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ScienceDirect
Phytochemistry 69 (2008) 403–410
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Seasonal variation in glucosinolate content in *Brassica oleracea* crops grown in northwestern Spain

María Elena Cartea [a,*], Pablo Velasco [a], Sara Obregón [b], Guillermo Padilla [a], Antonio de Haro [b]

[a] *Department of Plant Genetics, Misión Biológica de Galicia, Spanish Council for Scientific Research (CSIC), Apartado 28, E-36080 Pontevedra, Spain*

[b] *Department of Agronomy and Plant Breeding, Institute of Sustainable Agriculture, Spanish Council for Scientific Research (CSIC), Alameda del Obispo s/n, 14080 Córdoba, Spain*

Received 22 June 2007; received in revised form 7 August 2007
Available online 21 September 2007

Abstract

Brassica oleracea L. crops including kales, cabbages, and Tronchuda cabbages are widely grown in northwestern Spain and Portugal but little information is available on leaf glucosinolate content of these crops. The objectives were to determine the diversity for the total glucosinolate content and profile on leaves in a collection of 153 kales, 26 cabbages, and three Tronchuda cabbages varieties grown at two growing seasons and to determine the seasonal variation of glucosinolates in cabbages and Tronchuda cabbage varieties. Sinigrin, glucoiberin, and glucobrassicin were the major glucosinolates found in kales. Glucoiberin was the most common glucosinolate in Tronchuda cabbages in both planting seasons and in cabbages sown in fall season whereas glucobrassicin and glucoiberin were the most common glucosinolates in cabbages in spring season. In kales the total glucosinolate content ranged from 11.0 to 53 μmol g^{-1} dw, with a mean value of 26.3 μmol g^{-1} dw. Four kale varieties (MBG-BRS0468, MBG-BRS0476, MBG-BRS0060 and MBG-BRS0223) showed the highest total sinigrin or glucobrassicin contents. So, they could be good candidates for future breeding programs. In cabbages, the total glucosinolate content ranged from 10.9 to 27 g^{-1} dw. Total glucosinolate concentration during spring sowing (22 μm g^{-1} dw) was higher than those in fall sowing (13 μm g^{-1} dw). Regarding both high glucosinolate content and the agronomic value, MBG-BRS0057 and MBG-BRS0074 could be good sources of beneficial glucosinolates. The presence of high concentrations of sinigrin, glucoiberin, and glucobrassicin warrant further search into their potential use to enhance the level of these important phytochemicals in these edible crops.
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Keywords: *Brassica oleracea*; Cabbage; Glucosinolates; Kale; Sinigrin; Tronchuda cabbage

1. Introduction

Brassica vegetables are a family of six agricultural important species of vegetables which are consumed in high quantities throughout the world, being very important in human nutrition. Within *Brassica* genus, the *Brassica oleracea* species has evolved into a number of varieties of which different parts of the plant have become the edible constituents. They are used extensively in Europe and Central Asia and are an important source of vegetables in many countries.

A collection of 250 local populations of *B. oleracea* crops from northwestern Spain is maintained at the Misión Biológica de Galicia (MBG), part of the Spanish Council for Scientific Research (Padilla et al., 2007a,b). The *B. oleracea* collection includes kale (*B. oleracea L. acephala* group), cabbage (*B. oleracea* L. *capitata* group), and Portuguese Tronchuda cabbage (*B. oleracea* L. *costata* group), representing most of the region. They are known to be

* Corresponding author. Tel.: +34 986854800; fax: +34 986841362.
E-mail address: ecartea@mbg.cesga.es (M.E. Cartea).

0031-9422/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.08.014

cultivars well adapted to the soil and climate conditions of Galicia (northwestern Spain) and generally grown with little or no agrochemical inputs (Cartea et al., 2003).

Kale is by far the most well-represented variety of the species (214 accessions), being an important vegetable and forage crop. Twenty one of such accessions were identified as cabbages and two accessions as Tronchuda cabbages, a typical Portuguese horticultural crop that is occasionally grown in the south of Galicia in areas close to Portugal. Growers follow the same cultural practices for both cabbages and Tronchuda cabbages and they are also consumed in the same way. In northwestern Spain, cabbage and Tronchuda cabbage production can be accomplished almost all year long and landraces can be characterized by their ecological origin but by their growing period as well. Varieties can be grown the whole year around, primarily in two growing seasons, Spring/Summer and Fall/Winter, with different effects on plant growth and chemical composition. Previous works have shown that nutritional quality as well as the yield of cabbage crops can be affected by date of planting, environmental conditions and length of growing seasons (Rosa and Heaney, 1996; Wszelaki and Kleinhenz, 2003; Charron et al., 2005; Padilla et al., 2007b). Rosa et al. (1996) reported the variation in glucosinolate content in kales and Tronchuda cabbages at different growing season grown in Portugal.

