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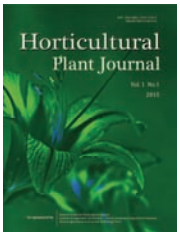

Glucosinolates in Self-crossed Progenies of Monosomic Cabbage Alien Addition Lines in Chinese Cabbage

GU Aixia, ZHAO Jianjun, WANG Yanhua, LI Xiaofeng, XUAN Shuxin, SHEN Shuxing*

Key Laboratory of Vegetable Germplasm Innovation and Utilization of Hebei, College of Horticulture, Agricultural University of Hebei, Baoding 071001, China

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Abstract

Brassica species have been reported to possess cancer preventive activity due to glucosinolates (GLS) and their derived properties. Many studies on GLS have focused on *Brassica oleracea* and *Brassica rapa*. However, information on GLS in progeny between Chinese cabbage (*B. rapa* ssp. *pekinensis*) and cabbage (*B. oleracea* var. *capitata*) remains limited. In this study, eight GLS were detected in the self-crossed progenies of monosomic cabbage alien addition lines in Chinese cabbage (Chinese cabbage – cabbage MAALs) and parental Chinese cabbage, and nine GLS were detected in the parental cabbage. The variation of GLS content ranges was greater in the progeny than in the parental Chinese cabbage. The nine GLS identified were subjected to PCA to evaluate the differences among progeny and parents. Eight progeny samples had a comprehensive principal component score closer to or greater than that of cabbage, and four of them exhibited glucoraphanin (GRA) and total GLS contents greater than that of Chinese cabbage with the relative content of total indolic GLS was greater than 50%. These results offered new opportunity to improve GLS-containing of Chinese cabbage using genes from cabbage.

Keywords: Chinese cabbage; cabbage; glucosinolate; monosomic alien addition lines

1. Introduction

GLS are important plant secondary metabolites that mainly exist in Cruciferae (Fahey et al., 2001), and their breakdown products effect on plant defense against pests, human healthy, vegetable flavor. Many studies have demonstrated that some breakdown products derived from GLS induce phase Ⅱ detoxification enzyme activity increasing the body's cancer defense mechanisms and even act as anticarcinogens (Mithen et al., 2003; Keum et al., 2004; Brew et al., 2009). Therefore, GLS have recently attracted intense research interest.

Brassica vegetables, the main edible members of Cruciferae, have naturally occurring GLS in edible structures, which have been monitored. Many studies on GLS have been performed, especially in *B. rapa* and *B. oleracea* (Cartea et al., 2008; Jia et al., 2009; Kim et al., 2010; Sun et al., 2011). Genetic and environmental factors, variation in GLS types and concentration among plant organs have been reported in *Brassica* vegetables (Sang et al., 1984; Clossais-Besnard, 1991). However, genotypic effects outweigh environmental effects in variation GLS (Kang et al., 2006; Chen et al., 2008).

* Corresponding author.
E-mail address: shensx@hebau.edu.cn

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With the increasing interest in human diet and health, the most promising varieties for future breeding purposes are those with high contents of beneficial GLS. Chen et al. (2008) reported that the total GLS content in Chinese cabbage ranges from 0.14 to 0.35 μmol · g^{-1} fresh weight (FW), and Cartea et al. (2008) reported that the total GLS content in cabbage ranges from 10.9 to 27.0 μmol · g^{-1} dry weight (DW). Many previous studies have also demonstrated that the total GLS content in cabbage is greater than that in Chinese cabbage. In order to transfer the characteristics of higher GLS contents into Chinese cabbage from cabbage, a series of Chinese cabbage – cabbage MAALs have been derived from backcrossing an allotriploid hybrid which was produced by crossing tetraploid Chinese cabbage and diploid cabbage with diploid Chinese cabbage (Gu et al., 2006, 2009a, 2009b), and MAALs have been further self-crossed. The added cabbage chromosomes in the Chinese cabbage MAALs include nine different chromosomes from cabbage. The self-crossed progeny of MAALs were used in this study, and the parent Chinese cabbage and cabbage were used as controls. The GLS contents were analyzed by high-performance liquid chroma-tography (HPLC) and evaluated by principle component analysis (PCA). The variation of GLS content ranges was greater in the progeny than in the parental Chinese cabbage. Eleven samples with high content of beneficial GLS or close to or higher than the comprehensive principal component scores of cabbage were obtained. These results will lay the foundation to breed new Chinese cabbage variety aimed at GLS and will provide support to reveal the Chinese cabbage synthesis mechanism of glucosinolate.

