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Survey of aliphatic glucosinolates in Sicilian wild and cultivated Brassicaceae

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Abstract

In the frame of the activities carried out to exploit Sicilian local cultivars of brassicas, we focused our attention on some of the potential health compounds of various local cruciferous crops. These compounds are of interest to improve the quality of the produce with the aim to develop new cultivars capable of providing functional foods able to prevent disease. In this context, we surveyed for the presence of specific glucosinolates in local cultivars of broccoli, cauliflower, kale, and in some wild species widespread in Sicily, using as control various commercial cultivars. Glucosinolate composition varied extensively among species and crops of the same species, such as cauliflower, broccoli and kale. Cultivar variation for glucosinolate profile was also observed for some crops. For example, Sicilian cultivars of cauliflower possessing colored curds displayed a high content of glucosinolates, glucoraphanin in particular, compared to white curd commercial cultivars. Also some wild species had a high content of other glucosinolates. © 2002 Published by Elsevier Science Ltd.

Keywords: *Brassica*; Broccoli; Cauliflower; Glucosinolate; Health compounds; Kale; Nutraceuticals

1. Introduction

In the frame of the activities carried out to characterize Sicilian local cultivars of brassicas (Branca and Iapichino, 1997), we focused our attention on the content of potentially health-inducing compounds, which could improve the value of the produce. Our objective was to evaluate local cultivars with the goal of improving genotypes for developing functional foods aimed at the prevention of human diseases. Kohlmeier and Su (1997) reported that consumption of *B. oleracea* vegetables results in lower probability of acquiring colon and rectal cancers. Similarly, Michaud et al. (1999) observed reduction of incidence of cancer to the bladder, and Cohen et al. (2000) for prostate cancer. In this context, we surveyed local cultivars of broccoli, cauliflower, kale and a few *Brassica* wild species for aliphatic glucosinolates (GSL).

Glucosinolates are a diverse class of compounds found mostly in the Brassicaceae. The glucosinolate molecule consists of two parts, a common glycone moiety and a variable aglycone side chain (Fenwick et al., 1983; Rosa et al., 1997). The aglycone part may contain aliphatic, indolyl, or aromatic side chains derived from a corresponding α-amino acid. Glucosinolates are hydrolyzed into isothiocyanates by the enzyme myrosinase, which is present in both plant cells and in human gut microflora (Shapiro et al., 1998). Many isothiocyanates have been shown to inhibit tumor formation when induced by chemical carcinogens in several animal systems (Zhang and Talalay, 1994; Hecht, 1995). One of these isothiocynantes is sulforaphane, resulting from the hydrolysis of glucoraphanin (GR = 4-methylsulfinyl-butyl glucosinolate), which is a potent inducer of phase II enzymes involved in carcinogen detoxification. Zhang et al. (1992, 1994) demonstrated that sulforaphane protects rats against tumorigenesis after treatment with dimethyl benzanthracene, a carcinogenic agent. For the purpose of our study, we collected local cultivars both in east and west Sicily whereas the wild species analyzed came mostly from west Sicily, where

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they occur naturally. Two of these wild species, *B. macrocarpa* and *B. rupestris,* form part of the primary gene pool of *B. oleracea*, which occurs in Sicily and is maintained by local growers (Branca and Iapichino, 1997; Gomez-Campo and Gustafsson, 1991). More specifically speaking, the aim of the present study was to determine primarily the variability of aliphatic GSL profiles in local cultivars compared with those from the related wild species. Although certain GSL derivatives have a protective effect against cancer (Rosa et al., 1997), there are some that may have detrimental effects, such as progoitrin and others derived from alkenyl GSL. These compounds mostly reported in rapeseed meal act as antinutrients affecting not only animal growth and development, but also lowering food intake. Additionally, modified isothiocyanates derived from progoitrin may have goitrogenic effects in animals (Rosa et al., 1997). Therefore, among the GSL surveyed in the present study, of particular interest are glucoraphanin because of its role as a cancer protecting agent, sinigrin, which has been reported as an inhibitor of fungi and nematodes (Rosa et al., 1997), and progoitrin, which is considered an antinutrient for its possible involvement in goiter.

2. Results and discussion

Glucosinolate quantity varied extensively among species and crops, with a global mean of 1.00 $\mu M^{-g\ FW\ leaves}$ (Fig. 1). All local and commercial broccoli and kale accessions showed higher amounts of total GSL, with means of approximately 1.20 and 1.32 $\mu M^{-g\ FW\ leaves}$ respectively, than cauliflower, which on the average all accessions contained 0.77 $\mu M^{-g\ FW\ leaves}$.

