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Effect of Genotype and Environmental Conditions on Health-Promoting Compounds in *Brassica rapa*

Marta Francisco,* María Elena Cartea, Pilar Soengas, and Pablo Velasco

Misión Biológica de Galicia (CSIC), P.O. Box 28, E-36080 Pontevedra, Spain

ABSTRACT: It is well-known that a variety of factors (genetic and environmental) affect the ultimate metabolite levels in brassica vegetables, although there is still little information about the role that genetics and environment play on glucosinolates and phenolic levels. Total glucosinolates were more abundant in turnip tops (26.02 μmol g^{-1} dw) than in turnip greens (17.78 μmol g^{-1} dw). On the other hand, total phenolic content was found in higher quantities in turnip greens (43.81 μmol g^{-1} dw) than in turnip tops (37.53 μmol g^{-1} dw). Aliphatic glucosinolates were clearly regulated by genotype; in contrast, the effects of environment and genotype × environment interaction on the indolic glucosinolate and phenolic compounds content appeared to be the main effects of variation. Identification of genotypes with enhanced and stable levels of these compounds would provide a value-added opportunity for marketing this crop with superior health promotion to consumers.

KEYWORDS: *Brassica rapa*, turnip greens, turnip tops, plant organ, climatic factors, glucosinolates, phenolic compounds

INTRODUCTION

Brassica vegetables, which are commonly known as crucifers, include a variety of horticultural crops (e.g., broccoli, Brussels sprouts, cabbage, cauliflower, turnip), which play a significant role in worldwide vegetable production and consumption. Brassica vegetables are low-fat and low-protein foods and have a high content in vitamins, fiber, and minerals. Besides, they show high quantities of phytochemicals, such as glucosinolates and phenolic compounds, which are widely studied for their beneficial properties.[1−3]

Glucosinolate diversity varies widely among families and species, suggesting that diversification has accompanied speciation.[4] Distribution of glucosinolates and phenolic compounds has been the target of several comprehensive reviews[1,4,5] showing that the profiles and amounts of these phytochemicals vary widely among families, species, and cultivars. Besides, the type and content of glucosinolate and phenolic levels depend on the plant part and may vary in vegetative and floral tissues during ontogeny.[4,6,7] In addition, there are many environmental factors that play a role in regulating the expression of these metabolites. Nitrogen and sulfur applications to the soil have a different effect on glucosinolate and phenolic content in the edible parts of brassicas.[8] Related to climatic conditions, winter or autumn seasons seem to lead to lower glucosinolate and flavonoid levels, due to short days, wetter conditions, cool temperatures, and less radiation.[6,9] Moreover, it has been reported that a higher disease and pest pressure influenced the concentration of these compounds.[6]

Sites regression (SREG)[10] has been suggested as an appropriate model to study the influence of genotype (G), environment (E), and genotype × environment interaction (GE) when large variation is due to E.[11] Although this method was mainly used for yield studies, nowadays it has been extended to other types of studies conducted in breeding programs to study the host−pathogen relationship[12] or gene × environment correlations.[13] The SREG method provides a graphical display called GGE (G plus GE interaction) biplot that facilitates visual cultivar evaluation.

In northwestern Spain and Portugal, *Brassica rapa* subsp. *rapa* L. includes turnip greens and turnip tops for culinary profit as well as turnips for fodder.[14] Turnip greens are the leaves harvested during the vegetative period, whereas turnip tops are the fructiferous stems with the flower buds and surrounding leaves, which are consumed before opening and while still green. Agriculture is still very traditional in these countries, and even today farmers continue to grow landraces in vegetable gardens for their own consumption.

A collection of *B. rapa* subsp. *rapa* from northwestern Spain is currently kept at the Misión Biológica de Galicia (CSIC, Spain). In a preliminary work, part of this collection was evaluated on the basis of agronomical and morfological traits,[14] finding that in many cases, the same landrace is sown for more than one purpose. This fact allows the existence of local populations with high levels of variability. Further studies determined the variation of desulfoglucosinolates among varieties,[15] and recently, Francisco et al.[16] determined the profile of intact glucosinolates and phenolic compounds in two different organs, leaves and shoots, in representative varieties of this collection. However, little information is available about the stability of glucosinolate and phenolic compounds profiles among varieties across environments and developmental stages.

Because a variety of factors affect the ultimate bioactive compounds levels in vegetables, it is necessary to study the role that genetics and environment play on these levels to improve the health benefit of functional foods. This study will determine which plant part contains the highest concentration of these beneficial compounds for human health and their environmental influence. The objectives of this study were (i) to evaluate the

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content and distribution of glucosinolates and phenolic compounds in two distinct edible parts of turnip (turnip greens and turnip tops) from *B. rapa* varieties grown in different production areas and (ii) to study the environmental influence on those health-promoting bioactive compounds.

MATERIALS AND METHODS

Plant Material. Twelve local varieties of *B. rapa* were evaluated in this study. From these 12 varieties, 10 were chosen on the basis of the study carried out by Padilla et al.[14] because of their agronomic performance to produce turnip tops and/or turnip greens: MBG-BRS0082, MBG-BRS0143, MBG-BRS0173, MBG-BRS0184, MBG-BRS0401, MBG-BRS0433, MBG-BRS0451, MBG-BRS0461, MBG-BRS0472, MBG-BRS0550, and two varieties derived from three cycles of masal selection for fresh yield, MBG-BRS0163(S)C3 and MBG-BRS0197-(S)C3. Varieties were transplanted in three years (2006, 2007 and 2008) at three locations that represent standard *B. rapa* production areas in northwestern Spain: Oroso (A Coruña) (43° 1′ N, 8° 26′ W, 280 masl), Guitiriz (Lugo) (43° 12′ N, 7° 53′ W, 516 masl), and Salcedo (Pontevedra) (42° 24′ N, 8° 38′ W, 20 masl). In Salcedo, trials were lost due to unfavorable climatic conditions in 2006 and to plant damage caused by *Delia radicum* L. immediately after transplanting in 2007. Varieties were planted in multipot trays, and seedlings were transplanted into the field at the five- or six-leaf stage. Transplanting dates were from September to October. Varieties were transplanted in a randomized complete block design with three replications. The experimental plots consisted of 3 rows with 10 plants per row. Rows were spaced 0.8 m apart, and plants within rows were spaced 0.5 m apart. Cultural operations, fertilization, and weed control were made according to local practices. Three samples of healthy leaves (turnip greens) and young shoots (turnip tops) from five plants per plot were used. Turnip greens harvest ranged from 44 to 64 days after planting, whereas turnip top harvest ranged from 98 to 229 days after planting according to the maturity cycle of each variety at the optimum time for consumption, just after flower bud formation and before flower opening. After harvesting on dry ice, the material was immediately transferred to the laboratory and frozen at −80 °C, prior to its lyophilization. The dried material was powdered by using an IKA-A10 (IKA-Werke GmbH & Co. KG) mill, and the powder obtained was used for analysis.

Extraction and Determination of Glucosinolates and Phenolic Compounds. The LC gradient for glucosinolate and phenolic analyses is a multipurpose chromatographic method that simultaneously separates glucosinolates and phenolics, and it was recently applied to Galician brassicas.[16] A portion of 150 mg of each sample was extracted in 4 mL of 70% MeOH at 70 °C for 30 min with vortex mixing every 5 min to facilitate the extraction. The samples were centrifuged (13000*g*, 15 min), 1 mL of supernatants was collected, and methanol was completely removed by using a sample concentrator (DB-3D, Techne, U.K.) at 70 °C. The dry material obtained was redissolved in 1 mL of ultrapure water and filtered through a 0.20 μm syringe filter (Acrodisc Syringe Filters, Pall Life Sciences). Chromatographic analyses were carried out on a Luna C18 column (250 mm × 4.6 mm, 5 μm particle size; Phenomenex, Macclesfield, U.K.). The mobile phase was a mixture of (A) ultrapure water/trifluoroacetic acid (TFA) (99.9:0.1) and (B) methanol/TFA (99.9:0.1). The flow rate was 1 mL min^{-1} in a linear gradient starting with 0% B after 0−5 min, reaching 17% B after 15−17 min, 25% B after 22 min, 35% B after 30 min, 50% B after 35 min, 99% B after 50 min, and 0% B after 55−65 min. The injection volume was 20 μL, and chromatograms were recorded at 330 nm for phenolic derivatives and at 227 nm for glucosinolates in a model 600 HPLC instrument (Waters) equipped with a model 486 UV tunable absorbance detector (Waters). Glucosinolates were quantified by using sinigrin (sinigrin monohydrate from Phytoplan, Diehm and Neuberger GmbH, Heidelberg, Germany) as standard. Caffeoylquinic and *p*-coumaroylquinic acid derivatives were quantified as chlorogenic acid (5-caffeoylquinic acid, Sigma-Aldrich Chemie GmbH, Steinheim, Germany), flavonoids as kaempferol 3-rutinoside (Extrasynthese, Genay, France), and sinapic acid and derivatives as sinapic acid (Sigma).

