± 0.307	3.108 ± 0.366	3.161 ± 0.400	1.150 ± 0.125	24.015 ± 2.582	4.063 ± 0.447	1.120 ± 0.102	0.319 ± 0.039	0.034 ± 0.004
B101	1.578 ± 0.143	0.758 ± 0.080	0.016 ± 0.002	ND	ND	ND	1.311 ± 0.143	19.692 ± 2.117	8.015 ± 0.881	0.710 ± 0.065	0.059 ± 0.007	0.018 ± 0.002
B102	2.942 ± 0.267	0.525 ± 0.055	0.128 ± 0.016	ND	ND	ND	0.172 ± 0.019	9.042 ± 0.972	1.140 ± 0.125	0.618 ± 0.056	0.087 ± 0.011	0.011 ± 0.001
B103	0.405 ± 0.037	0.039 ± 0.004	ND	ND	ND	ND	0.602 ± 0.065	1.604 ± 0.172	12.884 ± 1.416	0.041 ± 0.004	0.026 ± 0.003	ND
B104	5.884 ± 0.535	0.537 ± 0.057	ND	0.579 ± 0.065	1.993 ± 0.234	ND	0.456 ± 0.050	8.646 ± 0.930	3.022 ± 0.332	0.504 ± 0.046	0.056 ± 0.007	0.023 ± 0.003
B105	0.459 ± 0.042	0.210 ± 0.022	0.878 ± 0.110	ND	ND	0.014 ± 0.002	0.501 ± 0.054	8.102 ± 0.871	2.662 ± 0.292	0.228 ± 0.021	0.123 ± 0.015	0.009 ± 0.001
B106	5.810 ± 0.528	0.165 ± 0.017	0.018 ± 0.002	ND	ND	ND	0.321 ± 0.035	8.896 ± 0.957	3.260 ± 0.358	0.337 ± 0.031	0.043 ± 0.005	0.009 ± 0.001
B107	10.773 ± 0.979	0.694 ± 0.073	0.022 ± 0.003	1.665 ± 0.187	3.429 ± 0.403	0.034 ± 0.004	0.251 ± 0.027	7.774 ± 0.836	5.530 ± 0.608	1.238 ± 0.113	0.094 ± 0.012	0.022 ± 0.003
B108	1.354 ± 0.123	0.092 ± 0.010	ND	ND	ND	ND	0.137 ± 0.015	0.600 ± 0.064	0.249 ± 0.027	0.603 ± 0.055	0.025 ± 0.003	ND
B109	0.229 ± 0.021	0.024 ± 0.003	ND	ND	ND	ND	0.040 ± 0.004	0.268 ± 0.029	0.245 ± 0.027	0.095 ± 0.009	0.014 ± 0.002	ND

(*continued on next page*)

Table 1 (*continued*)