Brassica species are reported to possess cancer preventive activity, due to glucosinolates and their derived properties. Glucosinolate are a major group of natural plant compounds present in the family Brassicaceae which are largely responsible for the hot and pungent flavours of crucifers. Upon cellular disruption glucosinolates are hydrolyzed to various bioactive breakdown products (isothiocyanates, nitriles, thiocyanates, epithionitriles, and oxazolidines) which have positive and negative effects (Fenwick et al., 1983). Some glucosinolates have been shown to have health beneficial effects related to a reduction in the risk of certain cancers in humans (Zhang and Talalay, 1994; Fahey et al., 2001; Mithen et al., 2003) while other glucosinolates are detrimental for human and animal consumption (Rosa et al., 1997). Detailed reviews on glucosinolates in *Brassica* have been reported by several authors (Fenwick et al., 1983; Rosa et al., 1997; Mithen, 2001).

Many studies on glucosinolate content on cruciferous crops have been focussed in *B. oleracea* (Zhang et al., 1992; Nastruzzi et al., 1996; Farnham et al., 2000, 2004), being most of them in broccoli. Sulforaphane, an isothiocyanate derived from glucoraphanin and present at high levels in broccoli, has been proved to inhibit chemically induced breast cancer in rats (Fahey et al., 1994; Mithen et al., 2003). Although the most characterized isothiocyanate compound is sulforaphane, other isothiocyanates may also contribute to the anti-carcinogenic properties of crucifers. Glucosinolate hydrolysis products from glucoiberin, sinigrin and progoitrin have also been identified as suppressing agents, protecting human and animal cells against carcinogenesis. These glucosinolates may well exert comparable levels of biological activity to sulforaphane by either inducing Phase II detoxification enzymes or inhibiting Phase I enzymes (Fahey et al., 1998).

The *B. oleracea* collection maintained at MBG has been studied by Padilla et al. (2007a,b) and Vilar et al. (2007) based on morphological and agronomical traits. However, no information is available on the glucosinolate content of this collection. With the increased interest in diet and human health it is necessary to have information of profiles and levels of glucosinolates in this food *Brassica* species. These compounds are of interest to improve the quality of the product with the aim to develop new cultivars with an appropriate glucosinolate profile, from which high quality products with an added value can be produced. The most promising varieties for future breeding purposes would be those with the highest total glucosinolate content and, especially, with the highest content of glucosinolates related to beneficial effects.

The objectives of this study were: (i) to determine the diversity for the total glucosinolate content and profile on leaves of *B. oleracea* varieties from northwestern Spain and (ii) to determine the influence of growing seasons in the glucosinolate content in cabbage and Tronchuda cabbage varieties.

2. Results and discussion

2.1. Kale experiment

Ten glucosinolates were detected in the collection of kale varieties studied, belonging to the three chemical classes (Table 1). Five glucosinolates (sinigrin, glucoiberin, progoitrin, glucobrassicin, and neoglucobrassicin) were detected in almost all varieties. Gluconasturtiin, glucoraphanin and 4-hydroxyglucobrassicin were detected between the 60% and 70% of the varieties, while glucoiberverin and epiprogoitrin were present only in the 20% of the collection.

Kale varieties showed significant differences for total glucosinolate content and for all individual glucosinolates ($P \leqslant 0.01$) except for gluconasturtiin. The total glucosinolate content ranged from 11.0 to 52.8 $\mu mol\ g^{-1}$ dw, with a mean value of 26.3 $\mu mol\ g^{-1}$ dw (Table 2). These contents are higher than the ones found in other kale varieties (Ciska et al., 2000; Charron et al., 2005; Nilsson et al., 2006), but lower than the value found by Rosa and Heaney (1996) in Portuguese kales.

Aliphatic glucosinolates were predominant, representing 69.2% of the total glucosinolate content, followed by indolyl glucosinolates (30.4%) and aromatic glucosinolates (0.4%). Sinigrin was the major glucosinolate followed by glucoiberin and glucobrassicin (Table 2, Fig. 1). The rest of the glucosinolates were found in lower proportions (less than 3% of total glucosinolate content). Most previous studies have shown a similar glucosinolate pattern in leaves

Table 1
Glucosinolates identified in 153 kale varieties, 26 cabbage varieties, and three Tronchuda cabbage varieties grown in northwestern Spain at two growing seasons, their trivial name, and percentage of varieties with the glucosinolate

