Development and Validation of High-glucoraphanin Broccoli F₁ Hybrids and Parental Lines

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ABSTRACT. Sulforaphane is an anticarcinogenic isothiocyanate derived from 4-methylsulfinylbutyl glucosinolate (glucoraphanin), which is abundant in broccoli (*Brassica oleracea* var. *italica*) florets. However, some breakdown products from alkenyl glucosinolates present in many broccoli cultivars, particularly oxazolidine-2-thione hydrolyzed from 2-(R)-hydroxy-3-butenyl glucosinolate (progoitrin), have potentially harmful effects on human and animal health. The main objective of this study was to improve the glucoraphanin concentration in F₁ hybrids by cross-breeding with inbred lines and doubled haploids. Glucoraphanin concentrations in 31 of the 61 F₁ hybrids were significantly higher ($P = 0.05$) than that of the commercial cultivar (Youxiu) with the highest concentration of glucoraphanin ($4.18 \mu\text{mol}\cdot\text{g}^{-1}$ dry weight) among eight reference cultivars. Sixteen of the F₁ hybrids had glucoraphanin concentrations 3-fold higher than that of 'Youxiu'. Alkenyl glucosinolates were not detected in the new hybrids as a result of the parents having few of these compounds but were found in five reference cultivars. Most F₁ hybrids showed moderate indole glucosinolate concentrations and acceptable commercial traits. IL609 and IL702.2 were determined to be promising parental lines as a result of the high glucoraphanin concentration that they and their offspring contained. The findings also indicated that some F₁ hybrids do not show the high-glucoraphanin character of their parents; consequently, evaluation of these F₁ hybrids for their glucosinolate content is required for breeding high-glucoraphanin broccoli.

Broccoli is receiving increased attention because its florets are rich in many chemoprotective phytochemicals such as glucosinolates (GSs). Epidemiological studies have shown that diets rich in cruciferous (*Brassicaceae*) vegetables, especially broccoli, may provide protection against various forms of cancer, including colon and prostate cancer, particularly in the early initiation stages (Higdon et al., 2007). In addition, protective effects of broccoli against cardiovascular diseases have also been noted (Zhang et al., 2011).

Current research has demonstrated that the chemoprotective properties of cruciferous vegetables are attributed to GS hydrolysis products, primarily, isothiocyanates [ITCs (Traka and Mithen, 2009; Verkerk et al., 2009)]. Glucosinolates, consisting of β -thioglucoside *N*-hydroxysulfate, a side chain, and a β -D-glucopyranose moiety, are divided into three groups based on their amino acid precursors: aliphatic GSs (methionine, leucine, iso-leucine), indole GSs (tryptophan), and aromatic GSs (phenylalanine) (Table 1) (Sønderby et al., 2010b). On tissue damage, GSs can be hydrolyzed to several classes of bioactive breakdown products, including ITCs, thiocyanates, and nitriles, by typical plant myrosinases [β -thioglucoside glucohydrolase (EC 3.2.1.147)], which are compartmentalized either in specialized

myrosin cells in the phloem parenchyma or in stomata cells (Bürow and Wittstock, 2010). In addition, some bacteria in the gastrointestinal tract show myrosinase activity (Traka and Mithen, 2009). Among the hydrolysis products, many ITCs, particularly sulforaphane (SF), derived from the hydrolysis of aliphatic GSs, have shown high anticarcinogenic activity in mammalian cells (Traka and Mithen, 2009). SF has been studied extensively; this ITC is hydrolyzed from glucoraphanin, which is abundant in broccoli florets, seeds, and sprouts. The anticarcinogenic mechanism of ITCs, and particularly of SF, have been reviewed in previous studies (Traka and Mithen, 2009; Verkerk et al., 2009). Recent studies have suggested that SF prevents vascular diseases by acting as an indirect antioxidant through the activation of Nrf-2 (Xue et al., 2008) and protects against ischemic–reperfusion injury of the heart through the antioxidant pathway and mitochondrial K_{ATP} channels (Piao et al., 2010). It is notable that hepatic, colonic mucosal, and pancreatic quinone reductase and glutathione *S*-transferase activities were induced by high doses of SF but not by SF nitrile (Matusheski and Jeffery, 2001). Moreover, the activity of specifier proteins such as epithiospecifier protein in broccoli could inhibit the formation of SF (Matusheski et al., 2004). However, proper cooking (e.g., blanching for 2 min, short-term boiling, or microwave treatment) without macerating broccoli florets can efficiently inactivate endogenous *S*-glycosyl hydrolases and specifier proteins; intact GSs can then be degraded by colonic microflora to promote the formation of health-benefitting ITCs (Matusheski et al., 2004; Sarikamis et al., 2006).

Some hydrolysis products derived from alkenyl GSs, including 2-(R)-hydroxy-3-butenyl GS, 2-propenyl GS (sinigrin),

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Table 1. Trivial and chemical names of main glucosinolates identified in broccoli.

Trivial names	Chemical names of R groups	Breeding objective
Aliphatic glucosinolates	Alkyl or alkenyl	
Glucobrassicin	3-methylsulfinylpropyl	Increase ^z
Glucoraphanin	4-methylsulfinylbutyl	Increase
Glucorucin	4-methylthiobutyl	Increase
Glucosylsin	5-methylsulfinylpentyl	Increase
Progoitrin	2-hydroxy-3-butenyl	Decrease ^y
Sinigrin	2-propenyl	Moderate ^x
Gluconapin	3-butenyl	Moderate
Indole glucosinolates	Indoles	
4-hydroxyglucobrassicin	4-hydroxy-3-indolylmethyl	Moderate
Glucobrassicin	3-indolylmethyl	Moderate
4-methoxyglucobrassicin	4-methoxy-3-indolylmethyl	Moderate
Neoglucobrassicin	1-methoxy-3-indolylmethyl	Moderate
Aromatic glucosinolates	Phenyl	
Gluconasturtiin	2-phenylethyl	Increase

^zMore than 2-fold higher than that of major commercial cultivars.

^yLess than one-tenth the mean concentration of major commercial cultivars.

^xThe effects of these compounds on human health or vegetable flavor are controversial; high-glucoraphanin hybrids with lower concentrations of these compounds are preferred.

and 3-butenyl GS (gluconapin), also possess a degree of anticarcinogenic activity (Fahey et al., 1997), but these compounds may have adverse health effects (Verkerk et al., 2009). For example, oxazolidine-2-thiones formed from progoitrin and found in rapeseed have been associated with goiter and other negative effects in animals, including depressed growth, poor egg production, and liver damage (Tripathi and Mishra, 2007). To date, despite a lack of evidence for a goitrogenic effect of progoitrin and its breakdown products in humans (Traka and Mithen, 2009), the potential health risks may discourage people from consuming foods containing relatively high concentrations of this chemical. The alkenyl GSs and neoglucobrassicin also contribute to the pungent, bitter taste of some *Brassica* vegetables (Drewnowski and Gomez-Carneros, 2000; Schonhof et al., 2004). Some broccoli lines (e.g., 'Shogun', Eu8-1, and 'Lvxiang90') with relatively higher concentrations of alkenyl GSs (particularly progoitrin) were identified in previous studies (Brown et al., 2002; Kushad et al., 1999; Rosa and Rodrigues, 2001; Schonhof et al., 2004; Wang et al., 2012).

Indole-3-carbinol and diindolylmethane, two major hydrolysis products of glucobrassicin that are abundant in cruciferous plants, exhibit protective activities against many types of cancer, particularly hormone-responsive conditions, including breast, prostate, and ovarian cancers (Higdon et al., 2007). However, these compounds might also promote carcinogenesis by inducing phase-I enzymes, which can oxidize inert polyaromatic hydrocarbons to DNA-binding products (Baird et al., 2005). A recent study further demonstrated that the neoglucobrassicin/myrosinase complex showed strongly mutagenic properties in bacterial and mammalian cells (Glatt et al., 2011). Considering these findings, consumption of large amounts of indole glucosinolates should be approached with caution.

