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

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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).

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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		