2. Materials and methods

2.1. Material

Sixty three self-crossed progeny of Chinese cabbage – cabbage MAALs added individual chromosome from cabbage (Table 1), MAALs parents that were inbred Chinese cabbage and inbred cabbage.

2.2. Separation and desulphation of GLS

For each leaf sample, 200 mg of freeze-dried powder was placed in a 15 mL plastic tube containing 0.25 mL of glucotropaeolin (TRO), and preheated 100% methanol was then added. The samples were incubated in an 80 ℃ water bath for 20 min. After centrifugation at 3 000 r · min^{-1} for 10 min, the supernatants were collected and put into 15 mL plastic tubes, and the precipitate was extracted twice with 70% methanol, and the three supernatants were combined as 1 sample. This procedure was repeated 3 times for each specimen.

A Sephadex column was prepared as follows: glass wool was placed in a disposable syringe, which was then tightly plugged and placed on a tube, and 2 mL of activated DEAE Sephadex A25 was then added. The column was washed with 2 mL of ultra-pure water, and 2 mL of the sample solution was then added. When the sample solution stopped dripping off the column, 0.02 mol · L^{-1} sodium acetate was added. After the liquid was no longer dripping, the syringe was transferred to another tube. Desulfation was carried out by the addition of 75 μL of sulfatase, and the tube was then sealed and incubated overnight. The desulfated GLS were eluted with 1.5 mL of ultra-pure water, and the eluates were then filtered through a 0.45 μm filter membrane. The eluates were analyzed immediately by HPLC or stored at – 20 ℃ until analyzed.

2.3. Desulfo GLS analysis by HPLC

HPLC analysis was performed at room temperature on a Nova-Pak® with a C18 column (150 mm × 3.9 mm; 50 μm) with the following conditions: UV–Visible detector wavelength of 229 nm, flow rate of 1.0 mL · min^{-1} and injection volume of 20 μL. The elution buffers consisted of Buffer A (1 g of tetramethylammonium chloride was dissolved in 2 L of ultra-pure water, mixed and filtered by pumping filtration) and Buffer B (1 g of tetramethylammonium chloride was dissolved in 1.6 L of ultra-pure water followed by the addition of 400 mL of chromatographically pure acetonitrile, and the solution was mixed and filtered by pumping filtration). The following elution program was applied: 0 min, 100% A/0% B; 1 min, 100% A/0% B; 21 min, 0% A/100% B; 26 min, 100% A/0% B; and 31 min, 100% A/0% B.

Table 1 The Chinese cabbage – cabbage MAALs and its self-crossed progeny

Self-crossed progeny of Chinese cabbage – cabbage MAALs	Chinese cabbage – cabbage MAALs	Self-crossed progeny of Chinese cabbage – cabbage MAALs	Chinese cabbage – cabbage MAALs
1, 2, 11, 12, 13, 35, 36	D4	9, 10, 14, 44, 50, 51	D12
29, 30, 31, 40, 54, 55, 56	b17	20, 21, 39, 45, 46, 47, 67	D7
5, 6, 15, 23, 24, 25, 26	05①-6-2	4, 7, 8, 32, 33, 34, 42, 52	d51
27, 28, 41, 48, 49, 65, 66	d26	18, 19, 22, 43, 53, 57, 58	S6
3, 59, 60, 61, 62, 63, 64	B-1-5-2		

2.4. Principal component analysis (PCA)

The GLS content was analyzed using PCA, which was performed using the SPSS 16.0 software package.

3. Results

In this study, eight types of GLS were detected in the self-crossed progeny of Chinese cabbage – cabbage MAALs and parental Chinese cabbage leaves, and nine types of GLS were detected in the parental cabbage leaves. These GLS belonged to the aliphatic and indolic groups but not the aromatic group. Four aliphatic GLS, including progoitrin (PRO), glucoraphanin (GRA), gluconapin (NAP) and glucobrassicanapin (GBN), were detected in Chinese cabbage, cabbage and progeny. Sinigrin (SIN) was only detected in cabbage (Fig. 1). Four indole GLS, including 4-hydroxyglucobrassicin (4OH), glucobrassicin (GBC), 4-methoxyglucobrassicin (4ME) and neoglucobrassicin (NEO), were detected in all of the tested samples.

