Analysis on Composition and Content of Glucosinolates in Broccoli Flowers and Leaves

Guangmin LIU, Yue MA, Yunhua DING, Liping HU, Xuezhi ZHAO, Hongju HE*

Beijing Vegetable Research Center, Beijing Academy of Agriculture and Forestry Sciences/Beijing Key Laboratory of Fruits and Vegetables Preservation and Processing/Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (North China), Ministry of Agriculture/Key Laboratory of Urban Agriculture (North), Ministry of Agriculture, Beijing 100097, China

Abstract Glucosinolate composition and content were evaluated in flowers and leaves of 12 different broccoli varieties. The results indicated that there were 9 glucosinolates in broccoli, namely Glucoiberin (IBE); Progoitrin (PRO); Sinigrin (SIN); Glucoraphanin (RAA); Gluconapin (NAP); 4-Hydroxyglucobrassicin (4OH); Glucobrassicin (GBC); 4-Methoxyglucobrassicin (4ME); Neoglucobrassicin (NEO). Total glucosinolate content in flowers was 1 – 5 times higher than in leaves. The predominant glucosinolate in broccoli was glucoraphanin.

Key words Broccoli, Flowers, Leaves, Glucosinolates

1 Introduction

Broccoli (*Brassica oleracea* L. var. *bo tryti* L.) is an edible green plant in the cabbage family whose large flowering head is eaten as a vegetable. Glucosinolates (GLS) are sulfur-containing compounds and important secondary metabolites in cruciferous vegetables. All cruciferous plants are capable of synthesizing glucosinolates which exist in the roots, stems, leaves and seeds of such plants[1]. When the vegetables are eaten or mechanically broken, the glucosinolates in vegetables are hydrolyzed by endogenous mustard enzymes into a variety of degradation products with physiological activity, and isothiocyanate as one of the products can effectively prevent cancer, especially bladder cancer, colon cancer and lung cancer[2–4]. It is reported that broccoli is rich in Glucoiberin, Glucoraphanin, Glucobrassicin and Neoglucobrassicin[5]. British scientists have bred new broccoli varieties with high glucosinolate content, whose cancer-fighting ability is 80 times that of ordinary broccoli[4,6]. There are differences in the content and composition of glucosinolates for different vegetable species, different growth environment, different growth stages of the same plant and different parts of the same plant[7–10]. In this study, we analyzed and measured the content and composition of glucosinolates in flowers and leaves of 12 broccoli varieties common in the market and analyzed cultivation traits and yield, aimed at providing a reference for broccoli breeding and its by-product utilization.

2 Materials and methods

2.1 Materials The 12 test broccoli varieties were from Beijing Vegetable Research Center, Beijing Academy of Agriculture and Forestry Sciences (Table 1). They were sown on February 1, 2013, and planted in Tongzhou Farm of Beijing Vegetable Research Center on March 25. The open field direct planting method was used, with row spacing of 50 cm and plant spacing of 50 cm, repeated three times. The fresh flowers and leaves were taken on July 24 to determine the content of glucosinolates.

2.2 Methods

2.2.1 Extraction of glucosinolates[11]. The fresh broccoli flowers and leaves were cut into balls or pieces, and dried in the vacuum freeze dryer. 0.2 g of crushed sample was placed into 15 mL plastic tube. 0.25 mL of phenylmethyl glucosinolate was added and 100% preheated methanol was added rapidly. It was under water bath at 80 ℃ for 20 min and there was a whirlpool every 4 to 5 min. It was centrifuged for 10 min at 3000r/min, and the supernatant was poured into 15 mL plastic tube and placed in an ice tub. The precipitate was extracted twice with 70% methanol, and with the above-described processing, supernatants were combined to get sample solution. A disposable syringe was taken and glass wool was plugged in it, and it was placed on the test tube. 2 mL of DEAE gum solution was added, it was washed with 2 mL of distilled water and then 2 mL of sample solution was added. When the sample solution did not drip, 0.02 M NaAc solution was added. When the liquid did not drip, the syringe was transferred to another test tube, 75 μL of sulfatase solution was added, and it was sealed overnight. The syringe was washed with distilled water three times, 0.5 mL each time. The syringe was pressed with injection head so that the liquid was transferred to the tube as much as possible. The liquid in tube was transferred into a glass vial with 0.45 μm filter membrane, frozen and set aside.