Systematic name	Trivial name	Kale varieties (%)	Cabbage varieties (%)	Tronchuda varieties (%)
Aliphatic				
3-Methylsulfinylpropyl	Glucoiberin	100	100	100
2 (*R*)-2-Hydroxy-3-butenyl	Progoitrin	100	100	100
2 (*S*)-2-Hydroxy-3-butenyl	Epiprogoitrin	22	100	100
2-Propenyl	Sinigrin	100	100	100
4-Methylsulphinylbutyl	Glucoraphanin	60	97	100
3-Methylthiopropyl	Glucoiberverin	20	100	100
5-Methylsulphinylpentyl	Glucoalyssin	0	79	100
3-Butenyl	Gluconapin	0	90	66
4-Pentenyl	Glucobrassicanapin	0	41	66
4-Methylthiobutyl	Glucoerucin	0	38	0
Indolyl				
3-Indolylmethyl	Glucobrassicin	100	100	100
1-Methoxy-3-indolylmethyl	Neoglucobrassicin	99	100	100
4-Hydroxy-3-indolylmethyl	4-Hydroxyglucobrassicin	73	100	100
4-Methoxy-3-indolylmethyl	4-Metoxyglucobrassicin	0	38	100
Aromatic				
2-Phenylethyl	Gluconasturtiin	66	62	33

Table 2
Mean and range (μmol g^{-1} dw) for total glucosinolate content and for the major individual glucosinolates found in 153 kale varieties grown in northwestern Spain

	Mean	Range	LSD (5%)
Total glucosinolates	26.32	11.00–52.79	10.21
Aliphatic	18.24	7.22–46.62	
Glucoiberin	7.90	2.38–25.07	5.12
Progoitrin	0.72	0.09–2.51	0.94
Sinigrin	9.44	2.77–19.66	6.03
Glucoraphanin	0.13	0.00–1.27	0.23
Indolyl	7.99	1.99–18.45	
Glucobrassicin	7.12	1.75–16.76	4.07
Neoglucobrassicin	0.70	0.00–3.39	0.83
Aromatic	0.09	0.00–1.38	
Gluconasturtiin	0.09	0.00–1.38	–

LSD = Least significant difference.

of kales where the sinigrin was the dominant glucosinolate (Rosa and Heaney, 1996; Kushad et al., 1999).

Nilsson et al. (2006) detected glucobrassicin and glucoiberin as the main glucosinolates in kale while sinigrin was found as the major glucosinolate in white cabbage. Glucoiberin was reported as the only important glucosinolate in kales (Charron et al., 2005) while glucoraphanin, sinigrin, glucobrassicin, and glucoiberin were found to be the most abundant in cabbage. In our work, sinigrin was the most abundant glucosinolate but the glucosinolate profile varies depending on the variety. So, from the 153 varieties studied, sinigrin was found as the main glucosinolate in 63 varieties while glucoiberin was the main glucosinolate in 30 varieties and glucobrassicin in 23 varieties. Leaves of kale crops had high sinigrin and glucoiberin contents, which represented 36% and 29% of the total glucosinolate content, respectively. Isothiocyanates derived from the hydrolysis of sinigrin and glucoiberin have been found to have anti-carcinogenic and anti-mutagenic effects (Fahey et al., 1998; Farnham et al., 2004). Moreover, it is known that isothiocyanates derived from sinigrin can cause a reduction in the cholesterol levels in mice (Balasinska et al., 2005). Another beneficial effects attributed to sinigrin is its role as suppressor of the growth of nematodes, fungi, and other soil microorganisms (Rosa et al., 1997), although this glucosinolate also contribute, as well as glucoiberin and gluconasturtiin, to the presence of some specialist pests.

The third glucosinolate in abundance was glucobrassicin, which represented 27% of total glucosinolate content. Glucobrassicin is the parent compound of indole-3-carbinol which has been proven along with the sulforaphane as the most potent anticancer compounds found in cruciferous vegetables (Zhang and Talalay, 1994). Smaller amounts of other minor glucosinolates, such as, progoitrin, gluconasturtiin and neoglucobrassicin were also detected.

Because in this species, the amounts of the progoitrin, described as potentially goitrogenic are very low, there does not appear to have a health risk associated with consumption of these vegetables. This result agrees to previously reported levels of progoitrin in leaves of edible cabbage and kale (Kushad et al., 1999). Because both sinigrin and glucobrassicin have been reported as important glucosinolates for cancer chemoprotection, plant stages and plant parts should be considered when planning harvest or when making breeding selections for glucosinolate concentrations, because glucosinolate concentration changes with plant age and developmental stage (Velasco et al., 2007).

