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Research Report

Comparative Analysis of Individual Glucosinolates, Phytochemicals, and Antioxidant Activities in Broccoli Breeding Lines

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Abstract. The aim of this research was to evaluate the profile and concentration of individual glucosinolates (GSL), and the total phenol content (TPC), total flavonoid content (TFC), ascorbic acid content, and antioxidant activity of broccoli florets and flower stalks (10 commercial cultivars, 19 F1 hybrids, and 20 inbred lines). All broccoli heads were harvested at their marketable stage, and their flower stalks and florets were subjected to phytochemical analysis. GSL, TPC, TFC, and ascorbic acid content varied significantly depending on broccoli genotype. Altogether, nine GSLs were identified, four of which (glucoraphanin, progoitrin, glucoerucin, and glucobrassicin) were the most common in both broccoli flower stalks and florets. In florets, glucobrassicin was the most abundant GSL (4.46 μmol·g^{-1} DW), followed by glucoraphanin (1.93 μmol·g^{-1} DW), whereas glucoraphanin was the most abundant in flower stalks (1.47 μmol·g^{-1} DW). The concentrations of total GSLs, TPC, and TFC in florets were relatively higher than those in the flower stalks, whereas the concentration of ascorbic acid was higher in the flower stalks than the florets. Almost all F1 hybrids and inbred lines exhibited higher TPC, TFC, ascorbic acid concentration, and antioxidant activities than those in the commercial cultivars. Three F1 hybrids; 5075, 5078, and 5079, and one inbred line (5308) had the highest glucoraphanin and total GSL content. Three inbred lines, 5307, 5311, and 5409 had the higher concentration of glucobrassicin and total GSLs, superior antioxidant activity with low PRO+EPI content. These results suggest that these genotype selections had desirable compositions of individual GSLs and higher nutritional value for commercialization as functional vegetables.

Additional key words: ascorbic acid, flavonoid, glucobrassicin, glucoraphanin, phenolic, plant part

Introduction

Broccoli (*Brassica oleracea* L. var. *italica*) originated in the eastern Mediterranean region of Europe and has been cultivated throughout the world as a highly nutritious vegetable since the 15th century. Broccoli is also cultivated throughout Korea, with a cultivation area and total production mass of 1692 ha and 3705 tons, respectively, in 2013 (MIFAFF, 2012). To meet consumer demand, broccoli is imported from China, Taiwan, and the USA. More than 98% of imported broccoli came from China in 2014, totaling 14 million US dollars in revenue (KATI, 2014). Broccoli has several properties that are beneficial to human health, such as anti-obesity, cholesterol-lowering (Lee et al., 2009), anti-carcinogenic (Traka and Mithen, 2009) and anti-inflammatory properties (Jang and Ha, 2012). It also increases antioxidant activity (Perez-Balibrea et al., 2011; Lopez-Cervantes et al., 2013). These properties are conferred by many health-promoting phytochemicals such as glucosinolates (GSLs), vitamins, phenols, flavonoids, carotenoids, minerals, phytosterols, rutin, and glutathione (Granado et al., 2006; Cartea et al., 2011; Latte et al., 2011; Bhandari and Kwak, 2015a). GSLs, the most important phytochemicals present in broccoli, are plant secondary metabolites implicated in decreasing cancer risk as a part of a vegetable-rich diet.

More than 200 GSLs have been reported in different *Brassica* species, and the intake of individual GSLs may prevent prostate and lung (Spitz et al., 2000; Latte et al., 2011) cancers. These beneficial effects are mainly attributed to the products of hydrolysis reactions, such as sulforaphane, iberin, phenethyl alcohol, and allylisothiocyanate, which are derived from glucoraphanin (1-isothiocyanato-4-methylsulphinylbutane), glu-

coiberin, gluco-nastrutiin, and sinigrin, respectively (Fahey et al., 2001; Sarikamis et al., 2006; Shofran et al., 2006), and can prevent cardiovascular disorders (Dinkova-Kostova and Kostov, 2012) and inhibit the proliferation of cancer cells (Chung et al., 2000). In addition, indole-3-carbinol (I3C) and 3,3′-diindolylemthane (DIM), both glucobrassicin hydrolysis products, were reported to have various anticancer activities (Bonnesen et al., 2001; Nachshon-Kedmi et al., 2004). Vitamin C (ascorbic and dehydro-ascorbic acid) is another health-promoting, water-soluble antioxidant compound in broccoli that protects against singlet oxygen, hydroxyl radicals, and cell death, and acts as a lipid peroxidation chain-breaking agent (Gliszczynska-Swiglo et al., 2006). Likewise, phenolic compounds are a large group of antioxidants present in considerable quantities in broccoli that can neutralize or quench free radicals (Cartea et al., 2011). Flavonoids and their derivatives are the largest and most prominent group of plant polyphenols, possessing strong antioxidant activity due to their ability to scavenge reactive oxygen species and inhibit oxidative stress (Pourcel et al., 2006).

Because of the presence of various health-promoting compounds, the consumption of broccoli is increasing worldwide. Consumers generally use broccoli florets, discarding the flower stalks and leaves; however, the leaves and flower stalks may contain even more phytochemicals than florets. For example, Park et al. (2014) found higher antibacterial activity against *Listeria monocytogenes* and *Salmonella enteritidis* in broccoli leaf extracts than in florets. Furthermore, higher amounts of water-soluble sugars were present in broccoli stems than in florets (Bhandari and Kwak, 2015b), and the *n*-hexane fraction of broccoli stems showed greater nitrite scavenging ability, highlighting the potential of broccoli flower stalks to serve as an alternative food source (Kim et al., 2014).

The concentration and profile of GSLs and other phytochemicals in broccoli are known to be affected by several abiotic and biotic factors, such as plant genotype (Bhandari and Kwak, 2014), storage conditions (Schreiner et al., 2006), the sulforaphane biosynthesis process (Williams et al., 2008), plant part (Bhandari et al., 2015), postharvest conditions (Nath et al., 2011), and several other key environmental factors (Perez-Balibrea et al., 2001; Rosa and Rodrigues, 2001). In Korea, there have been several reports of variations in the profile and concentrations of GSLs in broccoli depending on the genotype, plant parts and growing season (Lee et al., 2012; Bhandari and Kwak, 2014; Bhandari et al., 2015). Furthermore, many studies have reported on selecting broccoli genotypes that contain a higher concentration of GSLs, and evaluating the variation in individual GSLs to elucidate the individual functionality of each GSL (Clarke, 2010; Wang et al., 2012). However, information on the profiles of GSLs, phenolics, and flavonoids, and antioxidant activity in domestic broccoli cultivars and breeding lines is limited.

Therefore, the aim of this study was to evaluate the individual GSL profile and concentration, total phenol content (TPC), total flavonoid content (TFC), ascorbic acid content, and antioxidant activities in broccoli florets and flower stalks, to select superior hybrids and breeding lines to be used as functional vegetables.