The composition and concentration of aliphatic GSs are mainly determined by the genotype of *B. oleracea* vegetables, although they can also be affected by many exogenous factors (Brown et al., 2002; Farnham et al., 2004; Ku et al., 2013; Schonhof et al., 2004). Compared with other *Brassica* vegetables, broccoli florets contain higher concentrations of glucoraphanin and comparatively low concentrations of other methionine-derived GSs (Verkerk et al.,

2009). Some pure lines high in glucoraphanin were found in previous investigations of GSs in broccoli germplasm (Farnham et al., 2000; Kushad et al., 1999; Wang et al., 2012). Attempts have been made to breed broccoli with enhanced concentrations of 3-methylsulfinylpropyl GS (glucobrassicin) and glucoraphanin to enhance health benefits (Faulkner et al., 1998; Mithen et al., 2003; Sarikamis et al., 2006). Sarikamis et al. (2006) bred a hybrid (48-13-4 × Br9) that shows an ≈2-fold increase in glucoraphanin concentration compared with commercial cultivars.

Various strategies for enhancing concentrations of chemoprotective GSs and decreasing concentrations of antinutritional GSs in *Brassica* vegetables were proposed by Verkerk et al. (2009). Previously, 14 pure

broccoli lines with glucoraphanin concentrations more than 2-fold higher than those of commercial hybrids were obtained as candidates for breeding high-GS cultivars (Wang et al., 2012). In the present study, 10 parental lines—eight high-glucoraphanin lines screened previously and two lines with good agronomic characteristics—were used to breed high-glucoraphanin F₁ hybrids (Table 2). We first evaluated two major commercial cultivars, Youxiu and Lvxiang90, which exhibited the highest concentrations of glucoraphanin and progoitrin, respectively, among commercial cultivars in our previous investigation (Wang et al., 2012), and six trial cultivars (Table 2). The reference cultivar with the highest concentration of glucoraphanin was determined as the final control, and the high-glucoraphanin hybrids were evaluated in comparison with this control. We also identified and analyzed 16 F₁ hybrids with more than 3-fold higher glucoraphanin content than the controls for individual GSs and groups and two parental lines were validated as high-glucoraphanin types.

Materials and Methods

PLANT MATERIALS. We evaluated 79 broccoli materials: two commercial cultivars, Youxiu (CK1) and Lvxiang90 (CK2), six trial hybrids, 10 pure lines, and 61 F₁ hybrids (GS hybrids), that were newly created for selection of high-glucoraphanin broccoli. The parents of the GS hybrids comprised four inbred lines (IL) and six doubled-haploid (DH) lines that were obtained through microspore culture (Gu et al., 2014) and were evaluated for GSs in our previous study (Wang et al., 2012). Seeds of the GS hybrids were produced by artificial emasculation and bud pollination.

CULTURE AND SAMPLING. All broccoli materials were seeded in growing matrix for plug seedlings in a greenhouse at the Zhejiang Academy of Agricultural Science experimental station (Hangzhou, China) on 10 Aug. 2010. The seedlings were transplanted after 1 month; parental lines were planted in a greenhouse as described previously (Wang et al., 2012) and hybrids were planted in the field (soil pH ≈7.0). Before transplanting, yard manure (22,500 kg·ha⁻¹), 45% potassium

Table 2. Code, name, source, planting location, and type of commercial cultivars, trial cultivars, and parental lines used in this study of high-glucoraphanin broccoli.

Code	Name	Source ^z	Planting location	Type ^y
CK1	Youxiu	SSC	Field	Commercial cultivar, F ₁ hybrid
CK2	Lvxiong90	TSC	Field	Commercial cultivar, F ₁ hybrid
B947	B10947	CAAS	Field	Trial cultivar, F ₁ hybrid
B949	B10949	CAAS	Field	Trial cultivar, F ₁ hybrid
B001	B10001	ZAAS	Field	Trial cultivar, F ₁ hybrid
B416	B10416	ZAAS	Field	Trial cultivar, F ₁ hybrid
B427	B10427	ZAAS	Field	Trial cultivar, F ₁ hybrid
B863	B10863	ZAAS	Field	Trial cultivar, F ₁ hybrid
DH004.3	DH004.3	ZAAS	Greenhouse	Parental line, DH
DH016.1	DH016.1	ZAAS	Greenhouse	Parental line, DH
DH020.33	DH020.33	ZAAS	Greenhouse	Parental line, DH
DH030.1	DH030.1	ZAAS	Greenhouse	Parental line, DH
DH030.2	DH030.2	ZAAS	Greenhouse	Parental line, DH
DH035	DH035	ZAAS	Greenhouse	Parental line, DH
IL503.2	IL603.2	ZAAS	Greenhouse	Parental line, IL, F ₆
IL609	IL701	ZAAS	Greenhouse	Parental line, IL, F ₇
IL702.2	IL801.2	ZAAS	Greenhouse	Parental line, IL, F ₈
IL707.1	IL802.1	ZAAS	Greenhouse	Parental line, IL, F ₈

^zSSC = Sakata Seed Corp., Yokohama, Japan; TSC = Tokita Seed Co., Omiya-shi, Japan; CAAS = Chinese Academy of Agricultural Sciences, Beijing, China; ZAAS = Zhejiang Academy of Agricultural Science, Hangzhou, China.

^yDH = doubled haploid line; IL = inbred line.

sulfate complex fertilizer (1500 kg·ha⁻¹ 15N–6.6P–12.5K), and boron fertilizer (300 kg·ha⁻¹ borax) were applied as a basal dressing. Urea (300 kg·ha⁻¹ 46N–0P–0K) was applied during the growth stage. On floret appearance, 45% potassium sulfate complex fertilizer (450 kg·ha⁻¹ 15N–6.6P–12.5K) and calcium superphosphate (150 kg·ha⁻¹ 0N–7P–0K) were applied to all plots. All plants were cultivated using identical agronomic practices (e.g., insecticide application and irrigation). We used a randomized complete block experimental design with four replicates (n = 24 plants per entry). Individual plots consisted of double rows. The width of each border was ≈130 cm. Spacing between double rows was ≈55 cm, and spacing between plants within single rows was ≈45 cm.

Time to harvest was generally less than 70 d for early-mature cultivars (e.g., Youxiu and B416), 70 to 80 d for middle-mature cultivars (e.g., B001 and B427), and greater than 80 d for late-mature cultivars (e.g., Lvxiong90 and B863). When broccoli heads were mature (i.e., small buds were not loose and the head remained tight), four heads without visible insect or mechanical damage were cut from each block and immediately sent to the laboratory. For hybrids, the stalks were cut to ≈12 cm and the diameter and weight of each head were measured and recorded. A small floret was selected from the top, middle, and lateral parts of each head; the florets were combined, covered with aluminum foil, and immediately frozen in liquid nitrogen. The entire sampling process for each genotype was completed in 1 d. After lyophilization, the freeze-dried tissue was ground into a powder for direct extraction of GS or for storage in a sealed bag at –20 °C.

GLUCOSINOLATE EXTRACTION AND ANALYSIS. Extraction and quantification of GSs were performed according to the protocol described by Wang et al. (2012). Briefly, for each sample, ≈100 mg powdered broccoli was weighed in a 10-mL capped tube. Boiled water (10 mL) was then added and the tube was capped, mixed, and immediately placed in boiling water for 10 min. After centrifuging for 10 min at 4000 g_n at room temperature,

1 mL of the supernatant was transferred into a 6 × 0.5-cm polypropylene column filled with diethylaminoethyl DEAE-Sephadex A-25 (Sigma, St. Louis, MO), which was activated with 0.5 M pyridine acetate to a height of 1 cm. The bed was equilibrated twice with water, washed with 20 mM pyridine acetate three times, and again washed twice with water. The GSs were treated overnight or for 16 h with 1.4 U sulfatase (Sigma). The desulfo-GSs were eluted with 1 mL water.