2.2.2 Analysis of glucosinolates. HPLC analysis conditions: Nova-Pak^R C18 chromatographic column, 3.9 × 150 mm, 50 μm, detection wavelength of 229 nm, flow rate of 1.0 mL/min, room temperature, injection volume of 20 μL. The gradient elution was shown in Table 2. Solution A: 1 g of TMACI was dissolved in 2 L

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* Corresponding author. E-mail: hehongju@nercv.org

distilled water, mixed and filtered. Solution B: 1 g of TMACI was dissolved in 1.6 L distilled water, and 400 mL HPLC-grade acetonitrile was added, mixed and filtered. With Glucotropaeolin as internal standard, the glucosinolate composition was determined according to retention time and peak area. Using internal standard and response factors, the content of glucosinolates was calculated, with μmol · g^{-1} DW as unit. The content of glucosinolates was calculated as follows:

Glucosinolate content = (*Desulfurization glucosinolate peak area* × *Internal standard amount* × *Desulfurization glucoside relative response factor*)/*Internal standard peak area* × *Sample weight*.

Table 1 Information of 12 test broccoli varieties

Variety	Degree of maturity	Head weight//kg	Variety source
Zhonglu 1	Mid-maturity	0.4	China National Seed Group Co., Ltd.
Gongxianzhe	Early maturity	0.2	Beijing Hua'nai Agricultural Development Co., Ltd.
Youya	Mid-late maturity	0.4	Beijing Keruide Seed Co., Ltd.
Yelu	Mid-maturity	0.3	Shangqiu Aoxue Seed Industry Co., Ltd.
Zhonglu 6	Mid-maturity	0.3	China National Seed Group Co., Ltd.
Lusen	Mid-late maturity	0.3	Beijing Hua'nai Agricultural Development Co., Ltd.
Zhonglu 9	Mid-late maturity	0.4	China National Seed Group Co., Ltd.
Zhonglu 5	Mid-late maturity	0.4	China National Seed Group Co., Ltd.
Nanxiu 366	Mid-maturity	0.5	Beijing Hua'nai Agricultural Development Co., Ltd.
Sali 3	Early maturity	0.2	Beijing Hua'nai Agricultural Development Co., Ltd.
Xingyun	Mid-maturity	0.4	Shanghai Shimanfeng Seed Industry Co., Ltd.
Dadi	Mid-early maturity	0.5	Beijing Keruide Seed Co., Ltd.

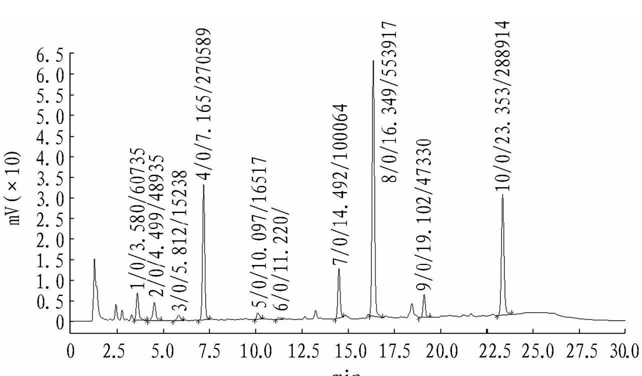
Table 2 The composition of mobile phase gradient

Time//min	Flow rate//mL/min	Pump A//%	Pump B//%
0	1	100	0
1	1	100	0
21	1	0	100
26	1	100	0
31	1	100	0

3 Results and discussions

3.1 Composition of glucosinolates in broccoli

According to different retention time and characteristic peak shape, the following components could be identified: (Glucoiberin, IBE); (Progoitrin, PRO); (Sinigrin, SIN); (Glucoraphanin, RAA); (Gluconapin, NAP); (4-Hydroxyglucobrassicin, 4OH); (Glucotropaeolin, TRO, internal standard); (Glucobrassicin, GBC); (4-Methoxyglucobrassicin, 4ME); (Neoglucobrassicin, NEO) (Fig. 1).



