J. Amer. Soc. Hort. Sci. 139(4):460–468. 2014.

Development and Validation of High-glucoraphanin Broccoli F_1 Hybrids and Parental Lines

Honghui Gu[1,2], Jiansheng Wang[1], Huifang Yu, Zhenqing Zhao, and Xiaoguang Sheng
Institute of Vegetables, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China

Jisuan Chen
Haitong Food Group Co., Ltd., Cixi 315300, China

Yingjun Xu
Cixi Agriculture Technology and Popularization Center, Cixi 315300, China

Additional index words. *Brassica oleracea*, breeding, glucosinolate, progoitrin

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 F_1 hybrids by cross-breeding with inbred lines and doubled haploids. Glucoraphanin concentrations in 31 of the 61 F_1 hybrids were significantly higher ($P = 0.05$) than that of the commercial cultivar (Youxiu) with the highest concentration of glucoraphanin (4.18 $\mu mol \cdot g^{-1}$ dry weight) among eight reference cultivars. Sixteen of the F_1 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 F_1 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 F_1 hybrids do not show the high-glucoraphanin character of their parents; consequently, evaluation of these F_1 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 (Burow 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),

Received for publication 4 Sept. 2013. Accepted for publication 16 May 2014. This work was funded by the Science and Technology Department of Zhejiang Province (2011C22009, 2012C12903-3-1), Ningbo Science and Technology Bureau (2009C10009), National Natural Science Foundation of China (31200230), and Zhejiang Academy of Agricultural Sciences.
We thank Dr. Jia Wei at Zhejiang Academy Agricultural Sciences for revising the manuscript.
[1]These authors contributed equally to this work.
[2]Corresponding author. E-mail: guhh@mail.zaas.ac.cn.

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	
Glucoiberin	3-methylsulfinylpropyl	Increase[z]
Glucoraphanin	4-methylsulfinylbutyl	Increase
Glucoerucin	4-methylthiobutyl	Increase
Glucoalysiin	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-phenyllethyl	Increase

[z]More than 2-fold higher than that of major commercial cultivars.
[y]Less than one-tenth the mean concentration of major commercial cultivars.
[x]The 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 'Lvxiong90') 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 (glucoiberin) 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 F_1 hybrids (Table 2). We first evaluated two major commercial cultivars, Youxiu and Lvxiong90, 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 F_1 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 Lvxiong90 (CK2), six trial hybrids, 10 pure lines, and 61 F_1 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

[z]SSC = 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.
[y]DH = 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 F_1 hybrids. Glucoraphanin concentration varied significantly between 'Youxiu' and 34 F_1 hybrids (Table 4), whereas there were no

significant differences in glucoraphanin concentration between 'Youxiu' and the other 27 F_1 hybrids, eight of which contained less than 4.18 μmol·g^{-1} DW glucoraphanin. Progoitrin (1.05 to 4.53 μmol·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) μmol·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 μmol·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 μmol·g^{-1} DW) or total aliphatic GS (21.77 to 31.79 μmol·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 × IL609 contained significantly more glucoerucin than the other GS hybrids. Glucoiberin was only observed in a few F_1 hybrids, primarily in the offspring of DH004.3. The concentrations of four individual indole GSs differed significantly among these high-gluroraphanin hybrids [Tukey's honestly significant difference test (P = 0.05)]. The concentrations of two major indole GSs, glucobrassicin and neoglucobrassin, ranged from 3.74 to 9.13 μmol·g^{-1} DW and from 0.45 to 6.92 μmol·g^{-1} DW, respectively.

Our results showed that the concentration of glucoraphanin ranged from 0.14 to 10.82 (average 4.33) μmol·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 μmol·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) μmol·g^{-1} DW, respectively, which were higher than the average value of all GS hybrids (8.84 μmol·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 × DH035 (9.41 μmol·g^{-1} DW glucoraphanin), other F_1 hybrids derived from IL702.2 belonged to the high-glucoraphanin type. In addition, no significant differences in glucoraphanin concentrations were found between the reciprocal F_1 hybrids of high-glucoraphanin hybrids with the exception of IL702.2 × 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 F_1 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]

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

[z]Data are the means of four replications.
[y]Not detected.
DW = dry weight.