3.1. GLS in parental Chinese cabbage

The mean total glucosinolate content in the Chinese cabbage was 3.82 μmol · g^{-1}DW. Aliphatic GLS represented 56.54% of the total GLS content. The contents of individual GLS ranged from 0 to 1.49 μmol · g^{-1}DW in the Chinese cabbage. The PRO content was the highest with a mean value of 1.46 μmol · g^{-1}DW, and the relative content was predominant, representing 38.22%. The NEO content was the lowest detectable GLS species (0.07 μmol · g^{-1}DW; relative content only 1.83%), and SIN was not detected at all (Table 2).

3.2. GLS in parental cabbage

The mean total GLS content in the parental cabbage was 11.68 μmol · g^{-1}DW. Aliphatic GLS represented 60.19% of total GLS. The individual GLS content in the cabbage ranged from 0.01 to 6.49 μmol · g^{-1}DW. The predominant GLS was SIN (5.98 μmol · g^{-1}DW), which represented 51.20% of total GLS (Table 2).

3.3. GLS in the self-crossed progeny of Chinese cabbage – cabbage MAALs

In the self-crossed progeny of Chinese cabbage – cabbage MAALs (Table 2), the total GLS contents ranged from 1.02 to 11.07 μmol · g^{-1}DW with a mean value of 4.13 μmol · g^{-1}DW. The average percent of aliphatic GLS in total GLS was 56.17%. There were significant differences in the content of individual GLS among progeny, for example, the content of GBN ranged

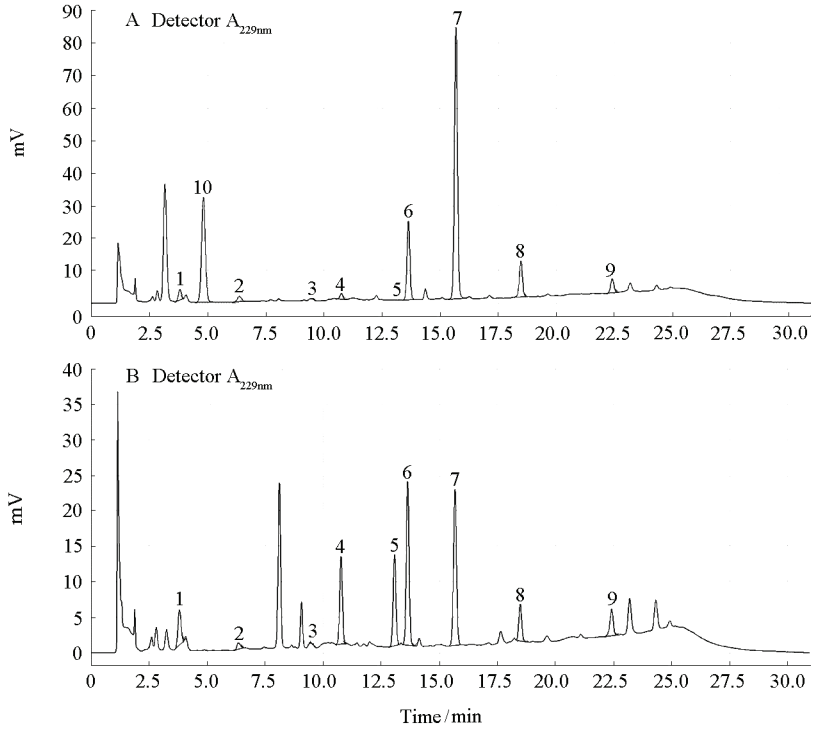

Fig. 1 HPLC chromatogram of glucosinolates

A: Cabbage; B: One self-crossed progeny of Chinese cabbage – cabbage MAAL.

1. PRO; 2. GRA; 3. NAP; 4. 4OH; 5. GBN; 6. TRO; 7. GBC; 8. 4ME; 9. NEO; 10. SIN

Table 2 Composition and content of glucosinolates in parental Chinese cabbage, cabbage and self-crossed progeny of Chinese cabbage – cabbage MAALs