No	Aliphaticsglucosinolates						Indolesglucosinolates				Phenylglucosinolates	
	GRA[a]	GER	GIB	NAP	PRO	SIN	4MGBS	GBS	NGBS	4HGBS	GNS	GTL
B110	1.653 ± 0.150	0.026 ± 0.003	0.016 ± 0.002	ND	ND	ND	0.051 ± 0.006	1.564 ± 0.168	0.223 ± 0.025	0.244 ± 0.022	0.028 ± 0.003	ND
B111	7.056 ± 0.641	6.186 ± 0.651	ND	ND	ND	ND	1.540 ± 0.167	7.740 ± 0.832	3.306 ± 0.363	2.517 ± 0.229	0.203 ± 0.025	0.010 ± 0.001
B112	3.692 ± 0.336	0.145 ± 0.015	0.009 ± 0.001	ND	ND	ND	0.184 ± 0.020	8.762 ± 0.942	13.444 ± 1.477	0.788 ± 0.072	0.045 ± 0.006	ND
B113	14.123 ± 1.284	0.469 ± 0.049	0.522 ± 0.065	ND	ND	ND	0.960 ± 0.104	5.117 ± 0.550	13.644 ± 1.499	1.555 ± 0.141	0.268 ± 0.033	0.018 ± 0.002
B114	3.015 ± 0.274	0.194 ± 0.020	ND	ND	ND	ND	0.307 ± 0.033	2.905 ± 0.312	0.314 ± 0.035	0.281 ± 0.026	0.053 ± 0.007	ND
B115	2.118 ± 0.193	0.129 ± 0.014	0.121 ± 0.015	ND	ND	ND	0.187 ± 0.020	1.113 ± 0.120	1.163 ± 0.128	0.272 ± 0.025	0.017 ± 0.002	0.011 ± 0.001
B116	5.408 ± 0.492	0.337 ± 0.036	ND	ND	ND	ND	0.652 ± 0.071	4.118 ± 0.443	6.239 ± 0.686	0.739 ± 0.067	0.207 ± 0.026	0.013 ± 0.002
B117	9.310 ± 0.846	1.054 ± 0.111	ND	0.418 ± 0.047	0.824 ± 0.097	ND	0.398 ± 0.043	13.881 ± 1.493	5.788 ± 0.636	3.289 ± 0.299	0.024 ± 0.003	0.034 ± 0.004
B118	4.080 ± 0.371	0.052 ± 0.005	0.010 ± 0.001	ND	ND	ND	0.289 ± 0.031	3.793 ± 0.408	0.652 ± 0.072	0.262 ± 0.024	0.019 ± 0.002	0.007 ± 0.001
B119	7.540 ± 0.685	1.737 ± 0.183	0.017 ± 0.002	ND	ND	ND	0.105 ± 0.011	13.938 ± 1.499	2.967 ± 0.326	1.998 ± 0.182	0.069 ± 0.009	ND
B120	1.247 ± 0.113	0.351 ± 0.037	0.044 ± 0.006	0.151 ± 0.017	0.503 ± 0.059	0.154 ± 0.019	0.214 ± 0.023	7.406 ± 0.796	5.331 ± 0.586	0.685 ± 0.062	0.058 ± 0.007	0.010 ± 0.001
B121	1.203 ± 0.109	0.244 ± 0.026	0.008 ± 0.001	ND	ND	ND	0.628 ± 0.068	12.955 ± 1.393	6.972 ± 0.766	0.192 ± 0.017	0.054 ± 0.007	0.009 ± 0.001
B122	1.280 ± 0.116	0.070 ± 0.007	0.122 ± 0.015	ND	ND	ND	0.148 ± 0.016	1.716 ± 0.185	2.659 ± 0.292	0.366 ± 0.033	0.030 ± 0.004	ND
B123	4.070 ± 0.370	0.047 ± 0.005	0.021 ± 0.003	ND	ND	ND	0.206 ± 0.022	8.378 ± 0.901	1.310 ± 0.144	0.168 ± 0.015	0.044 ± 0.005	0.021 ± 0.003