Local cultivars showed on average twice the amount of GSL than that observed for the commercial cultivars; with the exception cauliflower 'Geant d'Automne primis', which had approximately 1.0 $\mu M^{-g\ FW\ leaves}$. Two Sicilian broccoli accessions from W. Sicily (20 and 21, Table 1) showed 300% higher (ranging approximately between 4.40 and 4.31 $\mu M^{-g\ FW\ leaves}$) total GSL content than the general mean of all the accessions analyzed, while one accession from E. Sicily (16, Table 1) had close to 1.0 $\mu M^{-g\ FW\ leaves}$. Concerning local kale and cauliflower, high GSL content was observed for accessions 3 and 4, and for accessions 7, 10, and 11, respectively (Fig. 1). For cauliflower, the highest amounts were observed for local cultivars collected in the E. whereas for kale in the accessions collected both in the E. and W. (Fig. 1).

Amongst the wild species, MA showed the highest GSL content, 10.20 $\mu M^{-g\ FW\ leaves}$, which is approximately ten times higher than the mean GSL for all other accessions (Fig. 3). Also some accessions of RA showed high amounts of GSL, ranging between 1.49 and 3.06 $\mu M^{-g\ FW\ leaves}$, whereas for RU accession had a content of 1.71 $\mu M^{-g\ FW\ leaves}$.

Regarding specific GSL composition, we observed great differences among accessions for both aliphatic

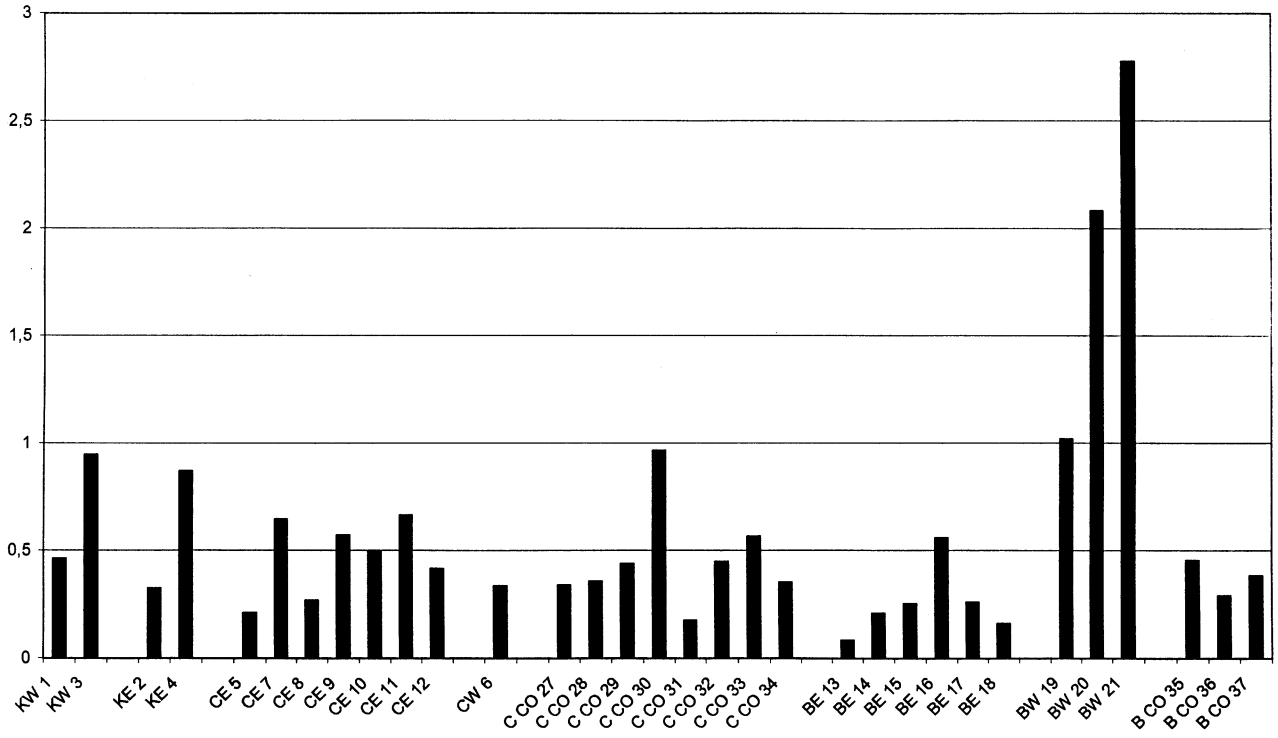

Fig. 1. Amount of glucosinolates in the young leaves of the analyzed accessions ($\mu mol^{-g\ FW\ leaves}$).

and indole glucosinolates detected in young leaves of the different crops and wild species. In all accessions analyzed the indole glucosinolate glucobrassicin represented approximately 46% of all glucosinolates (Fig. 2), except for RA and RU where it represented approximately 12%. For the latter two species more than 70% of GSL were represented by the aliphatic GSL gluconapin. On the other hand, MA lacked glucobrassicin, but instead it contained approximately 97% of the aliphatic GSL sinigrin and approximately 3% of glucoiberin (Fig. 2).