Materials and Methods

Plant Materials and Cultural Condition

Ten commercial cultivars (SK3-085, Very Dome, Koyosi, Ace Dome, Woosu, Neahanwoosu, Yeomsu, Kanghan, Engmu, and Supergrace), 19 F1 hybrids, and 20 inbred lines of broccoli were used. Seeds of SK3-085, Woosu, Neahanwoosu, and Yeonmu were obtained from Sakata Co. (Yokohama, Japan); seeds of Very Dome and Ace Dome were obtained from Takii Co. (Kyoto, Japan); seeds of Engmu and Kanghan were obtained from China; seeds of Koyosi and Supergrace were obtained from Heusungseedplus Co. (Busan, Korea) and Teawoo Co. (Seoul, Korea), respectively. In addition, three maternal genotypes that were crossed with two paternal genotypes and six F1 hybrids were used. All plants were grown in a greenhouse at the Breeding Research Institute of Koregaon, Gimje, Korea (35°40.9′N 126°11.8′E). Broccoli seeds were sown on July 15, 2014, and florets were harvested after reaching maturation. After harvesting, the inflorescences were divided into two parts: florets and flower stalks. Both parts were then freeze-dried, ground into fine powder, and stored at -20°C until they were analyzed for their phytochemical composition and antioxidant activities.

GSL Analysis

Freeze-dried powder samples (100 mg) of florets and flower stalks were mixed with 1 mL 70% methanol, incubated in a water bath at 70°C for 60 min, centrifuged at 10 000 × g for 15 min at 4°C and the supernatant was transferred to a vial. Thereafter, the pellet was re-extracted one more time under the same conditions and the supernatants were combined. Desulfoglucosinolates were then prepared using purified sulfatase isolated from *Helix pomatia* and then quantitatively determined by High Performance Liquid Chromatography (HPLC), following the procedure based on Lee et al. (2013) and Bhandari et al. (2015). Briefly, the crude GSL extract was loaded onto a Mini Bio-Spin Chromatography Column (Bio-Rad Laboratories, Hercules, CA, USA), containing 0.7 mL diethylaminoethyl (DEAE)-Sephadex A-25 (Sigma-Aldrich, St. Louis, MO, USA), and pre-activated with 0.1 M sodium acetate (pH 4.0). Desulfation was then conducted by adding 200 μL purified aryl sulfatase (EC 3.1.6.1, type H-1 from *H. pomatia*; Sigma-Aldrich Co., St. Louis, MO, USA). The column was capped and allowed to stand at 20°C for 18 h, and the

desulfated GSLs were eluted three times with 0.5 mL distilled water and filtered through a 0.2-μm syringe filter. The sample was then injected into an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with a photodiode array (PDA) detector set at 229 nm. GSLs were separated using an Acquity UPLC® BEH-C18 Column (1.7 μm, 2.1 × 100 mm; Waters Co., Milford, MA, USA), with gradient elution of solvent A (100% distilled water) and solvent B (20% acetonitrile) at a flow rate of 0.2 mL·min^{-1}. The gradient programs were conducted as follows: a linear step from 1% to 99% solvent B within 6 min, followed by constant conditions for up to 14 min, and then a rapid drop to 1% solvent B at 15 min, and isocratic conditions of 1% solvent B for up to 25 min. Pure standards of GSLs were desulfated and used to identify and quantify peaks. Concentrations of individual GSLs were calculated from experimental peak areas by analytical interpolation, using a standard calibration curve of each GSL across different ranges depending on the GSL concentration, which was expressed as micromoles per gram (μmol·g^{-1}) of dry weight.

Analysis of Total Phenol Content (TPC)

The total phenol content was analyzed by the Folin-Ciocalteu colorimetric method, based on the procedure described by Singleton and Rossi (1956) with some modifications. Floret or flower stalk powder (200 mg) was mixed with 5 mL 80% methanol and extracted in a water bath shaker set at 50°C and 120 rpm for 60 min, followed by centrifugation at 4000 × *g* for 15 min at 4°C. Briefly, 400 μL of extract was mixed with 400 μL water, to which 200 μL Folin-Ciocalteu reagent was added. Then, 200 μL Na_2CO_3 (15%) was added, vortexed, and kept in dark conditions. After 1 h, the absorbance was measured at 640 nm using a Multiskan GO Microplate Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Different concentrations of gallic acid were used as standards to generate a calibration curve. The results are expressed in milligrams of gallic acid equivalent per gram on a dry weight basis (mg GAE·g^{-1} DW).

Analysis of Total Flavonoid Content (TFC)

The total flavonoid content was analyzed using the colorimetric method as described by Menichini et al. (2009) with some modifications. The same extract obtained from the TPC analysis was also used for flavonoid analysis. Briefly, 200 μL extract was mixed with 800 μL water, followed by the addition of 60 μL $NaNO_2$. After 5 min, 60 μL $AlCl_3·6H_2O$ (10%) and 400 μL NaOH (1 M) were added, and the absorbance was measured at 510 nm using a Multiskan GO Microplate Spectrometer (Thermo Fisher Scientific Inc.). Different concentrations of catechin hydrate were used as a standard. The results are expressed in milligrams of catechin hydrate equivalent per gram on a dry weight basis (mg CE·g^{-1}, DW).

Analysis of Ascorbic Acid Content

Ascorbic acid content was determined following the methods of Bhandari and Kwak (2015a) with some modifications. Briefly, a 0.2 g freeze-dried powder sample was mixed with 10 ml 5% metaphosphoric acid solution and extracted. After centrifugation at 4000 × *g* for 5 min, the supernatant was filtered through a 0.20 μm syringe filter, and the sample was immediately analyzed using an Agilent 1200 HPLC (Agilent Technologies) equipped with an Acquity UPLC® HSS T3 (1.8 μm, 2.1 × 100 mm) column and a PDA detector (Agilent, USA) set at 254 nm. The mobile phase was composed of 99% 0.05 M metaphosphoric acid and 1% acetonitrile (ACN) at a flow rate of 0.2 mL·min^{-1}. An authentic ascorbic acid standard was used to identify and quantify the peak.

Measurement of Antioxidant Activity

***Ferric reducing antioxidant power (FRAP) assay*:** Antioxidant activity was measured by the ferric reducing antioxidant power (FRAP) method as described by Thaipong et al. (2006) with some modifications. First, different stock solutions were prepared containing 300 mM acetate buffer (3.1 g $C_2H_3NaO_2·3H_2O$ and 16 mL $C_2H_4O_2$; pH 3.6), 10 mM 2,3,6-Tris(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3·6H_2O$. Then, fresh FRAP working solution was prepared by mixing acetate buffer, TPTZ solution, and $FeCl_3·6H_2O$ solution at ratio of 10:1:1 (v/v/v) and warmed at 37°C. Broccoli extract (50 μL) in a 1.5-mL tube was allowed to react with 950 μL FRAP working solution for 10 min, vortexed, and the absorbance was measured at 593 nm using a Multiskan GO Microplate Spectrometer (Thermo Fisher Scientific, Vantaa, Finland). Different concentrations of trolox (100-500 μmol) were used as standards, and the results were expressed in μmol TE·g^{-1} on a dry weight basis.

***2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic Acid) (ABTS) assay*:** A 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Assay assay was performed following the methods of Thiapong et al. (2006). First, ABTS radical cations ($ABTS^{·+}$) were produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate, and allowing the mixture to stand in the dark at 20ºC for 16 h before use. The $ABTS^{·+}$ solution was diluted 20 times with methanol (100%) until an absorbance of 0.9 ± 0.02 was reached at 734 nm. The sample extract (50 μL) was then added to 950 μL $ABTS^{·+}$ solution, and the absorbance was recorded at 734 nm using a Multiskan GO Microplate Spectrometer (Thermo Fisher Scientific, Vantaa, Finland), after incubation in the dark for 2 h. Different concentrations of trolox (100-1000 μmol)

were used as standards, and the results were expressed as trolox equivalent antioxidant capacity (μmol TE·g^{-1}) on a dry weight basis.