In a previous work, Padilla et al. (2007a) based on agronomical and morphological traits, grouped the same set of kale varieties in five clusters using the Ward-MLM method. Cluster A included 20 varieties with tall plants and white flowers. Cluster B was the most numerous, including 51 varieties with a low early vigor. Thirty eight plants with the lowest yield were grouped in cluster C. The early flowering varieties (25) were included in cluster D and, finally, cluster E grouped the shortest varieties (9) which had the highest number of leaves and scars. In our study there were significant differences among these groups ($P \leqslant 0.01$) for total glucosinolate content and for all individual glucosinolates, except for sinigrin and epi-progoitrin (Table 3). Cluster A was one of the two clusters with less total glucosinolate content but it showed more glucoiberin than the rest of the clusters. Cluster E had the varieties with the highest total glucosinolate concentration. Among the three main glucosinolates, varieties grouped in the cluster E had the highest glucobrassicin content and showed three times more gluconasturtiin than the rest of the clusters (Table 3). It could be a relationship between the agronomic characteristics and the glucosinolate content.

Because the beneficial effects on human health attributed to sinigrin, glucoiberin, and glucobrassicin, the most promising varieties for future breeding purposes would be those with the highest contents in the glucosinolates abovementioned. The variety MBG-BRS0205 had the highest total glucosinolate content (52.8 μmol g^{-1} dw) and the highest sinigrin and glucoiberin contents (19.7 μmol g^{-1} dw and 25.1 μmol g^{-1} dw, respectively). Focusing on the sinigrin content, MBG-BRS0205 along with other 20 varieties could be selected. The varieties MBG-BRS0468, MBG-BRS0445, and MBG-BRS0476 had the highest glucobrassicin content (17 μmol g^{-1} dw).

In a previous study, MBG-BRS0468 and MBG-BRS0476 displayed a good agronomic performance for fresh production, MBG-BRS0060 and MBG-BRS0223 were the most resistant varieties to attack by Lepidoptera pest, and MBG-BRS0366 had the best early vigor (Vilar et al. 2007). Regarding both high glucosinolate content and the agronomic value of this crop, MBG-BRS0060 and MBG-BRS0223, which had high sinigrin content, along with MBG-BRS0468 and MBG-BRS0476, which had the highest for glucobrassicin content, have potential to develop genotypes with increased glucosinolate levels that have beneficial health effects. The presence of glucoraphanin in most varieties (60%) should be studied more extensively, because this aliphatic glucosinolate is the precursor of sulforaphane, a potent anti-cancer isothiocyanate.

Table 3
Mean (μmol g^{-1} dw) for total glucosinolate content and for major individual glucosinolates for five clusters grouping the 148 kale local varieties from northwestern Spain

	Cluster					LSD (5%)
	A	B	C	D	E	
Total glucosinolate	25.73cd	27.77bc	24.19d	28.42b	32.28a	2.29
Glucoiberin	9.64a	7.72bc	6.85c	8.43b	8.28b	1.15
Progoitrin	0.47d	0.70bc	0.60cd	0.83b	1.22a	0.21
Sinigrin	8.55a	9.68a	8.89a	9.86a	10.48a	–
Glucoraphanin	0.05c	0.13b	0.11b	0.23a	0.20a	0.05
Glucobrassicin	5.54c	7.87b	5.65c	7.92b	10.28a	0.91
Neoglucobrassicin	0.49c	0.78ab	0.61bc	0.95a	0.86a	0.19
Gluconasturtiin	0.03b	0.08b	0.06b	0.10b	0.34a	0.09

In each row, numbers followed by the same letter are not significant different.
LSD = Least significant difference.

2.2. Cabbage experiment

Fifteen glucosinolates were detected in the cabbage collection and 14 glucosinolates in the Tronchuda cabbage varieties belonging to the three chemical classes (Table 1). Nine glucosinolates (glucoiberin, progoitrin, epiprogoitrin, sinigrin, glucoraphanin, glucoiberverin, glucobrassicin, neoglucobrassicin, and 4-hydroxyglucobrassicin) were detected in almost all cabbage and Tronchuda cabbage varieties. Other aliphatic glucosinolates, such as glucoalyssin, gluconapin and glucobrassicanapin were present in some varieties.

Aliphatic glucosinolates were predominant, with glucoiberin as the most common glucosinolate followed by glucobrassicin and sinigrin in Tronchuda cabbages evaluated at both planting seasons and cabbages evaluated at fall season while glucobrassicin was the most common glucosinolate followed by glucoiberin and sinigrin in cabbages evaluated at spring season (Table 4, Fig. 1).

The variety × sowing season interaction was not significant for total glucosinolate concentration and for most of glucosinolates, including the three main glucosinolates. For this reason, the study of the glucosinolate profile and concentration was made combining both sowing seasons. There were significant differences ($P \leqslant 0.05$) between sowing seasons for total glucosinolate content and for five glucosinolates, including glucoiberin and glucobrassicin (Table 4). Differences in total glucosinolate content were found among varieties, with a total glucosinolate content which ranged from 27 μm g^{-1} dry to 10.9 g^{-1} dw.