The eluate was filtered through a 0.45-μm syringe filter for high-performance liquid chromatography (HPLC) analysis after addition of *ortho*-nitrophenyl-D-galactopyranoside (Sigma) as an internal standard. A HPLC system (Breeze; Waters, Milford, MA) with an autosampler, binary pump, and dual-absorbance detector (Models 717 Plus, 1525, and 2487, respectively; Waters) was used for GS detection. A Spherisorb C18 column (5 μm, 250 ×

4.6-mm i.d.; Elite Analytical Instruments, Dalian, China) protected by a guard column was used for separation at 25 °C. The mobile phases were water and acetonitrile (Tedia Co., Fairfield, OH). Separation was achieved using the following program: 5 min at 1.5% acetonitrile, 15-min gradient from 1.5% to 20% acetonitrile, 5 min at 20% acetonitrile, 1-min gradient to 100% acetonitrile, 5 min at 100% acetonitrile, 1-min gradient to 1.5% acetonitrile, and 8 min at 1.5% acetonitrile. The flow rate was 1.0 mL·min⁻¹ and the injection volume was 20 μL. Concentrations of GSs were determined from HPLC peak areas, the internal standard, and the published ultraviolet response factors of individual desulfo-GSs at 226 nm (Wang et al., 2012) and expressed as μmol·g⁻¹ dry weight (DW). Abbreviations for the GS compounds are presented in Table 1.

DATA ANALYSIS. Calculations were performed using Excel 2003 (Microsoft, Redmond, WA). The highest concentration of glucoraphanin among commercial and trial plants was used as the control against which each cross was compared using Student's *t* tests (significance levels of *P* = 0.05 and *P* = 0.01). Mean separation of individual GS profiles among high-glucoraphanin hybrids was performed using Tukey's honestly significant difference test after one-way analysis of variance (*P* = 0.05) using SPSS (Version 16.0 for Windows; IBM, Armonk, NY).

Results

To better compare floret concentrations of glucoraphanin by genotype, two major commercial Chinese cultivars (Youxiu and Lvxiong90) and six trial hybrids with good commercial characteristics were used as references for GS analysis (Table 3). The maximum glucoraphanin concentration among the commercial and trial hybrids was recorded in 'Youxiu' (4.18 μmol·g⁻¹ DW) and was compared with that of the F₁ hybrids. Glucoraphanin concentration varied significantly between 'Youxiu' and 34 F₁ hybrids (Table 4), whereas there were no

significant differences in glucoraphanin concentration between ‘Youxiu’ and the other 27 F₁ hybrids, eight of which contained less than 4.18 $\mu\text{mol}\cdot\text{g}^{-1}$ DW glucoraphanin. Progoitrin (1.05 to 4.53 $\mu\text{mol}\cdot\text{g}^{-1}$ DW) was detected in five reference cultivars including the commercial hybrids (Table 3), but not in GS hybrids (Table 4). The major GSs were glucoraphanin and glucobrassicin, the concentrations of which ranged from 2.39 to 18.95 (average 8.89) and from 1.06 to 9.13 (average 4.27) $\mu\text{mol}\cdot\text{g}^{-1}$ DW, respectively, in GS hybrids. In contrast to the GS hybrids, the major GSs varied among reference cultivars but the predominant indole GS was glucobrassicin (2.69 to 7.40 $\mu\text{mol}\cdot\text{g}^{-1}$ DW). Generally, the GS hybrids contained higher concentrations of aliphatic GSs than the reference cultivars, mainly because of the higher concentration of glucoraphanin. Indole GS concentrations were similar between the hybrids and cultivars.

Previously, we proposed that a 2-fold increase of glucoraphanin in broccoli heads was a basic requirement for high-glucoraphanin selection based on our broccoli germplasms (Wang et al., 2012). In the present study, 26 GS hybrids met that criterion, whereas 16 had more than 3-fold higher glucoraphanin (Table 4). No significant differences in glucoraphanin (13.62 to 18.95 $\mu\text{mol}\cdot\text{g}^{-1}$ DW) or total aliphatic GS (21.77 to 31.79 $\mu\text{mol}\cdot\text{g}^{-1}$ DW) concentration were observed in the high-glucoraphanin hybrids (Supplemental Table 1). Glucoerucin was another methionine-derived GS detected in the high-glucoraphanin hybrids; IL702.2 $\times$ IL609 contained significantly more glucoerucin than the other GS hybrids. Glucoiberin was only observed in a few F₁ hybrids, primarily in the offspring of DH004.3. The concentrations of four individual indole GSs differed significantly among these high-glucoraphanin hybrids [Tukey’s honestly significant difference test ($P = 0.05$)]. The concentrations of two major indole GSs, glucobrassicin and neoglucobrassicin, ranged from 3.74 to 9.13 $\mu\text{mol}\cdot\text{g}^{-1}$ DW and from 0.45 to 6.92 $\mu\text{mol}\cdot\text{g}^{-1}$ DW, respectively.

Our results showed that the concentration of glucoraphanin ranged from 0.14 to 10.82 (average 4.33) $\mu\text{mol}\cdot\text{g}^{-1}$ DW in 10 parental lines (Fig. 1A). The lines IL609, IL702.2, DH030.1, and DH030.2 exhibited higher concentrations of glucoraphanin than the other pure lines. In contrast, DH016.1, IL707.1, and DH004.3 contained relatively lower concentrations of glucoraphanin (0.14, 1.31, and 1.68 $\mu\text{mol}\cdot\text{g}^{-1}$ DW, respectively) and no detectable alkenyl GSs were found in these parental lines. Of the high-glucoraphanin hybrids, five were progeny of IL609 and 10 were progeny of IL702.2 (Supplemental Table 2). The average glucoraphanin concentrations in the progenies of IL609 and IL702.2 were 11.86 (5.24 to 18.95) and 14.93 (9.41 to 17.86) $\mu\text{mol}\cdot\text{g}^{-1}$ DW, respectively, which were higher than the average value of all GS hybrids (8.84 $\mu\text{mol}\cdot\text{g}^{-1}$ DW) (Fig. 1B). Nine of 14 progenies of IL609 showed concentrations of glucoraphanin higher than the average value (Table 4). Except for IL702.2 $\times$ DH035 (9.41 $\mu\text{mol}\cdot\text{g}^{-1}$ DW glucoraphanin), other F₁ hybrids derived from IL702.2 belonged to the high-glucoraphanin type. In addition, no significant differences in glucoraphanin concentrations were found between the reciprocal F₁ hybrids of high-glucoraphanin hybrids with the exception of IL702.2 $\times$ DH035. Thus, our data suggest that IL609 and IL702.2, particularly the latter, are high-glucoraphanin parental lines suitable for breeding high-glucoraphanin cultivars.

Discussion

We did not find remarkably higher concentrations of glucoraphanin in the trial hybrids compared with the major commercial cultivars Youxiu and Lvxiong90, which account for more than 80% of the market in China. However, most F₁ hybrids derived from high-glucoraphanin parents showed higher concentrations of glucoraphanin than either commercial or trial hybrids. Significant variation in glucoraphanin concentration has been observed

Table 3. Concentrations of glucosinolates in the florets of eight reference broccoli cultivars (two commercial cultivars and six trial cultivars) grown in the field.^z