B124	6.278 ± 0.571	0.163 ± 0.017	0.009 ± 0.001	ND	ND	ND	0.463 ± 0.050	8.501 ± 0.914	9.060 ± 0.996	1.133 ± 0.103	0.074 ± 0.009	0.007 ± 0.001
B125	7.184 ± 0.653	0.880 ± 0.093	0.439 ± 0.055	2.069 ± 0.232	3.235 ± 0.381	2.238 ± 0.283	1.342 ± 0.146	18.092 ± 1.945	4.638 ± 0.510	0.988 ± 0.090	0.348 ± 0.043	0.033 ± 0.004
B126	11.409 ± 1.037	0.081 ± 0.009	0.030 ± 0.004	ND	ND	ND	0.263 ± 0.029	4.186 ± 0.450	14.485 ± 1.592	0.438 ± 0.040	0.040 ± 0.005	0.010 ± 0.001
B127	2.999 ± 0.273	0.044 ± 0.005	0.233 ± 0.029	0.319 ± 0.036	1.438 ± 0.169	0.422 ± 0.053	0.197 ± 0.021	2.446 ± 0.263	11.431 ± 1.256	0.244 ± 0.022	0.043 ± 0.005	0.013 ± 0.002
B128	0.175 ± 0.016	0.033 ± 0.004	0.012 ± 0.002	ND	ND	ND	0.060 ± 0.007	0.560 ± 0.060	0.095 ± 0.010	0.067 ± 0.006	0.004 ± 0.000	0.001 ± 0.000
B129	4.533 ± 0.412	0.347 ± 0.037	ND	ND	ND	ND	2.474 ± 0.269	5.549 ± 0.597	8.791 ± 0.966	0.772 ± 0.070	0.098 ± 0.012	0.021 ± 0.003
B130	4.583 ±	0.191 ± 0.020	0.012 ± 0.002	ND	ND	ND	1.155 ± 0.126	4.624 ± 0.497	45.954 ± 5.050	0.593 ± 0.054	0.029 ± 0.004	0.015 ± 0.002
B131	7.356 ± 0.669	0.228 ± 0.024	0.010 ± 0.001	ND	ND	ND	3.915 ± 0.426	12.814 ± 1.378	3.362 ± 0.369	2.522 ± 0.229	0.208 ± 0.026	0.009 ± 0.001
B132	11.891 ± 1.081	0.496 ± 0.052	0.016 ± 0.002	ND	ND	ND	0.602 ± 0.065	18.573 ± 1.997	0.309 ± 0.034	2.927 ± 0.266	0.217 ± 0.027	0.011 ± 0.001
B133	5.197 ± 0.472	2.208 ± 0.232	0.011 ± 0.001	ND	ND	ND	1.395 ± 0.152	23.937 ± 2.574	2.478 ± 0.272	2.428 ± 0.221	0.167 ± 0.021	0.013 ± 0.002
B134	4.099 ± 0.373	0.182 ± 0.019	ND	ND	ND	ND	0.759 ± 0.083	0.929 ± 0.100	7.723 ± 0.849	0.058 ± 0.005	0.076 ± 0.009	0.013 ± 0.002
B135	5.105 ± 0.464	0.178 ± 0.019	ND	0.973 ± 0.109	2.872 ± 0.338	ND	0.220 ± 0.024	3.695 ± 0.397	11.122 ± 1.222	1.181 ± 0.107	0.028 ± 0.003	0.019 ± 0.002
B136	8.878 ± 0.807	1.020 ± 0.107	0.779 ± 0.097	ND	ND	ND	0.169 ± 0.018	0.940 ± 0.101	9.159 ± 1.006	0.825 ± 0.075	0.039 ± 0.005	0.010 ± 0.001
Mean[c]	4.192 ± 3.742	0.528 ± 1.014	0.078 ± 0.167	0.167 ± 0.509	0.344 ± 0.907	0.084 ± 0.433	0.599 ± 0.613	7.003 ± 6.043	5.691 ± 7.089	0.873 ± 0.749	0.093 ± 0.090	0.010 ± 0.009
Range	0.136–14.973	0.008–6.273	0–0.878	0–2.728	0–4.537	0–3.161	0.014–3.915	0.103–27.690	0.018–45.954	0.014–3.289	0.004–0.441	0–0.040