Name	Chinese cabbage			Cabbage			Self-crossed progeny		
	Percent/%	Mean	Range	Percent/%	Mean	Range	Percent/%	Mean	Range
Total glucosinolates		3.82 ± 0.24	3.59 – 4.06		11.68 ± 1.26	10.43 – 12.95		4.13	1.02 – 11.07
Aliphatic	56.54			60.19			56.17		
PRO	38.22	1.46 ± 0.03	1.43 – 1.49	5.39	0.63 ± 0.02	0.61 – 0.65	27.60	1.14	0.11 – 2.77
GRA	2.09	0.08 ± 0.04	0.04 – 0.13	2.57	0.30 ± 0.01	0.29 – 0.31	2.42	0.10	0.02 – 0.76
NAP	3.66	0.14 ± 0.06	0.09 – 0.20	0.94	0.11 ± 0.02	0.09 – 0.13	4.60	0.19	0.02 – 0.88
GBN	12.57	0.48 ± 0.02	0.46 – 0.50	0.09	0.01 ± 0.00	0.01	21.55	0.89	0.02 – 6.25
SIN	0	0	0	51.20	5.98 ± 0.51	5.47 – 6.49	0	0	0
Indolic	43.46			39.81			43.83		
4OH	7.33	0.28 ± 0.01	0.27 – 0.29	0.51	0.06 ± 0.01	0.06 – 0.07	4.36	0.18	0.00 – 1.06
GBC	18.85	0.72 ± 0.11	0.61 – 0.83	34.76	4.06 ± 0.74	3.32 – 4.80	19.37	0.80	0.06 – 2.54
4ME	15.45	0.59 ± 0.08	0.52 – 0.67	3.17	0.37 ± 0.01	0.36 – 0.38	16.95	0.70	0.15 – 1.57
NEO	1.83	0.07 ± 0.03	0.04 – 0.10	1.37	0.16 ± 0.04	0.13 – 0.20	3.15	0.13	0.01 – 2.62

from 0.02 to 6.25 μmol · g^{-1}DW. The PRO content was also the highest ranging from 0.11 to 2.77 μmol · g^{-1} DW with a mean value of 1.14 μmol · g^{-1}DW. However, the relative content of PRO (27.60%) in the progeny was lower than that in Chinese cabbage, and the relative GRA, NAP, GBN, GBC, 4ME and NEO contents were higher to different degrees than that in Chinese cabbage (Table 2).

The breakdown products of GRA, SIN and indole GLS have been suggested to have beneficial effects on human health (Mithen et al., 2000, 2003; Fahey et al., 2001; Farnham et al., 2004). Considering these beneficial effects, Chinese cabbage, which contains high levels of GRA, SIN and indole GLS, should be of high dietary value (Kim et al., 2010). Sulforaphen, which is an isothiocyanate derived from GRA and thought to be the most promising anticancer compound, inhibits phase Ⅰ enzymes responsible for the activation of carcinogens and induces phaseⅡ, detoxification enzyme systems, thereby increasing the cancer defense mechanisms of the body. In the present study, the mean GRA content was 0.08 μmol · g^{-1}DW in the parental Chinese cabbage and 0.30 μmol · g^{-1}DW in the parental cabbage. Moreover, the GRA content ranged from 0.02 to 0.76 μmol · g^{-1}DW in the self-crossed progeny of the Chinese cabbage – cabbage MAALs. Fourteen progeny samples had GRA contents greater than 0.13 μmol · g^{-1} DW that was the highest content in parental Chinese cabbage. SIN were not detected in the parental Chinese cabbage and self-crossed progeny, and the mean SIN content in the parental cabbage was 5.98 μmol · g^{-1}DW. These results may have been caused by low homology between the genes related to the dynamic accumulation of SIN in cabbage and Chinese cabbage, thereby resulting in a low rate of relevant genes being transferred to progeny. In the self-crossed progeny of the Chinese cabbage – cabbage MAALs, there were 27 samples in which the total GLS contents were more than the highest level in the parental Chinese cabbage (4.06 μmol · g^{-1}DW) with the highest content being 11.07 μmol · g^{-1}DW, which was close to the mean total GLS content of 11.68 μmol · g^{-1}DW in the parental cabbage. Of these 27 samples, 26 of them exhibited a relative total indolic GLS content greater than 50% (the mean relative content of total indolic GLS in the Chinese cabbage was 43.46%)(Table 2). The samples in which the GRA and total GLS contents were more than that of Chinese cabbage with relative total indolic GLS contents more than 50% were respectively 2, 5, 14, 15, 28, 45 and 48 (Table 3).

Table 3 Composition and content concentration of glucosinolates in selected self-crossed progeny of Chinese cabbage – cabbage MAALs