Note: 1 (IBE); 2 (PRO); 3 (SIN); 4 (RAA); 5 (NAP); 6 (4OH); 7 (internal standard); 8 (GBC); 9 (4ME); 10 (NEO).

Fig. 1 HPLC chromatogram of broccoli glucosinolates

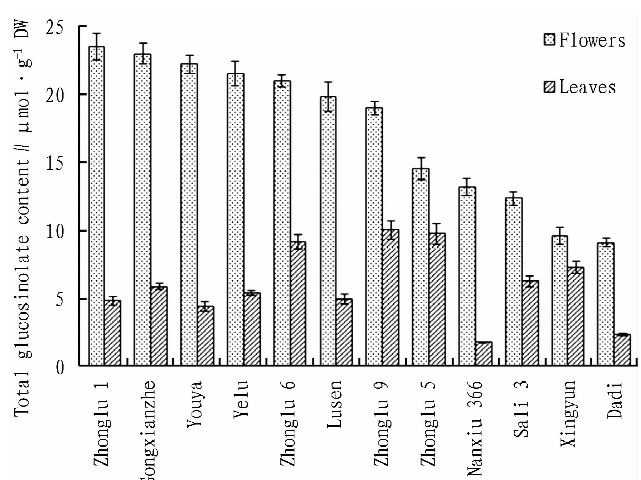


Fig. 2 Total glucosinolate content in flowers and leaves of different broccoli varieties

3.2 Total glucosinolate content in different varieties of broccoli

As shown in Fig. 2, there were great differences in total glucosinolate content in flowers and leaves of different broccoli varieties. As for broccoli flowers, Zhonglu 1 had the highest total glucosinolate content (23.53μmol · g^{-1} DW), followed by Gongxianzhe (23.03 μmol · g^{-1} DW); Dadi had the lowest content (9.19 μmol · g^{-1} DW), only 39% of that of Zhonglu 1. As for broccoli leaves, Zhonglu 9 had the highest total glucosinolate content (10.11 μmol · g^{-1} DW), followed by Zhonglu 5 (9.86 μmol · g^{-1} DW); Nanxiu 366 had the lowest content (1.93 μmol · g^{-1} DW). Overall, the glucosinolate content in broccoli flowers was 1 – 5 times that in broccoli leaves and there were differences between various varieties.