[a] Glucosinolates abbreviations: GS: glucosinolates, GRA: glucoraphanin (4-methylsulphinylbutyl GS); GER: glucoerucin (4-methylthiobutyl GS); GIB: glucoiberin (3-methylsulfinylpropyl GS); NAP: gluconapin (3-butenyl GS); PRO: progoitrin (2-R-hydroxy-3-butenyl GS); SIN: sinigrin (2-propenyl GS); 4MGBS: 4-methoxyglucobrassicin (4-methoxy-3-indolylmethyl GS); GBS: glucobrassicin (3-indolylmethyl GS); NGBS: neoglucobrassicin (1-methoxy-3-indolylmethyl GS); 4HGBS:4-hydroxyglucobrassicin (4-hydroxy-3-indolylmethyl GS); GNS: Gluconasturtiin (2-phenylethyl GS); GTL: glucotropaeolin (Benzyl glucosinolate).

[b] Values are expressed as mean ± standard deviation (SD) (n = 3), and "ND" stands for not detected.

[c] Mean presented as the average ± SD.

In our work, the principal component analysis presented three factors that explained 60.53% of the variance, and the corresponding contributions were 34.28%, 17.17% and 9.08% (Table S3), and the glucosinolate concentration varied depending on the genotype. From Table S4, component 1 presented a positive correlation among the twelve factors, directly corresponding to total glucosinolate amounts. Component 2 showed four factors with a negative correlation among glucoiberin, gluconapin, progoitrin and sinigrin of the downstream products of C3 and C4 aliphatic glucosinolates, which corresponded to the up- and downstream regulation of glucosinolates. In component 3, most of the factors were negatively correlated, and a complex relationship was observed among them. From Figure S2, we could clearly find the principal glucosinolates in 80 broccoli genotypes, and as well as the positive and negative relationships of total glucosinolate and independent component.

From the correlation based on the Pearson test, we found positive correlations among hydroxyglucobrassicin, methoxyglucobrassicin, glucobrassicin, glucoerucin, gluconasturtiin, glucoraphanin, and glucotropaeolin ($P < 0.05$). The aliphatic glucosinolates, glucoiberin, gluconapin, gluconasturtiin, glucotropaeolin, progoitrin, and sinigrin, were related correspondingly ($P < 0.05$), providing validation and evidence regarding the regulatory pathways of aliphatic glucosinolates (Table S5). However, it must be noted that there was still a problem regarding glucoraphanin and gluconapin in this study. As shown in previous reports, *AOP2* (*BoGSL-ALK*) could play a role in *B. rapa* and collard, producing gluconapin from glucoraphanin, but in broccoli, there is the presence of a nonfunctional allele *BoGSL-ALK* (GenBank no. AY044424) originating from a 2-bp deletion in exon 2 (G. Li & Quiros, 2003; Sønderby, Geu-Flores, & Halkier, 2010); thus, gluconapin should be absent in broccoli florets, but in some previous reports and from 18 genotypes in this study, gluconapin was detected in florets (B. G. Hansen, Kliebenstein, & Halkier, 2007; G. Li & Quiros, 2003; Stewart, Nho, & Jeffery, 2004), which is an obvious contradiction in broccoli (Fig. 3). As mutant materials, 18 broccoli genotypes have been studied regarding *AOP2* regulatory function.

In addition, glucosinolates are usually influenced by environmental factors such as soil, climate and cultivation conditions including fertilization, harvest time, plant organ, and physical damage. However, wide genetic variations in the glucosinolate contents and compounds have been reported from previous studies, and glucosinolates are affected by genotype and environment (Carlson, Daxenbichler, VanEtten, Tookey, & Williams, 1981; Ishida, Nagata, Ohara, Kakizaki, Hatakeyama, & Nishio, 2012; G. Li & Quiros, 2003). The accumulation of aliphatic glucosinolates in *B. rapa* is enhanced by low nitrogen and high sulfur supply, and high temperature can enhance the accumulation of aliphatic glucosinolates (Volker, Freeman, Banuelos, & Jeffery, 2010). However, the change and induction of molecular and transport mechanisms of glucosinolates remain to be further studied in the future.

3.4. Evaluation of glucosinolates in different broccoli organs

Among the many varieties of vegetables, *Brassicaceae* vegetables have received the most attention because their unique constituents, glucosinolates, are abundant in edible parts and are regarded as most likely to support human health through continuous consumption. For the future, the breeding of *Brassicaceae* vegetables by particularly addressing beneficial glucosinolates is expected to grow in importance (Gorissen, Kraut, de Visser, de Vries, Roelofsen, & Vonk, 2011; Mithen, Bennett, & Marquez, 2010).

To better define the relationship between glucosinolate content and leaf development, six genotypes and their eight organs were planted and treated in this study. From the distribution of glucosinolate compounds in different organs of broccoli B1-B6 (Fig. 4), we found that the total glucosinolate content ranged from 0.214 to 73.527 μmol/g DW in different organs, with the highest content being approximately 343-fold than the lowest value and much higher than that in florets. In these materials, a total of eight glucosinolate compounds were found except in B4, which had nine compounds in roots. Among the organs, seven glucosinolates, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin, glucobrassicin, glucoerucin, gluconasturtiin, glucoraphanin and neoglucobrassicin, were found, but with the following exceptions: all were found in roots except glucotropaeolin, which was just found in B4; all