***2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay*:** The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is based on a change in color (from violet to yellow) of DPPH. First, a mixture of 400 μM DPPH solution in 80% methanol was prepared. Aliquots of the reaction mixture, consisting of 100 μL sample extract, or methanol, and 100 μL DPPH dissolved in methanol, were placed in the wells of 96-well microplates. The absorbance was measured at 518 nm using a Multiskan GO Microplate Spectrometer (Thermo Fisher Scientific, Vantaa, Finland), and free radical-scavenging activity (%) was calculated as described by Bhandari and Kwak (2014).

Authentic Standards and Chemicals

Twelve GSL standards, glucoiberin (IBR), progoitrin (PRO), epiprogoitrin (EPI), glucoraphanin (GRA), glucoraphenin (GRE), sinigrin (SIN), gluconapin (NAP), glucobrassicanapin (BCN), glucoerucin (ERU), glucobrassicin (BRA), glucobarbarin (BAR), and gluconasturtiin (NAS), were purchased from Cfm Oskar Co. (Marktredwitz, Germany). DEAE, Sephadex-A25, aryl sulfatase (EC 3.1.6.1, type H-1) from *H. pomatia*, gallic acid, catechin hydrate, (±) 6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid (Trolox), Folin-Ciocalteu phenol, sodium carbonate, aluminum chloride, sodium hydroxide, ABTS, L-ascorbic acid, DPPH, and TPTZ were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals, including acetonitrile (HPLC grade) and methanol (HPLC grade), were purchased from Avantor Performance Materials (Center Valley, PA, USA). Metaphosphoric acid was purchased from Yakuri Pure Chemicals Co. (Uji, Kyoto, Japan).

Statistical Analyses

Means of three independent sample replications were used. To cluster broccoli breeding lines based on phyto-chemicals including GSLs and antioxidant activity, Ward's minimum variance cluster analysis was performed using SAS 9.2, based on Euclidean distances. Principal component analysis (PCA) was carried out to establish the statistical discrimination of all the measured phytochemical parameters between commercial cultivars, F1 hybrids, and inbred lines.

Results and Discussion

Comparison of Individual and Total GSL Concentrations in Broccoli

Nine desulfoglucosinolates in broccoli were analyzed by HPLC using 12 pure standards (Fig. 1). Among the nine

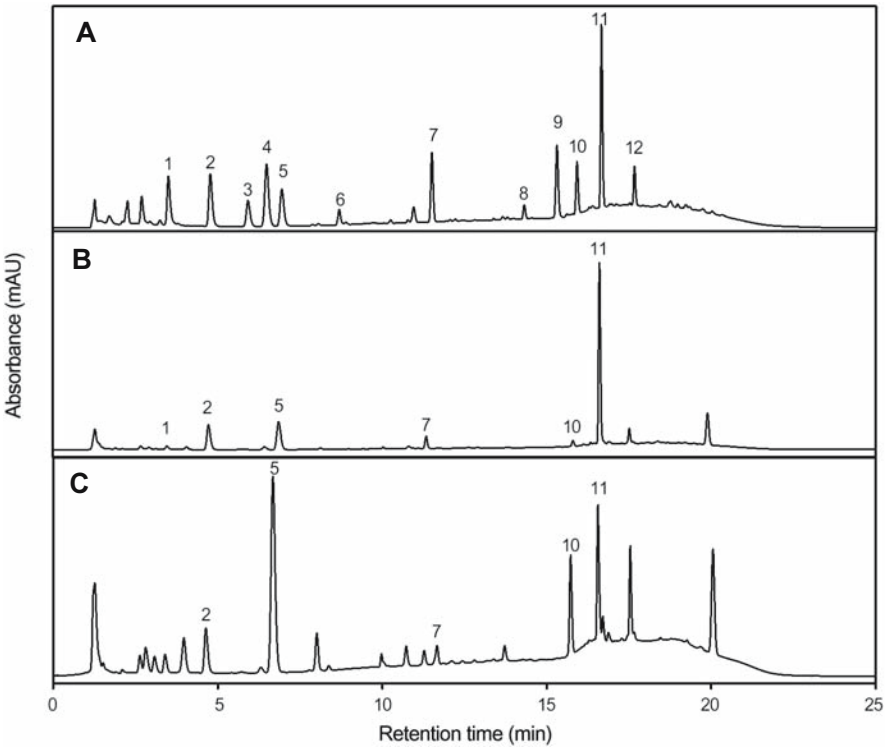

Fig. 1. HPLC chromatogram of GSL standards (A) and extracts from broccoli F1 hybrid 5075 floret (B) and flower stalk (C) identified using standard materials of 12 GSLs (1, glucoiberin; 2, progoitrin; 3, epiprogoitrin; 4, sinigrin; 5, glucoraphanin; 6, glucoraphenin; 7, gluconapin; 8, glucobarbarin; 9, glucobrassicanapin; 10, glucoerucin; 11, glucobrassicin; 12, gluconasturtiin).

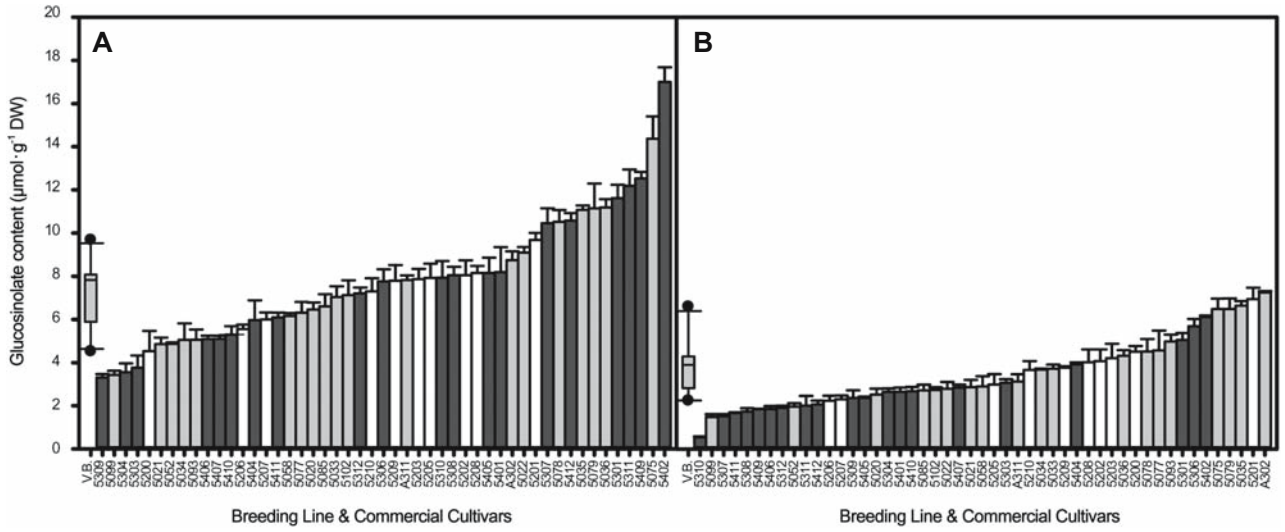

Fig. 2. Total glucosinolate (GSL) content in florets (A) and flower stalks (B) of 49 broccoli breeding lines. The white, gray, and black colors in each bar represent commercial cultivar, F1 hybrid, and inbred lines, respectively. Each vertical box represents the average GSL content of ten commercial cultivars. Each vertical bar represents mean ± SD of the three replications.