3.3 Analysis of composition of glucosinolates in different broccoli varieties Currently, more than 120 kinds of glucosinolates have been isolated and identified from nature, and different cruciferous plants have different composition of glucosinolates. 15 – 20 kinds of glucosinolates are found in Brassica vegetables[12]. It has been reported that there are 16 kinds of glucosinolates in broccoli[5]. This experiment determined the composition of glucosinolates in flowers and leaves of 12 broccoli varieties, and a total of 9 kinds of glucosinolates were detected (Table 3, 4). The 9 kinds of glucosinolates belonged to fats and indoles, respectively, and aromatic glucosinolates were not detected. As shown in Table 3, in 9 kinds of glucosinolates, RAA was the glucosinolate component with the highest content in broccoli flowers, accounting for 20.14 – 69.42% of total content. For Nanxiu 366, the total glucosinolate content was only 13.31 μmol · g^{-1} DW, but RAA had the content of 9.24 μmol · g^{-1} DW, with the largest proportion in all varieties. For Yelu, the total glucosinolate content was 21.55 μmol · g^{-1} DW, but RAA had the content of 4.34 μmol · g^{-1} DW, accounting for 20.14% of total content. The results showed that different cultivars of broccoli affected the percentage of RAA glucosinolates in total glucosinolates, which was consistent with the detection results of Schonhof *et al.* [13]. The studies of Abercrombie *et al.* found that in the diallel cross population formed by double haploid parent for two consecutive years, the RAA content exhibited significant conventional genetic binding force, but there was no significant specific genetic binding force, and the mean square of conventional genetic binding force was 14 times that of the specific genetic binding force, indicating that the conventional binding force was more important than the specific binding force in predicting RAA content, so the combination of a given selfed seed with other selfed seeds could produce the hybrids whose RAA content could be predicted[14]. The broccoli flower was the main edible part, so the potential health care effect of broccoli depended mainly on the cultivars selected. As shown in Table 4, RAA had the largest share (9.33 – 56.57%) in the composition of glucosinolates in broccoli leaves. Zhonglu 6 had total glucosinolate content of 9.25 μmol · g^{-1} DW and RAA content of 4.76 μmol · g^{-1} DW; Nanxiu 366 had the lowest total glucosinolate content, only 1.93 μmol · g^{-1} DW, and the RAA proportion was also smallest. The use of leaves was different from that of flowers, and leaves were by-products of broccoli production, and could be re-used as feed. Studies showed that the glucosinolates containing β-OH could be degraded into two products that caused goiter: isothiocyanate and oxazolidin-2-thiophenyl; they harmed human thyroid in different ways[3–4]. PRO was the main glucosinolate component having adverse effect on nutrition[15]. As seen from Table. 4, PRO accounted for 11.89% – 42.83% of total glucosinolate content, and Xingyun had the PRO content of 0.88 μmol · g^{-1} DW and total glucosinolate content of 7.4 μmol · g^{-1} DW. In this regard, the leaves of Xingyun were most suitable for feed processing. Broccoli was one of the cruciferous vegetables with the richest content of 4-methylthio-3-butenyl glucosinolate (Glucoraphanin, RAA)[16]. RAA was the precursor of sulforaphane, and sulforaphane was proved to be the intensest phase II enzyme inducer ever found, which could reduce the incidence of esophageal cancer, colon cancer and breast cancer[2–5, 17–19]. The anticancer efficacy of homologous isothiocyanates of other glucosinolates was also reported, for example, the hydrolyzate indole-3-methanol of 3-indolyl glucosinolate (Glucobrassicin, GBC) could modulate the activity of biotransformation enzymes, thereby inhibiting the activity of breast cancer and prostate cancer[20–21]. The differences in content of glucosinolates in different vegetables and varieties could be attributed to different genotype[22], and it was affected by environmental factors. Farnham studied the glucosinolate content of 9 kinds of broccoli with different genotypes under three different conditions, and found the effect of environment on glucosinolate content. RAA was the only glucosinolate significantly affected by genotypes, among the factors affecting RAA content, the genotype effects accounted for 52.8%, and genotype and environment interaction effect was less than genotype effect[10]. In this experiment, all varieties were grown in the same environment, and the cultivation conditions were basically the same, so the difference of glucosinolate content mainly depended on genetic difference of the variety itself.

Table 3 Composition and total content of glucosinolates in flowers of different broccoli varieties