Sample	Aliphatic glucosinolates				Indolic glucosinolates			
	PRO	GRA	NAP	GBN	4OH	GBC	4ME	NEO
2	1.05 ± 0.01	0.16 ± 0.00	0.15 ± 0.00	1.61 ± 0.01	0.46 ± 0.02	0.93 ± 0.02	0.18 ± 0.00	0.12 ± 0.00
5	1.74 ± 0.01	0.17 ± 0.00	0.06 ± 0.00	6.25 ± 0.05	0.06 ± 0.00	2.13 ± 0.01	0.56 ± 0.00	0.11 ± 0.00
14	1.23 ± 0.00	0.16 ± 0.01	0.16 ± 0.01	1.09 ± 0.00	0.01 ± 0.00	1.64 ± 0.01	0.30 ± 0.00	0.21 ± 0.01
15	1.67 ± 0.03	0.16 ± 0.00	0.11 ± 0.00	1.47 ± 0.02	0.05 ± 0.00	0.57 ± 0.00	0.39 ± 0.01	0.20 ± 0.00
28	0.64 ± 0.00	0.15 ± 0.01	0.02 ± 0.00	0.13 ± 0.00	1.06 ± 0.01	0.62 ± 0.01	0.63 ± 0.01	2.62 ± 0.04
41	1.57 ± 0.02	0.09 ± 0.00	0.07 ± 0.00	0.23 ± 0.01	0.39 ± 0.00	1.53 ± 0.01	0.96 ± 0.00	0.13 ± 0.01
45	1.85 ± 0.00	0.20 ± 0.01	0.20 ± 0.01	0.93 ± 0.01	0.77 ± 0.01	1.62 ± 0.02	0.82 ± 0.01	0.07 ± 0.00
47	1.69 ± 0.01	0.05 ± 0.00	0.88 ± 0.01	0.99 ± 0.00	0.62 ± 0.00	1.70 ± 0.01	1.34 ± 0.01	0.10 ± 0.00
48	2.28 ± 0.02	0.76 ± 0.02	0.16 ± 0.01	1.05 ± 0.02	0.18 ± 0.00	0.57 ± 0.00	1.57 ± 0.03	0.37 ± 0.01
49	2.59 ± 0.01	0.11 ± 0.01	0.07 ± 0.00	0.38 ± 0.01	0.11 ± 0.00	2.54 ± 0.02	1.40 ± 0.00	0.10 ± 0.00
67	1.93 ± 0.01	0.02 ± 0.00	0.11 ± 0.00	1.26 ± 0.00	0.87 ± 0.01	1.17 ± 0.01	0.37 ± 0.00	0.04 ± 0.00

3.4. GLS content was analyzed by PCA

PCA can serve to align, visualize and differentiate the components in large data sets, and PCA allows easy visualization of complex data. The data obtained for the nine types of GLS detected were subjected to PCA to outline the GLS profile differences among samples. The PCA revealed that four of the principal component eigenvalues were more than one that is usually as extraction level of principal component. The corresponding loadings of individual GLS in the four principal components resulted in the following trends: SIN, GRA and GBC ranked higher in the first principal component; PRO, GBN and 4OH ranked higher in the second principal component; NEO and 4OH ranked higher in the third principal component; and GRA, NAP and 4ME ranked higher in the fourth principal component (Table 4).

Table 4 The principal component analysis (PCA) of glucosinolates

Glucosinolates	Principal component			
	1	2	3	4
PRO	0.383	0.706	−0.281	0.144
GRA	0.550	−0.178	0.089	0.429
NAP	−0.338	−0.248	0.048	0.545
4OH	0.215	0.497	0.663	0.012
GBN	0.311	0.545	−0.393	−0.345
GBC	0.857	−0.200	−0.172	−0.023
4ME	0.054	0.306	−0.342	0.725
NEO	0.276	0.235	0.788	0.097
SIN	0.708	−0.626	0.006	−0.068
Pecent of variance	22.444	19.070	16.124	12.901
Cumulative pecent/%	22.444	41.514	57.639	70.540

These results also suggested that the 4 principal components can reflect information regarding all individual GLS. The four principal component expressions were derived such that the eigenvector must be multiplied by the corresponding individual GLS concentration. The proportions of every principal component eigenvalue to the sum of principal component eigenvalues were as weight to further obtain the comprehensive principal component expression (F) as follows: $F = 0.20X_1$ (PRO) + $0.18X_2$ (GRA) − $0.03X_3$ (NAP) + $0.28X_4$ (4OH) + $0.05X_5$ (GBN) + $0.24X_6$ (GBC) + 0.13 X_7(4ME) + $0.27X_8$ (NEO) + $0.02X_9$ (SIN); The self-crossed progeny of the Chinese cabbage − cabbage MAALs, parental Chinese cabbage and cabbage were analyzed by comprehensive principal component. The comprehensive principal component scores of the Chinese cabbage and most of the progeny ranged from − 1.0 to 0.5, and the comprehensive principal component scores of the cabbage ranged from 0.70 to 1.20. The progeny with comprehensive principal component scores ranging from 0.68 to 1.38 were respectively 41 (0.68), 5 (0.75), 67 (0.92), 47 (1.00), 49 (1.14), and 45 (1.38). Sample 28 had the maximum comprehensive principal component score of 3.09, and sample 48 had the second highest comprehensive principal component score of 1.98 (Fig. 2). The contents of individual GLS in the 8 aforementioned samples are shown in Table 3.