Soil Analyses and Climate Data. Soil samples were collected at the three above-mentioned environments. Samplings were carried out by using a hollow cylindrical corer with an internal diameter of 7 cm. Six subsamples, 25 cm deep each, were taken by following a zigzag path across the center of each plot. Subsamples were mixed to obtain a homogeneous sample, about 500−1000 g, to be analyzed. The soil properties examined were pH, percentage of organic matter, available phosphorus, available potassium, exchangeable magnesium, exchangeable cations (Ca, Mg, Na, K, and Al) and cation exchange capacity. Soil analyses were performed at Estación Fitopatológica do Areeiro (Salcedo, Spain). Glucosinolate and phenolic contents were related to several climatic covariables: precipitation, degree days, mean of the maximum temperature, mean of the minimum temperature, mean of the mean temperature, number of days with maximum temperature over 30 and 20 °C, number of days with a mean temperature over 20 and below 10 °C, and number of days with a minimum temperature below 10 and 0 °C. Climatic data were obtained from meteorological stations located close to the experimental fields.

Statistical Analyses. Analyses of variance were performed for each trait according to a randomized complete block design. Years, locations, and varieties were considered to be a fixed effect. Comparisons of means among varieties in each plant organ were performed by using Fisher's protected least significant difference (LSD) at $P = 0.05$.[17] Simple correlation coefficients ($P < 0.05$) between secondary metabolites and climatic data were made to establish the relationships between them. To study the genotype × location (GE) interaction, SREG was used.[10] Each environment was defined as the combination of a year and a location resulting in seven different environments under study. Because this method does not allow missing data, 11 varieties were evaluated for turnip greens assessment and 9 varieties for turnip tops at 5 locations. For this method, principal component (PC) analysis was made on residuals of an additive model with locations as the only main effects. A two-dimensional biplot called the GGE biplot (G plus GE interaction) of the two first PCs was used to display genotypes and environments simultaneously.[11] Genotypes and locations were displayed in the same plot. Each genotype and location was defined by the scores of genotypes and locations in the two PCs, respectively. All statistical analyses were made by the SAS program.[18]

RESULTS AND DISCUSSION

The glucosinolate and phenolic profile of *B. rapa* varieties studied in this work was composed by eight glucosinolates belonging to the 3 chemical classes (progoitrin, glucoraphanin, gluconapin, 4-hydroxyglucobrassicin, glucobrassicanapin, glucobrassicin, neoglucobrassicin and gluconasturtiin) and 17 phenolic compounds, of which 9 were flavonoids and 8 were hydroxycinnamic acids. Flavonoids were glycosylated in the 3-position with sophoroside, with some compounds simultaneously acylated with cinnamic acids, and in the 7-position with glucose: (**1**) quercetin-3-*O*-(methoxycaffeoyl) sophoroside-7-*O*-glucoside; (**2**) quercetin-3-*O*-(caffeoyl) sophoroside-7-*O*-glucoside; (**3**) kaempferol-3-*O* (methoxycaffeoyl) sophoroside-7-*O*-glucoside; (**4**) kaempferol-3-*O* (caffeoyl) sophoroside-7-*O*-glucoside; (**5**) quercetin-3,7-di-*O*-glucoside; (**6**) kaempferol-3-*O*-(sinapoyl) sophoroside-7-*O*-glucoside; (**7**) kaempferol-3-*O*-(feruloyl) sophoroside-7-*O*-glucoside; (**8**) kaempferol-3,7-di-*O*-glucoside; (**9**) isorhamnetin-3,7-di-*O*-glucoside. Hydroxycinnamic acids were quinic acids and sinapic acids derivatives: (3CQA) 3-caffeoyl quinic acid;

Table 1. Mean Squares of the Combined Analysis of Variance for the Individual and Total Glucosinolate Content in the *B. rapa* Varieties Evaluated in Northwestern Spain[a]

	Glucosinolates										
trait	PRO	GRA	GNA	OHGBS	GBN	GBS	GST	NGBS	ALIPH	INDOL	GLUCT
location (L)	1.14	0.29	3740**	0.05	3.03	0.89**	0.32*	0.15	482.33**	0.93**	513.68**
year (Y)	9.89**	6.99**	102.71*	9.38**	2.41	10.68**	4.37**	6.69**	168.85**	65.24**	322.36**
variety (V)	6.48**	0.34**	513.20**	0.30**	8.12**	0.20**	0.11**	0.05	440.81**	0.36*	443.99**
plant organ (P)	23.81**	0.60*	5000**	0.17*	21.29**	0.33*	0.82**	0.89**	6551**	0.01	6408**
L × Y	0.02	0.41	150.84**	0.24*	7.64**	0.12	0.56**	0.05	217.12**	0.04	224.40**
V × L	0.44	0.18**	6.43	0.039	0.44	0.04	0.05	0.10*	7.62	0.29*	6.8
V × Y	1.27**	0.21**	24.82**	0.07*	1.78**	0.11	0.07	0.06	40.35**	0.29*	43.09**
P × L	1.00**	1.20**	25.16	0.05	0.35	0.19	0.31**	0.47**	16.03	1.49**	25.13
P × Y	3.49**	0.06	802.67**	0.88**	18.15**	3.06**	0.13	3.16**	46.44**	14.93**	1422**
P × V	2.84**	0.14	61.37**	0.09**	1.48**	0.17**	0.15**	0.04	104.75**	0.32*	48.58**
V × L × Y	0.51*	0.08	44.59**	0.033	1.20**	0.05	0.09**	0.03	44.49**	0.14	47.95**
P × L × Y	1.18*	0.04	75.90**	0.17**	4.63**	0.09	0.24**	0.01	20.72	0.58*	110.46**
P × V × L	0.72**	0.12	17.15	0.03	0.71	0.05	0.03	0.13**	23.51	0.32**	22.65
P × V × Y	1.05**	0.18*	20.36*	0.07**	1.05**	0.07	0.16**	0.05	17.32	0.23	27.32*
P × V × L × Y	0.5	0.15	14.57	0.02	0.91*	0.07	0.08*	0.04	32.90**	0.16	19.52
error	0.43	0.15	23.05	0.05	0.85	0.10	0.07	0.05	32.53	0.12	35.21

[a] PRO, progoitrin; GRA, glucoraphanin; GNA, gluconapin; 4-OHGBS, 4-hydroxyglucobrassicin; GBN, glucobrassicanapin; GBS, glucobrassicin; GNT, gluconasturtiin; NGBS, neoglucobrassicin; ALIPH, total aliphatics; INDOL, total indolics; GLUCT, total glucosinolates. *, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$.

Table 2. Mean Squares of the Combined Analysis of Variance for the Major Individual and Total Flavonoid and Hydroxycinnamic Acid Content in the *B. rapa* Varieties Evaluated in Northwestern Spain*

	Phenolic compounds													
trait	**3**	**4**	**6**	**7**	**8**	**9**	FLAVt	**3CQA**	**SG**	**SA**	**A1**	**A2**	HIDRt	PHENt
location (L)	6.25*	5.42*	5.58**	2.48*	6.73**	22.69**	255.24**	0.24**	38.05**	192.03*	4.60	5.52	440.24**	1291**
year (Y)	24.87**	69.84**	3.26**	1.35	38.30**	61.79**	866.30**	0.39**	380.06	233.28*	19.54**	55.06**	477.02**	2739**
variety (V)	1.99**	2.16**	3.24**	1.18	3.01**	1.79	53.83**	0.24**	2.45**	40.68*	3.89**	14.51**	23.78	106.80
plant organ (P)	2.17	39.54**	1.29*	0.14	201.73**	0.28	40.69	0.01	157.62**	15170**	244.72**	441.42**	4412**	3432**
L × Y	1.25	1.34	0.25	0.09	1.10	2.58	16.49	0.16*	3.29	174.17*	3.03	0.26	135.79	281.13
V × L	0.67	0.53	0.15	0.30	0.94	0.95	9.03	0.02	2.27**	18.47	0.89	1.67	24.25	44.96
V × Y	1.21*	0.80	0.33	0.50	1.21	1.69	17.86	0.30*	0.55	23.11	1.28	4.31**	51.92	110.53
P × L	2.03	1.39	1.43**	0.90	7.26**	6.02**	89.83**	0.06*	4.72**	232.30**	2.86	2.16	131.19*	151.57
P × Y	6.93**	33.53**	7.21**	10.42**	22.23**	0.03	464.39**	0.21	86.84**	704.31**	51.71**	121.00**	2159**	4464**
P × V	1.14	0.29	0.49**	0.6*	0.92	1.77	12.32	0.33*	2.37**	23.25	1.25	1.92	34.31	68.42
V × L × Y	1.27*	1.12*	0.60**	0.45	1.14*	1.63	22.31*	0.03	0.36	29.40	1.26	1.45	56.99*	126.47
P × L × Y	2.35*	1.93	1.09**	0.02	7.64**	3.51	55.70*	0.10**	1.00	102.88**	4.79	8.84**	179.26**	354.62**
P × V × L	0.49	0.53	0.22	0.30	1.02	0.88	7.83	0.01	2.49**	10.32	1.31	1.98	15.68	36.02
P × V × Y	1.51	0.79	0.47*	0.27	1.43**	1.33	23.14*	0.01	0.77	14.67	0.93	2.18	26.76	87.05
P × V × L × Y	0.40	0.31	0.20	0.20	0.88	1.59	3.81	0.03*	0.57	11.84	0.59	1.89	23.56	35.55
error	1.06	1.23	0.35	0.40	0.81	1.19	19.18	0.02	0.97	48.72	2.85	3.35	61.86	138.71

* **3**, kaempferol-3-*O* (methoxycaffeoyl) sophoroside-7-*O*-glucoside; **4**, kaempferol-3-*O* (caffeoyl) sophoroside-7-*O*-glucoside; **6**, kaempferol-3-*O*-(sinapoyl) sophoroside-7-*O*-glucoside; **7**, kaempferol-3-*O*-(feruloyl) sophoroside-7-*O*-glucoside; **8**, kaempferol-3,7-di-*O*-glucoside; **9**, isorhamnetin-3,7-di-*O*-glucoside; FLAVt, total flavonoids; **3CQA**, 3-caffeoyl quinic acid; **SA**, sinapic acid; **SG**, sinapoylglucoside; **A1**, 1,2-disinapoylgentiobioside; **A2**, 1-sinapoyl-2-feruloylgentiobioside; HIDRt, total hydroxycinnamic acids; PHENt, total phenolics. *, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$.