Table 4. Concentrations of glucosinolates in the florets of 61 field-grown broccoli F_1 hybrids developed by cross-breeding with 10 parental lines.[z]

Glucosinolate hybrid FP×MP[y]	Glucoiberin	Glucoraphanin[x]	4-Hydroxy glucobrassicin	Glucoerucin	Gluco brassicin	4-Methoxy glucobrassicin	Neoglu cobrassicin	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 ± 0.0.09	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 ± 0.53NS	0.19 ± 0.03	0.29 ± 0.04	2.55 ± 0.42	0.60 ± 0.08	1.60 ± 0.50	3.35 ± 056	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	Glucoerucin	Gluco brassicin	4-Methoxy glucobrassicin	Neoglu cobrassicin	Aliphatic glucosinolate	Indole glucosinolate	Total glucosinolate
	Glucosinolate concn [mean ± SD (μmol·g^{-1} DW)]									
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

[z]Data are means of four replications.
[y]FP×MP = female parent × male parent; DH = doubled haploid line; IL = inbred line.
[x]Comparison 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.
[w]Not detected.
DW = dry weight.

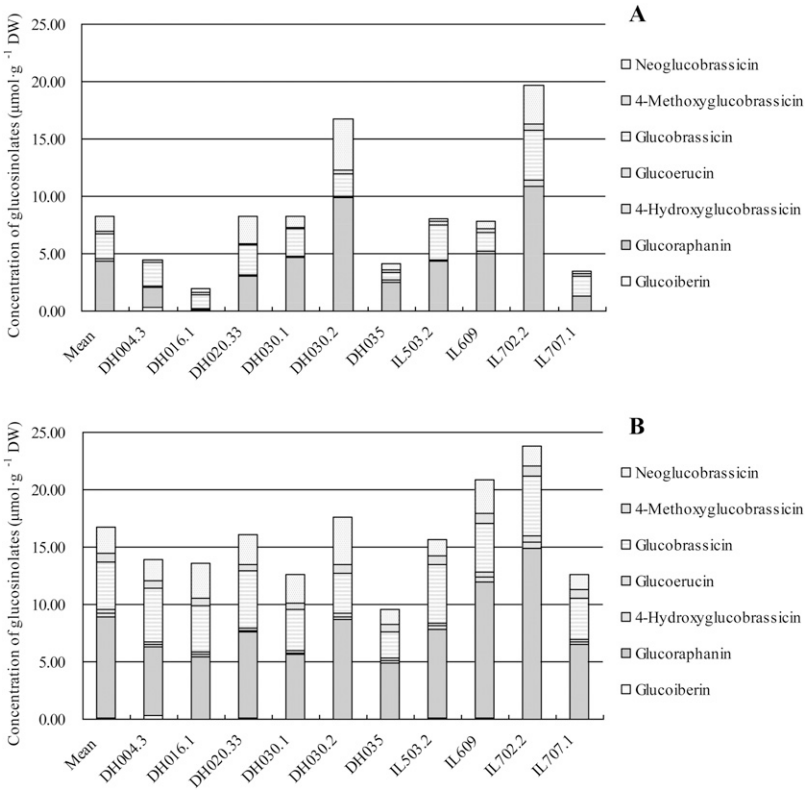

Fig. 1. The overall patterns and mean concentrations of glucosinolates in florets of 10 broccoli parental double haploid (DH) and inbred (IL) lines grown in the greenhouse (**A**) and in florets of F_1 hybrids of each broccoli parental line grown in the field (**B**). The number of progeny of the parental 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 F_1 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 $\mu mol \cdot g^{-1}$ DW in 'Youxiu' and 'Lvxiong90', respectively (Wang et al., 2012), relatively higher and lower concentrations (4.18 and 2.49 $\mu mol \cdot g^{-1}$ 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 'Lvxiong90' and B863, high-glucoiberin 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 F_1 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 F_1 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-glucoraphnin 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 F_1 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 F_1 hybrids from other parents (data not shown) and that most of the high-glucoraphanin hybrids, particularly the F_1 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 apoplasm 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.

Literature Cited

Abercrombie, J.M., M.W. Farnham, and J.W. Rushing. 2005. Genetic combining ability of glucoraphanin level and other horticultural traits of broccoli. Euphytica 143:145–151.

Baird, W.M., L.A. Hooven, and B. Mahadevan. 2005. Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. Environ. Mol. Mutagen. 45:106–114.

Brown, A.F., G.G. Yousef, E.H. Jeffery, B.P. Klein, M.A. Wallig, M.M. Kushad, and J.A. Juvik. 2002. Glucosinolate profiles in broccoli: Variation in levels and implications in breeding for cancer chemoprotection. J. Amer. Soc. Hort. Sci. 127:807–813.

Burow, M. and U. Wittstock. 2010. Glucosinolate breakdown in *Arabidopsis*: Mechanism, regulation and biological significance. The arabidopsis book. 8:e0134. 16 May 2014. <http://www.bioone.org/doi/abs/10.1199/tab.0134>.