Variety	Aliphatic glucosinolates // μmol · g^{-1} DW					Indolyl glucosinolates // μmol · g^{-1} DW				Total content
	RAA (share in total glucosinolates // %)	IBE	PRO	NAP	SIN	NEO	4ME	4OH	GBC	
Zhonglu 1	13.62(57.88)	0.05	0.01	0.71	0.01	4.43	0.35	0.3	4.05	23.53
Gongxianzhe	12.22(53.06)	3.17	1.82	0.35	0.44	1.73	0.26	0.45	2.59	23.03
Youya	7.57(34.04)	0.90	2.01	0.29	0.26	8.20	0.46	0.15	2.40	22.24
Yelu	4.34(20.14)	0.01	0.08	0.78	0.01	11.83	0.91	0.28	3.31	21.55
Zhonglu 6	10.46(49.64)	1.40	0.13	0.31	–	4.25	0.35	0.22	3.95	21.07
Lusen	8.82(44.37)	3.23	0.36	0.38	–	3.59	0.21	0.19	3.10	19.88
Zhonglu 9	6.75(35.45)	0.87	0.86	0.61	0.34	5.22	0.33	0.07	3.99	19.04
Zhonglu 5	6.64(45.45)	1.00	0.01	0.40	–	3.07	0.26	0.13	3.10	14.61
Nanxiu 366	9.24(69.42)	0.08	0.17	0.63	–	1.13	0.24	0.29	1.53	13.31
Sali 3	5.35(43.04)	1.36	1.27	0.73	0.38	1.58	0.48	0.15	1.13	12.43
Xingyun	3.64(37.41)	0.73	–	0.35	0.01	2.87	0.20	0.05	1.88	9.73
Dadi	4.67(50.82)	0.74	0.54	1.19	0.19	0.81	0.13	0.03	0.89	9.19

Note: The values in brackets indicate the percentage of one kind of glucosinolate in total glucosinolates, the same in the following table.

Table 4 Composition and total content of glucosinolates in leaves of different broccoli varieties

Variety	Aliphatic glucosinolates // μmol · g⁻¹ DW					Indolyl glucosinolates // μmol · g⁻¹ DW				Total content
	RAA (share in total glucosinolates // %)	IBE	PRO (share in total glucosinolates // %)	NAP	SIN	NEO	4ME	4OH	GBC	
Zhonglu 9	3.00(26.70)	3.27	2.13(21.07)	0.50	0.54	0.41	0.06	0.10	0.10	10.11
Zhonglu 5	4.28(43.41)	2.05	1.95(19.78)	0.33	0.74	0.35	0.10	0.06	–	9.86
Zhonglu 6	4.76(51.46)	1.85	1.13(12.22)	0.53	0.76	0.17	0.01	0.03	0.01	9.25
Xingyun	3.23(43.65)	1.30	0.88(11.89)	0.80	0.89	0.25	0.01	0.03	0.01	7.40
Sali 3	1.34(21.04)	1.50	2.14(33.59)	0.76	0.24	0.25	0.08	0.01	0.05	6.37
Gongxianzhe	1.6(26.71)	1.78	1.42(23.71)	0.42	0.29	0.35	0.02	0.08	0.03	5.99
Yelu	0.86(15.58)	1.89	1.65(29.89)	0.40	0.01	0.60	0.05	0.05	0.01	5.52
Lusen	1.88(37.08)	0.66	0.73(14.40)	0.35	1.09	0.18	0.13	0.04	0.01	5.07
Zhonglu 1	2.80(56.57)	0.73	0.78(15.76)	0.33	0.02	0.21	0.04	0.03	0.01	4.95
Youya	0.73(16.11)	0.82	1.94(42.83)	0.63	0.08	0.24	0.08	0.01	–	4.53
Dadi	0.32(13.06)	0.57	0.73(29.80)	0.62	0.06	0.13	0.02	–	–	2.45
Nanxiu 366	0.18(9.33)	0.32	0.51(26.42)	0.65	0.01	0.12	0.01	0.12	0.01	1.93

4 Conclusions

The results of this study showed that there were 9 glucosinolates in broccoli, namely Glucoiberin (IBE); Progoitrin (PRO); Sinigrin (SIN); Glucoraphanin (RAA); Gluconapin (NAP); 4-Hydroxyglucobrassicin (4OH); Glucobrassicin (GBC); 4-Methoxyglucobrassicin (4ME); Neoglucobrassicin (NEO). These glucosinolates belonged to indoles and fats, and the aromatic glucosinolates were not detected. RAA was the main glucosinolate in broccoli. The flowers of Nanxiu 366 had the highest RAA content while the leaves of Nanxiu 366 had the lowest RAA content, with potential application prospect.

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