(**3*p*CoQA**) 3-*p*-coumaroylquinic acid; (**SA**) sinapic acid; (**SG**) sinapoylglucoside; (**A1**) 1,2-disinapoylgentiobioside; (**A2**) 1-sinapoyl-2-feruloylgentiobioside; (**A3**) 1,2,2′-trisinapoylgentiobioside; (**A4**) 1,2′-disinapoyl-2-feruloylgentiobioside.

Combined analysis of variance showed significant differences among varieties in seven glucosinolates (Table 1) and in most of the phenolic compounds mentioned above (Table 2). Year × variety, locality × variety, and year × locality × variety interactions were highly significant for most of these compounds (Tables 1 and 2), showing the great environmental influence on these compounds. Velasco et al.[6] in kale and Ciska et al.[19] in different cruciferous vegetables found that low temperatures

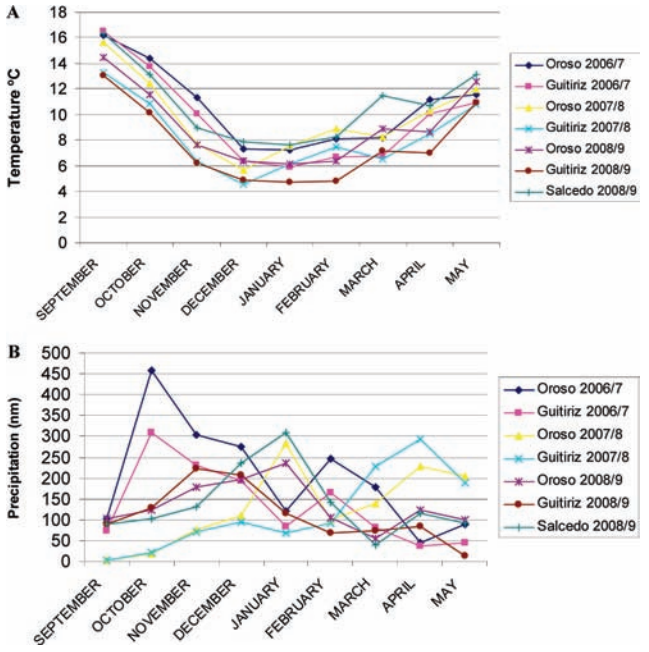

Figure 1. Mean temperature (A) and precipitation (B) from September to May in seven environments from northwestern Spain (Oroso, Guitiriz, and Salcedo) for 3 years (2006−2009).

caused a reduction on glucosinolate content, whereas a high average temperature increased glucosinolate concentration in a significant way. Moreover, lower average rainfall increased glucosinolate content.[4] With regard to phenolic compounds, there is a lack of information about the influence of climatic effects on these compounds in brassica crops, although Vallejo et al.[9] found that flavonoid content in broccoli was influenced by season.

Furthermore, significant differences were found among plant organs (turnip greens and turnip tops) for most compounds analyzed (glucosinolates and phenolics). As has been recently reported, concentrations of glucosinolates and phenolics in *B. rapa* vary across stage development.[20] Similarly to what happened with varieties, year × plant organ, locality × plant organ, and year × locality × plant organ interactions were significant for most compounds (Tables 1 and 2), showing once again the great environmental influence on these compounds. Despite these interactions, varieties and plant organs showed similar behaviors across locations and years for most compounds, mainly for the total glucosinolate and gluconapin content as well as for the total phenolics and SA content, which are the major glucosinolates and phenolics found in both organs, respectively. Therefore, a combined analysis of variance for each plant organ was made, focusing on the main effects, which are location, year, and variety (data not shown).

Variation of Glucosinolates among Plant Organs. In the individual analysis of variance of each plant organ, significant differences ($P \leq 0.01$) were found among varieties, locations, and years for most individual and total glucosinolate contents in both plant organs. Year × plant organ interactions were also significant. These interactions were mainly due to the different climatic conditions in each location and year, throughout the crop cycle (see Figure 1). Total glucosinolate content was higher in turnip tops than in turnip greens. For turnip greens, total glucosinolate content ranged from 14.35 to 23.60 μmol g^{-1} dw with a mean value of 17.78 μmol g^{-1} dw. For turnip tops, total glucosinolate content ranged from 20.18 to 36.36 μmol g^{-1} dw with a mean value of 26.02 μmol g^{-1} dw (Table 3). Similar values were found by Padilla et al.[15] and Francisco et al.[16] in *B. rapa* varieties from northwestern Spain. Differences in glucosinolate concentrations among different plant organs have also been reported by other authors. In kale, Velasco et al.[6] detected an increasing concentration of aliphatic glucosinolates in kale leaves from the early stage until the prebolting stage.

Glucosinolate quantification showed that aliphatic glucosinolates were predominant, representing 72 and 82% of the total glucosinolate content in turnip greens and turnip tops, respectively. Gluconapin was by far the most abundant glucosinolate in these cultivars, followed by glucobrassicanapin. Yang and Quiros[21] studied glucosinolate variation in more than 80 crops of *B. rapa*, and they found that the major glucosinolate was gluconapin. In our varieties, gluconapin levels represented between 49 and 68% and between 56 and 78% of the total glucosinolate content in turnip greens and turnip tops, respectively. The mean values of gluconapin were 10.21 μmol g^{-1} dw in turnip greens and 17.39 μmol g^{-1} dw in turnip tops. These contents are consistent with those previously found by Padilla et al.[15] and Francisco et al.[16] Some reports found that the pungent and bitter flavor of some brassica crops is related to gluconapin content.[4,15,22] The second glucosinolate in abundance, glucobrassicanapin, represented between 5 and 15% of the total glucosinolate content in both plant stages. The mean values of this glucosinolate were 1.90 μmol g^{-1} dw in turnip greens and 2.38 μmol g^{-1} dw in turnip tops.

Other aliphatic glucosinolates such as glucoraphanin and progoitrin were found in minor quantities (Table 3). Among glucosinolates present in *Brassica* crops, the most studied of them is glucoraphanin, the main glucosinolate in broccoli, which is thought to be a good source of cancer-protective compounds.[2] It is well-known that glucoraphanin, progoitrin, and gluconapin are in the same pathway of biosynthesis of aliphatic glucosinolates.[23] The fact that our varieties are very rich in gluconapin could offer future prospects to further modify glucosinolate composition and get glucoraphanin accumulating plants as a source of anticarcinogens. Biosynthesis of gluconapin requires a functional allele, *Brgsl-Alk*, that converts glucoraphanin to its alkenyl homologous, that is, gluconapin. Li and Quiros[23] obtained *Arabidopsis* plants with a reduced concentration of glucoraphanin, which was converted into gluconapin. Some approaches for developing a variety of *B. rapa* containing glucoraphanin are to produce *Brgsl-Alk* knockout lines to efficiently accumulate glucoraphanin in the side-chain modification pathway or to use gene-silencing methods such as RNAi to accomplish the same objective.[21]

The indole group of glucosinolates represented between 19 and 13% of total glucosinolate content in turnip greens and turnip tops, respectively. Glucosinolates belonging to this class found in our samples were 4-hydroxyglucobrassicin, glucobrassicicin, and neoglucobrassicin. In this group of compounds, differences among plant organs were not found, with means of 3.30 μmol g^{-1} dw in turnip greens and 3.21 μmol g^{-1} dw in turnip tops. It is interesting to note that, when these classes of compounds are hydrolyzed, they give rise to a range of involatile indole compounds that have been implicated in the anticarcinogenic activities of brassica vegetables.[2]

Gluconasturtiin was the only aromatic glucosinolate found, in concentrations of 1.52 and 1.43 μmol g^{-1} dw in turnip greens and turnip tops, respectively. Phenethyl isothiocyanate (PEITC) is the degradation product of gluconasturtiin, which appears in great quantities in watercress. A protective effect of PEITC has