Broccoli and colored cauliflower contained GR whereas white cauliflower practically lacked this compound, except for 'D' Albenga precoce' (No. 32), which is an old Italian commercial cultivar and displayed a GSL profile similar to that found in colored cauliflower (Figs. 3 and 4). All other white cauliflowers showed a high content of sinigrin and an absence or low presence of GR . Most colored "cauliflowers" are actually intermediate types between broccoli and cauliflower (Kop et al., 2000), so it is not surprising that they have intermediate GSL profiles between broccoli and white cauliflower. The highest GR amount was observed for local colored cauliflower cultivars collected in the W. whereas the lowest was observed for the commercial ones (Fig. 5). In particular, violet curded local cultivars 8, 11 and 12, and green curded cultivar 6 showed higher contents of GR than the rest of cauliflower accessions (Fig. 5).

Broccoli and cauliflower are believed to have a common genetic background, originating in the Eastern Mediterranean region, whereas the rest of the cole crops seem to have originated in Western Europe (Gray, 1989).

Table 1
Accessions analyzed and their origin[a]

Name	Species	Origin	
Sicilian materials			
1. Cavolo vecchio	*B. oleracea* conv. *acephala*	Polizzi generosa	West Sicily
2. Cavolo vecchio	*B. oleracea* conv. *acephala*	Nicolosi	East Sicily
3. Cavolo vecchio	*B. oleracea* conv. *acephala*	Isnello	West Sicily
4. Cavolo vecchio	*B. oleracea* conv. *acephala*	Trecastagni	East Sicily
5. Austina	*B. oleracea* conv. *botrytis*^{V}	Piazza Armerina	East Sicily
6. Marzuddu	*B. oleracea* conv. *botrytis*^{G}	Palermo	West Sicily
7. Sammartinaro	*B. oleracea* conv. *botrytis*^{V}	Rosolini	East Sicily
8. Natalino	*B. oleracea* conv. *botrytis*^{V}	Noto	East Sicily
9. Sammartinaro	*B. oleracea* conv. *botrytis*^{V}	Francavilla	East Sicily
10. Natalisi	*B. oleracea* conv. *botrytis*^{V}	Modica	East Sicily
11. Natalisi	*B. oleracea* conv. *botrytis*^{V}	Modica	East Sicily
12. Maiarolo	*B. oleracea* conv. *botrytis*^{V}	Modica	East Sicily
13. N'tagghiu	*B. oleracea* conv. *italica*^{C}	Adrano	East Sicily
14. Natalino	*B. oleracea* conv. *italica*^{C}	Adrano	East Sicily
15. Precoce	*B. oleracea* conv. *italica*^{C}	Messina	East Sicily
16. Natalino	*B. oleracea* conv. *italica*^{C}	Messina	East Sicily
17. Natalino	*B. oleracea* conv. *italica*^{C}	Francavilla	East Sicily
18. Sammartinaro	*B. oleracea* conv. *italica*^{V}	Catania	East Sicily
19. Nostrale	*B. oleracea* conv. *italica*^{S}	Isnello	West Sicily
20. Sparaceddu	*B. oleracea* conv. *italica*^{S}	Cefalü	West Sicily
21. Smuzzatura	*B. oleracea* conv. *italica*^{S}	Isnello	West Sicily
22. Cauleddu	*B. rapa*	Isnello	West Sicily
23. Cauleddu	*B. rapa*	Collesano	West Sicily
24. Cauleddu	*B. rapa*	Trapani	West Sicily
25. Caulu sarvaggiu	*B. macrocarpa*	Favignana	West Sicily
26. Caulu di rocca	*B. rupestris*	Gratteri	West Sicily
Commercial materials			
27. Precoce di Jesi	*B. oleracea* conv. *botrytis*^{W}	Ingegnoli	Seed C.
28. Palla di neve	*B. oleracea* conv. *botrytis*^{W}	Ingegnoli	Seed C.
29. Godrai	*B. oleracea* conv. *botrytis*^{W}	Ingegnoli	Seed C.
30. Geant d'Automne primis	*B. oleracea* conv. *botrytis*^{W}	Ingegnoli	Seed C.
31. Romanesco	*B. oleracea* conv. *botrytis*^{R}	ingegnoli	Seed C.
32. Alpha 5	*B. oleracea* conv. *botrytis*^{W}	Ingegnoli	Seed C.
33. Cristina F_1	*B. oleracea* conv. *botrytis*^{W}	Ingegnoli	Seed C.
34. Timpurie de Bacau	*B. oleracea* conv. *botrytis*^{W}	SCLB	Seed C
35. Ramoso calabrese	*B. oleracea* conv. *italic*^{a}	SCLB	Seed C.
36. Sarnese	*B. oleracea* conv. *italica*^{C}	Pontecagnano	ISPORT
37. Mariner F_1	*B. oleracea* conv. *italica*^{C}	Peto seeds	Seed C.