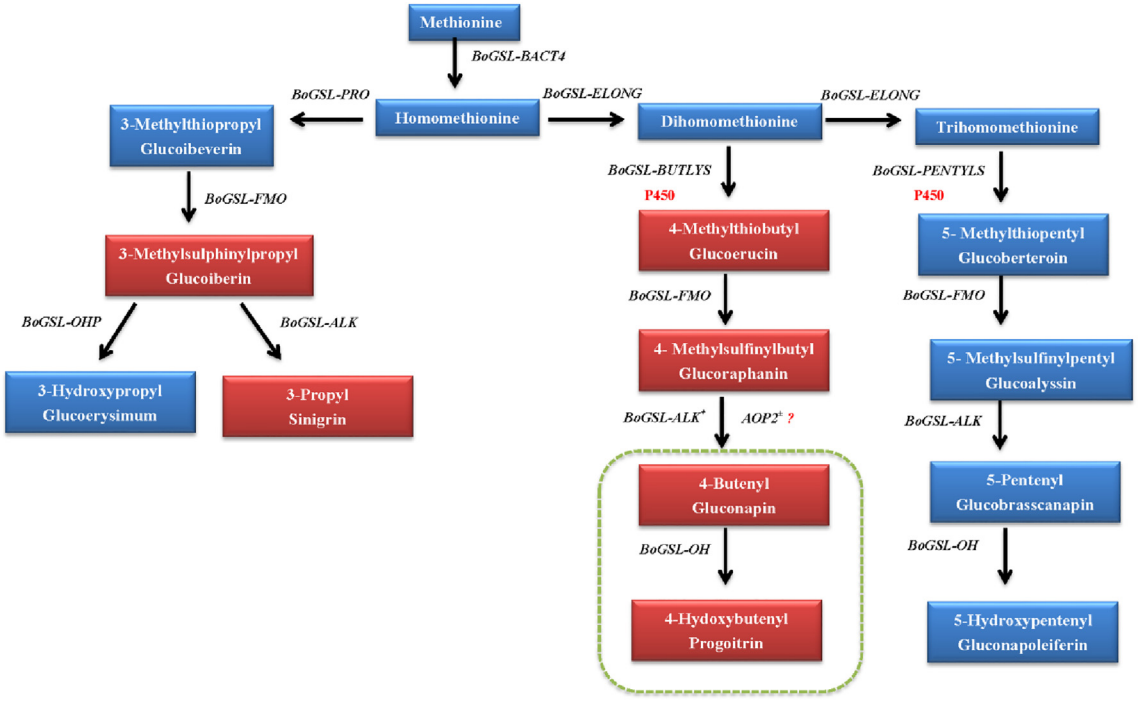

Fig. 3. Glucosinolate core structure and side-chain modification pathway for 3C, 4C and 5C aliphatic glucosinolates. The red frame presents glucosinolates detected in broccoli florets, and the dotted green line indicates the potential query depending on the *AOP2* $^{\pm}$ gene (G. Li & Quiros, 2003; Mithen, Bennett, & Marquez, 2010). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