Table 3. Mean (Micromoles per Gram dw) Glucosinolate Content in Turnip Greens and Turnip Tops from the *B. rapa* Varieties Evaluated in Northwestern Spain[a]

variety MBG-	Glucosinolates										
	PRO	GRA	GNA	OHGBS	GBN	GBS	GST	NGBS	ALIPH	INDOL	total GS
Turnip Greens											
BRS0082	0.88 ± 0.55	0.36 ± 0.46	8.47 ± 5.07	0.86 ± 0.40	2.09 ± 0.90	1.31 ± 0.26	1.37 ± 0.30	1.04 ± 0.17	11.81 ± 6.05	3.21 ± 0.68	16.38 ± 6.35
BRS0143	0.73 ± 0.59	0.25 ± 0.40	8.27 ± 3.37	0.96 ± 0.29	1.53 ± 0.19	1.18 ± 0.19	1.61 ± 0.24	1.06 ± 0.36	10.78 ± 3.54	3.21 ± 0.50	15.60 ± 3.33
BRS0163	0.63 ± 0.55	0.24 ± 0.36	13.52 ± 8.08	1.22 ± 0.09	2.26 ± 0.76	1.53 ± 0.30	1.49 ± 0.28	1.09 ± 0.20	16.65 ± 9.11	3.83 ± 0.36	21.98 ± 9.57
BRS0173	0.62 ± 0.71	0.29 ± 0.43	15.35 ± 5.75	0.99 ± 0.23	1.69 ± 0.49	1.19 ± 0.20	1.43 ± 0.23	1.05 ± 0.17	17.94 ± 5.93	3.23 ± 0.50	22.60 ± 6.02
BRS0184	1.06 ± 0.67	0.30 ± 0.47	9.15 ± 3.50	0.98 ± 0.24	2.78 ± 1.31	1.41 ± 0.28	1.63 ± 0.28	1.05 ± 0.32	13.31 ± 4.31	3.43 ± 0.59	18.37 ± 4.43
BRS0197	0.86 ± 0.74	0.40 ± 0.39	14.99 ± 5.89	1.20 ± 0.10	2.27 ± 0.80	1.27 ± 0.12	1.51 ± 0.20	1.09 ± 0.11	18.53 ± 7.00	3.55 ± 0.18	23.60 ± 7.12
BRS0401	0.75 ± 0.80	0.14 ± 0.24	10.93 ± 4.58	0.71 ± 0.40	1.70 ± 0.55	1.20 ± 0.20	1.50 ± 0.33	1.06 ± 0.13	13.52 ± 5.07	2.97 ± 0.50	17.99 ± 5.30
BRS0433	0.78 ± 0.55	0.00 ± 0.00	7.07 ± 3.76	1.01 ± 0.25	1.80 ± 0.60	1.22 ± 0.14	1.42 ± 0.28	1.08 ± 0.18	9.64 ± 3.87	3.29 ± 0.29	14.35 ± 3.45
BRS0451	0.59 ± 0.52	0.09 ± 0.24	9.55 ± 3.64	1.03 ± 0.20	1.94 ± 0.71	1.22 ± 0.17	1.59 ± 0.26	1.07 ± 0.18	12.18 ± 4.22	3.32 ± 0.42	17.09 ± 4.20
BRS0461	0.99 ± 0.46	0.24 ± 0.22	8.16 ± 3.33	1.08 ± 0.27	1.57 ± 0.27	1.23 ± 0.22	1.49 ± 0.32	1.07 ± 0.13	10.96 ± 3.69	3.38 ± 0.52	15.84 ± 3.78
BRS0472	0.67 ± 0.44	0.09 ± 0.24	8.52 ± 3.04	0.91 ± 0.29	1.58 ± 0.30	1.22 ± 0.21	1.55 ± 0.31	1.09 ± 0.15	10.86 ± 3.09	3.21 ± 0.55	15.61 ± 3.32
BRS0550	1.16 ± 0.66	0.24 ± 0.38	8.57 ± 4.15	1.05 ± 0.25	1.62 ± 0.44	1.22 ± 0.23	1.61 ± 0.44	1.05 ± 0.24	11.59 ± 4.67	3.31 ± 0.42	16.52 ± 4.78
Mean	0.80 ± 0.64	0.22 ± 0.37	10.21 ± 5.15	0.98 ± 0.29	1.90 ± 0.75	1.26 ± 0.23	1.52 ± 0.30	1.06 ± 0.20	12.96 ± 5.67	3.30 ± 0.52	17.78 ± 5.81
LSD (5%)	0.19	0.09	1.19	0.07	0.19	0.05	0.08	0.05	1.29	0.11	1.39
Turnip Tops											
BRS0082	2.02 ± 0.77	0.48 ± 0.60	12.29 ± 3.65	0.95 ± 0.40	2.64 ± 1.28	1.25 ± 0.34	1.37 ± 0.24	1.03 ± 0.24	17.43 ± 4.37	3.22 ± 0.86	22.02 ± 4.85
BRS0143	1.38 ± 0.82	0.46 ± 0.46	12.42 ± 4.57	0.94 ± 0.43	1.78 ± 0.66	1.52 ± 1.11	1.58 ± 0.32	0.96 ± 0.43	16.02 ± 5.72	3.40 ± 1.53	21.01 ± 6.32
BRS0163	0.75 ± 0.47	0.28 ± 0.35	24.58 ± 3.31	1.26 ± 0.10	3.16 ± 0.29	1.64 ± 0.28	1.58 ± 0.31	0.90 ± 0.51	28.78 ± 3.53	3.79 ± 0.62	34.15 ± 3.77
BRS0173	0.60 ± 0.53	0.23 ± 0.38	24.87 ± 8.61	1.04 ± 0.27	1.70 ± 0.59	1.23 ± 0.28	1.48 ± 0.27	0.85 ± 0.37	27.39 ± 9.23	3.11 ± 0.73	31.98 ± 9.60
BRS0184	1.42 ± 0.94	0.44 ± 0.48	15.85 ± 4.16	1.06 ± 0.39	3.73 ± 1.89	1.31 ± 0.43	1.42 ± 0.50	0.70 ± 0.52	21.44 ± 4.54	3.05 ± 1.07	25.91 ± 5.50
BRS0197	0.72 ± 0.42	0.35 ± 0.31	28.21 ± 5.94	1.20 ± 0.09	2.09 ± 0.38	1.38 ± 0.17	1.62 ± 0.20	0.80 ± 0.55	31.36 ± 5.96	3.38 ± 0.74	36.36 ± 0.20
BRS0401	0.41 ± 0.44	0.08 ± 0.22	20.21 ± 4.83	0.84 ± 0.37	1.85 ± 0.46	1.37 ± 0.43	1.38 ± 0.25	0.83 ± 0.30	22.55 ± 5.33	3.04 ± 0.88	26.97 ± 0.25
BRS0433	1.63 ± 0.82	0.06 ± 0.21	13.18 ± 2.15	0.80 ± 0.28	2.53 ± 0.70	0.94 ± 0.43	1.15 ± 0.26	0.98 ± 0.27	17.40 ± 2.66	2.71 ± 0.91	21.26 ± 2.34
BRS0451	1.03 ± 0.48	0.16 ± 0.24	14.25 ± 4.19	1.02 ± 0.40	2.17 ± 0.55	1.13 ± 0.38	1.45 ± 0.28	0.89 ± 0.46	17.60 ± 4.76	3.02 ± 1.00	22.08 ± 5.56
BRS0461	1.28 ± 0.65	0.40 ± 0.49	14.87 ± 4.70	1.02 ± 0.43	2.76 ± 1.90	1.32 ± 0.45	1.37 ± 0.30	0.86 ± 0.40	19.31 ± 6.43	3.20 ± 1.05	23.88 ± 7.53
BRS0472	0.99 ± 0.71	0.57 ± 0.51	12.70 ± 1.69	0.91 ± 0.28	1.70 ± 0.32	1.12 ± 0.31	1.41 ± 0.15	0.79 ± 0.39	15.95 ± 2.14	2.82 ± 0.74	20.18 ± 2.50
BRS0550	2.49 ± 1.47	0.57 ± 0.50	15.25 ± 5.50	1.14 ± 0.30	2.46 ± 1.20	1.18 ± 0.49	1.29 ± 0.26	0.91 ± 0.49	20.77 ± 6.74	3.23 ± 1.06	25.30 ± 7.50
Mean	1.28 ± 1.01	0.35 ± 0.46	17.39 ± 7.12	1.02 ± 0.36	2.38 ± 1.22	1.30 ± 0.51	1.43 ± 0.30	0.89 ± 0.42	21.38 ± 7.35	3.21 ± 0.99	26.02 ± 7.91
LSD (5%)	0.20	0.13	1.31	0.06	0.33	0.13	0.07	0.11	1.38	0.18	1.42

[a] PRO, progoitrin; GRA, glucoraphanin; GNA, gluconapin; 4-OHGBS, 4-hydroxyglucobrassicin; GBN, glucobrassicanapin; GBS, glucobrassicin; GNT, gluconasturtiin; NGBS, neoglucobrassicin; ALIPH, total aliphatics; INDOL, total indolics; total GS, total glucosinolates; LSD, least significant difference.

been reported as a inhibitor of tumor growth inducing the apoptosis of human prostate cancer cells.[2]

The cancer chemopreventive effect of cruciferous vegetables is mainly attributed to the degradation products of glucosinolates.[1,2] The most promising varieties for future breeding purposes would be those with the highest total glucosinolate content and, particularly, glucosinolates with beneficial effects related to human health. In the present study we found a high variability on glucosinolate content among varieties in both plant organs, MBG-BRS0197, MBG-BRS0163 and MBG-BRS0173 being the varieties that showed the highest values on total glucosinolate, total aliphatic, and gluconapin contents for both plant organs (Table 3). Variety MBG-BRS0163 also had the highest content of indolic glucosinolates (Table 3). In turnip greens, varieties with the highest gluconasturtiin content were MBG-BRS0184 and MBG-BRS0550, whereas in turnip tops these were varieties MBG-BRS0197 and MBG-BRS0163. Besides, variety MBG-BRS0143 had high levels of this glucosinolate in the two plant organs (Table 3). Apart from the medicinal value of isothiocyanates, these compounds also play a significant organoleptic role in brassica products.[4,14,22]