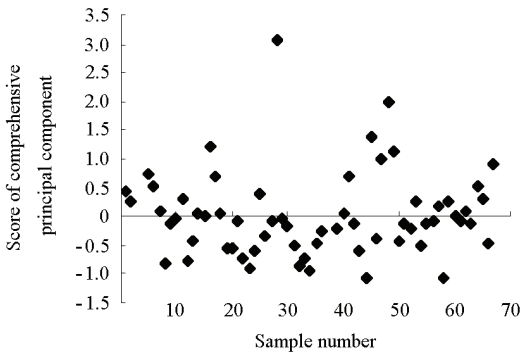

Fig. 2 The comprehensive principal component scores of the PCA

4. Discussion

Chromosome engineering can be used to transfer beneficial genes from related species to cultivated species to genetically improve cultivated species, and this process has been widely performed in wheat (Kuraparthy et al., 2007; Ren et al., 2009; Zhao et al., 2010). Genes from cabbage could be transferred into Chinese cabbage by chromosome engineering due to the close relationship between the two plants (Lilivelt et al., 1993). The GLS contents in the self-cross progeny of Chinese cabbage − cabbage MAALs showed a wide range of variation, which offered an opportunity to screen GLS-containing materials that meet the breeding aims, and preliminarily confirmed the feasibility of transferring beneficial characteristics from cabbage into Chinese cabbage. In this study, the selected plants from the self-cross progeny of the Chinese cabbage − cabbage MAALs exhibited higher GRA and total GLS contents than that in the Chinese cabbage. Moreover, the relative content of total indolic GLS of the selected self-cross progeny was greater than 50%, and these selected progeny had higher comprehensive principle component scores. These selected progeny lines will be further screened to obtain genetically stable Chinese cabbage new germplasm with a high content of beneficial GLS, and study of related genes GLS synthesis. The ranges of variation of both individual and total GLS contents were greater in the self-cross progeny, which may be caused by added new alien genes and dose-response of homologous genes. However, which genes were played important role in influencing the specific individual GLS content needs further study. There are 52 GLS biosynthetic genes in *Arabidopsis*, to 44 of them, there are 102 homologous

genes in Chinese cabbage (Wang, 2011; Wang et al., 2011). Compared with *Arabidopsis*, multi-copy of GLS biosynthetic genes in *B. rapa* improved the variation range of GLS content (Wang, 2011). Li et al. (2008) reported that gene replication may lead to functional redundancy of a gene in biological systems as well as provide a potential basis for specific quantitative trait variation.

These selected samples which had a comprehensive principal component score closer to or greater than that of cabbage were 5, 28, 41, 45, 47, 48, 49, 67. Samples 28, 41, 48 and 49 were from the monosomic alien addition line d26 (2n = 21). Samples 45, 47 and 67 were from the monosomic alien addition line D7 (2n = 21). Sample 5 was from the monosomic alien addition line 05①-6-2 (2n = 21). The d26, D7 and 05①-6-2 were the lines in which Chinese cabbage had added one different chromosome from cabbage. Thus, we inferred that the 3 chromosomes from cabbage included the main genes that participated in GLS synthesis. Wang (2011) reported that the 8 QTLs controlling GLS accumulation were distributed in 4 linkage groups using 3 population of *B. rapa* as materials, with major QTLs on linkage group A03 comprising 5 QTLs. Chromosome segment substitution lines (CSSLs) are the excellent materials to detection and fine mapping of quantitative trait loci (QTLs) for target trait (Yano et al., 2001; Kubo et al., 2002; Ebitani et al., 2005), which can be used for the detection of QTLs with small additive effects that are masked by QTLs with larger effects in primary populations such as F_2 populations and recombinant inbred lines (Takai et al., 2007). So far, a number of QTLs for biological and economic traits have been detected through the use of CSSLs (Mei et al., 2001; Kubo et al., 2002; Ando et al., 2004; Ebitani et al., 2005; Ishinaru et al., 2005; Salem et al., 2012). The self-cross progeny from the Chinese cabbage – cabbage MAALs will be further back-crossed and self-crossed through generations to development of CSSLs, which will be innovate way to fine mapping of QTLs for GLS.