[a] Broccoli: *C*, calabrese group, *S*, sparaceddu group; Cauliflower: *G*, green group, *R*, romanesco; *V*, violet group; *W*, white group (Branca and Iapichino, 1997).

The *Botrytis* group, which encompasses cauliflowers, most likely originated from the *Italica* group, which includes broccoli. The curd forming cauliflowers display low reproductive fitness, since most of the meristematic tissue forming the curd aborts and fails to develop into flowers. Therefore, it is likely that cauliflowers were the result of intense human selection (Crisp, 1989). Genetically, the cauliflower phenotype can be explained by the interaction of two recessive alleles of the genes *AP1* and *CAL* (Kop et al., 2000). The selection for curd might have resulted in the unintended selection for specific glucosinolates, where in cauliflower, GR was mostly

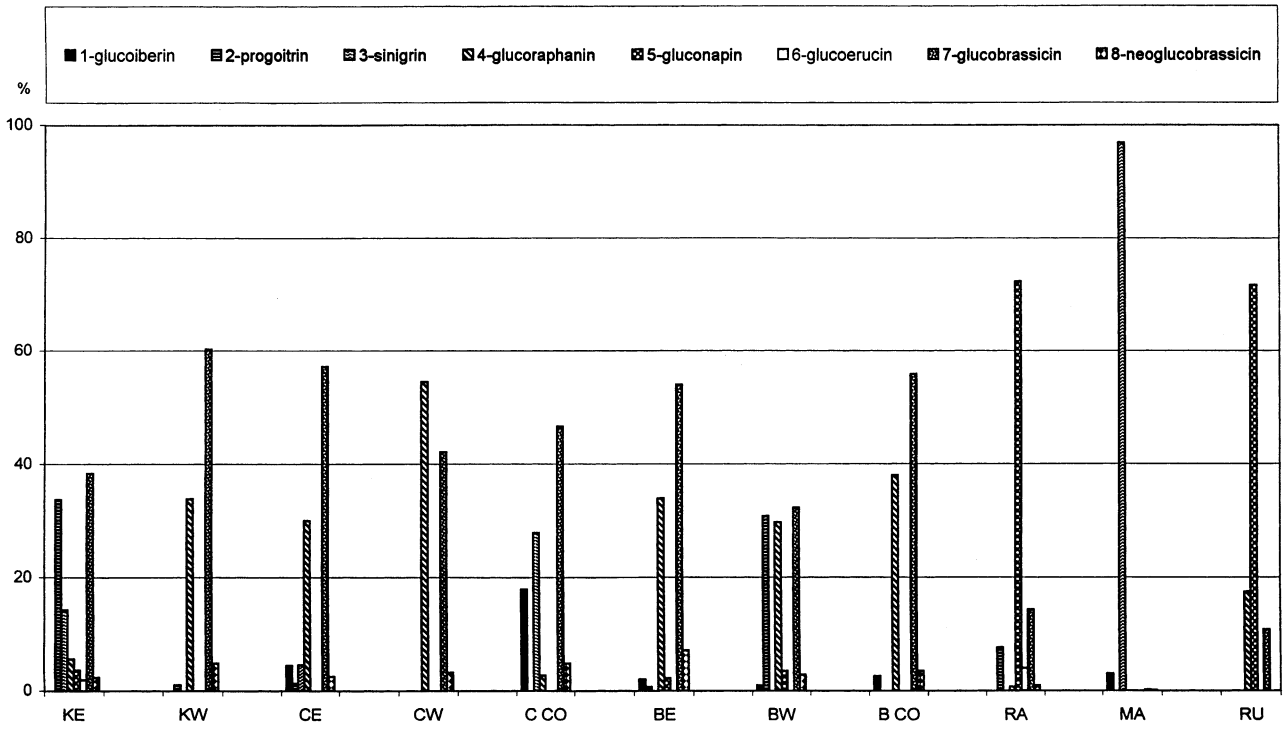

Fig. 2. Relative amount of glucosinolates in the young leaves with reference to crops and wild species and to area of diffusion.