Variation of Phenolic Compounds among Plant Organs. For turnip tops, analysis of variance showed significant differences ($P \leq 0.01$) among varieties, locations, and years for most of the individual and total phenolics. For turnip greens, significant differences ($P \leq 0.01$) were found among the main effects for most of the individual phenolic compounds. Nevertheless, no differences were found among varieties in this plant organ for total flavonoids, total hydroxycinnamic acids, and total phenolics. Likewise, it happened on glucosinolate analysis; variety × year interaction was also significant in both plant organs. Total phenolic content was found in higher quantities in turnip greens than in turnip tops, and values ranged from 41.16 to 47.58 μmol g^{-1} dw with a mean value of 43.81 μmol g^{-1} for turnip greens and from

Table 4. Mean (Micromoles per Gram dw) for the Major Flavonoid and Hydroxycinnamic Acid Contents in Turnip Greens and Turnip Tops from the *B. rapa* Varieties Evaluated in Northwestern Spain[a]

variety	Phenolic compound														
MBG-	**3**	**4**	**6**	**7**	**8**	**9**	FLAVt	**3CQA**	**SG**	**SA**	**A1**	**A2**	**A3**	HIDRt	PHENt
							Turnip Greens								
BRS0082	3.49 ± 0.67	3.45 ± 0.89	1.85 ± 0.45	1.92 ± 0.46	2.04 ± 0.84	3.08 ± 1.40	18.01 ± 3.72	0.32 ± 0.11	1.19 ± 1.00	21.75 ± 5.66	1.91 ± 1.40	3.07 ± 2.82	0.70 ± 0.50	29.65 ± 7.22	47.58 ± 10.35
BRS0143	3.26 ± 0.53	2.85 ± 0.93	2.07 ± 0.55	1.98 ± 0.45	1.91 ± 0.93	2.75 ± 0.70	17.55 ± 2.75	0.46 ± 0.14	1.45 ± 0.89	19.67 ± 5.68	1.48 ± 0.82	2.20 ± 1.45	0.67 ± 0.81	26.85 ± 6.20	44.26 ± 8.34
BRS0163	3.13 ± 1.49	2.86 ± 1.26	1.80 ± 0.76	1.95 ± 0.95	1.78 ± 1.08	2.75 ± 0.82	16.36 ± 0.92	0.37 ± 0.16	1.70 ± 1.73	17.90 ± 8.45	1.85 ± 1.64	1.68 ± 1.30	0.75 ± 0.35	24.90 ± 9.08	41.26 ± 14.81
BRS0173	2.50 ± 0.59	2.51 ± 0.70	1.70 ± 0.46	1.45 ± 0.41	1.82 ± 0.75	3.10 ± 1.96	15.54 ± 3.69	0.45 ± 0.11	1.18 ± 0.90	22.29 ± 7.80	1.49 ± 0.85	1.38 ± 0.82	0.56 ± 0.31	28.07 ± 8.97	43.50 ± 11.48
BRS0184	3.06 ± 1.16	2.81 ± 0.76	1.91 ± 0.52	1.70 ± 0.43	2.01 ± 0.96	3.04 ± 1.35	17.10 ± 4.85	0.60 ± 0.18	1.46 ± 1.12	21.07 ± 6.03	1.78 ± 0.88	2.20 ± 1.46	0.85 ± 0.70	28.82 ± 7.74	45.80 ± 11.40
BRS0197	3.28 ± 0.90	3.03 ± 0.63	1.66 ± 0.47	1.70 ± 0.47	1.85 ± 0.95	3.34 ± 1.64	17.22 ± 4.02	0.39 ± 0.10	1.29 ± 0.93	20.34 ± 6.72	1.48 ± 1.30	1.33 ± 0.48	0.54 ± 0.16	25.98 ± 7.45	43.20 ± 10.83
BRS0401	3.06 ± 1.09	2.48 ± 0.95	1.90 ± 0.66	1.90 ± 0.67	2.33 ± 1.50	2.93 ± 1.00	16.83 ± 5.10	0.45 ± 0.12	1.17 ± 0.87	20.79 ± 7.87	1.11 ± 0.67	1.57 ± 1.08	0.68 ± 0.21	26.51 ± 9.19	43.21 ± 13.27
BRS0433	2.61 ± 0.38	2.46 ± 0.50	1.80 ± 0.48	2.27 ± 1.52	1.49 ± 0.26	2.99 ± 2.07	15.89 ± 2.78	0.62 ± 0.12	0.64 ± 0.30	18.39 ± 4.55	2.11 ± 0.93	3.00 ± 1.29	0.71 ± 0.26	26.32 ± 6.07	41.91 ± 7.48
BRS0451	3.00 ± 0.61	3.03 ± 1.59	2.21 ± 0.46	1.81 ± 0.39	1.79 ± 0.83	2.59 ± 0.94	17.00 ± 2.92	0.46 ± 0.13	1.57 ± 1.14	18.50 ± 5.62	2.15 ± 1.19	2.82 ± 1.83	0.71 ± 0.38	27.03 ± 5.29	43.89 ± 4.91
BRS0461	3.12 ± 0.66	2.52 ± 0.51	1.62 ± 0.31	1.65 ± 0.36	1.75 ± 0.47	2.36 ± 0.82	14.94 ± 2.60	0.45 ± 0.08	0.93 ± 0.70	20.22 ± 4.00	1.51 ± 0.90	1.68 ± 1.17	0.60 ± 0.30	26.37 ± 5.39	41.16 ± 6.78
BRS0472	3.51 ± 0.84	2.75 ± 0.82	2.47 ± 0.58	1.82 ± 0.37	2.10 ± 0.92	2.78 ± 1.06	17.69 ± 3.74	0.50 ± 0.15	0.92 ± 0.58	22.30 ± 5.25	1.80 ± 0.94	1.77 ± 1.12	0.53 ± 0.28	28.67 ± 5.74	46.23 ± 8.92
BRS0550	3.09 ± 1.52	2.74 ± 1.01	2.06 ± 0.75	1.73 ± 0.71	1.85 ± 0.92	2.78 ± 2.00	16.47 ± 6.07	0.58 ± 0.28	1.18 ± 1.09	18.45 ± 7.11	2.14 ± 1.40	2.10 ± 1.68	0.57 ± 0.36	25.76 ± 9.43	42.10 ± 15.27
mean	3.11 ± 0.95	2.79 ± 0.96	1.93 ± 0.59	1.80 ± 0.68	1.90 ± 0.92	2.88 ± 1.35	17.73 ± 4.16	0.47 ± 0.16	1.22 ± 0.99	20.25 ± 6.35	1.73 ± 1.12	2.08 ± 1.59	0.66 ± 0.38	27.21 ± 7.42	43.81 ± 10.66
LSD (5%)	0.33	0.31	0.18	0.24	0.31	0.45	1.44	0.05	0.22	2.16	0.33	0.43	0.12	3.67	2.43
							Turnip Tops								
BRS0082	2.99 ± 1.00	2.49 ± 1.34	2.08 ± 0.55	1.79 ± 0.52	3.51 ± 1.16	2.97 ± 1.03	17.94 ± 5.17	0.45 ± 0.15	2.12 ± 1.59	6.24 ± 5.10	4.26 ± 2.03	5.30 ± 2.03	1.47 ± 0.72	20.80 ± 6.33	38.73 ± 10.48
BRS0143	3.23 ± 0.88	1.95 ± 1.39	2.60 ± 0.86	2.23 ± 0.67	3.57 ± 1.41	3.39 ± 1.09	19.78 ± 5.40	0.50 ± 0.11	3.26 ± 2.25	7.54 ± 2.85	3.43 ± 1.05	4.82 ± 1.74	1.38 ± 0.54	21.95 ± 6.66	41.72 ± 11.52
BRS0163	2.90 ± 0.85	2.16 ± 0.96	2.01 ± 0.66	2.17 ± 0.67	3.00 ± 0.94	2.84 ± 1.39	17.33 ± 4.98	0.37 ± 0.14	3.43 ± 2.22	7.86 ± 1.99	3.97 ± 1.64	4.96 ± 2.14	1.72 ± 1.12	23.34 ± 4.13	40.66 ± 8.43
BRS0173	2.83 ± 1.03	1.70 ± 1.32	1.70 ± 0.68	1.59 ± 0.51	2.87 ± 1.03	2.52 ± 0.65	15.46 ± 4.67	0.37 ± 0.07	2.25 ± 1.74	7.48 ± 5.47	3.63 ± 1.41	3.93 ± 1.53	1.17 ± 0.34	19.68 ± 7.19	35.14 ± 11.46
BRS0184	2.66 ± 1.16	2.20 ± 1.76	1.72 ± 0.61	1.52 ± 0.59	3.43 ± 1.66	3.32 ± 1.73	16.75 ± 7.49	0.67 ± 0.24	2.05 ± 2.24	5.98 ± 3.31	3.65 ± 2.07	4.66 ± 2.85	1.45 ± 0.68	19.46 ± 8.36	36.21 ± 15.78
BRS0197	3.14 ± 0.66	2.00 ± 0.92	1.86 ± 0.49	1.91 ± 0.50	3.46 ± 1.01	2.82 ± 1.00	17.76 ± 3.72	0.38 ± 0.07	4.53 ± 2.34	8.85 ± 2.79	3.49 ± 1.46	3.84 ± 1.21	1.32 ± 0.80	23.27 ± 4.47	41.03 ± 7.00
BRS0401	2.76 ± 0.84	1.66 ± 1.03	1.99 ± 0.70	1.94 ± 0.40	3.94 ± 1.05	3.11 ± 1.03	17.78 ± 4.52	0.42 ± 0.09	3.39 ± 2.59	7.67 ± 4.41	2.86 ± 0.92	3.94 ± 0.97	1.22 ± 0.27	20.40 ± 6.37	38.18 ± 10.36
BRS0433	1.99 ± 0.67	1.53 ± 1.15	1.69 ± 0.32	1.30 ± 0.46	2.45 ± 1.20	2.61 ± 0.87	13.42 ± 5.32	0.59 ± 0.17	0.60 ± 0.30	5.93 ± 4.43	2.99 ± 1.12	3.93 ± 1.81	1.15 ± 0.37	16.07 ± 8.03	29.50 ± 13.15
BRS0451	3.43 ± 0.94	2.18 ± 1.08	2.54 ± 0.58	2.08 ± 0.58	3.49 ± 1.05	3.14 ± 1.55	19.20 ± 4.58	0.42 ± 0.06	3.03 ± 2.17	4.02 ± 3.61	3.67 ± 0.96	5.21 ± 2.10	1.31 ± 0.53	18.59 ± 5.61	37.79 ± 9.62
BRS0461	2.81 ± 1.10	1.86 ± 1.20	1.49 ± 0.53	1.39 ± 0.71	2.93 ± 1.32	2.54 ± 1.13	14.95 ± 5.71	0.47 ± 0.23	2.15 ± 1.95	7.41 ± 4.13	2.60 ± 1.46	3.14 ± 2.12	1.13 ± 0.65	17.89 ± 7.46	32.84 ± 12.72
BRS0472	3.04 ± 0.74	1.92 ± 1.55	2.43 ± 0.58	1.61 ± 0.54	3.62 ± 0.97	2.87 ± 1.12	17.34 ± 4.01	0.44 ± 0.08	3.16 ± 2.70	5.58 ± 3.47	2.83 ± 0.33	3.55 ± 1.37	0.89 ± 0.34	17.33 ± 4.90	34.66 ± 8.46
BRS0550	3.28 ± 0.20	1.86 ± 1.39	2.54 ± 0.84	1.97 ± 0.54	3.95 ± 1.36	2.70 ± 1.35	18.20 ± 3.31	0.55 ± 0.20	3.58 ± 3.57	4.94 ± 2.62	3.56 ± 1.57	4.78 ± 2.30	1.38 ± 0.65	19.71 ± 6.52	37.91 ± 8.07
mean	2.98 ± 1.02	2.04 ± 1.24	2.10 ± 0.73	1.82 ± 0.62	3.42 ± 1.24	2.96 ± 1.22	17.49 ± 5.19	0.47 ± 0.11	2.83 ± 2.40	6.63 ± 3.99	3.47 ± 1.48	4.40 ± 2.04	1.30 ± 0.64	20.04 ± 6.57	37.53 ± 10.99
LSD (5%)	0.28	0.27	0.18	0.15	0.29	0.33	1.16	0.05	0.30	0.98	0.45	0.54	0.17	1.40	2.20