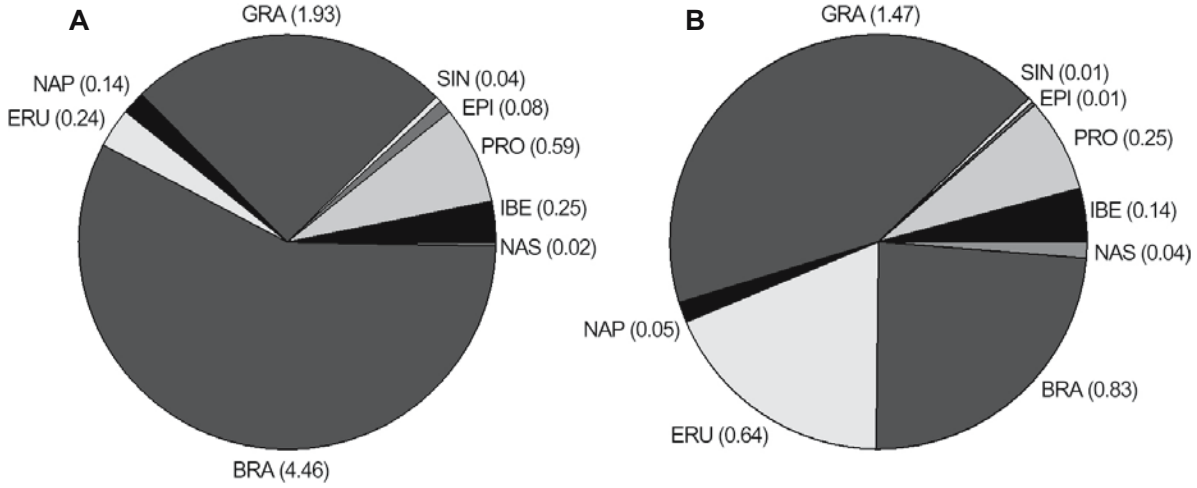

Fig. 3. Total glucosinolate concentrations (μmol·g^{-1} DW) in floret (A) and flower stalk part (B). The abbreviations outside the graphs represent individual glucosinolates. (IBE, glucoiberin; PRO, progoitrin; EPI, epiprogoitrin; SIN, sinigrin; GRA, glucoraphanin; NAP, gluconapin; ERU, glucoerucin; BRA, glucobrassicin; NAS, gluconasturtiin).

GSLs identified, four (GRA, ERU, PRO, and BRA) were the most common in both the florets and flower stalks. In this study, BRA (an indolyl GSL) was present in the highest concentrations (0.84-10.76 μmol·g^{-1} DW) compared to the other GSLs in most broccoli florets, followed by GRA, an aliphatic GSL (0.19-6.43 μmol·g^{-1} DW), PRO (0.00-3.51 μmol·g^{-1} DW) and ERU (0.02-1.91 μmol·g^{-1} DW). However, the concentration of total aliphatic GSLs was higher than indolyl GSL. These results concurred with those reported by Jeffery et al. (2003) and Vallejo et al. (2003), but were different from other previous studies (e.g., Rosa and Rodrigues, 2001; Lee et al., 2012; Bhandari et al., 2015), where GRA was observed as a major GSL in broccoli, possibly because of differences in genotypes, growing conditions, and experimental conditions (Brown et al., 2002; Jeffery et al., 2003).

In broccoli flower stalks, GRA was the major GSL (0.06-3.21 μmol·g^{-1}) identified in most of the breeding lines, followed by BRA (0.22-2.55 μmol·g^{-1}), ERU (0.02-2.14 μmol·g^{-1}) and PRO (0.00-1.74 μmol·g^{-1}). Bhandari and Kwak (2014) also found GRA to be the predominant GSL in broccoli flower stalks; however, the other GSLs (i.e., BRA and ERU) were not present at the same concentrations. The concentration of major GSLs was higher in florets than in flower stalks of the respective breeding lines and, consequently, a higher (2.2 times) total GSL concentration was found in florets (3.32-16.99; average: 7.75 μmol·g^{-1}) than in flower stalks (0.56-7.26; average: 3.44 μmol·g^{-1}) (Figs. 2 and 3). The values obtained in this study were within the

Table 1. Individual glucosinolate (GSL) profiles, total GSL, total phenol, total flavonoid, and ascorbic acid contents and antioxidant activities in the florets of 11 broccoli breeding lines

Breeding Line		Concentration (μmol·g^{-1} DW)				TPC (mg GAE·g^{-1} DW)	TFC (mg CE·g^{-1} DW)	Ascorbic acid (mg·g^{-1} DW)	FRAP (μmol TE·g^{-1} DW)	ABTS (μmol TE·g^{-1} DW)	DPPH (%)
		PRO+EPI	GRA	BRA	Total GSL						
F1 Hybrids	5035	3.09 ± 0.11[z]	2.48 ± 0.06	3.91 ± 0.30	11.08 ± 0.20	2.99 ± 0.24	1.54 ± 0.03	3.41 ± 0.07	5.59 ± 0.68	11.32 ± 0.27	37.64 ± 3.71
	5036	2.10 ± 0.16	2.22 ± 0.20	6.19 ± 0.04	11.19 ± 0.38	2.44 ± 0.09	1.22 ± 0.04	2.93 ± 0.04	3.93 ± 0.01	10.09 ± 0.26	21.61 ± 0.83
	5075	2.95±0.12	4.05 ± 0.68	5.04 ± 0.44	14.37 ± 1.04	2.73 ± 0.18	1.76 ± 0.07	3.15 ± 0.03	4.91 ± 0.31	11.08 ± 0.28	30.13 ± 0.75
	5078	0.03 ± 0.03	6.19 ±0.15	2.27 ± 0.25	10.52 ± 0.54	2.77 ± 0.07	1.85 ± 0.05	2.68 ± 0.01	4.97 ± 0.18	10.91 ± 0.20	28.74 ± 1.20
	5079	N/D[y]	6.42 ± 1.24	2.77 ± 0.28	11.15 ± 1.15	2.79 ± 0.20	1.68 ± 0.02	2.66 ± 0.03	4.96 ± 0.09	11.16 ± 0.24	32.63 ± 0.53
Inbred Lines	5301	1.31 ± 0.02	0.96 ± 0.07	6.84 ± 0.68	11.62 ± 0.62	3.29 ± 0.48	1.60 ± 0.07	3.02 ± 0.03	3.65 ± 0.12	10.39 ± 0.27	20.86 ± 2.53
	5307	0.09 ± 0.00[z]	3.21 ± 0.61	6.75 ± 0.52	10.45 ± 0.69	5.94 ± 0.08	2.55 ± 0.08	2.78 ± 0.03	7.32 ± 0.41	21.31 ± 0.59	82.15 ± 1.37
	5311	0.05 ± 0.02	3.04 ± 0.73	8.60 ± 0.03	12.19 ± 0.76	6.20 ± 0.59	2.27 ± 0.04	2.78 ± 0.03	6.73 ± 0.07	21.40 ± 0.20	77.30 ± 0.87
	5402	3.63 ± 0.03	1.58 ± 0.81	10.76 ± 0.34	16.99 ± 0.68	3.20 ± 0.19	1.52 ± 0.02	3.55 ± 0.03	5.16 ± 0.05	11.38 ± 0.38	33.23 ± 1.65
	5409	0.99 ± 0.02	0.70 ± 0.13	10.43 ± 0.32	12.53 ± 0.29	4.61 ± 0.27	1.95 ± 0.07	3.57 ± 0.03	6.30 ± 0.15	14.87 ± 0.20	51.88 ± 2.19
	5412	0.07 ± 0.00	1.62 ± 0.35	8.78 ± 0.23	10.58 ± 0.35	3.25 ± 0.14	1.50 ± 0.04	2.54 ± 0.01	4.21 ± 0.09	10.70 ± 0.22	25.05 ± 0.96

[z]Values are mean ± SD of three replications on a dry weight basis.
[y]N/D: Not detected. DW, dry weight; TPC, total phenol content; TFC, total flavonoid content; FRAP, ferric reducing antioxidant power; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; PRO, progoitrin; EPI, epiprogoitrin; GRA, glucoraphanin; BRA, glucobrassicin.

range of those previously reported by Wang et al. (2012), who analyzed 148 broccoli breeding lines. Other GSLs, such as IBE, EPI, NAS, SIN, and NAP, were present at low concentrations, or not observed, depending on the breeding line. Although the PRO+EPI content of florets was generally lower in most breeding lines, it was higher in florets compared to flower stalks.