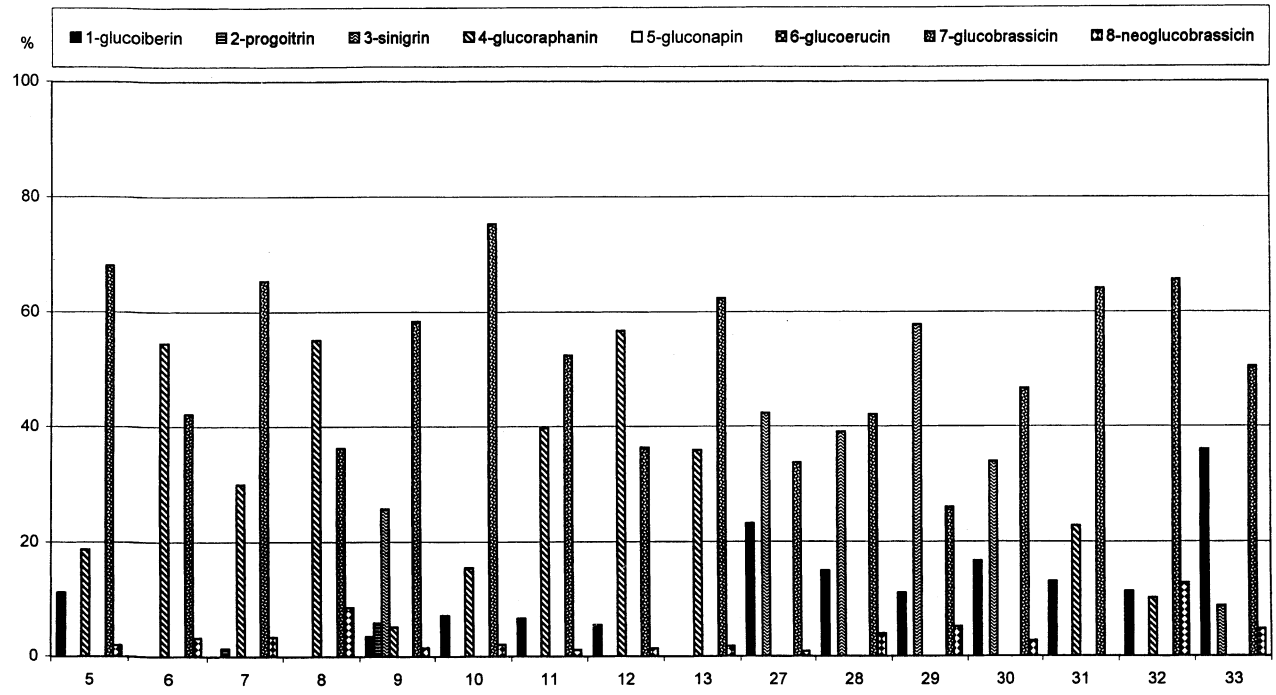

Fig. 3. Glucosinolate profile of cauliflower cultivars.

replaced by sinigrin. This replacement is due to the action of the alleles of two independent genes (Li et al., 2001).

The presence of high sinigrin in *B. macrocarpa* was not surprising, considering that two other species belonging to the oleracea gene pool, *B. villosa* var. *drepanensis* and *B. atlantica* were reported to contain high amounts of total GSL (11.88 $\mu M^{-g\ FW\ leaves}$), and sinigrin in particular (Mithen et al., 1987). This finding is certainly not universal for all related wild species,