Glucosinolate	Commercial cultivar		Trial cultivar					
	Youxiu	Lvxiong90	B10001	B10416	B10427	B10863	B10947	B949
Glucosinolate concn [mean $\pm$ SD ($\mu\text{mol}\cdot\text{g}^{-1}$ DW)]								
<i>Aliphatic glucosinolates</i>								
Aliphatic glucosinolates	6.60 $\pm$ 0.28	6.50 $\pm$ 0.20	4.22 $\pm$ 0.50	0.51 $\pm$ 0.02	4.68 $\pm$ 0.18	6.27 $\pm$ 0.17	4.40 $\pm$ 0.25	7.80 $\pm$ 0.62
Glucoiberin	0.41 $\pm$ 0.07	ND ^y	ND	ND	3.08	ND	ND	ND
Progoitrin	1.05 $\pm$ 0.04	3.10 $\pm$ 0.03	2.13 $\pm$ 0.15	ND	ND	4.53 $\pm$ 0.15	ND	3.85 $\pm$ 0.20
Sinigrin	0.25 $\pm$ 0.01	0.11 $\pm$ 0.01	ND	ND	ND	ND	ND	ND
Glucoraphanin	4.18 $\pm$ 0.27	2.49 $\pm$ 0.18	1.82 $\pm$ 0.35	0.39 $\pm$ 0.02	1.44 $\pm$ 0.12	1.03 $\pm$ 0.00	4.02 $\pm$ 0.20	3.46 $\pm$ 0.55
Gluconapin	0.48 $\pm$ 0.08	0.58 $\pm$ 0.03	0.10 $\pm$ 0.03	ND	ND	0.70 $\pm$ 0.01	ND	0.49 $\pm$ 0.05
Glucoerucin	0.24 $\pm$ 0.01	0.21 $\pm$ 0.01	0.21 $\pm$ 0.02	0.12 $\pm$ 0.00	0.16 $\pm$ 0.05	ND	0.38 $\pm$ 0.05	ND
<i>Indole glucosinolates</i>								
Indole glucosinolates	7.78 $\pm$ 0.35	5.44 $\pm$ 0.22	9.01 $\pm$ 0.86	6.75 $\pm$ 0.33	7.18 $\pm$ 0.62	4.74 $\pm$ 0.06	8.12 $\pm$ 0.07	12.75 $\pm$ 1.48
4-Hydroxyglucobrassicin	0.32 $\pm$ 0.01	ND	0.22 $\pm$ 0.08	0.09 $\pm$ 0.02	ND	ND	ND	0.15 $\pm$ 0.03
Glucobrassicin	5.05 $\pm$ 0.19	2.69 $\pm$ 0.02	5.42 $\pm$ 0.43	4.16 $\pm$ 0.27	4.42 $\pm$ 0.34	2.82 $\pm$ 0.11	6.49 $\pm$ 0.1	7.40 $\pm$ 1.09
4-Methoxyglucobrassicin	0.88 $\pm$ 0.10	0.20 $\pm$ 0.02	0.52 $\pm$ 0.08	0.57 $\pm$ 0.05	0.67 $\pm$ 0.03	0.25 $\pm$ 0.01	0.38 $\pm$ 0.02	0.54 $\pm$ 0.10
Neoglucobrassicin	1.53 $\pm$ 0.07	2.50 $\pm$ 0.20	2.84 $\pm$ 0.64	1.93 $\pm$ 0.01	2.08 $\pm$ 0.26	1.64 $\pm$ 0.01	1.25 $\pm$ 0.01	4.65 $\pm$ 0.33
<i>Total glucosinolates</i>								
Total glucosinolates	14.38 $\pm$ 0.63	11.93 $\pm$ 0.09	13.22 $\pm$ 0.87	7.26 $\pm$ 0.31	11.86 $\pm$ 0.45	11.01 $\pm$ 0.13	12.53 $\pm$ 0.18	20.54 $\pm$ 1.36

^zData are the means of four replications.

^yNot detected.

DW = dry weight.

Table 4. Concentrations of glucosinolates in the florets of 61 field-grown broccoli F₁ hybrids developed by cross-breeding with 10 parental lines.^z