Almost all of the commercial cultivars had similar GSL profiles, and the concentration of total GSLs ranged from 4.53 to 9.68 μmol·g^{-1} and 2.25 to 6.60 μmol·g^{-1} in florets and flower stalks, respectively. However, the highest genotypic effect was observed among inbred lines, with BRA and GRA displaying the highest variation, followed by PRO, NAP, and ERU. Altogether, 11 breeding lines were selected as superior genotypes with higher total glucosinolate content (Fig. 2, Table 1). Among them, five F1 hybrids, namely 5035, 5036, 5075, 5078, and 5079 (10.52-14.37 μmol·g^{-1} DW), and six inbred lines, 5301, 5307, 5311, 5402, 5409, and 5412 (10.45-16.99 μmol·g^{-1} DW), had relatively higher total GSL concentrations in their florets than that in commercial cultivars (3.49-10.04 μmol·g^{-1} DW) (Table 1 and Fig. 2), suggesting their superiority to other breeding lines. Furthermore, many researchers are interested in determining the concentrations of GRA, IBE and BRA in broccoli because of their health-promoting effects (Chung et al., 2000; Nachshon-Kedmi et al., 2004). In this study (Fig. 4), three F1 hybrids (i.e., 5075, 5078, and 5079), and one inbred line (5308), which is not included in selection line (Table 1), had higher concentrations of GRA in their florets compared to those in commercial cultivars. This indicates the high pharmacological value of these cultivars, as GRA is transformed into sulforaphane, a compound with anticancer activity (Latte et al., 2011). IBE was present only in some breeding lines at a range of 0.00-1.23 μmol·g^{-1} DW, which was significantly lower than that reported by Wang et al. (2012) who found a range of IBE of 0.00-2.09 μmol·g^{-1} among 143 pure lines and 5 commercial cultivars. The concentration of BRA was 5.1 times higher in florets than flower stalks (Fig. 3), and breeding lines 5311, 5402, 5409 and 5412 exhibited significantly higher BRA concentrations than other breeding lines, suggesting the potential use of these cultivars for breeding programs. These specific GSL profiles, and the wide variation of individual GSLs under identical agricultural and environmental conditions, revealed that broccoli genotype plays a crucial role in the accumulation of GSLs.

Variation in TPC, TFC, Ascorbic Acid Content, and Antioxidant Activities in Broccoli Breeding Lines

The TPC and TFC of florets were the highest in inbred lines, followed by F1 hybrids, and relatively lower concentrations were found in commercial cultivars. All F1 hybrids, inbred lines, and commercial cultivars had higher concentrations of both TPC and TFC in florets than in flower stalks (Fig. 5A-D). The TPC of florets and flower stalks ranged from 2.06 to 6.27 mg GAE·g^{-1} DW and 1.42 to 4.01 mg GAE·g^{-1} DW, respectively. A similar TPC value was obtained by Bhandari and Kwak (2014); however, the value obtained in this study was lower than that found in a recent study by Lopez-Cervantes et al. (2013), who reported

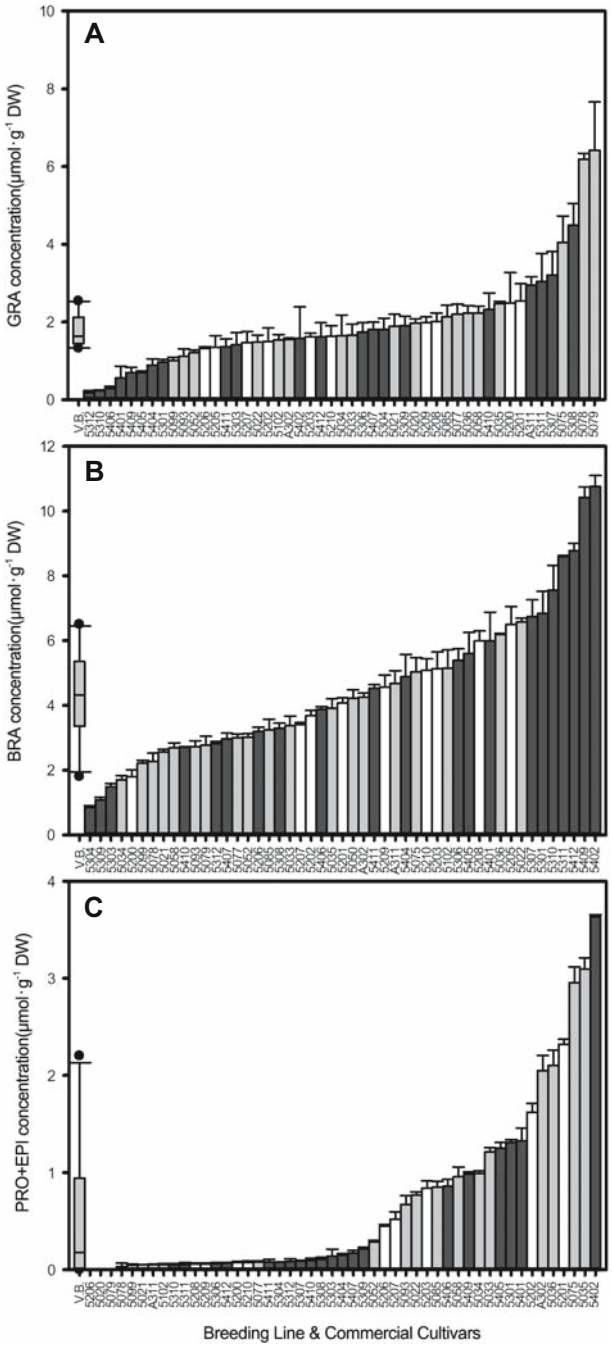

Fig. 4. Glucoraphanin (A), glucobrassicin (B), and progoitrin+ epiprogoitrin (C) content in florets of 49 broccoli breeding lines. The white, gray, and black colors in each bar represent commercial cultivar, F1 hybrid, and inbred line, respectively. Each vertical box represents the average glucosinolate concentration of ten commercial cultivars. Each vertical bar represents mean ± SD of the three replications. GRA, glucoraphanin; BRA, glucobrassicin; PRO, progoitrin; EPI, epiprogoitrin.

the range of TFC in florets and flower stalks to be 1.13 to 2.55 mg CE·g^{-1} DW and 1.41 to 4.01 mg CE·g^{-1} DW, respectively. Inbred lines 5307, 5311, and 5312 possessed higher TPC and TFC in florets compared to those in other inbred lines, F1 hybrids, and commercial cultivars. Similarly, the ascorbic acid content was also higher in inbred lines than in commercial cultivars and F1 hybrids (Fig. 5E-F). The ascorbic acid content in florets was relatively lower than that in flower stalks, ranging from 2.20 to 3.88 mg·g^{-1} DW and 2.59 to 7.04 mg·g^{-1} DW in the florets and flower stalks, respectively. These values are lower than those reported by Bhandari and Kwak (2015a), who obtained ascorbic acid values ranging from 4.03 to 4.75 mg·g^{-1} in the florets of various F1 broccoli hybrids; however, the ascorbic acid content of flower stalks was similar. Inbred line 5404 and F1 hybrid 5052 had higher concentrations of ascorbic acid in the flower stalks and florets, respectively.