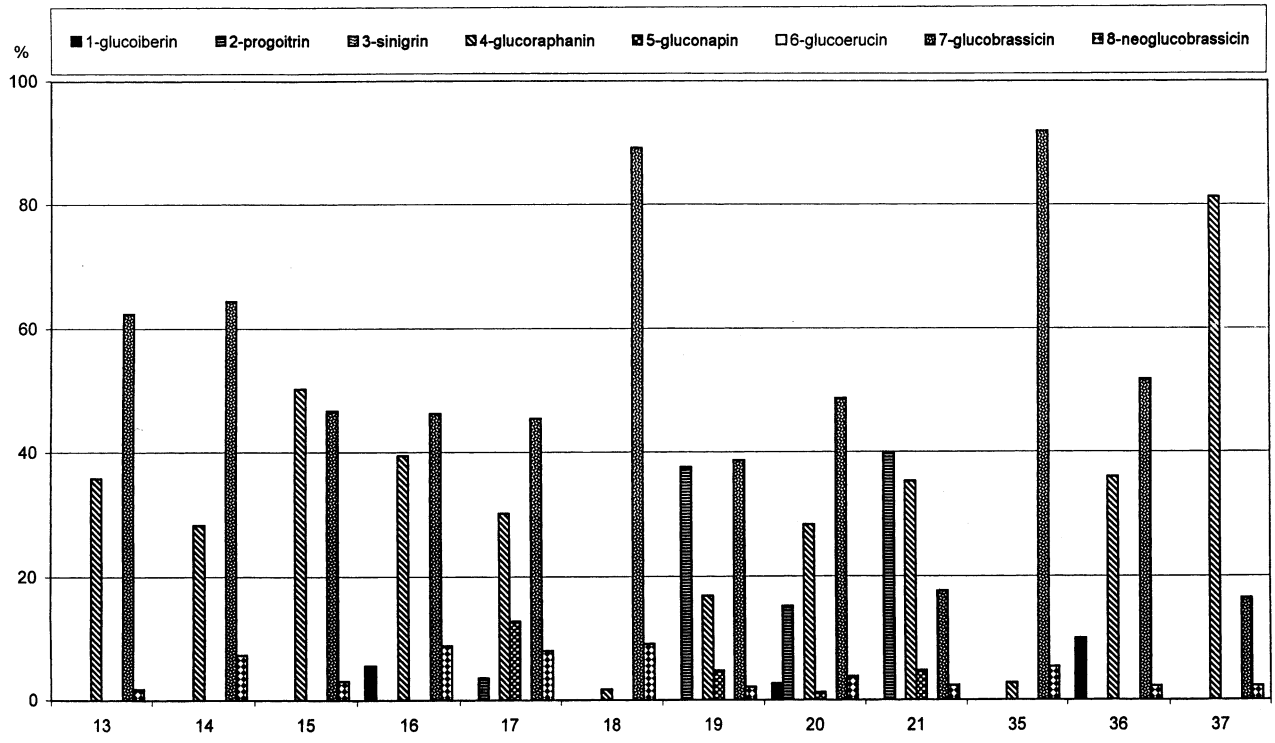

Fig. 4. Glucosinolate profile of broccoli cultivars.