Glucosinolate hybrid FP×MP ^y	Glucobriferin	Glucoraphanin [*]	4-Hydroxy glucobrassicin	Glucorucin	Glucobrassicin	4-Methoxy glucobrassicin	Neoglucobrassicin	Aliphatic glucosinolate	Indole glucosinolate	Total glucosinolate
Glucosinolate concn [mean ± SD (μmol·g ⁻¹ DW)]										
DH004.3 × DH020.33	0.65 ± 0.05	8.15 ± 0.78**	0.10 ± 0.00	0.15 ± 0.01	6.11 ± 0.40	0.35 ± 0.02	0.38 ± 0.04	8.95 ± 0.83	6.93 ± 0.45	15.88 ± 1.28
DH004.3 × DH035	0.33 ± 0.03	3.15 ± 0.07 NS	0.21 ± 0.06	0.10 ± 0.04	3.04 ± 0.19	0.57 ± 0.15	0.22 ± 0.01	3.55 ± 0.00	4.04 ± 0.12	7.59 ± 0.12
DH004.3 × IL503.2	0.84 ± 0.10	6.92 ± 0.95*	0.26 ± 0.08	0.27 ± 0.02	4.69 ± 0.34	0.53 ± 0.05	0.43 ± 0.04	8.03 ± 1.07	5.92 ± 0.51	13.94 ± 1.57
DH004.3 × IL609	0.69 ± 0.03	6.90 ± 0.61*	0.42 ± 0.07	0.29 ± 0.02	8.53 ± 0.46	0.88 ± 0.04	0.66 ± 0.08	7.88 ± 0.64	10.49 ± 0.50	18.37 ± 0.98
DH004.3 × IL707.1	0.41 ± 0.05	3.70 ± 0.36 NS	0.09 ± 0.06	0.19 ± 0.05	4.10 ± 1.14	0.48 ± 0.12	0.39 ± 0.11	4.30 ± 0.42	5.06 ± 1.41	9.36 ± 1.79
DH016.1 × DH030.1	ND ^w	8.91 ± 0.75**	0.31 ± 0.03	0.22 ± 0.02	4.97 ± 0.44	0.53 ± 0.04	2.66 ± 0.28	9.13 ± 0.77	8.47 ± 0.65	17.60 ± 1.40
DH016.1 × IL707.1	ND	6.59 ± 0.84 NS	0.38 ± 0.03	0.15 ± 0.01	3.10 ± 0.58	0.97 ± 0.04	1.52 ± 0.09	6.74 ± 0.83	5.97 ± 0.74	12.71 ± 0.10
DH020.33 × DH004.3	0.68 ± 0.03	7.10 ± 0.40*	0.19 ± 0.01	0.19 ± 0.00	6.58 ± 0.23	0.42 ± 0.01	0.36 ± 0.03	7.97 ± 0.42	7.55 ± 0.31	15.52 ± 0.74
DH020.33 × DH016.1	ND	6.04 ± 0.45 NS	0.19 ± 0.36	0.28 ± 0.01	5.76 ± 0.25	0.80 ± 0.01	7.95 ± 1.72	6.31 ± 0.46	14.69 ± 1.93	21.01 ± 1.48
DH020.33 × DH030.1	ND	7.32 ± 0.63*	0.14 ± 0.03	0.27 ± 0.02	5.79 ± 0.73	0.48 ± 0.05	3.47 ± 0.45	7.59 ± 0.65	9.88 ± 0.88	17.47 ± 1.49
DH020.33 × DH030.2	ND	6.68 ± 0.55*	0.27 ± 0.02	0.28 ± 0.04	5.18 ± 0.30	0.78 ± 0.06	5.62 ± 0.58	6.95 ± 0.51	11.84 ± 0.96	18.80 ± 0.52
DH020.33 × DH035	ND	3.49 ± 0.38 NS	0.10 ± 0.01	ND	2.16 ± 0.02	0.45 ± 0.03	0.88 ± 0.03	3.49 ± 0.39	3.56 ± 0.06	7.05 ± 0.36
DH020.33 × IL503.2	ND	5.60 ± 0.94 NS	0.20 ± 0.06	0.28 ± 0.03	7.25 ± 0.46	0.54 ± 0.07	1.5 ± 0.27	5.88 ± 0.96	9.51 ± 0.71	15.40 ± 0.73
DH020.33 × IL609	ND	14.24 ± 0.55**	0.17 ± 0.03	0.43 ± 0.02	5.44 ± 0.86	0.61 ± 0.09	2.25 ± 0.38	14.67 ± 0.56	8.46 ± 1.36	23.13 ± 1.61
DH020.33 × IL707.1	ND	8.79 ± 0.23**	0.13 ± 0.08	0.23 ± 0.02	3.89 ± 0.75	0.57 ± 0.12	1.64 ± 0.32	9.01 ± 0.22	6.23 ± 1.27	15.24 ± 1.39
DH030.1 × DH004.3	ND	7.72 ± 1.53**	0.13 ± 0.05	0.24 ± 0.03	4.27 ± 0.98	0.70 ± 0.09	3.20 ± 0.63	7.96 ± 1.56	8.30 ± 1.61	16.27 ± 0.30
DH030.1 × DH016.1	ND	5.39 ± 0.33 NS	0.16 ± 0.02	0.23 ± 0.03	4.67 ± 0.65	0.52 ± 0.06	2.81 ± 0.26	5.61 ± 0.35	8.15 ± 1.48	13.77 ± 1.75
DH030.1 × DH020.33	ND	5.71 ± 0.55 NS	0.10 ± 0.02	0.10 ± 0.01	3.52 ± 0.09	0.38 ± 0.07	1.23 ± 0.03	5.80 ± 0.56	5.21 ± 0.19	11.01 ± 0.73
DH030.1 × DH030.2	ND	2.81 ± 0.21 NS	0.10 ± 0.00	ND	1.36 ± 0.14	0.59 ± 0.13	1.06 ± 0.28	2.82 ± 0.22	3.06 ± 0.55	5.88 ± 0.35
DH030.1 × DH035	ND	2.39 ± 0.48 NS	ND	0.10 ± 0.05	1.06 ± 0.20	0.30 ± 0.08	0.80 ± 0.14	2.49 ± 0.52	2.16 ± 0.31	4.64 ± 0.42
DH030.1 × IL503.2	ND	6.82 ± 0.49*	0.14 ± 0.02	0.24 ± 0.02	4.90 ± 0.92	0.48 ± 0.10	1.57 ± 0.64	7.06 ± 0.50	7.10 ± 1.68	14.16 ± 1.83
DH030.1 × IL609	ND	5.92 ± 1.57 NS	0.10 ± 0.05	0.29 ± 0.03	4.00 ± 0.62	0.49 ± 0.08	2.60 ± 0.40	6.21 ± 1.60	7.17 ± 1.06	13.38 ± 2.59
DH030.1 × IL702.2	ND	13.81 ± 2.49**	0.23 ± 0.009	0.43 ± 0.06	4.81 ± 0.34	0.56 ± 0.07	4.01 ± 1.37	14.24 ± 2.53	9.60 ± 1.51	23.84 ± 1.59
DH030.1 × IL707.1	ND	2.87 ± 0.46 NS	0.10 ± 0.03	0.17 ± 0.02	3.83 ± 0.68	0.33 ± 0.08	0.53 ± 0.12	3.04 ± 0.48	4.79 ± 0.65	7.83 ± 0.92
DH030.2 × DH004.3	ND	5.97 ± 0.33 NS	0.13 ± 0.02	0.20 ± 0.03	4.42 ± 0.60	0.56 ± 0.72	8.10 ± 0.31	6.18 ± 0.32	13.21 ± 0.81	19.39 ± 1.06
DH030.2 × DH016.1	ND	5.55 ± 0.70 NS	0.14 ± 0.02	0.23 ± 0.03	2.47 ± 0.40	0.68 ± 0.13	3.21 ± 0.40	5.79 ± 0.73	6.51 ± 0.91	12.30 ± 1.63
DH030.2 × DH030.1	ND	7.66 ± 0.26**	0.10 ± 0.01	0.17 ± 0.00	3.74 ± 0.26	0.62 ± 0.06	10.37 ± 1.78	7.83 ± 0.25	14.79 ± 2.12	22.62 ± 1.91
DH030.2 × DH035	ND	3.96 ± 0.19 NS	0.15 ± 0.01	0.24 ± 0.01	1.82 ± 0.09	0.54 ± 0.03	2.46 ± 0.14	4.19 ± 0.20	4.97 ± 0.25	9.17 ± 0.44
DH030.2 × IL503.2	ND	9.88 ± 1.92**	0.15 ± 0.03	0.30 ± 0.04	4.39 ± 0.40	0.79 ± 0.22	3.12 ± 1.12	10.18 ± 1.95	8.44 ± 1.34	18.63 ± 2.47
DH030.2 × IL609	ND	6.21 ± 0.17 NS	0.44 ± 0.01	0.31 ± 0.01	3.72 ± 0.05	1.22 ± 0.06	8.15 ± 0.28	6.52 ± 0.16	13.53 ± 0.34	20.05 ± 0.27
DH030.2 × IL702.2	ND	13.62 ± 1.65**	0.68 ± 0.02	0.46 ± 0.05	5.28 ± 0.86	1.02 ± 0.23	2.05 ± 0.48	14.08 ± 1.70	9.02 ± 1.50	23.10 ± 3.20
DH030.2 × IL707.1	ND	4.26 ± 0.27 NS	0.12 ± 0.02	0.23 ± 0.04	1.77 ± 0.34	0.52 ± 0.08	1.53 ± 0.53	4.48 ± 0.31	3.94 ± 0.96	8.43 ± 1.26
DH035 × DH016.1	ND	3.06 ± 0.53 NS	0.19 ± 0.03	0.29 ± 0.04	2.55 ± 0.42	0.60 ± 0.08	1.60 ± 0.50	3.35 ± 0.56	4.94 ± 0.95	8.29 ± 1.11
DH035 × DH020.33	ND	8.81 ± 2.40**	0.16 ± 0.09	0.16 ± 0.09	3.23 ± 0.48	0.55 ± 0.11	1.78 ± 0.26	9.04 ± 2.43	5.72 ± 0.61	14.76 ± 2.55
DH035 × DH030.1	ND	4.49 ± 0.56 NS	0.20 ± 0.01	0.22 ± 0.01	2.44 ± 0.20	0.66 ± 0.05	2.01 ± 0.41	4.70 ± 0.57	5.31 ± 0.63	10.02 ± 0.61
DH035 × DH030.2	ND	4.62 ± 0.32 NS	0.30 ± 0.03	0.20 ± 0.03	1.62 ± 0.07	0.73 ± 0.05	1.18 ± 0.18	4.81 ± 0.34	3.83 ± 0.15	8.64 ± 0.48
DH035 × IL503.2	ND	6.16 ± 1.34 NS	0.42 ± 0.14	0.39 ± 0.14	3.53 ± 0.59	0.88 ± 0.20	0.72 ± 0.13	6.55 ± 1.35	5.55 ± 0.94	12.10 ± 1.93
DH035 × IL609	ND	8.84 ± 0.87**	0.12 ± 0.06	0.44 ± 0.05	1.88 ± 0.15	0.61 ± 0.07	1.71 ± 0.50	9.29 ± 0.86	4.32 ± 0.62	13.61 ± 1.25
DH035 × IL702.2	ND	17.86 ± 0.60**	0.59 ± 0.06	0.61 ± 0.17	5.08 ± 0.25	1.01 ± 0.09	2.48 ± 0.38	18.47 ± 0.55	9.17 ± 0.66	27.63 ± 1.02
DH035 × IL707.1	ND	5.48 ± 0.028 NS	0.25 ± 0.01	0.24 ± 0.03	2.54 ± 0.23	0.71 ± 0.05	1.00 ± 0.50	5.72 ± 0.30	4.49 ± 0.43	10.22 ± 0.70
IL609 × DH004.3	ND	5.24 ± 0.29 NS	0.48 ± 0.02	0.22 ± 0.01	2.08 ± 0.20	0.73 ± 0.02	1.33 ± 0.02	5.47 ± 0.28	4.62 ± 0.21	10.09 ± 0.07

Continued next page

Table 4. Continued.