The influence of sowing season on glucosinolate content and profile in cabbages was clear. Total glucosinolate concentration in leaves harvested at spring season was higher (22 μm g^{-1} dry tissue) than the concentration in fall season (13 μm g^{-1} dry tissue) which is in agreement with findings by Rosa et al. (1996) for Portuguese kales. Charron et al. (2005) also found variation on glucosinolate content in cabbage leaves harvest at spring and fall seasons but the highest concentrations of total glucosinolates generally occurred when crops were harvested during periods of high temperatures and long day length. Aliphatic glucosinolates were the most common during fall season whereas the percentage of indole glucosinolates increased during the spring season (Fig. 1). These results agree with Charron et al. (2005) and could be related with temperature as it was already stated by Velasco et al. (2007), who studied the glucosinolate concentration under different environments and development plant stages in kales.

Table 4
Mean (μmol g^{-1} dw) for total glucosinolate content and individual glucosinolates for 26 cabbage varieties and three Tronchuda cabbage varieties from northwestern Spain grown in two sowing seasons

Varieties	GST	GIB	PRO	EPI-PRO	SIN	GRA	GAL	GNA	GIV	GBS	4-OHGBS	NGBS
MBG-BRS0057	23.23ba	8.89b–d	0.23j	0.08fg	2.92bc	0.21	0.01c	0.00c	0.07	10.18a	0.33a	0.21f–h
MBG-BRS0072	12.85e–i	3.03ij	1.23b–e	0.71c–g	1.74c–f	0.19	0.09bc	0.08c	0.04	5.18b–h	0.18e–g	0.29e–h
MBG-BRS0074	21.44a–c	9.40a–c	0.39i–h	0.23fg	2.46b–d	0.14	0.00c	0.00c	0.07	8.16bac	0.21c–g	0.23e–h
MBG-BRS0076	17.17b–i	5.48e–j	1.09b–g	1.85a–c	2.85bc	0.04	0.06bc	0.04c	0.04	5.05c–h	0.21b–g	0.36e–h
MBG-BRS0083	19.85b–e	9.25bac	0.37i–h	0.56d–g	2.23b–e	0.19	0.03c	0.01c	0.10	6.52b–g	0.14g	0.33e–h
MBG-BRS0120	17.79b–h	5.60e–j	1.20b–e	1.14a–g	2.12b–f	0.07	0.02c	0.12c	0.05	6.68b–f	0.28a–e	0.26e–h
MBG-BRS0152	17.96c–i	7.38b–f	0.91c–i	1.41a–e	1.92b–f	0.09	0.00c	0.05c	0.05	5.27b–h	0.18d–g	0.60c–e
MBG-BRS0176	19.08b–f	5.32e–j	2.77a	1.14avg	2.91bc	0.07	0.13bc	0.73a	0.05	5.58b–h	0.16fg	0.11h
MBG-BRS0397	15.67c–i	4.93e–j	0.82c–j	2.02a	1.69c–f	0.07	0.08bc	0.20c	0.06	5.18b–g	0.16fg	0.36d–g
MBG-BRS0400	11.24hi	2.66j	0.91c–i	1.94ab	0.52e–f	0.00	0.19ab	0.06c	0.06	4.53d–h	0.20c–g	0.11h
MBG-BRS0402	15.68c–i	3.79g–j	1.42b–d	1.86a–c	2.15b–e	0.09	0.29a	0.32b	0.05	5.28b–g	0.22a–g	0.13h
MBG-BRS0404	18.63b–g	6.86c–g	0.41i–h	0.09fg	2.71bc	0.12	0.01c	0.00c	0.09	7.55a–d	0.19c–g	0.42d–h
MBG-BRS0408	19.33b–f	5.87d–i	0.92c–i	0.42d–g	2.84bc	0.14	0.03c	0.02c	0.07	8.32ab	0.31a–c	0.24e–h
MBG-BRS0411	14.30d–i	6.88 c–g	0.57f–j	0.19gf	2.02b–f	0.06	0.03c	0.05c	0.03	4.19e–h	0.17fg	0.06h
MBG-BRS0425	20.80 a–d	5.52e–j	1.56bx	0.78 c–g	5.71a	0.04	0.07bc	0.19b	0.09	6.34b–g	0.26a–f	0.15h
MBG-BRS0449	19.19b–f	6.71c–g	0.92 c–i	1.45a–d	2.24b–e	0.05	0.03c	0.14b	0.09	6.75b–f	0.17e–g	0.55c–g
MBG-BRS0452	15.33 c–i	4.72f–j	0.76e–j	0.40d–g	2.04b–f	0.02	0.00c	0.02c	0.05	6.14b–g	0.23a–g	0.84bc
MBG-BRS0534	15.73 c–i	6.51c–h	0.30ij	0.12fg	2.09b–f	0.02	0.00c	0.01c	0.06	6.05b–g	0.23a–g	0.21f–h
MBG-BRS0535	12.68f–i	5.26e–j	0.51g–j	0.03g	2.62bc	0.01	0.03c	0.01c	0.00	3.57f–h	0.18d–g	0.36d–h
MBG-BRS0536	22.02a–c	9.59a–c	0.44h–j	0.26e–g	2.69bc	0.13	0.01c	0.02c	0.05	7.30a–e	0.28 a–f	1.12b
MBG-BRS0537	18.51b–g	6.63c–g	0.47g–j	0.11fg	3.16bc	0.15	0.02c	0.04c	0.07	7.276a–e	0.21b–g	0.22e–h
Col repollo pie liso[a]	11.57g–i	3.45hi	1.44bc	0.82c–g	2.36b–d	0.01	0.12bc	0.07c	0.02	2.79h	0.13g	0.30d–h
Virtudes[a]	17.22b–i	6.67c–g	0.48g–j	0.65d–g	0.77d–f	0.04	0.01c	0.01c	0.09	8.00a–c	0.23a–g	0.18gh
Corazón de Buey[a]	15.13c–i	6.72c–g	0.80d–j	0.02g	3.62b	0.01	0.02c	0.09bc	0.06	3.41gh	0.16gf	0.19gh
Leo[a]	10.94i	3.90g–j	0.39i–h	0.29e–g	0.61ef	0.06	0.00c	0.03c	0.04	5.18b–g	0.18d–g	0.15h
Brunswick[a]	26.95a	12.06a	1.01c–h	1.25a–f	3.49b	0.15	0.02c	0.03c	0.04	8.12a–c	0.29a–d	0.40d–h
MBG-BRS0121	19.49b–f	10.07ba	0.48g–j	0.17fg	1.72c–f	0.13	0.01c	0.02c	0.05	5.91b–g	0.30a–c	0.59c–f
MBG-BRS0226	20.31a–d	7.92b-e	0.34ij	0.16fg	3.56b	0.13	0.00c	0.01c	0.05	5.72b–g	0.17fg	2.14a
Col asa de cántaro[a]	18.14b–g	9.28a–c	0.24j	0.34e–g	0.43f	0.03	0.00c	0.00c	0.07	6.66b–g	0.32ab	0.67cd
LSD (5%)	7.08	3.15	0.64	1.18	1.72	–	0.14	0.25	0.08	3.26	0.12	0.39
Mean	17.52	6.57	0.81	0.78	2.35	0.08	0.04	0.08	0.06	6.10	0.22	0.41
Fall sowing	13.14b	5.18b	0.75	0.77	2.14	0.07	0.05	0.07	0.03b	3.85b	0.12b	0.15b
Spring sowing	21.88a	7.87a	0.87	0.79	2.56	0.10	0.04	0.10	0.09a	8.37a	0.32a	0.66a
LSD (5%)	1.86	0.82	–	–	–	–	–	–	0.02	0.86	0.03	0.10