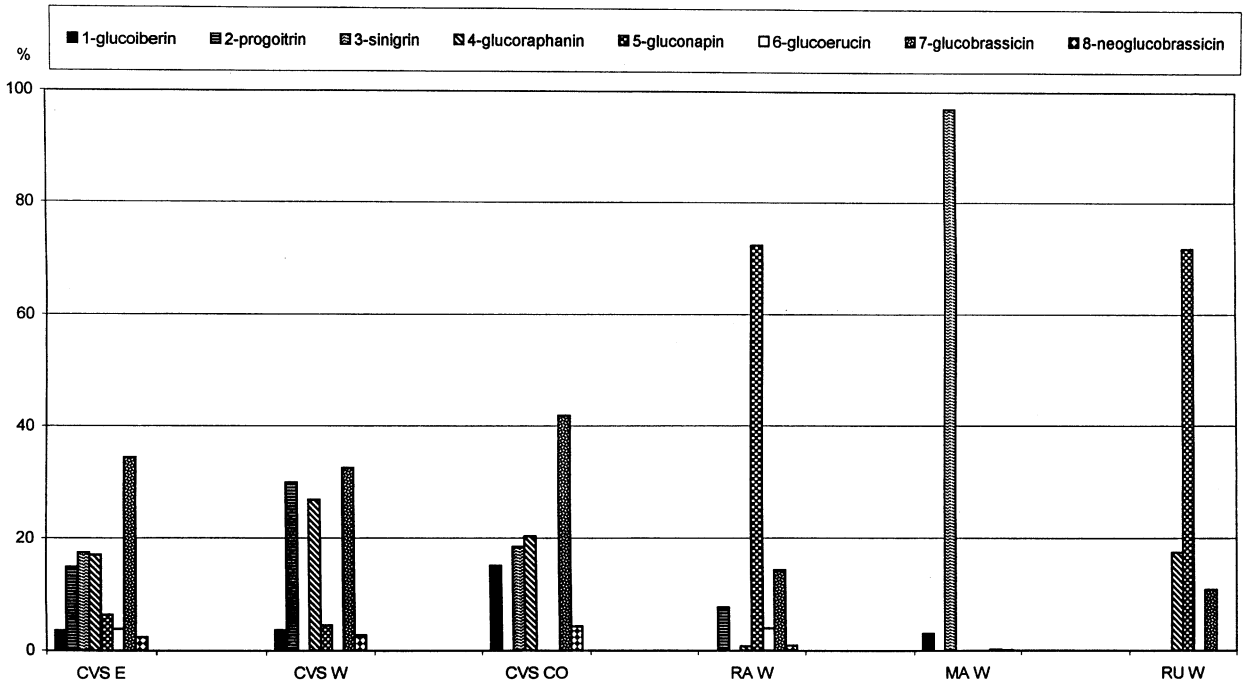

Fig. 5. Relative amount of glucosinolates in the young leaves of crops and wild species with reference to the area of diffusion.

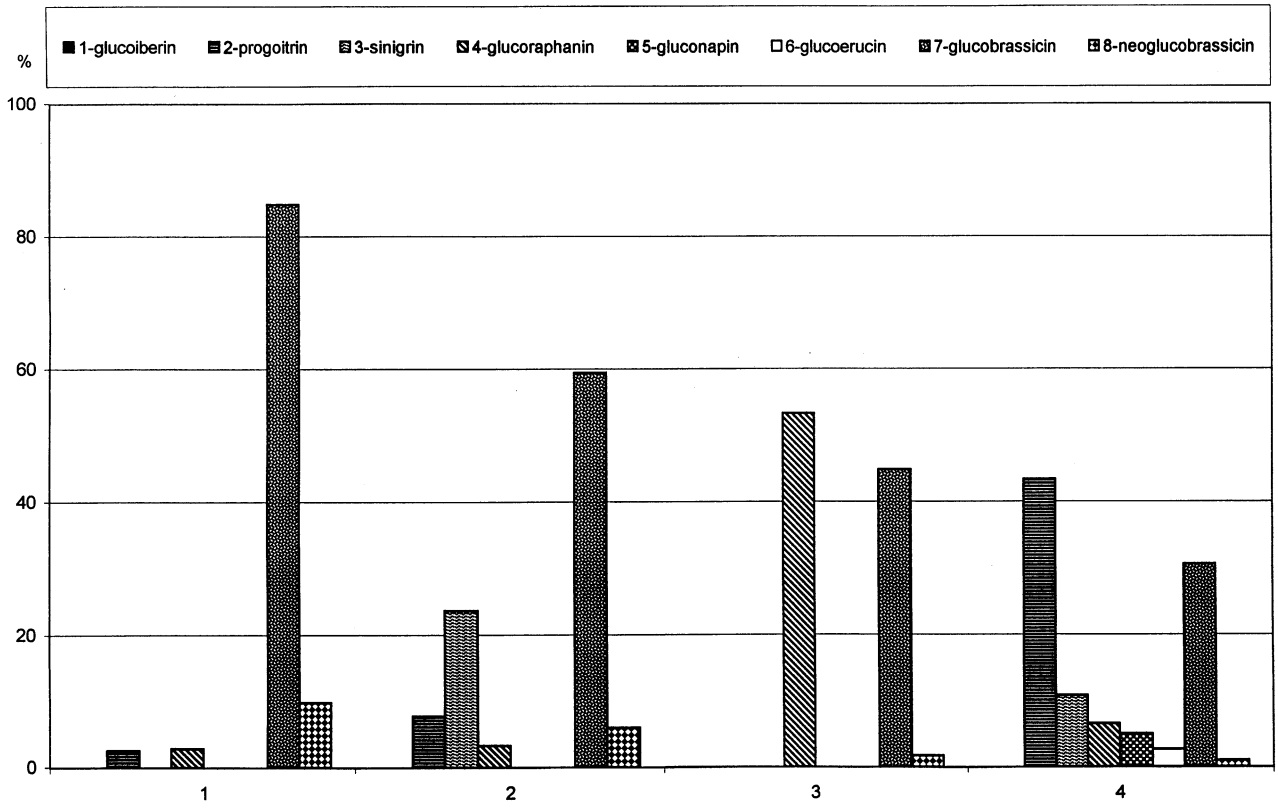

Fig. 6. Glucosinolate profile of kale cultivars.

considering that the RU accessions we tested did not have high GSL content in particular.

Our results show different GR amounts than those reported by Kushad et al. (1999) and Farnham et al. (2000). This discrepancy can be explained by the different plant organs analyzed, which for us were young leaves whereas for the above-cited authors were immature florets. It is a known fact that glucosinolate content increases as the plant develops reaching maximum concentration in the seeds (Rosa et al., 1997).