In this study, we evaluated antioxidant activity in broccoli florets and flower stalks by performing three different assays (i.e., FRAP, ABTS, and DPPH), using different concentrations of broccoli-methanol extracts (Fig. 6). In all samples, antioxidant activities in flower stalks were much lower than in florets (data not shown). This finding may be caused by higher TPC and TFC in florets, as phenols and flavonoids are major contributors to total antioxidant activity (Zhou and Yu, 2006). Antioxidant activity analyzed by FRAP and DPPH assays in broccoli florets ranged from 2.86 to 7.67 μmol TE·g^{-1} and 17.32 to 82.89%, respectively. The values measured in this study were higher than those obtained by Kaur et al. (2007), who found an antioxidant activity range of 2.86-7.67 μmol TE·g^{-1} and antioxidant capacities of 54.49-74.64% in various F1 broccoli hybrids. In the florets, the values from the FRAP, ABTS, and DPPH assays were higher in F1 hybrids and inbred lines (e.g., inbred line 5307) than in commercial cultivars, indicating their potential value for health. Overall, the values obtained for TPC, TFC, ascorbic acid, and antioxidant activities in this study were different from those reported in the literature (e.g., Kaur et al., 2007; Bhandari and Kwak, 2014), potentially because of differences in genotype, growing conditions, and experimental conditions (Kurilich et al., 2002; Perez-Balibrea et al., 2011).

Effects of Maternal and Paternal Lines on GSL Content in F1 Hybrids of Broccoli

Three maternal genotypes (5402, 5405, and 5411) were crossed with four paternal genotypes (5301, 5302, 5304, and 5307) to obtain F1 hybrids (Table 2). The major GSL concentrations, TPC, TFC, ascorbic acid, and antioxidant activities in florets were compared among maternal, paternal, and F1 hybrids. The F1 hybrids, obtained from crosses between different maternal and paternal genotypes, showed significant accumulation of BRA and total GSLs; however, in most of the F1 hybrids, PRO+EPI and GRA showed no relationship with paternal lines. Among the F1 hybrids, 5035 and 5036 had relatively higher total GSL contents compared to those in other F1 hybrids. F1 hybrid 5035 had the highest TPC, TFC, and antioxidant activity among hybrids, and F1 hybrid

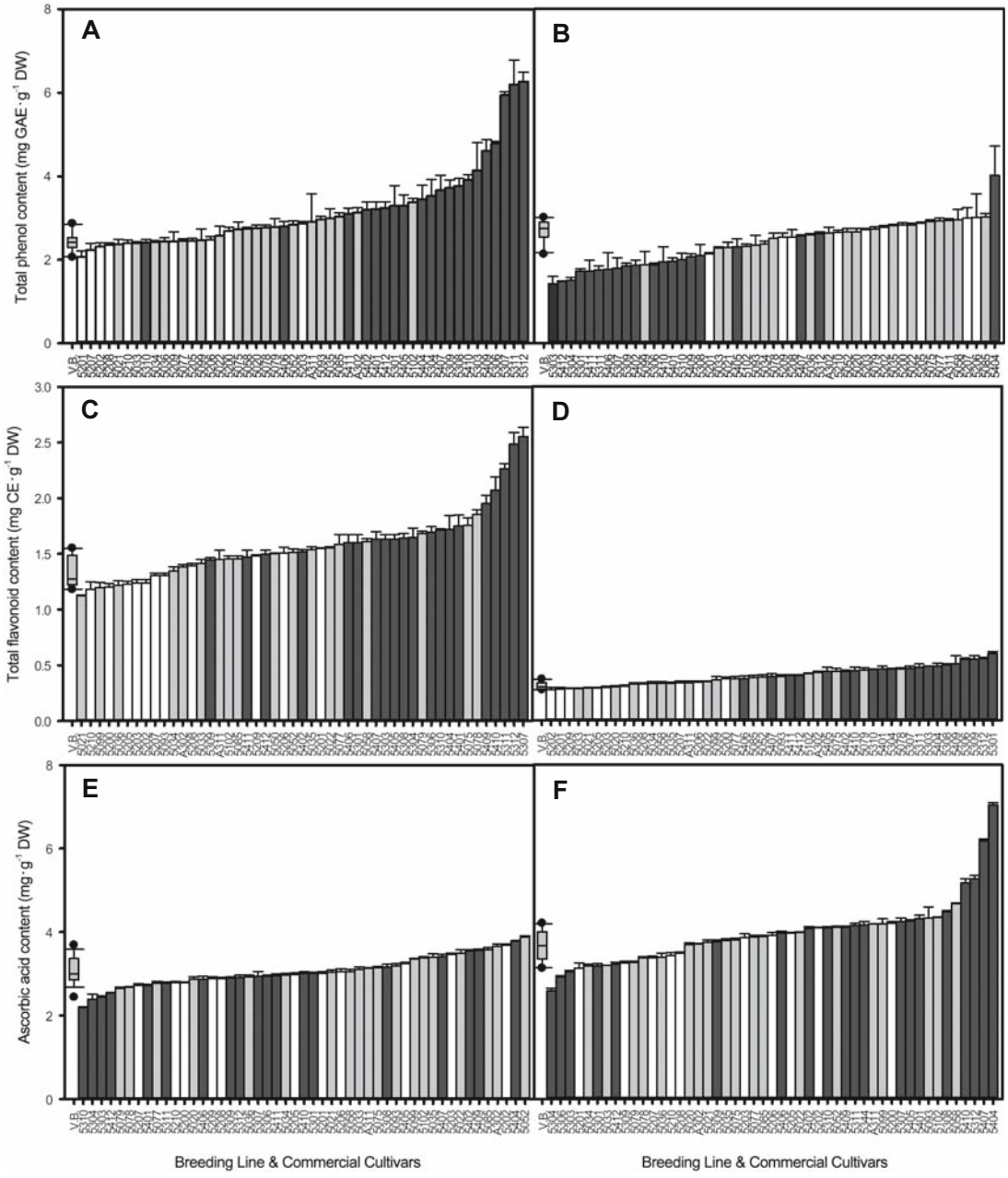

Fig. 5. Total phenol (A and B), total flavonoid (C and D) and ascorbic acid (E and F) content in florets (A, C and E) and flower stalks (B, D and F) of 49 broccoli breeding lines. The white, gray, and black colors in each bar represent commercial cultivar, F1 hybrid, and inbred line, respectively. Each vertical box represents the average total phenol content of ten commercial cultivars. Each vertical bar represents mean ± SD of the three replications.

5052 had the highest ascorbic acid content. Furthermore, only F1 hybrids 5035 and 5099 showed significant transfer of antioxidant activities and ascorbic acid content from their parental lines, respectively. These results suggest that the accumulation and transfer of phytochemicals in broccoli depends on the nature of the compounds.