Glucosinolate hybrid FP×MP ^y	Glucoiberin	Glucoraphanin ^x	4-Hydroxy glucobrassicin	Glucorucin	Glucobrassicin concn [mean ± SD (μmol·g ⁻¹ DW)]	4-Methoxy glucobrassicin	Neoglucobrassicin	Aliphatic glucosinolate	Indole glucosinolate	Total glucosinolate
IL609 × DH016.1	ND	15.84 ± 1.60**	0.44 ± 0.07	0.54 ± 0.08	5.71 ± 0.35	1.00 ± 0.08	5.32 ± 0.08	16.37 ± 1.68	12.47 ± 0.53	28.84 ± 2.20
IL609 × DH020.33	ND	18.95 ± 0.33**	0.61 ± 0.06	0.40 ± 0.01	3.93 ± 0.10	0.99 ± 0.11	6.92 ± 0.15	19.35 ± 0.33	12.44 ± 0.20	31.79 ± 0.52
IL609 × DH030.1	1.27 ± 0.15	11.65 ± 0.43**	0.69 ± 0.13	0.29 ± 0.06	3.71 ± 0.21	0.66 ± 0.11	0.31 ± 0.01	13.21 ± 0.63	5.36 ± 0.44	18.56 ± 1.07
IL609 × DH030.2	ND	12.23 ± 0.87**	0.12 ± 0.01	0.41 ± 0.04	4.09 ± 0.52	0.65 ± 0.07	4.52 ± 0.94	12.64 ± 0.91	9.38 ± 1.54	22.02 ± 2.32
IL609 × IL503.2	ND	17.65 ± 2.33**	0.60 ± 0.19	0.37 ± 0.06	3.97 ± 0.66	0.85 ± 0.15	2.49 ± 0.73	18.01 ± 2.35	7.90 ± 1.66	25.92 ± 3.50
IL609 × IL707.1	ND	11.25 ± 1.55**	0.33 ± 0.17	0.14 ± 0.05	3.54 ± 0.46	0.91 ± 0.55	1.95 ± 0.23	11.39 ± 1.56	6.73 ± 0.69	18.12 ± 1.85
IL702.2 × DH004.3	ND	15.90 ± 1.19**	0.43 ± 0.04	0.44 ± 0.02	5.82 ± 0.46	0.62 ± 0.04	1.40 ± 0.05	16.34 ± 1.18	8.27 ± 0.44	24.61 ± 1.62
IL702.2 × DH020.33	ND	13.82 ± 1.57**	0.32 ± 0.03	0.44 ± 0.04	6.91 ± 0.59	0.63 ± 0.05	0.94 ± 0.10	14.26 ± 1.58	8.80 ± 0.77	23.06 ± 1.80
IL702.2 × DH030.1	ND	14.28 ± 0.63**	0.47 ± 0.00	0.45 ± 0.01	5.53 ± 0.40	0.61 ± 0.03	1.54 ± 0.11	14.73 ± 0.64	8.15 ± 0.48	22.88 ± 1.12
IL702.2 × DH030.2	ND	15.43 ± 1.98**	0.61 ± 0.01	0.42 ± 0.02	4.93 ± 0.54	1.04 ± 0.04	3.76 ± 1.04	15.85 ± 2.00	10.33 ± 1.54	26.18 ± 3.54
IL702.2 × DH035	ND	9.41 ± 0.51**	0.62 ± 0.09	0.82 ± 0.10	3.94 ± 1.13	1.08 ± 0.31	0.62 ± 0.17	10.23 ± 0.60	6.26 ± 1.70	16.49 ± 2.19
IL702.2 × IL609	ND	17.09 ± 1.80**	0.37 ± 0.02	1.47 ± 0.44	6.16 ± 0.49	0.89 ± 0.06	1.24 ± 0.05	18.56 ± 1.42	8.66 ± 0.48	27.22 ± 1.89
IL702.2 × IL707.1	ND	16.15 ± 2.83**	0.55 ± 0.11	0.36 ± 0.05	5.41 ± 0.83	1.04 ± 0.21	0.45 ± 0.08	16.52 ± 2.85	7.45 ± 1.22	23.96 ± 3.16
IL707.1 × DH016.1	ND	2.56 ± 0.57 NS	ND	0.17 ± 0.02	4.58 ± 0.84	0.52 ± 0.03	1.71 ± 0.60	2.73 ± 0.59	6.80 ± 0.31	9.54 ± 0.56
IL707.1 × DH020.33	ND	14.47 ± 2.06**	0.33 ± 0.06	0.21 ± 0.02	5.57 ± 0.26	0.82 ± 0.09	3.11 ± 0.55	14.69 ± 1.06	9.82 ± 0.54	24.51 ± 1.58
IL707.1 × DH030.1	ND	5.70 ± 0.53 NS	0.38 ± 0.02	0.20 ± 0.01	2.19 ± 0.09	0.73 ± 0.05	1.07 ± 0.16	5.90 ± 0.52	4.36 ± 0.32	10.26 ± 0.20
IL707.1 × DH035	ND	6.25 ± 0.14 NS	0.44 ± 0.04	0.23 ± 0.00	2.08 ± 0.08	0.94 ± 0.02	0.84 ± 0.04	6.49 ± 0.14	4.30 ± 0.19	10.79 ± 0.16
IL707.1 × IL503.2	ND	10.81 ± 0.80**	0.51 ± 0.02	0.26 ± 0.01	5.87 ± 1.08	1.15 ± 0.09	1.06 ± 0.04	11.07 ± 0.88	8.59 ± 1.23	19.66 ± 2.11
IL707.1 × IL609	ND	13.97 ± 1.03**	0.46 ± 0.12	0.24 ± 0.03	3.74 ± 0.53	1.16 ± 0.24	2.21 ± 0.19	14.21 ± 1.05	7.56 ± 1.04	21.77 ± 1.91
IL707.1 × IL702.2	ND	16.85 ± 0.61**	0.59 ± 0.07	0.28 ± 0.20	3.82 ± 0.34	1.05 ± 0.10	0.74 ± 0.20	17.13 ± 0.67	6.20 ± 0.51	23.33 ± 0.85
Range	ND–1.27	2.39–18.95	ND–0.69	ND–1.47	1.06–9.13	0.30–1.22	0.22–10.37	2.49–19.35	2.16–14.79	4.64–31.79
Average	0.08	8.84	0.29	0.30	4.15	0.71	2.35	9.21	7.48	16.70

^yData are means of four replications.^yFP×MP = female parent × male parent; DH = doubled haploid line; IL = inbred line.^xComparison of glucoraphanin concentration of broccoli florets between glucosinolate hybrids and the commercial cultivar Youxiu. Asterisks indicate significant differences (Student's *t* test):**P* = 0.05, ***P* = 0.01, NS = nonsignificant.^wNot detected.

DW = dry weight.

Fig. 1. The overall patterns and mean concentrations of glucosinolates in florets of 10 broccoli parental double haploid (DH) and inbred (IL) lines grown in the greenhouse (A) and in florets of F₁ hybrids of each broccoli parental line grown in the field (B). The number of progeny of the parental lines is as follows: 10 (DH004.3), 8 (DH016.1), 14 (DH020.33), 16 (DH030.1), 13 (DH030.2), 14 (DH035), seven (IL503.2), 14 (IL609), 11 (IL702.2), and 15 (IL707.1), respectively. The “Mean” column on the x-axis indicates the average value of each glucosinolate.

among commercial cultivars by numerous authors (Carlson et al., 1987; Kushad et al., 1999; Matusheski et al., 2006; Rosa and Rodrigues, 2001). Nevertheless, in most studies, only a few cultivars or F₁ hybrids (e.g., ‘Shogun’) have shown higher concentrations of glucoraphanin than the commercial cultivar Marathon (Abercrombie et al., 2005; Matusheski et al., 2006; Rosa and Rodrigues, 2001; Schonhof et al., 2004; Vallejo et al., 2003). In other investigations, commercial cultivars (e.g., Brigadier, Majestic, Belstar, and Cronado) contained high concentrations of glucoraphanin, but the absolute concentrations were not as high as those observed by Brown et al. (2002), Kushad et al. (1999), and Matusheski et al. (2004), possibly because of environmental factors. Recently, Ku et al. (2013) observed that glucoraphanin concentrations in florets of five commercial hybrids over two growing seasons were significantly influenced by environmental conditions and that genotype × environment interactions accounted for nearly 40% of the total variation. Similar significant variations in GS concentrations were observed in our studies. Although we previously observed glucoraphanin concentrations of 2.60 and 5.95 $\mu\text{mol}\cdot\text{g}^{-1}$ DW in ‘Youxiu’ and ‘Lvxiang90’, respectively (Wang et al., 2012), relatively higher and lower concentrations (4.18 and 2.49 $\mu\text{mol}\cdot\text{g}^{-1}$ DW, respectively) were detected in these cultivars in the present study. Although these studies have failed to show significant differences in glucoraphanin concentration between major cultivars from China, Europe, or North America (e.g., ‘Marathon’ and ‘Shogun’), comparative studies over multiple years and sites are required to validate the stability of