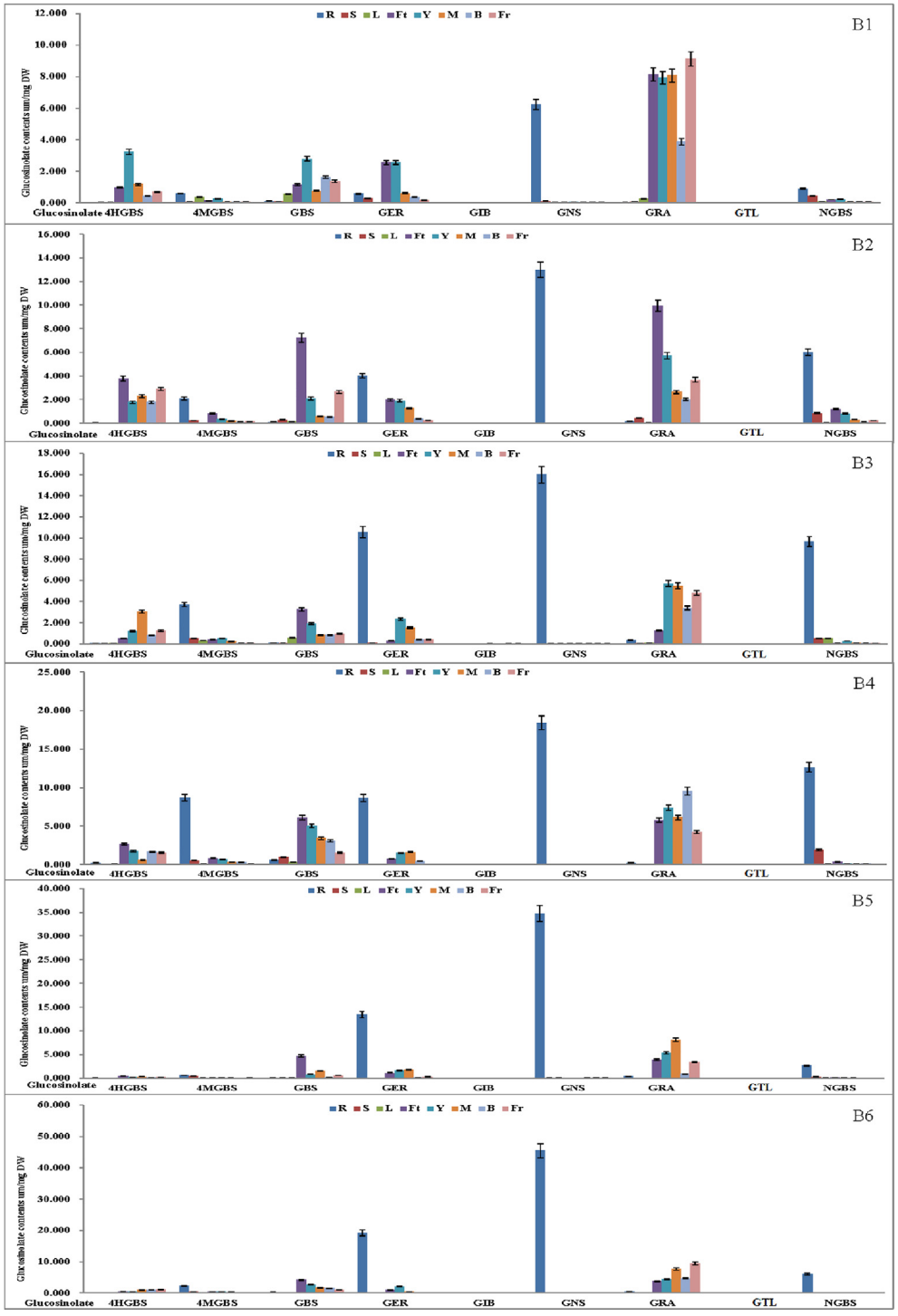

Fig. 4. The contents of nine glucosinolates in eight organs of six broccoli genotypes (B1-B6), the organs were roots (R), stalks (S), leaves (L), florets (Ft), young buds (Y), mature buds (M), buds before flowering (B) and flowers (Fr), and nine individual glucosinolate was glucoraphanin (GRA), glucoerucin (GER), glucoiberin (GIB), glucobrassicin (GBS), neoglucobrassicin (NGBS), 4hydroxyglucobrassicin (4MGBS), 4-Hydroxyglucobrassicin (4HGBS), glucotropaeolin (BGS), and gluconasturtiin (GNS).

were found in stalks, except in B4 and B5; all were found in leaves, except glucoerucin; all florets presented glucoiberin, except in B1; young buds also presented glucoiberin; mature buds also presented glucoiberin, except in B1-B3; buds before flowering also presented glucoiberin, except in B1 and B5; and flowers also presented glucoiberin. The glucosinolate content ranged from 8.482 to 73.527 μmol/g DW in roots with an average value of 41.518 μmol/g DW, 0.486 to 3.475 μmol/g DW in stalks with an average value of 1.503 μmol/g DW, 0.214 to 1.563 μmol/g DW in leaves with an average value of 0.682 μmol/g DW, 5.859 to 24.948 μmol/g DW in florets with an average value of 13.383 μmol/g DW, 8.144 to 17.009 μmol/g DW in florets with an average value of 12.664 μmol/g DW, 7.262 to 12.122 μmol/g DW in young buds with an average value of 10.647 μmol/g DW, 1.363 to 15.093 μmol/g DW in buds before flowering with an average value of 6.790 μmol/g DW, and 4.568 to 11.878 μmol/g DW in buds before flowering with an average value of