Carlson, D.G., M.E. Daxenbichler, C.H. Van Ettern, W.F. Kwolek, and P.H. Williams. 1987. Glucosinolates in crucifer vegetables: Broccoli, brussels sprouts, cauliflower, collards, kale, mustard greens, and kohlrabi. J. Amer. Soc. Hort. Sci. 112:173–178.

Drewnowski, A. and C. Gomez-Carneros. 2000. Bitter taste, phytonutrients, and the consumer: A review. Amer. J. Clin. Nutr. 72:1424–1435.

Fahey, J.W., Y.S. Zhang, and P. Talalay. 1997. Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc. Natl. Acad. Sci. USA 94:10367–10372.

Farnham, M.W., K.K. Stephenson, and J.W. Fahey. 2000. Capacity of broccoli to induce a mammalian chemoprotective enzyme varies among pure lines. J. Amer. Soc. Hort. Sci. 125:482–488.

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

Faulkner, K., R. Mithen, and G. Williamson. 1998. Selective increase of the potential anticarcinogen 4-methylsulphinylbutyl glucosinolate in broccoli. Carcinogenesis 19:605–609.

Gigolashvili, T., B. Berge, and U. Flügge. 2009. Specific and coordinated control of indolic and aliphatic glucosinolate biosynthesis by R2R3-MYB transcription factors in *Arabidopsis thaliana*. Phytochem. Rev. 8:3–13.

Glatt, H., C. Baasanjav-Gerber, F. Schumacher, B.H. Monien, M. Schreiner, H. Frank, A. Seidel, and W. Engst. 2011. 1-Methoxy-3-indolylmethyl glucosinolate: A potent genotoxicant in bacterial and mammalian cells: Mechanisms of bioactivation. Chemico-Biol. Interaction 192:81–86.

Gu, H., Z. Zhao, X. Sheng, H. Yu, and J. Wang. 2014. Efficient doubled haploid production in microspore culture of loose-curd cauliflower (*Brassica oleracea* var. *botrytis*). Euphytica 195:467–475.

Higdon, J.V., B. Delage, D.E. Williams, and R.H. Dashwood. 2007. Cruciferous vegetables and human cancer risk: Epidemiologic evidence and mechanistic basis. Pharmacol. Res. 55:224–236.

Ku, K.M., E.H. Jeffery, and J.A. Juvik. 2013. Influence of seasonal variation and methyl jasmonate mediated induction of glucosinolate biosynthesis on quinone reductase activity in broccoli florets. J. Agr. Food Chem. 61:9623–9631.

Kushad, M.M., A.F. Brown, A.C. Kurilich, J.A. Juvik, B.P. Klein, M.A. Wallig, and E.H. Jeffery. 1999. Variation of glucosinolates in vegetable crops of *Brassica oleracea*. J. Agr. Food Chem. 47:1541–1548.

Li, G. and C.F. Quiros. 2003. In planta side-chain glucosinolate modification in *Arabidopsis* by introduction of dioxygenase *Brassica* homolog *BoGSL-ALK*. Theor. Appl. Genet. 106:1116–1121.

Matusheski, N.V. and E.H. Jeffery. 2001. Comparison of the bioactivity of two glucoraphanin hydrolysis products found in broccoli, sulforaphane and sulforaphane nitrile. J. Agr. Food Chem. 49:5743–5749.

Matusheski, N.V., J.A. Juvik, and E.H. Jeffery. 2004. Heating decreases epithiospecifier protein activity and increases sulforaphane formation in broccoli. Phytochemistry 65:1273–1281.

Matusheski, N.V., R. Swarup, J.A. Juvik, R. Mithen, M. Bennett, and E.H. Jeffery. 2006. Epithiospecifier protein from broccoli (*Brassica oleracea* L. ssp. *italica*) inhibits formation of the anticancer agent sulforaphane. J. Agr. Food Chem. 54:2069–2076.

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

Nour-Eldin, H.H., T.G. Andersen, M. Burow, S.R. Madsen, M.E. Jøgensen, C.E. Olsen, I. Dreyer, R. Hedrich, D. Geiger, and B.A. Halkier. 2012. NRT/PTR transporters are essential for translocation of glucosinolate defence compounds to seeds. Nature 488:531–534.

Piao, C.S., S. Gao, G.H. Lee, D.S. Kim, B.H. Park, S.W. Chae, and S.H. Kim. 2010. Sulforaphane protects ischemic injury of hearts through antioxidant pathway and mitochondrial K_{ATP} channels. Pharmacol. Res. 61:342–348.

Rosa, E.A.S. and A.S. Rodrigues. 2001. Total and individual glucosinolate content in 11 broccoli cultivars grown in early and late seasons. HortScience 36:56–59.