Selection of Functional Broccoli Breeding Lines

Results of the principal component analysis (PCA) for individual GSLs confirmed that the highest concentrations of GRA and BRA were in inbred lines 5079 and 5402, respectively (Fig. 7A). This finding is of particular significance, as these GSLs have been shown to inhibit carcinogenesis and tumor growth by inducing apoptosis and cell cycle arrest in cancer cell lines (Nachshon-Kedmi et al., 2004; Traka and Mithen, 2009; Latte et al., 2011). PRO was found in the highest quantities in 5402, while ERU was highest in 5301. Likewise, the PCA of antioxidant activity showed that

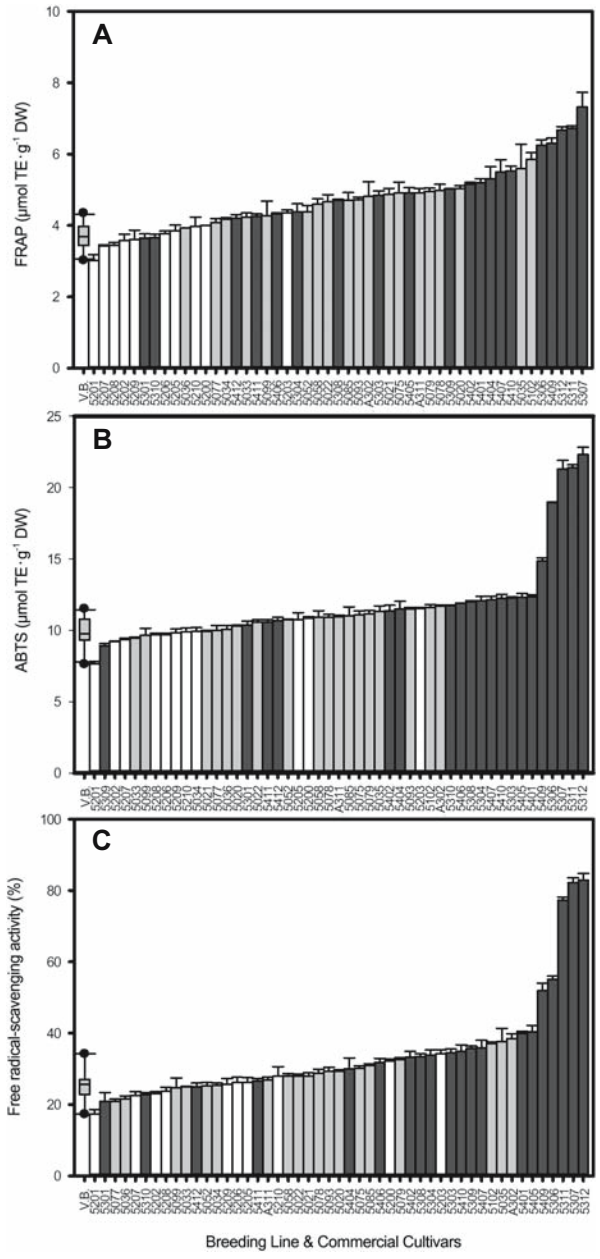

Fig. 6. Antioxidant activities of broccoli florets measured by ferric reducing antioxidant power (FRAP) (A), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (B), and free radical scavenging activity (C) assays. The white, gray, and black colors in each bar represent commercial cultivar, F1 hybrid, and inbred line, respectively. Each vertical box represents the average antioxidant activity of ten commercial cultivars. Each vertical bar represents mean ± SD of the three replications.

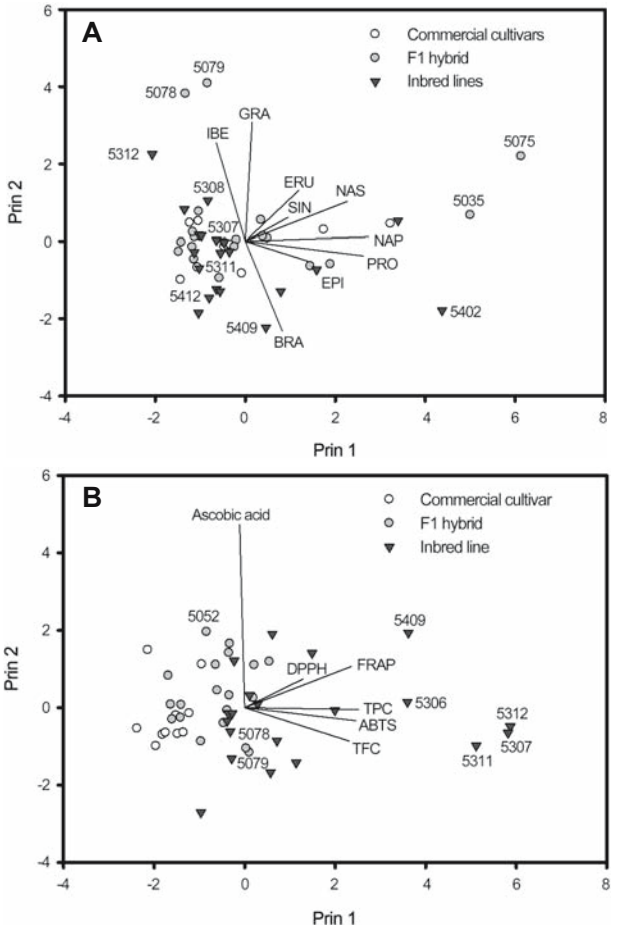

Fig. 7. Principal component analysis (PCA) of individual glucosinolates (A), and total phenol content (TPC), total flavonoid content (TFC), and ascorbic acid contents and antioxidant activities (B) in 49 broccoli breeding lines. IBE, glucoiberin; PRO, progoitrin; EPI, epiprogoitrin; SIN, sinigrin; GRA, glucoraphanin; NAP, gluconapin; ERU, glucoerucin; BRA, glucobrassicin; NAS, gluconasturtiin).

inbred lines 5306, 5307, 5311, 5312 and 5409 had the highest antioxidant activities (Fig. 7B). Similarly, total phenol and total flavonoid content were highest in 5307, 5311 and 5312. Results of the individual GSL cluster analyses displayed clear agglomeration of the GRA (5078, 5079, and 5308) and BRA groups (5311, 5402, 5409, and 5412) (Fig. 8A). Groups with high and low antioxidant activities were confirmed by cluster analysis. In addition, lines 5307, 5311, and 5312 were classified in a group with high antioxidant capacity (Fig. 8B). Likewise, four F1 hybrids and seven inbred lines showed higher total GSL concentrations than those in commercial cultivars. Among the four F1 hybrids, 5078 and 5079 had the highest concentrations of GRA and total GSL with low PRO+EPI content. Likewise, inbred lines 5307 and 5311 had the highest concentrations of GRA and total GSL, the highest antioxidant activity, and low PRO+EPI content. The selection of superior breeding lines is important for future commercialization. The superior health-promoting effects and nutritional qualities of these broccoli cultivars lead us to recommend their commercialization.