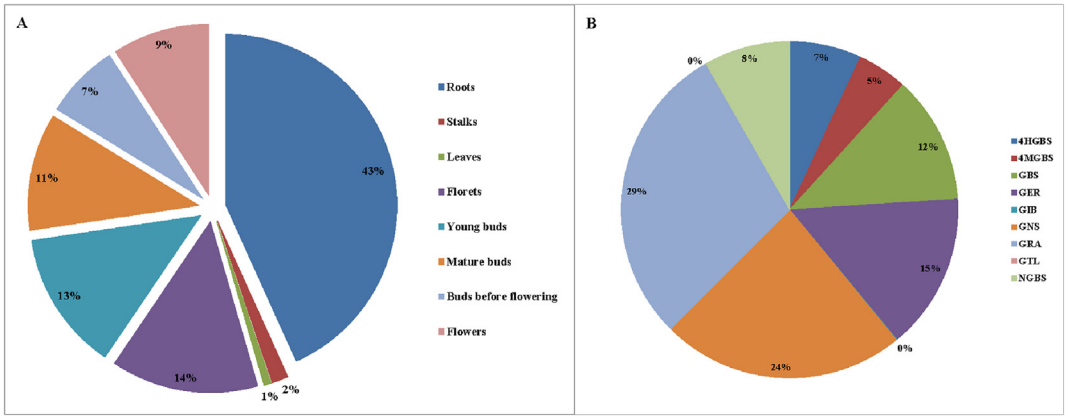

Fig. 5. The percentage of total glucosinolate in different organs, including roots, stalks, leaves, florets, young buds, mature buds, buds before flowering and flowers (A), and the average percentage of nine glucosinolate distributed in all the materials (B).

8.806 μmol/g DW.

In *Arabidopsis*, the highest glucosinolate concentrations are found in reproductive organs, including seeds, siliques, flowers and developing inflorescences, followed by young leaves, the root system and fully expanded leaves (Grubb & Abel, 2006; B. G. Hansen, Kliebenstein, & Halkier, 2007; Sønderby, Geu-Flores, & Halkier, 2010). Our data showed that the roots contained almost 43% of the total glucosinolates, which was different from that in *Arabidopsis*. According to several previous reports, under deficiency of nitrogen or sulfur, the levels of several glucosinolates decreased in leaves but increased in roots, which suggests that some glucosinolates are initially generated and accumulated in roots first (B. G. Hansen, Kliebenstein, & Halkier, 2007; Kushad, et al., 1999; Z. S. Li, et al., 2019). Meanwhile, some genes such as *MYB68*, *MYB34/ATR1*, *MYB51/HIG1* and *MYB122* can regulate the production of indolic glucosinolates in roots and late-stage rosette leaves, which usually cause changes in indolic glucosinolate levels (Gigolashvili, Yatusevich, Rollwitz, Humphry, Gershenzon, & Flugge, 2009). The florets and developmental buds contained 7% to 14% of the total glucosinolates, respectively, and the leaves and stalks contained 1% and 2% of the total glucosinolates (Fig. 5A). Tracer studies have demonstrated de novo synthesis in siliques and phloem transport of glucosinolates from mature and senescing leaves to inflorescences and developing fruits in *Arabidopsis* (Chen, Glawischnig, Jorgensen, Naur, Jorgensen, Olsen, et al., 2003; Mikkelsen, Petersen, Olsen, & Halkier, 2002). Active and specific glucosinolate uptake into *Brassica* leaf protoplasts is mediated by a proton-coupled symporter (Chen, et al., 2003).