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References

Ando, T., Shimizu, T., Shomura, A., Takeuchi, Y., Lin, S.Y., Yano, M., 2004. Mapping of QTLs controlling panicle structure using chromosome segment substitution lines of rice. Breeding Res, 6: 104.

Brew, C.T., Aronchik, I., Kosco, K., McCammon, J., Bjeldanes, L.F., Firestone, G.L., 2009. Indole-3-carbinol inhibits MDA-MB-231 breast cancer cell motility and induces stress fibers and focal adhesion formation by activation of Rho kinase activity. Int J Can, 124: 2294–2302.

Cartea, M.E., Velasco, P., Obergón, S., Padilla, G., de Haro, A., 2008. Seasonal variation in glucosnolate content in *Brassica oleracea* crops grown in northwestern Spain. Phytochemistry, 69: 403–410.

Chen, X.J., Zhu, Z.J., Gerendás, J., Zimmermann, N., 2008. Glucosinolates in Chinese *Brassica campestris* vegetables: Chinese cabbage, purple cai-tai, choysum, pakchoi, and turnip. HortScience, 43: 571–574.

Clossais-Besnard, N.L.F., 1991. Physiological role of glucosinolates in *Brassica napus*. concentration and distribution pattern of glucosinolates among plant organs during a complete life cycle. J Sci Food Agric, 56: 25–38.

Ebitani, T., Takeuchi, Y., Nonoue, Y., Yamamoto, T., Takeuchi, K., Yano, M., 2005. Construction and evaluation of chromosome segment substitution lines carrying overlapping chromosome segments of *indica* rice cultivar 'Kasalath' in genetic background of *Japonica* elite cultivar 'Koshihikari'. Breed Sci, 55: 65–73.

Fahey, J.W., Fahey, A.T., Talalay, P., 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry, 56: 5–51.

Farnham, M.W., Wilson, P.E., Stephenson, K.K., Fahey, J.W., 2004. Genetic and environmental effects on glucosinolate content and chemoprotective potency of broccoli. Plant Breed, 123: 60–65.

Gu, A.X., Shen, S.X., Chen, X.P., Zhang, C.H., Li, X.F., 2006. Allotriploid hybrids obtained from interspecific hybridization between Chinese cabbage and cabbage and the preliminary research on reproductive characters. Acta Hortic Sin, 33: 73–77.

Gu, A.X., Wang, J., Chen, X.P., Luo, S.X., Wang, Y.H., Shen, S.X., 2009a. Identification of Chinese cabbage – cabbage double alien monosomic addition line by SSR and studies on its characters. Sci Agric Sin, 42: 1698–1704.

Gu, A.X., Zheng, B.Z., Wang, Y.H., Xuan, S.X., Luo, S.X., Shen, S.X., 2009b. Obtaining and studies of Chinese cabbage monosomic alien addition line with Chromosome 3 of cabbage. Acta Hortic Sin, 36: 39–44.

Ishimaru, K., Kashiwagi, T., Hirotsu, N., Madoka, Y., 2005. Identification and physiological analysis of a locus for rice yield potential across the genetic background. J Exp Bot, 56: 2745–2753.

Jia, C.G., Xu, C.J., Wei, J., Yuan, J., Yuan, G.F., Wang, B.L., Wang, Q.M., 2009. Effect of modified atmosphere packaging on visual quality and glucosinolates of broccoli florets. Food Chem, 114: 28–37.

Kang, J., Ibrahim, K., Juvik, J., Kim, D., Kang, W., 2006. Genetic and environmental variation of glucosinolate content in Chinese cabbage. HortScience, 41: 1382–1385.

Keum, Y.S., Jeong, W.S., Kong, A.N.T., 2004. Chemoprevention by isothiocyanates and their underlying molecular signaling mechanisms. Mutat Res, 555: 191–202.

Kim, J.K., Chu, S.M., Kim, S.J., Lee,D.J, Lee, S.Y., Lim, S.H., Ha, S., Kweon, S.J., Cho, H.S., 2010. Variation of glucosinolates in vegetale crops of *Brassica rapa* L. ssp. *pekinensis*. Food Chem, 119: 423–428.

Kubo, T., Aida Y., Nakamura, Tsunematsu H., Doi K., Yoshimura, A., 2002. Reciprocal chromosome segment substitution series derived from *Japonica* and *Indica* cross of rice (*Oryza sativa* L.). Breeding Sci, 52: 319–325.