Sarikamis, G., J. Marquez, R. MacCormack, and R.N. Bennett. 2006. High glucosinolate broccoli: A delivery system for suforaphane. Mol. Breed. 18:219–228.

Schonhof, I., A. Krumbein, and B. Brückner. 2004. Genotypic effects on glucosinolates and sensory properties of broccoli and cauliflower. Food/Nahrung 1:25–33.

Sønderby, I.E., M. Burow, H.C. Rowe, D.J. Kliebenstein, and B.A. Halkier. 2010a. A complex interplay of three R2R3 MYB transcription factors determines the profile of aliphatic glucosinolates in *Arabidopsis*. Plant Physiol. 153:348–363.

Sønderby, I.E., F. Geu-Flores, and B.A. Halkier. 2010b. Biosynthesis of glucosinolates—Gene discovery and beyond. Trends Plant Sci. 15:283–290.

Traka, M. and R. Mithen. 2009. Glucosinolates, isothiocyanates and human health. Phytochem. Rev. 8:269–282.

Tripathi, M.K. and A.S. Mishra. 2007. Glucosinolates in animal nutrition: A review. Anim. Feed Sci. Technol. 13:21–27.

Vallejo, F., F.A. Tomás-Rarberán, A.G. Bennavente-Garacía, and C. García-Vguera. 2003. Total and individual glucosinolate contents in inflorescences of eight broccoli cultivars grown under various climatic and fertilization conditions. J. Sci. Food Agr. 83:307–313.

Verkerk, R., M. Schreiner, A. Krumbein, E. Ciska, B. Holst, I. Rowland, R.D. Schrijver, M. Hansen, C. Gerhäuser, R. Mithen, and M. Dekker. 2009. Glucosiolates in *Brassica* vegetables: The influence of the food supply chain on intake, bioavailability and human health. Mol. Nutr. Food Res. 53:S219–S265.

Wang, J., H. Gu, H. Yu, Z. Zhao, X. Sheng, and X. Zhang. 2012. Genotypic variation of glucosinolates in broccoli (*Brassica oleracea* var. *italica*) florets from China. Food Chem. 133:735–741.

Williams, D.J., C. Critchley, S. Pun, S. Nottingham, and T.J. O'Hare. 2008. Epithiospecifier protein activity in broccoli: The link between terminal alkenyl glucosinolates and sulphoraphane nitrile. Phytochemistry 69:2765–2773.

Xue, M., Q. Qian, A. Adaikalakoteswari, N. Rabbani, R. Babaei-Jadidi, and P.J. Thornalley. 2008. Activation of NF-E2-related factor-2 reverses biochemical dysfunction of endothelial cells induced by hyperglycemia linked to vascular disease. Diabetes 57:2809–2817.

Yan, X.F. and S.X. Chen. 2007. Regulation of plant glucosinolate metabolism. Planta 226:1343–1352.

Zhang, X., X.O. Shu, Y.B. Xiang, G. Yang, H. Li, J. Gao, H. Cai, Y.T. Gao, and W. Zheng. 2011. Cruciferous vegetable consumption is associated with a reduced risk of total and cardiovascular disease mortality. Amer. J. Clin. Nutr. 94:240–246.

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

Glucosinolate hybrid[y]	Glucoraphanin	4-Hydroxyglucobrassicin	Glucoerucin	Glucobrassicin	4-Methoxyglucobrassicin	Neoglucobrassicin	Neoglucobrassicin	Aliphatic glucosinolate	Indole glucosinolate
	Glucosinolate concn [mean ± SD (μmol·g^{-1} DW)]								
DH030.2 × 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	23.10 a
DH030.1 × 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	23.84 a
IL702.2 × 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	23.06 a
IL707.1 × IL609	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	21.77 a
DH020.33 × 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	23.13 a
IL702.2 × 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	22.88 a
IL707.1 × DH020.33	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	24.51 a
IL702.2 × 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	26.18 a
IL609 × 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	28.84 a
IL702.2 × 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	24.61 a
IL702.2 × 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	23.96 a
IL707.1 × 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	23.33 a
IL702.2 × 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	27.22 a
IL609 × 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	25.92 a
DH035 × IL702.2	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	27.63 a
IL609 × 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	31.79 a

[z]Data are the means of four replications.
[y]Hybrids in italic font represent reciprocal crosses; hybrids in bold font represent high-glucoraphanin reciprocal crosses; DH = doubled haploid line, IL = inbred line.
[x]Means 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 [mean ± SD (cm)]	Wt [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
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

[z]Data are the means of four replications.
[y]DH = doubled haploid line; IL = inbred line.