We found for most crops and wild species analyzed low content of glucoiberin and progoitrin with a few exceptions. The former was detected in three commercial cultivars of cauliflower (27 and 33) constituting 20% of the total GLS. Progoitrin was detected in one local cultivar of kale (4) and two of broccoli (19 and 21), constituting 43, 38 and 40% of total GSL, respectively (Figs. 4 and 6). The use of these certainly should be discouraged for food purposes.

3. Conclusions

Our results disclosed a great level of variability in GSL content in the Sicilian *Brassica* germplasm tested, including related wild species.

GSL profiles differed, above all between local and commercial cultivars of cauliflower, especially for glucoraphanin. For this crop the local colored cultivars seem to show a more desirable profile for beneficial GSL than the commercial ones. For broccoli and kale we found the same GSL as those reported by other authors. The wild *Brassica* screened had different GSL profiles, with *B. macrocarpa* showing a very high content of sinigrin whereas *B. rupestris* displayed a profile analogous to those of the crops analyzed.

The results obtained encourage us to continue the work started both by expanding the evaluation of GSL content to a larger collection of cultivars and wild species widespread in Italy, and to initiate a breeding program to develop lines from the material already analyzed. This activity is expected to result in the development of lines with different GSL content that could be used for different purposes. For example lines with high GR content for functional food development (cancer protection) and lines with high sinigrin content for biological pest control (nematodes and fungal pathogens).

4. Experimental

A total of 37 accessions were included in the present study. Out of these, 21 have been maintained at the Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari of Catania University (DOFATA),

representing a core collection of Sicilian local cultivars of kale (*Brassica oleracea* convar. *acephala*), cauliflower (*B. oleracea* convar. *botrytis*) and broccoli (*B. oleracea* convar. *italica*), and one each for *B. macrocarpa* (MA) and *B. rupestris* (RU). Eleven commercial cultivars (CO), consisting of eight cauliflower (seven white curded and one green romanesco) and three of broccoli varieties were included in the study. The remaining three accessions included in the analysis were existing wild forms of *B. rapa* (RA) gathered in countryside and utilized as a leafy vegetable both in east (E) and west (W) Sicily. The origin and distribution of the accessions included in the study are listed in Table 1.

Seeds were sown in trays at the experimental field of the Department of Vegetable Crops of California University (UC Davis) and seedlings for GSL analysis were grown in the greenhouse at an average temperature of 27 °C during the day and 20 °C in the night.

Total glucosinolates from young leaves (about 10 cm long) of 20 two-week-old seedlings per accession were extracted in methanol and converted into their desulfo analogs by sulfatase treatment (Kraling et al., 1990). After applying the supernatant to a DEAE-Sephadex A-25 column (Sigma Chemical Co., St. Louis, MO), the GSL were converted into desulfo-GSL with sulfatase (0.5% enzyme in water for 12 h at 24 °C, Sigma H-I type). The desulfo-GSLs were then eluted by adding 1.5 ml distilled water. The resulting mixture was separated by a high-performance liquid chromatography system (HPLC), which consisted of a pump (model LC-10AT), an auto sampler/injector (model SIL-10AD), a UV detector (model SPD-10A), a column temperature controller (model CTO-10AS), and a systems controller (model-SCL-10A) (all by Shimadzu Instrument Co., Columbia, MD). The detection was carried out at 230 nm and the column used was a Lichrosphere 100 RP-18 (Alltech Associates Inc., Deerfield, IL). A micro-computer with software *Class-VP*® (Shimadzu Instrument Co., Columbia, MD) was used for data collection and computations. A linear binary solvent gradient from 1 to 19% acetonitrile in water over 20 min was routinely used. The flow rate was 1.5 ml/min and the column was maintained at 32 °C. The HPLC chromatogram was compared to the desulfo-glucosinolateprofile of 'Linetta' rapeseed, a cultivar used widely as a standard for glucosinolate (GSL) identification to compare the peaks with the corresponding GSL. The presence of desulfosinigrin and desulfoglucoraphanin (GR) peaks was confirmed by using pure authentic sinigrin (Sigma Chemical Co., St. Louis, MO) as an internal standard. Qualitative assessment of GSL was done visually by the presence or absence of the specific peaks. GSL content was quantified with glucotropaeolin (E.M. Science, Gibbstown, NJ) as an internal standard. Glucosinolate content was expressed as uM of GSL per gram of fresh leaves. We corrected the data for UV response factors for different types of glucosinolates (ISO 9167–1, 1992; Wathelet et al., 2001).

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