Glucosinolate profiles have been systematically monitored in *Arabidopsis* during plant development and vary significantly between tissues and organs (P. D. Brown, Tokuhisa, Reichelt, & Gershenzon, 2003; Halkier & Gershenzon, 2006). However, previous research has not verified this finding in broccoli, so the result might provide new additional evidence for glucosinolate metabolism research in *Brassica* plants (Liang, Li, Yuan, & Vriesekoop, 2008). The distribution of each glucosinolate in all the materials suggested some differences among the compounds (Fig. 5B): glucoraphanin represented 29% of the total glucosinolates, followed by gluconasturtiin (24%), glucoerucin (15%), glucobrassicin (12%), neoglucobrassicin (8%), 4-hydroxyglucobrassicin (7%), and 4-methoxyglucobrassicin (5%). Glucoiberin and glucotropaeolin constituted 2% and 1% of the total glucosinolates, respectively, much less than that of glucoraphanin. The results were obviously different from those in florets of the 80 genotypes, and there are no similar reports in previous research that could provide useful evidence and a basis for studying the metabolism and diversity of glucosinolates in *Brassica* developmental organs.

In addition, in this study, the dendrogram, component plot (rotated space) and regression of glucosinolates in different broccoli organs were carried out based on statistical analysis. According to comparison of glucosinolate contents in different organs, some similar conclusions were revealed in this study based on representative broccoli genotype B1. From Figure S3, the related distance cluster combine of glucosinolates based on cluster analysis presented the root as an individual branch, the stalks and leaves were close branches, and the florets, developmental buds and flowers were relatively close branches. The cluster result clearly revealed that the vegetative and reproductive organs of broccoli should be divided into three parts to analyze glucosinolate concentrations and structures. The roots, as a single branch of vegetative organs, are different from other organs in glucosinolate products. All the reproductive organs exhibited one large branch variation based on genotype, including florets, buds, flowers, siliques and seeds, which usually contain more glucoraphanin in terms of total glucosinolates, which were consistent with the previous reports (L. P. Guo, Yang, & Gu, 2016; Z. S. Li, et al., 2019). More information about buds was also shown in this study, which were young buds with higher glucoraphanin content good for development of cruciferous tea. At the same time, the regression analysis of glucosinolates indicated that there were linear relationships between hydroxyglucobrassicin and glucobrassicin, glucoraphanin, glucobrassicin and glucoiberin, glucotropaeolin. This result was consistent with the correlation analysis of glucosinolates contents based on 80 genotypes, providing new insight into glucosinolate compounds in different organs and genotypes, which might also help us evaluate the concentration of glucosinolates based on a linear regression equation (Fig. S3). So far, few reports state similar results based on regression analysis. The principal component analysis was also carried out to reveal the characteristics of glucosinolates in different organs, and it was concluded that aliphatic glucosinolates were domain glucosinolates in broccoli, consistent with most previous reports; three components were basically described in the component plot in rotated space (Fig. S3), but small differences were also observed in the concentrations and compounds based on the B1-B6 genotypes (Nilsson, et al., 2006).

4. Conclusion

Our work firstly provide a fast and reliable method for the determination of glucosinolates in broccoli based on UHPLC-Triple-TOF-MS, and the correlations among glucosinolates, genotypes and organs are all thoroughly determined by investigating variations in glucosinolate profiles in 80 genotypes and eight developmental organs. Twelve glucosinolates constitute three major variation components from broccoli inbred lines, and the vegetative and reproductive organs contain different glucosinolate concentrations and components with respect to domain aliphatic glucosinolates. The statistical analysis of glucosinolates indicates that the root is different from the other vegetative organs with respect to glucosinolate metabolism, and the

reproductive organs might be clustered together with a higher level of glucoraphanin, which is helpful for humans. The study also might provide some insight into broccoli mutations for deep research on the *AOP2* gene, as well as some methods to evaluate the major components and concentrations of glucosinolates in *Brassica* plants based on statistical analysis.

Author contributions

Authors Y. Liu, Z. Fang, L. Yang, and S. Zheng conducted the experiments. Authors Z. Li, D. Xu, M. Zhuang, Y. Zhang, Y. Wang, Y. Wang and H. Lv designed the studies and interpreted the results. Author Z. Li wrote the manuscript. Yumei Liu; Zhiyuan Fang; Limei Yang: Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.127519.

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