high-glucoraphanin cultivars in comparison with major commercial cultivars. Here, no detectable alkenyl GSs were observed in the GS hybrids, whereas the reference cultivars (e.g., high-progoitrin types ‘Lvxiang90’ and B863, high-glucoraphanin type B427, and glucoraphanin-lacking type B414) showed diverse aliphatic GS patterns. ‘Shogun’ has previously been shown to contain a higher proportion of alkenyl GSs compared with ‘Marathon’ (Kushad et al., 1999; Rosa and Rodrigues, 2001; Schonhof et al., 2004). Previous studies in *B. oleracea* have demonstrated that modification of alkyl GSs is genetically determined and mostly unaffected by environmental factors (Li and Quiros, 2003; Schonhof et al., 2004). We also found that the F₁ progenies of parents with higher concentrations of progoitrin had similarly higher ratios of progoitrin (data not shown). Thus, it can be deduced that if alkenyl GSs were not detected in the GS hybrids, it was because their parents contained little of these compounds (Fig. 1B). Considering these results, the evaluation of parental lines is sufficient for the reduction of alkenyl GSs in F₁ hybrids. Nevertheless, significant variation in individual indole GSs was found among GS hybrids and reference cultivars, which is in agreement with some earlier reports (Rosa and Rodrigues, 2001; Schonhof et al., 2004; Wang et al., 2012). Numerous studies have shown that indole GSs in broccoli are more strongly affected by environmental factors than by

genotype (Brown et al., 2002; Rosa and Rodrigues, 2001; Schonhof et al., 2004; Vallejo et al., 2003). Therefore, the detection of individual GSs is required for functional foods derived from broccoli. Overall, it is better to prevent accumulation of indole GSs (particularly neoglucobrassicin) when breeding high-glucoraphanin hybrids because of the potential negative health effects of some of their breakdown products (Baird et al., 2005; Glatt et al., 2011). In addition, Matusheski et al. (2006) found that epithiospecifier protein from the Packman cultivar directed hydrolysis of glucoraphanin to form sulforaphane nitrile instead of SF, and some high-glucoraphanin commercial cultivars do not produce correspondingly high concentrations of SF. There is also a significant relationship between epithiospecifier protein activity and the formation of sulforaphane nitrile in broccoli seedlings (Williams et al., 2008). In the future, it will be necessary to clarify the breakdown products of glucoraphanin in the high-glucoraphanin hybrids developed in this study when intact tissue is damaged.

Our data demonstrated that the offspring of IL609 and IL702.2 generally showed higher glucoraphanin concentrations than those of other parental lines, indicating that these offspring may have had high general combining ability (GCA) for glucoraphanin. Abercrombie et al. (2005) observed significant GCA for glucoraphanin concentration using a diallelic population of 36 F₁ hybrids across two environments and suggested that the GCA was primarily a result of additive gene effects. In this study, some progenies of IL609 and IL702.2 did not contain as much glucoraphanin as the high-glucoraphanin hybrids,

indicating that the genetic mechanism of aliphatic GSs varies in broccoli. Because of limited seed availability, the GCA of individual GSs was not estimated through diallel analysis in this study. We observed that the progenies of IL702.2 were larger than the F₁ hybrids from other parents (data not shown) and that most of the high-glucoraphanin hybrids, particularly the F₁ progenies of IL702.2, showed marketable characteristics comparable to the commercial cultivars and trial hybrids (Supplemental Table 2). Much higher production of GSs in plant tissues may be attributable to the promotion of glucoraphanin accumulation in reproductive organs such as flowers and seeds. Nevertheless, in China, small plant size is pursued by breeders to increase broccoli planting density and yield, which may partially explain why the popular Chinese cultivars contain low concentrations of glucoraphanin. Head size and shape also varied among the parental lines: DH030.1, DH030.2, and IL605.1 produced relatively small broccoli heads with thick buds. If the head shape is not high and round or if the buds are thick, broccoli may not be well accepted by breeders and consumers. For example, 'Marathon' is a popular broccoli cultivar in the United States but not in China because of its appearance. For most broccoli cultivars, the concentration of GS in broccoli heads usually increases with floret growth. Thus, a primary task in breeding high-GS cultivars is to determine the harvest standard for broccoli heads.

To date, the methionine- and tryptophan-derived GS biosynthesis and breakdown pathways have generally been elucidated in *Arabidopsis thaliana* (Burow and Wittstock, 2010; Sønderby et al., 2010b) and some transcription factors involved in GS metabolism have been identified (Gigolashvili et al., 2009). MYB28, MYB29, and MYB76 regulate specific genes in aliphatic GS biosynthesis and interact as a complex to alter the distribution of aliphatic GS within *A. thaliana* leaves (Sønderby et al., 2010a). Several plant hormone-signaling networks (e.g., jasmonic acid, salicylic acid, ethylene, and abscisic acid) and protein phosphorylation, redox regulation, and nitrogen/sulfate metabolism also significantly influence GS composition and concentration (Yan and Chen, 2007). Thus, in addition to GS metabolism itself, pathways that interact with GS metabolism might produce significant variability in GS concentration among broccoli cultivars. Unlike leafy *Brassica* vegetables, transportability from synthetic tissue to inflorescences may play a more important role in the accumulation of glucoraphanin in the broccoli floret. Recently, Nour-Eldin et al. (2012) identified and characterized two members of the nitrate/peptide transporter family (GTR1 and GTR2) as high-affinity, proton-dependent, GS-specific transporters that control the loading of GSs from the apoplast into the phloem in *A. thaliana*. These studies indicate that the mechanism underlying the accumulation of glucoraphanin and aliphatic GSs in broccoli florets is complex. The genome sequence of a cabbage (*B. oleracea* var. *capitata*) was announced in 2011. The studies described provide strong support for the molecular breeding of broccoli containing specific GSs. The materials developed in this study will allow for identification of critical genes and markers that are responsible for the natural variability of glucoraphanin concentration in the inflorescence of *B. oleracea* crops.

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Supplemental Table 1. Comparison of individual glucosinolates and each group of glucosinolates among high-glucoraphanin broccoli hybrids.^z