Kuraparthy, V., Chhuneja, P., Dhaliwal, H.S., Kaur, S., Bowden, R.L., Gill B.S., 2007. Characterization and mapping of cryptic alien introgression from *Aegilops geniculata* with new leaf rust and stripe rust resistance genes *Lr57* and *Yr40* in wheat. Theor and Appl Genet, 114: 1379–1389.

Li, J., Hansen, B.G., Ober, J.A., Kliebenstein, D.J., Halkier, B.A., 2008. Subclade of flavin-monooxygenases involved in aliphatic glucosinolate biosynthesis. Plant Physiol, 148: 1721–1733.

Lilivelt, C., Lange, W., Dolstra O., 1993. Intergeneric crosses for the transfer of resistance to beet cyst nematode from *Rahanus sativus* to *Brassica napus*. Euphytica, 68: 111–120.

Mei, H.W., Xu, J.L., Li, Z.K., Yu, Q., Guo, L.B., Wang, Y.P., Ying, C.S., Luo, L.J., 2001. QTLs influencing panicle size detected in two reciprocal introgressive line (IL) populations in rice (*Oryza sativa* L.). Theor Appl Genet, 112: 648–656.

Mithen, R.F., Dekker, M., Verkerk, R., Rabot, S., Johnson, I.T., 2000. Review: the nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. J Sci Food Agric, 80: 967–984.

Mithen, R., Faulkner, K., Magrath, R., Rose, P., Williamson, G., Marquez, J., 2003. Development of isothiocyanate-enriched broccoli, and its enhanced ability to induce phase 2 detoxification enzymes in mammalian cells. Theor Appl Genet, 106: 727–734.

Nonoue, Y., Yamamoto, Y., Takeuchi, T., Yano, M.K., 2005. Construction and evaluation of chromosome segment substitution lines carrying overlapping chromosome segments of *Indica* rice cultivar ‘Kasalath’ in genetic background of *Japonica* elite cultivar ‘Koshihikari’. Breed Sci, 55: 65–73.

Ren, T.H., Yang Z.J., Yan, B.J., Zhang, H.Q., Fu, S.L., Ren, Z.L., 2009. Development and characterization of a new 1BL-1RS translocation line with resistance to stripe rust and powdery mildew of wheat. Euphytica, 169: 207–213.

Salem, M., Kazuhiko, S., Utako, Y., Masaki, S., Tomoki, H., Kiyosumi, H., Masatomo, K., Kanako, I., Masahiro, Y., 2012. Mapping and characterization of seed dormancy QTLs using chromosome segment substitution lines in rice. Theor Appl Genet, 124: 893–902.

Sang, J., Minchinton, I., Johnstone, P., Truscott, R., 1984. Glucosinolate profiles in the seed, root and leaf tissue of cabbage, mustard, rapeseed, radish and swede. Can J Plant Sci, 64: 77–93.

Sun, B., Lin, N., Zhao, Y.T., Yan, H.Z., Wang, Q.M., 2011. Variation of glucosinolates in three edible parts of Chinese kalea (*Brassica alboglabra* Bailey) varieties. Food Chem, 124: 941–947.

Takai, T., Nonoue, Y., Yamamoto, S., Yamanouchi, U., Matsubara, K., Liang, Z.W., Lin, H.X., Ono, N., Uga, Y., Yano, M., 2007. Development of chromosome segment substitution lines derived from backcross between *Indica* donor rice cultivar ‘Nona Bokra’ and *Japonica* recipient cultivar ‘Kodhihidari’. Breed Sci, 57: 257–261.

Wang, H., 2011. Identification of glucosinolate biosynthetic gene and mapping QTL for glucosinolate in *Brassica rapa* [Ph. D. Dissertation]. Beijing: China Academy Agricultural Sciences.

Wang, X.W., Wang, H.Z., Wang, J., Sun, R.F., Wu, J., Liu, S.Y., Bai, Y.Q., Mun, J.H., Bancroft, L., Cheng, F., et al., 2011. The genome of the mesopolyploid crop species *Brassica rapa.* Nature Genetics, 43: 1035–1039.

Yano, M., Kojima S., Takahashi Y., Lin H.X., Sasaki T., 2001. Genetic control of flowering time in rice, a short-day plant. Plant Physio, 127: 1425–1429.

Zhao, W.C., Qi, L.L., Gao, X., 2010. Development and characterization of two new *Triticum aestivum* – *Dasypyrum villosum* Robertsonian translocation lines T1DS·1V#3L and T1DL·1V#3S and their effect on grain quality. Euphytica, 175: 343–350.