Means in columns followed by the same letter are not significantly different at $P \leqslant 0.05$.
GST = total glucosinolate content; GIB: glucoiberin; PRO: progoitrin; E-PRO: epiprogoitrin; SIN: sinigrin; GRA: glucoraphanin, GAL: glucoalyssin, GNA: gluconapin, GIV: Glucoiberverin, GBS: glucobrassicin; 4OHGBS: 4-hydroxyglucobrassicin; NGBS: neoglucobrassicin.
Other glucosinolates as glucobrassicanapin, glucoerucin, 4-methoxyglucobrasssicn, and gluconasturtin are not shown because they were found in lower amounts (⩽0.03 μmol g^{-1} dw).
LSD = Least significant difference.
[a] Commercial variety.

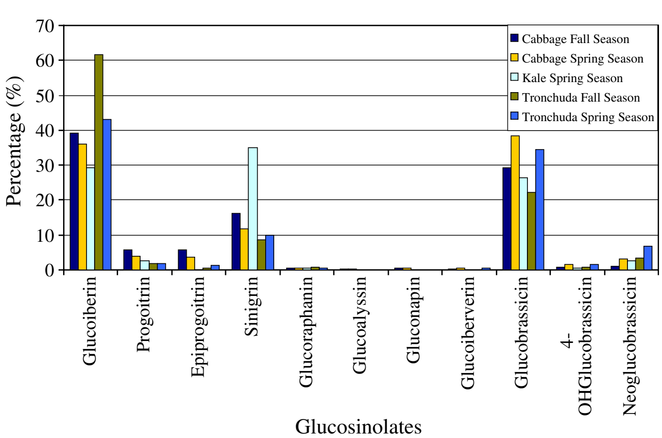

Fig. 1. Percentage of the main glucosinolates (%) in *Brassica oleracea acephala* group during the spring season and *capitata* and *costata* groups during spring and fall seasons from northwestern Spain.