In conclusion, the present study identified wide genotypic variation in the profiles (TPC, TFC, ascorbic acid, and antioxidant activities) and concentrations of various GSLs in the florets and flower stalks of broccoli. Broccoli florets had

Table 2. Glucosinolate (GSL), total phenol, total flavonoid, and ascorbic acid contents, and antioxidant activities in maternal, paternal, and F1 hybrid lines

Female × male	F1 Hybrid	Concentration (μmol·g-1 DW)				TPC (mg GAE·g^{-1} DW)	TFC (mg CE·g^{-1} DW)	Ascorbic acid (mg·g^{-1} DW)	FRAP (μmol TE·g^{-1} DW)	ABTS (μmol TE·g^{-1} DW)	DPPH (%)
		PRO+EPI	GRA	BRA	Total GSL						
5402×5301		3.51×1.22[z]	1.58×0.96	10.76×6.84	16.99×11.62	3.20×1.60	1.52×3.29	3.55×3.02	5.16×3.65	11.38×10.39	33.23×20.86
	5035	2.93	2.48	3.91	11.08	2.99	1.54	3.41	5.59	11.32	37.64
5402×5304		3.51×0.00	1.58×1.81	10.76×0.86	16.99×3.56	3.20×3.53	1.52×1.65	3.55×2.39	5.16×4.38	11.38×12.08	33.23×33.87
	5034	0.90	1.65	1.71	5.06	2.43	1.35	2.97	4.18	9,92	25.32
5402×5307		3.51×0.02	1.58×3.21	10.76×6.75	16.99×10.46	3.20×5.94	1.52×2.55	3.55×2.94	5.16×7.32	11.38×21.31	33.23×82.15
	5036	2.00	2.22	6.19	11.19	2.44	1.22	2.93	3.39	10.09	21.61
5402×5303		3.51×0.02	1.58×1.41	10.76×1.49	16.99×3.77	3.20×4.14	1.52×1.63	3.55×2.44	5.16×4,85	11.38×12.29	33.23×34.49
	5033	1.12	1.66	3.38	7.04	2.39	1.42	3.11	4.24	9.45	24.94
5405×5303		1.17×0.02	0.70×1.41	5.61×1.49	8.15×3.77	3.30×4.14	1.63×1.63	3.25×2.44	4.91×4.85	12.33×12.29	40.41×34.49
	5052	0.29	1.21	3.03	4.87	2.84	1.51	3.88	4.38	10.75	25.27
5411×5303		0.00×0.02	1.36×1.41	4.52×1.49	6.10×3.77	3.10×4.14	1.47×1.63	2.96×2.44	4.26×4.85	10.57×12.29	26.56×34.49
	5099	0.00	1.01	2.22	3.43	2.46	1.20	3.36	4.28	9.65	24.71

[z]Values are mean of three replications. DW, dry weight; TPC, total phenol content; TFC, total flavonoid content; FRAP, ferric reducing antioxidant power; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; PRO, progoitrin; EPI, epiprogoitrin; GRA, glucoraphanin; BRA, glucobrassicin.

relatively higher concentrations of phytochemicals and antioxidant activities compared to flower stalks, except for the concentration of ascorbic acid. Altogether, 11 breeding lines (five F1 hybrids and six inbred lines) were selected as superior genotypes with higher total glucosinolate content. Furthermore, three F1 hybrids (5075, 5078, and 5079) and one inbred line (5308) with highest GRA concentration, and four inbred lines (5311, 5402, 5409, and 5412) with the highest concentration of BRA compared to other breeding lines in their florets, make them ideal candidates for commercialization.

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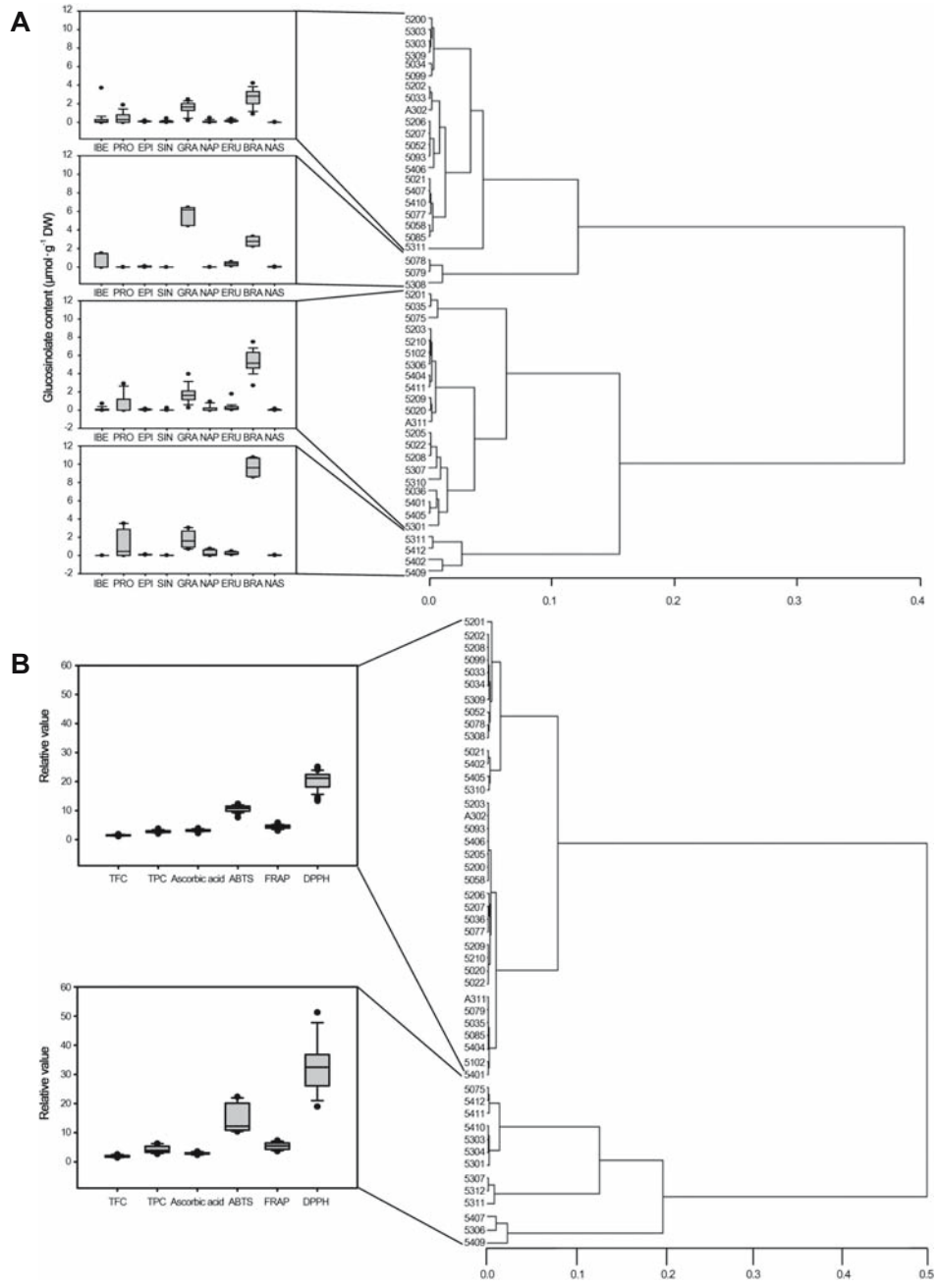

Fig. 8. Cluster analysis of individual glucosinolates (A), total phenol, total flavonoid, and ascorbic acid contents and antioxidant activities (B) in 49 broccoli breeding lines. IBE, glucoiberin; PRO, progoitrin; EPI, epiprogoitrin; SIN, sinigrin; GRA, glucoraphanin; NAP, gluconapin; ERU, glucoerucin; BRA, glucobrassicin; NAS, gluconasturtiin; TPC, total phenol content; TFC, total flavonoid content; FRAP, ferric reducing antioxidant power; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl.

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