[a] **3**, kaempferol-3-*O* (methoxycaffeoyl) sophoroside-7-*O*-glucoside; **4**, kaempferol-3-*O* (caffeoyl) sophoroside-7-*O*-glucoside; **6**, kaempferol-3-*O*-(sinapoyl) sophoroside-7-*O*-glucoside; **7**, kaempferol-3-*O*-(feruloyl) sophoroside-7-*O*-glucoside; **8**, kaempferol-3,7-di-*O*-glucoside; **9**, isorhamnetin-3,7-di-*O*-glucoside; FLAVt, total flavonoids; **3CQA**, 3-caffeoyl quinic acid; **SA**, sinapic acid; **SG**, sinapoylglucoside; **A1**, 1,2-disinapoylgentiobioside; **A2**, 1-sinapoyl-2-feruloylgentiobioside; **A3**, 1,2,2′-trisinapoylgentiobioside; HIDRt, total hydroxycinnamic acids; PHENt, total phenolics; LSD, least significant difference.

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29.50 to 41.72 μmol g^{-1} dw with a mean value of 37.53 μmol g^{-1} for turnip tops. These values were higher than those reported by other authors in different *Brassica oleracea* crops[7,24] and similar to those found in turnip tops by other authors.[20,24] According to our results, Fernandes et al.[20] found different concentrations of individual flavonoids and hydroxycinnamic acids among leaves and stems of *B. rapa*.

Hydroxycinnamic acids were the major phenolic compounds in *B. rapa* varieties evaluated in this work, representing 62 and 54% of the total phenolics in turnip greens and turnip tops, respectively. These percentages were higher than those reported by Sousa et al.[24] in the inflorescences of *B. rapa*. Hydroxycinnamic acids are found in higher quantities in turnip greens than in turnip tops, mainly determined by the content of SA, which reaches 74 and 33% of the total hydroxycinnamic acids in turnip greens and turnip tops, respectively. In turnip tops, SA content was 2−5 times the amount exhibited by turnip tops, with mean values of 20.25 and 6.63 μmol g^{-1} dw in turnip greens and turnip tops, respectively. On the contrary, sinapic acids derivatives (**A1**, **A2**, **A3**, and **A4**) were higher in turnip tops than in turnip greens, thus providing an added nutritional value to turnip tops. Plumb et al.[25] reported that the sinapoyl glucose and feruloyl glucose derivatives are highly effective in preventing lipid damage.

With regard to flavonoids, the most abundant ones were the kaempferol derivatives, which varied between 64 and 75% of the total flavonoid content in both plant organs. Flavonoids **3**, **4**, and **8** were the major kaempferol derivatives representing from 14 to 17% of the total flavonoid content. Despite not finding significant differences for total flavonoid content among plant organs, individual flavonoids were significantly different between these two plant organs. In turnip greens, flavonoids **3** and **4** reached maximum values. Mean values of flavonoid **3** were 3.11 μmol g^{-1} dw in turnip greens and 2.98 μmol g^{-1} dw in turnip tops, and the mean values for flavonoid **4** were 2.79 μmol g^{-1} dw in turnip greens and 2.04 μmol g^{-1} dw in turnip tops. These flavonoids were acylated with caffeic acid. The presence of an *O*-dihydroxy structure in the caffeoyl moiety confers great stability to their radical scavenging capacity.[26] On the other hand, flavonoid **8** reached maximum values in turnip tops, being also the major flavonoid in this plant organ with mean values of 3.42 μmol g^{-1} dw in turnip tops and 1.90 μmol g^{-1} dw in turnip greens. In contrast to other brassica vegetables, *B. rapa* varieties showed a high concentration of isorhamnetin (compound **9**), being the second flavonoid in abundance for most varieties, which represented between 15 and 20% of the total flavonoid content. This flavonoid did not show differences between plant organs and showed mean values of 2.88 and 2.96 μmol g^{-1} dw in turnip greens and tops, respectively. Isorhamnetin diglucoside, isolated from mustard leaf (*Brassica juncea*) showed a strong activity in reducing serum levels of glucose in diabetes mellitus through an antioxidant activity test.[27] Although intake of quercetin has been inversely linked to mortality from coronary heart disease,[3] quercetin derivatives (**1**, **2**, and **5**) were minor compounds in all varieties, having concentrations of <1 μmol g^{-1} dw.

Because phenolic compounds are important as health-protective agents in human nutrition, the development of varieties with an improved nutritional value would be useful. *B. rapa* varieties evaluated in this work showed similar contents of total phenolics, total hydroxycinnamic acids, and total flavonoids in turnip greens over years. Nevertheless, turnip tops showed differences among varieties. Varieties with the highest levels of total phenolic compounds were MBG-BRS143, MBG-BRS0197, and MBG-BRS0163 (Table 4). If we focus only on each group of phenolics, MBG-BRS0143 and MBG-BRS0163 were the varieties that showed the highest flavonoid and hydroxycinnamic acid concentrations, respectively (Table 4).

Table 5. Analysis of Variance of the Sites Regression (SREG) Multiplicative Model for Aliphatic Glucosinolates, Indolic Glucosinolates, Flavonoids, and Hydroxycinnamic Acids on Turnip Greens and Turnip Tops of *B. rapa* Varieties Evaluated in Five Different Environments[a]

	turnip greens			turnip tops		
	Df	SS	MS	Df	SS	MS
Aliphatic Glucosinolates						
E	4	2463.76	615.93**	4	807.60	201.90**
GGE	50	2161.46	43.22**	40	4512.30	112.81**
error	108	1482.87	13.73	81	1780.07	21.97
Indolic Glucosinolates						
E	4	3.36	0.84**	4	49.50	12.38**
GGE	50	10.15	0.20**	40	20.17	0.50*
error	108	11.24	0.10	81	25.76	0.31
Flavonoids						
E	4	585.12	146.28**	4	897.34	224.33**
GGE	50	669.86	13.39	40	1331.47	33.29**
error	108	2157.50	19.97	81	990.20	12.22
Hydroxycinnamic Acids						
E	4	2285.00	571.25**	4	758.87	189.71**
GGE	50	1942.53	38.85	40	1733.94	38.35**
error	108	6282.71	58.17	81	1557.74	19.23

[a] E, environmental main effects, where one E is the combination of a location and year; GGE, genotype plus genotype × environment interaction effects; Df, degree of freedom; SS, sum of squares; MS, mean of squares. *, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$.