In a recent study (Padilla et al., 2007b), discussed the influence on sowing date on traits related to plant morphology and earliness in the same set of cabbage varieties used for our study, which could also explain the differences in glucosinolate concentrations between the two sowing seasons. In the autumn/winter season, varieties had a better flavour and were sweeter (Padilla et al., 2007b). In our study, varieties showed lower total glucosinolate contents than in the spring/summer season. However, they were not significant differences for sinigrin content. This suggests that the characteristic flavour of these vegetables is probably due to other phytochemicals, not just of hydrolysis products derived from sinigrin. Moreover, the bitter taste in cabbages is less pronounced compared to other brassica crops as turnip greens (*Brassica rapa rapa* group) or Brussels sprouts (*B. oleracea gemmifera* group).

The hydrolysis products of glucoiberin, glucobrassicin, and sinigrin are isothiocyanates, nitriles, and epithionitriles. Isothiocyanates derived from glucosinolates have a chemoprotective effect, related to a reduction in the risk of certain cancers in humans. Therefore, the most promising varieties for future breeding purposes would be those with the highest total glucosinolate content. Four local cabbage varieties MBG-BRS0057, MBG-BRS0074, MBG-BRS0425, MBG-BRS0536, the local Tronchuda cabbage variety MBG-BRS0226, and the commercial white cabbage Brunswick had the highest total glucosinolate content ($\geqslant 20\ \mu mol\ g^{-1}$ dw) (Table 4). From commercial varieties examined, three are white cabbage (‘Col repollo pie liso’, ‘Corazón de Buey’, and ‘Brunswick’) and two are Savoy types (‘Leo’ and ‘Virtudes’). The white cabbage Brunswick showed higher mean values of total glucosinolates and specially, glucoiberin than Savoy cabbages, which is in disagreement with the work of Sones et al. (1984).

Commercial varieties are hybrids but the collection is formed by open-pollinated local varieties, so there is a high intravarietal diversity and then it would be possible to select varieties with the highest glucosinolate content. In general, the best varieties chosen by Padilla et al. (2007b) for their agronomic performance at both planting seasons had the highest total glucosinolate content as well as the highest content for some of the three main glucosinolates. Two varieties (MBG-BRS0057 and MBG-BRS0074) stood out at each season, autumn/winter and spring/summer, respectively. The variety MBG-BRS0074 had high glucoiberin content ($9.4\ \mu mol\ g^{-1}$ dw) while MBG-BRS0057 had the highest glucobrassicin and glucoraphanin contents ($10.2\ \mu mol\ g^{-1}$ dw and $0.21\ \mu mol\ g^{-1}$ dw, respectively). These two glucosinolates have been widely studied for their anticarcinogenic properties. Regarding both high glucosinolate content and the agronomic value of this crop, MBG-BRS0057 and MBG-BRS0074 could be good sources of beneficial glucosinolates.

Beneficial and detrimental effects of glucosinolates are attributed to isothiocyanates rather than to the glucosinolates themselves. Therefore, the determination of the glucosinolate content may be misleading when evaluating the beneficial properties of these crops. Cruciferous plants, including kale, cabbage and broccoli have been shown to produce not only isothiocyanates but other alternative bioactive breakdown products as nitriles and epithionitriles. So, glucosinolate levels do not necessarily reflect the amounts of the corresponding isothiocyanates that will be formed. Matusheski et al. (2006) demonstrated that different levels of glucoraphanin, the major glucosinolate component in broccoli, did not correlate with the formation of anti-carcinogenic sulforaphane (the isothiocyanate derived from glucoraphanin). Therefore, for a breeding program for varieties with enhanced cancer-preventive potential further analysis of glucosinolate breakdown products will be essential to confirm our results.

3. Conclusions

Sinigrin makes the major contribution of glucosinolates to kales, while glucobrassicin or glucoiberin do so to cabbage leaves. The presence of high concentrations of sinigrin, glucoiberin, and glucobrassicin in cabbage and kale warrant further search into their potential use to enhance the level of these important phytochemicals in these edible crops. The varieties MBG-BRS0468, MBG-BRS0476, MBG-BRS0060 and MBG-BRS0223 could be good candidates for future breeding programs since they had high total sinigrin or glucobrassicin contents and good agronomic performance. Regarding both high glucosinolate content and the agronomic value of cabbage collection, two varieties MBG-BRS0057 and MBG-BRS0074 could be good sources of beneficial glucosinolates since they had high glucoiberin and glucobrassicin contents, which have been widely reported to possess cancer preventive activity.