Glucosinolate hybrid ^y	Glucosinolate concn [mean $\pm$ SD ($\mu\text{mol}\cdot\text{g}^{-1}$ DW)]										Aliphatic glucosinolate	Indole glucosinolate
	Glucoraphanin	4-Hydroxyglucobrassicin	Glucobrassicin	4-Methoxyglucobrassicin	Neoglucobrassicin	Neoglucobrassicin	Neoglucobrassicin	Neoglucobrassicin	Neoglucobrassicin	Neoglucobrassicin		
DH030.2 $\times$ IL702.2	13.62 a ^x	0.68 cd	0.46 a	5.28 ab	1.02 ab	2.05 a–c	14.08 a	9.02 ab	14.08 a	23.10 a		
DH030.1 $\times$ IL702.2	13.81 a	0.23 ab	0.43 a	4.81 ab	0.56 a	4.01 b–d	14.24 a	9.60 a–c	14.24 a	23.84 a		
IL702.2 $\times$ DH020.33	13.82 a	0.32 a–d	0.44 a	6.91 bc	0.63 ab	0.94 a	14.26 a	8.80 ab	14.26 a	23.06 a		
<i>IL707.1</i> $\times$ <i>IL609</i>	13.97 a	0.46 a–c	0.24 a	3.74 a	1.16 bc	2.21 a–c	14.21 a	7.56 ab	14.21 a	21.77 a		
DH020.33 $\times$ IL609	14.24 a	0.17 a	0.43 a	5.44 ab	0.61 ab	2.25 a–c	14.67 a	8.46 ab	14.67 a	23.13 a		
IL702.2 $\times$ DH030.1	14.28 a	0.47 a–d	0.45 a	5.53 ab	0.61 ab	1.54 ab	14.73 a	8.15 ab	14.73 a	22.88 a		
<i>IL707.1</i> $\times$ <i>DH020.33</i>	14.47 a	0.33 a–d	0.21 a	5.57 ab	0.82 ab	3.11 a–d	14.69 a	9.82 a–c	14.69 a	24.51 a		
IL702.2 $\times$ DH030.2	15.43 a	0.61 b–d	0.42 a	4.93 ab	1.04 ab	3.76 b–d	15.85 a	10.33 a–c	15.85 a	26.18 a		
IL609 $\times$ DH016.1	15.84 a	0.44 a–d	0.54 a	5.71 ab	1.00 ab	5.32 de	16.37 a	12.47 bc	16.37 a	28.84 a		
IL702.2 $\times$ DH004.3	15.90 a	0.43 a–d	0.44 a	5.82 ab	0.62 ab	1.40 ab	16.34 a	8.27 ab	16.34 a	24.61 a		
IL702.2 $\times$ IL707.1	16.15 a	0.55 a–d	0.36 a	5.41 ab	1.04 ab	0.45 a	16.52 a	7.45 ab	16.52 a	23.96 a		
IL707.1 $\times$ IL702.2	16.85 a	0.59 b–d	0.28 a	3.82 a	1.05 ab	0.74 a	17.13 a	6.20 a	17.13 a	23.33 a		
IL702.2 $\times$ IL609	17.09 a	0.37 a–d	1.47 b	6.16 ab	0.89 ab	1.24 ab	18.56 a	8.66 ab	18.56 a	27.22 a		
IL609 $\times$ IL503.2	17.65 a	0.60 b–d	0.37 a	3.97 a	0.85 ab	2.49 a–c	18.01 a	7.90 ab	18.01 a	25.92 a		
<i>DH035</i> $\times$ <i>IL702.2</i>	17.86 a	0.59 b–d	0.61 a	5.08 ab	1.01 ab	2.48 abc	18.47 a	9.17 ab	18.47 a	27.63 a		
IL609 $\times$ DH020.33	18.95 a	0.61 b–d	0.40 a	3.93 a	0.99 ab	6.92 de	19.35 a	12.44 bc	19.35 a	31.79 a		

^zData are the means of four replications.^yHybrids in italic font represent reciprocal crosses; hybrids in bold font represent high-glucoraphanin reciprocal crosses; DH = doubled haploid line, IL = inbred line.^xMeans within a column followed by the same letter are not significantly different at $P = 0.05$ (Tukey's honestly significant difference test).

DW = dry weight.

Supplemental Table 2. The head diameter and head weight of broccoli lines planted in the field.^z

No.	Code ^y	Diam	Wt
		[mean ± SD (cm)]	[mean ± SD (g)]
1	CK1	13 ± 0.7	315 ± 18
2	CK2	13.6 ± 0.5	434 ± 21
3	B001	13 ± 1.0	340 ± 49
4	B416	12.8 ± 1.4	416 ± 20
5	B427	11.6 ± 0.5	375 ± 46
6	B863	12.8 ± 1.3	377 ± 58
7	B947	11.6 ± 0.4	332 ± 32
8	B949	12.4 ± 0.9	349 ± 35
9	DH004.3 × DH020.33	12.0 ± 0.8	230 ± 14
10	DH004.3 × DH035	14.5 ± 0.6	317 ± 19
11	DH004.3 × IL503.2	12.8 ± 0.8	320 ± 51
12	DH004.3 × IL609	13.3 ± 1.4	302 ± 55
13	DH004.3 × IL707.1	12.8 ± 2.0	355 ± 51
14	DH016.1 × DH030.1	12.0 ± 0	295 ± 16
15	DH016.1 × IL707.1	10.0 ± 0	242 ± 14
16	DH020.33 × DH004.3	12.8 ± 0.3	247 ± 14
17	DH020.33 × DH016.1	9.0 ± 0	155 ± 12
18	DH020.33 × DH030.1	13.0 ± 1	305 ± 41
19	DH020.33 × DH030.2	8.8 ± 0.3	160 ± 18
20	DH020.33 × DH035	12.8 ± 0.4	247 ± 14
21	DH020.33 × IL503.2	11.0 ± 0.6	182 ± 28
22	DH020.33 × IL609	12.3 ± 0.7	262 ± 22
23	DH020.33 × IL707.1	10.5 ± 0.6	160 ± 17
24	DH030.1 × DH004.3	11.8 ± 0.8	310 ± 23
25	DH030.1 × DH016.1	12.5 ± 0.4	362 ± 24
26	DH030.1 × DH020.33	11.0 ± 0.6	175 ± 17
27	DH030.1 × DH030.2	11.3 ± 0.9	250 ± 46
28	DH030.1 × DH035	13.0 ± 0.2	347 ± 13
29	DH030.1 × IL503.2	13.3 ± 0.7	352 ± 36
30	DH030.1 × IL609	14.5 ± 0.6	390 ± 14
31	DH030.1 × IL702.2	10.8 ± 0.4	270 ± 20
32	DH030.1 × IL707.1	13.5 ± 1.1	357 ± 32
33	DH030.2 × DH004.3	10.0 ± 0.6	210 ± 23
34	DH030.2 × DH016.1	12.8 ± 1.3	395 ± 63
35	DH030.2 × DH030.1	8.5 ± 0.6	157 ± 9
36	DH030.2 × DH035	12.3 ± 0.3	305 ± 6
37	DH030.2 × IL503.2	9.2 ± 1.0	170 ± 27
38	DH030.2 × IL609	10.0 ± 0.3	230 ± 16
39	DH030.2 × IL702.2	10.3 ± 0.9	252 ± 39
40	DH030.2 × IL707.1	12.5 ± 0.9	363 ± 42
41	DH035 × DH016.1	12.5 ± 0.6	353 ± 20
42	DH035 × DH020.33	13.5 ± 0.6	297 ± 14
43	DH035 × DH030.1	12.3 ± 0.3	347 ± 20
44	DH035 × DH030.2	10.0 ± 0	230 ± 16
45	DH035 × IL503.2	12.3 ± 0.3	320 ± 18
46	DH035 × IL609	11.3 ± 0.3	237 ± 12
47	DH035 × IL702.2	13.8 ± 0.7	390 ± 46
48	DH035 × IL707.1	12.5 ± 0.4	325 ± 40
49	IL609 × DH004.3	11.5 ± 0.9	282 ± 33
50	IL609 × DH016.1	13.5 ± 0.6	272 ± 3
51	IL609 × DH020.33	12.5 ± 0.6	255 ± 34
52	IL609 × DH030.1	10.8 ± 0.7	218 ± 33
53	IL609 × DH030.2	7.0 ± 0.3	108 ± 9
54	IL609 × IL503.2	9.3 ± 0.3	190 ± 10
55	IL609 × IL707.1	8.3 ± 0.2	152 ± 12
56	IL702.2 × DH004.3	12.3 ± 0.6	380 ± 28

Continued next column

Supplemental Table 2. Continued.

No.	Code ^y	Diam	Wt
		[mean ± SD (cm)]	[mean ± SD (g)]
57	IL702.2 × DH020.33	15.8 ± 0.3	475 ± 57
58	IL702.2 × DH030.1	13.5 ± 0.6	440 ± 23
59	IL702.2 × DH030.2	11.0 ± 0.2	280 ± 34
60	IL702.2 × DH035	14.8 ± 0.9	442 ± 36
61	IL702.2 × IL609	11.0 ± 0.6	260 ± 12
62	IL702.2 × IL707.1	10.3 ± 0.8	262 ± 20
63	IL707.1 × DH016.1	12.5 ± 0.6	390 ± 80
64	IL707.1 × DH020.33	9.3 ± 1.0	213 ± 36
65	IL707.1 × DH030.1	13.5 ± 1.4	415 ± 72
66	IL707.1 × DH035	14.5 ± 0.6	450 ± 69
67	IL707.1 × IL503.2	9.5 ± 0.6	248 ± 20
68	IL707.1 × IL609	11.0 ± 0.6	250 ± 6
69	IL707.1 × IL702.2	11.0 ± 1.0	271 ± 42

^zData are the means of four replications.

^yDH = doubled haploid line; IL = inbred line.