Climate and Soil Effects on Glucosinolate and Phenolic Content. Soil and climate differences across environments could be the cause of significant differences between environments and plant organ × environment interaction found for some traits. Climatic conditions throughout the crop cycle (between September and May) over the three years were very different at each location. Mean temperatures and precipitation among the cycle crop in 2006/2007, 2007/2008, and 2008/2009 are shown in Figure 1. Simple correlations were made to study the relationships between climatic factors and secondary metabolite levels. Results showed that in turnip greens, the number of days with a minimum temperature under 0 °C was negatively correlated (ranging from $R = -0.67$ to $R = -0.76$*) with total aliphatic, total glucosinolate, and gluconapin contents. On the other hand, in turnip tops, these traits were positively and highly correlated with the number of days with a maximum temperature over 20 °C, the mean of maximum temperature, and degree days of maximum temperatures (ranging from $R = 0.72$ to $R = 0.85$*). In turnip tops, total indolic glucosinolates were correlated with the number of days with a minimum temperature under 10 °C ($R = 0.79$*). For both plant organs it was found that precipitation had negative correlations with indolic glucosinolates, being highly significant in turnip greens ($R = -0.90$**). These results are in agreement with those reported by other authors, who found that brassica crops grown under cool temperatures and abundant rainfall seem to have a lower total glucosinolate content.[4,6,19]

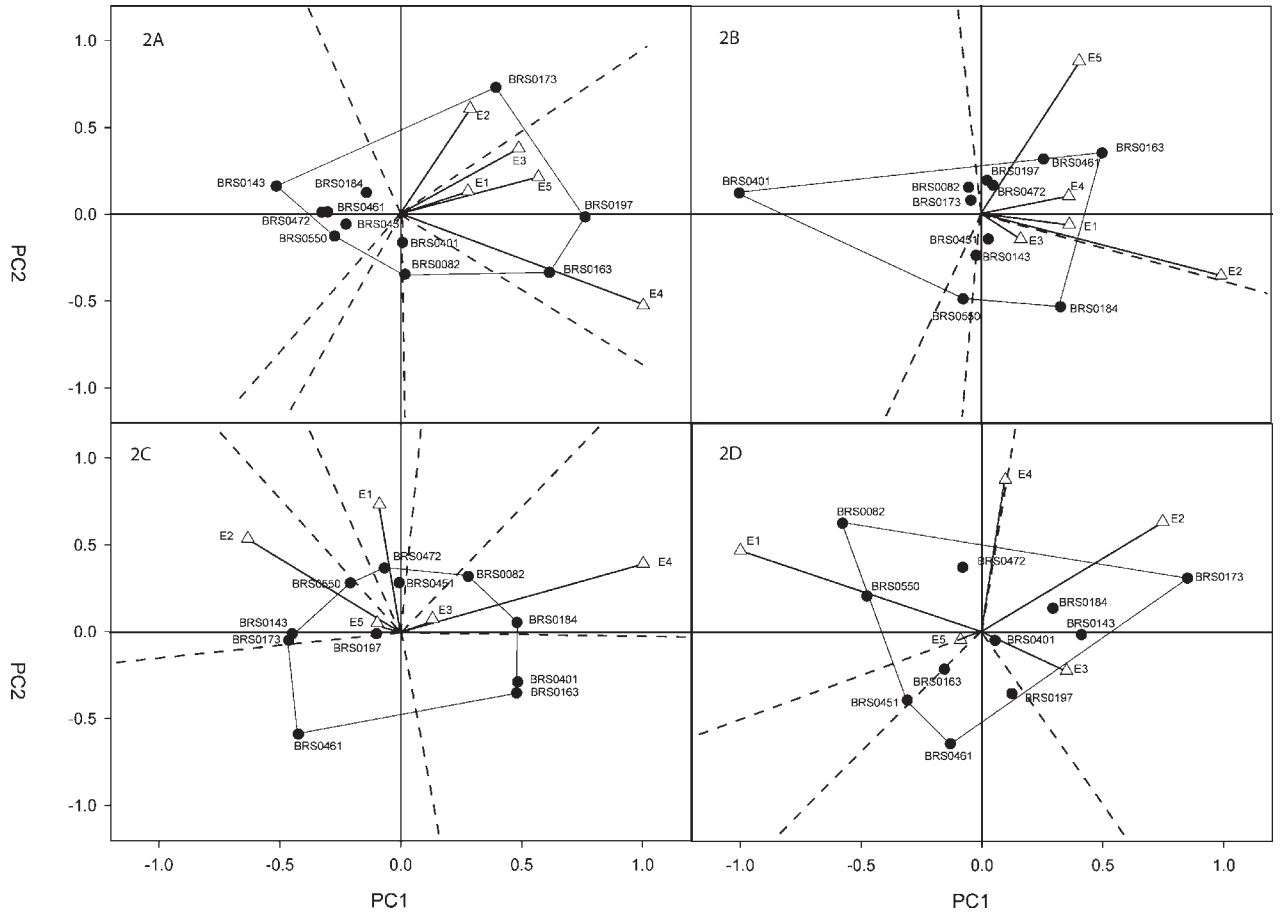

Figure 2. G + GE interaction (GGE) biplot based on the metabolite content of 11 *B. rapa* varieties for turnip greens in 5 environments. Environments are E1 (Guitiriz 2007), E2 (Oroso 2007), E3 (Guitiriz 2008), E4 (Oroso 2008), and E5 (Salcedo 2008). Metabolites are total alphatic glucosinolates (A), total indolic glucosinolates (B), total flavonoids (C), and total hydroxycinnamic acids (D). The polygon shown with tiny dots was made by joining the genotypes, which are on the vertices.

With regard to phenolics, no correlations between these compounds and climatic factors were found in turnip greens. On the contrary, for turnip tops there was a clear relationship between the number of days with a minimum temperature under 0 and 10 °C (ranging from $R = 0.77^{*}$ to $R = 0.95^{**}$) with total phenolics, total hydroxycinnamic acids, and total flavonoids. As it is a winter crop, much of the growing cycle takes place at temperatures below 10 °C and minimum temperatures under 0 °C. Stefanowska et al.[28] observed large phenolic deposits in the plasma membrane and membrane-bound organelles of winter oilseed (*Brassica napus*) plants grown in cold and freezing temperatures. This was related to pronounced ultrastructural changes in leaf epidermal and mesophyll cells due to low temperatures.

Differences in soil parameters were proved by edaphic analyses. The main characteristic of soils used in this study was their high acidity, with an average pH value of 5.3 in Guitiriz, 5.5 in Pontevedra, and 5.6 in Oroso. Soils were rich in organic matter with an average content ranging from 6.8% in Salcedo to 13.4% in Oroso. Available phosphorus was high in Guitiriz and Salcedo and medium in Oroso. Available potassium was high in Oroso and Salcedo and medium in Guitiriz. Results showed that both aliphatic and indolic glucosinolates presented the highest levels in Salcedo along with Oroso. Phenolic compound levels were also higher in Oroso. Therefore, for most of the compounds, the highest glucosinolate and phenolic compound contents occurred in locations with the highest soil pH and available potassium, thus suggesting some type of relationship between glucosinolate and phenolic content and soil effect. In addition, other soil factors may influence the content of these metabolites. Kim et al.[8] found that glucosinolate levels were strongly regulated by nitrogen and sulfur application in turnip. In field experiments, an increase in nitrogen availability favored the hydroxylation step on the aliphatic pathway.[29] On the other hand, it has been reported that flavonols of kaempferol and quercetin derivatives in *B. rapa* L. subsp. *Sylvestris* were reduced by sulfur availability.[30]

Genotype × Environment Interaction (SREG). Results of analyses of variance for SREG are presented in Table 5. Both aliphatic and indolic glucosinolates in the two plant organs were significantly affected by E and GGE. For aliphatic glucosinolates, the main effect E explained 53 and 17% of the total variation in turnip greens and turnip tops, whereas GGE accounted for 47 and 83% of the total sum of squares, respectively. Genotype main effects (G) were also significant and accounted for 63 and 70% of the GGE. Variation due to G was larger than variation due to GE interaction, and also this interaction was not significant in turnip greens, meaning that the genotypes had similar behaviors across environments. With regard to indolic glucosinolates the main effect E explained 25 and 71% of total variation in turnip greens and tops, respectively. The main effect G accounted for 45 and 21% of GGE in turnip greens and turnip tops, respectively.