4. Experimental

4.1. Plant material

Leaves for glucosinolate analyses were harvested from the experimental fields described in Padilla et al. (2007a,b). The kale trial comprised 148 varieties from Galicia (northwestern Spain) and five commercial varieties. The cabbage and Tronchuda cabbage trials comprised 21 cabbages varieties and two Tronchuda cabbages varieties, representing the landraces widely grown in this region. Five commercial hybrids of cabbages (‘Col repollo pie corto’, ‘Corazón de Buey’, ‘Brunswick’, ‘Virtudes’, and ‘Leo’) and one commercial variety of Tronchuda cabbage (‘Col asa de cántaro’) were used as checks. Varieties were planted in multiplot-trays and seedlings were transplanted into the field at the 5–6 leaf stage in April 2003 for kales. Transplanting dates for cabbages were on 10 September 2003 for the autumn/winter season and on 22 March 2004 for the spring/summer season. All trials were performed in Pontevedra, Spain (lat. 42°24′N, long. 8°38′W), 20 m above the sea level in acidic sandy loam soil.

For the kale experiment, a sample of healthy and fresh leaves was used from three to five plants from each plot. The five upper leaves/plant (the two next to the apical leaf along with the adjacent three leaves) were sampled because they corresponded to tender leaves used for human consumption. Harvest was made five months after transplanting. For the cabbage and Tronchuda cabbage experiment, the evaluations were carried out in two planting dates (autumn/winter and spring/summer). In each sowing, a sample of healthy and fresh leaves from heads was used from three to five plants from each plot. Leaves were collected four months after transplanting.

4.2. HPLC analysis

Leaf samples were frozen in situ and were taken immediately into the laboratory where they were stored at −80 °C. Then, the green material was ground in liquid N_2, freeze-dried, and milled to a fine powder for the glucosinolate extractions. Glucosinolate composition was determined by HPLC according to Font et al. (2005). For each leaf sample, 100 mg dry wt were weighed and grounded in a Janke and Kunkel, Model A10 mill (IKA-Labortechnik) for about 20 s and a two-step glucosinolate extraction was carried out in a water bath at 75 °C to inactivate myrosinase. In the first step the sample was heated for 15 min in 2.5 ml 70% aqueous methanol and 200 µl 10 mM glucotropaeolin (benzyl glucosinolate) as an internal standard. A second extraction was applied after centrifugation (5 min, 5000*g*) by using 2 ml of 70% aqueous methanol. One ml of the combined glucosinolate extracts was pipetted onto the top of an ion-exchange column containing 1 ml Sephadex DEAE-A25 in the formate form. Desulphation was carried out by the addition of 75 µl of purified sulphatase (E.C. 3.1.6.1, type H-1 from *Helix pomatia*) (Sigma) solution. Desulphated glucosinolates were eluted with 2.5 ml (0.5 ml × 5) Milli-Q (Millipore) ultra-pure water and analysed with a Model 600 HPLC instrument (Waters) equipped with a Model 486 UV tunable absorbance detector (Waters) at a wavelength of 229 nm. Separation was carried out by using a Lichrospher 100 RP-18 in Lichrocart 125-4 column, 5 µm particle size (Merck). HPLC solvents and gradient were according to the ISO protocol (ISO Norm, 1992). The HPLC chromatogram was compared to the desulpho-glucosinolate profile of three certified reference materials recommended by U.E. and ISO (CRMs 366, 190 and 367) (Wathelet et al., 1991). The amount of each individual glucosinolate present in the sample was calculated by mean of the internal standard, and expressed as $\mu mol\ g^{-1}$ of dry wt. The total glucosinolate content was computed as the sum of all the individual glucosinolates present in the sample. Data were corrected for UV response factors for different types of glucosinolates (ISO Norm, 1992).

4.3. Statistical analyses

Individual analyses of variance were made according to a completely random design for glucosinolate content in the kale experiment, with plants sampled in each plot as replications. In the cabbage experiment, analyses of variance were made according to a randomized block design. Varieties and sowing seasons were considered as fixed effects. Comparisons of means among varieties and between sowing seasons were performed for each trait using the Fisher's protected least significant difference (LSD) at $P = 0.05$ (Steel et al., 1997). These analyses were made using the GLM procedure from SAS (SAS Institute, 2000).

Acknowledgements

Research supported by the National Plan for Research and Development (AGL2003-01366 and AGL2006-04055) and Excma. Diputación Provincial de Pontevedra. G. Padilla acknowledges a fellowship from the Cabildo Insular de La Palma. Authors thank the invaluable help of Elisa Santiago, Rosaura Abilleira, and Gloria Fernández for all the laboratory work.

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