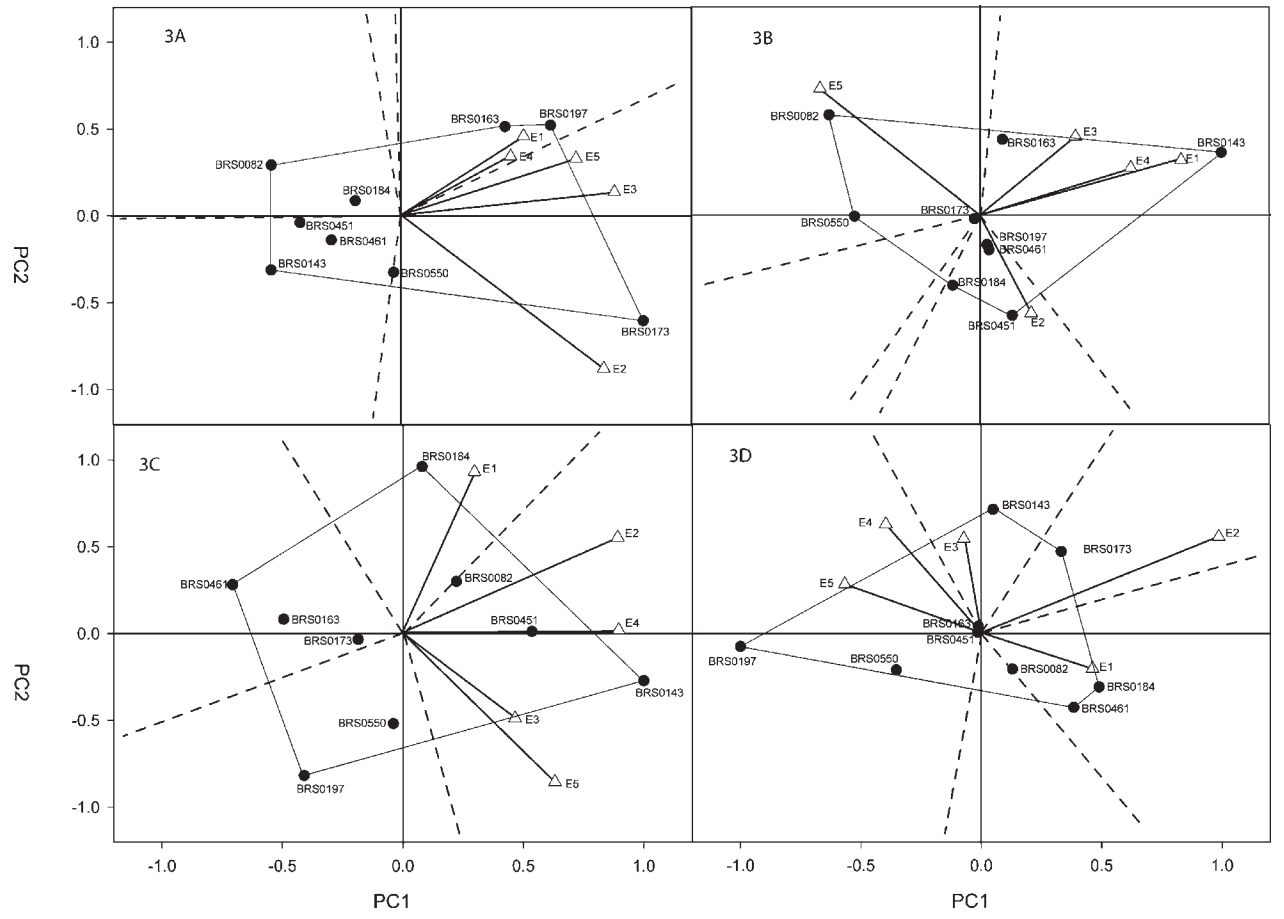

Figure 3. G + GE interaction (GGE) biplot based on the metabolite content of nine *B. rapa* varieties for turnip tops at five environments. Environments are E1 (Guitiriz 2007), E2 (Oroso 2007), E3 (Guitiriz 2008), E4 (Oroso 2008), amd E5 (Salcedo 2008). Metabolites are total alphatic glucosinolates (A), total indolic glucosinolates (B), total flavonoids (C), and total hydroxycinnamic acids (D). The polygon shown with tiny dots was made by joining the genotypes, which are on the vertices.

Despite the fact that GE interaction was not significant in any plant organ, the total percent of sums of squares attributable to GE was much higher than that attributable to G. Besides, for turnip tops, E had a larger influence, which suggests that these kinds of glucosinolates were highly influenced by environmental conditions.

For aliphatic and indolic gluocosinolates, PC1 and PC2 together, which make up a GGE biplot, explained >90% of total GGE variation in two plant organs (Table 5). The two-dimensional biplot showed that varieties MBG-BRS0197 and MBG-BRS0163 for turnip greens (Figure 2A) and MBG-BRS0173 for turnip tops (Figure 3A) presented the highest total aliphatic contents in most of the environments. Besides, MBG-BRS0197 for turnip greens appeared as a high and stable genotype because it showed a large PC1 score and a near-zero PC2 score (Figure 2A). With regard to indolic glucosinolates, varieties MBG-BRS0163 and MBG-BRS0143 had the highest mean of these compounds in turnip greens and tops, respectively (Figures 2B and 3B). The ideal test environments should have small (absolute) PC2 scores (more representative of the overall environment) and large PC1 scores (more power to discriminate genotypes in terms of the genotypic main effect.[11,13] Therefore, in turnip greens, Oroso 2008 was the ideal test environment for aliphatic glucosinolate content, whereas Salcedo 2008 was the most discriminatory. In turnip tops, Guitiriz 2008 was the ideal test and the most discriminatory environment. For indolic glucosinolates, Guitiriz 2007 was the best environment for both crops.

Few studies have contrasted the genetic versus environmental contribution to glucosinolate concentration, and most of them are focused on the genetic effects on glucoraphanin content. Brown et al.[31] evaluated a subset of 10 broccoli varieties grown over 4 seasons, finding that indolic glucosinolates were regulated very differently compared to the aliphatic ones. They reported that the synthesis of aliphatic glucosinolates was clearly regulated by G (60%), with E and GE interactions exerting smaller effects (5 and 10%, respectively). In contrast, regulation of indolic glucosinolates content was primarily environmental (G, 12%; E, 33%; and GE, 21%). Results from the current study confirm the relative importance of G in the expression of aliphatic glucosinolates, whereas indolic glucosinolates were mostly influenced by E and GE. These results indicate that turnip greens and tops should respond well to the selection for increasing aliphatic glucosinolates concentration.

With regard to phenolic compounds, analyses of variance for SREG showed that, in turnip greens, total flavonoids and total hydroxycinnamic acids were significantly affected only by the main effect E, which explained 46 and 53% of total variation (Table 5), respectively. GGE interaction was not significant in this plant organ. For turnip tops, E and GGE were significant for both kinds of compounds. The main effect E explained 40 and

30% of total variation of flavonoids and hydroxycinnamic acids contents, respectively. The main effect G and GE interaction were significant and accounted for 38 and 62% for flavonoids and 17 and 85% for hydroxycinnamic acids, respectively.

The study of GGE biplot of phenolic compounds showed that for both plant organs, PC1 and PC2 together explained >70% of the total GGE variation of total flavonoids and total hydroxycinnamates, respectively (Table 5). Except for flavonoids in turnip tops, different genotypes produced the highest metabolite content in different environments (Figures 2C,D and 3D). This fact complicates the selection of varieties for future breeding programs. For turnip tops, it was possible to identify the best genotypes for total flavonoids (Figure 3C). Variety MBG-BRS0143 presented the highest flavonoid levels in most of the environments, and MBG-BRS0451 reached good levels of these compounds and was also the most stable one. For these compounds, Oroso 2008 was the ideal test environment.

Several studies reviewed by Parr and Bolwell[32] have demonstrated that the change in phenolic composition of plant leaves is a consequence of environmental effects (biotic and abiotic estress). Detailed examination by molecular biological approaches has indicated that the phenomenon is largely due to an enhanced transcription of the phenolic biosynthetic genes following exposure to the inducing stimulus. On the other hand, genetic factors within crop populations may have important effects on the phenolic content of vegetables.[33] Nevertheless there are few works that study the influence of genotype and environment effects or the interaction between both effects independently. In the present study we found that both flavonoids and hydroxycinnamic acids are highly influenced by the E effects and GE interactions, which were predominant with respect to the main effect G. Therefore, if much of the variability is due to the environment, heritability of these compounds is probably low and selection strategies must take this into account.

In conclusion, the present work is an important step forward in the knowledge of the role that G, E, and their interaction GE play on the final concentrations of glucosinolate and phenolic compounds. Besides, it was also possible to identify varieties with a high and stable metabolite content. Varieties MBG-BRS0163, MBG-BRS0197, MBG-BRS0173, and MBG-BRS0143 were the most promising varieties for future breeding programs focused on varieties with high glucosinolate contents. Moreover, due to their stability and high content in flavonoids, varieties MBG-BRS0143 and MBG-BRS0401 could also be good candidates for breeding. Because bioactivity of turnip greens and tops is putatively associated with the concentration of glucosinolate and phenolic compounds, identification of genotypes with enhanced and stable levels of these compounds would provide a value-added opportunity for marketing this crop with superior health promotion to consumers.

AUTHOR INFORMATION

Corresponding Author

*Phone: 0034-986854800. Fax: 0034-986841362. E-mail: mfrancisco@mbg.cesga.es.

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