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Identification and Quantification of Glucosinolates in Sprouts Derived from Seeds of Wild *Eruca sativa* L. (Salad Rocket) and *Diplotaxis tenuifolia* L. (Wild Rocket) from Diverse Geographical Locations

RICHARD N. BENNETT,*,† ROSA CARVALHO,† FRED A. MELLON,‡ JOHN EAGLES,‡ AND EDUARDO A. S. ROSA†

CECEA-Departamento de Fitotecnia e Engenharia Rural, Universidade de Trás-os-Montes e Alto Douro (UTAD), Apartado 1013, 5001-801 Vila Real, Portugal, and Mass Spectrometry Group, Institute of Food Research (IFR), Norwich Research Park, Colney, Norwich NR47UA, United Kingdom

The Brassicaceae rocket species *Eruca sativa* L. (salad rocket) and *Diplotaxis tenuifolia* L. (wild rocket) are consumed throughout the world in salads, predominantly the leaves but also the flowers and more recently the sprouts (seedlings). Ontogenic profiling of glucosinolates and flavonoids in plants derived from commercial seed of these species has previously been done, but no studies have been conducted to determine how geographical origin affects glucosinolate composition in rocket species. Seeds from wild *E. sativa* L. and *D. tenuifolia* L. from diverse regions of the world were obtained from gene banks and grown under controlled conditions. Sprouts were harvested when they would normally be harvested for consumption, and glucosinolates were extracted and profiled in these accessions. All of the sprouts from Italian *E. sativa* L. had consistently high total glucosinolate content, with only a few exceptions, and also the highest percentage contents of 4-mercaptobutylglucosinolate. In contrast, sprouts produced from Central and Eastern European seeds had a much higher percentage of 4-methylthiobutylglucosinolate. With a single exception, Tunisia, all sprouts produced from North African seeds had very high 4-methylthiobutylglucosinolate contents. The single sample from China had a high total glucosinolate content and glucosinolate profile that was very similar to the accessions from Uzbekistan and Pakistan. All of the *D. tenuifolia* L. sprouts had consistently high total glucosinolate contents, and a high percentage of this was 4-mercaptobutylglucosinolate. This glucosinolate variation in levels and profiles of the rockets can be used for genetic studies, selected breeding, and human intervention studies.

KEYWORDS: ***Eruca sativa*; *Diplotaxis tenuifolia*; rocket; glucosinolates; geographical origin; seedlings; sprouts; LC/MS**

INTRODUCTION

Plants from the Brassicaceae family play a major role in worldwide vegetable production and consumption, ranking second after the Solanaceae. The Brassicaceae is a large family of plants, within the plant order Capparales, which includes both major vegetable crops such as broccoli and cabbage and also salad species such as *Eruca sativa* L. (salad rocket) and *Diplotaxis tenuifolia* L. (wild rocket) (*1*). These species, well represented in the Mediterranean area, have gradually spread to other latitudes, and there has been increasing interest in the past decade for their use in salads, although cooked leaves, flowers, and more recently sprouts (seedlings) are also used. Leaves of salad rocket (*Eruca sativa* L.) have a characteristic pungent taste and odor that is dependent on species, genetic diversity, and environment (*2*). Rocket has been reported as having high vitamin C content and also various medicinal properties including diuretic, anti-inflammatory, and effects on blood circulation (*3*, *4*). Eating the fresh raw material is the best way of gaining all of the health benefits claimed for this vegetable because only minor losses in health-promoting components are likely to occur, whereas losses are greater during cooking of related species. In India and Pakistan, special ecotypes are cultivated for their inedible and pungent seed oil (*5*). Historically, *Eruca* spp were also used as a biological control to inhibit various pests (*6*).

Rocket contains glucosinolates that are β-thioglucoside *N*-hydroxysulphates with a side chain (R) and a sulfur-linked β-D-

* Author to whom correspondence should be addressed [telephone +351-259350750; fax +351-259350327; e-mail richard.bennett@bbsrc.ac.uk].
† UTAD.
‡ IFR.

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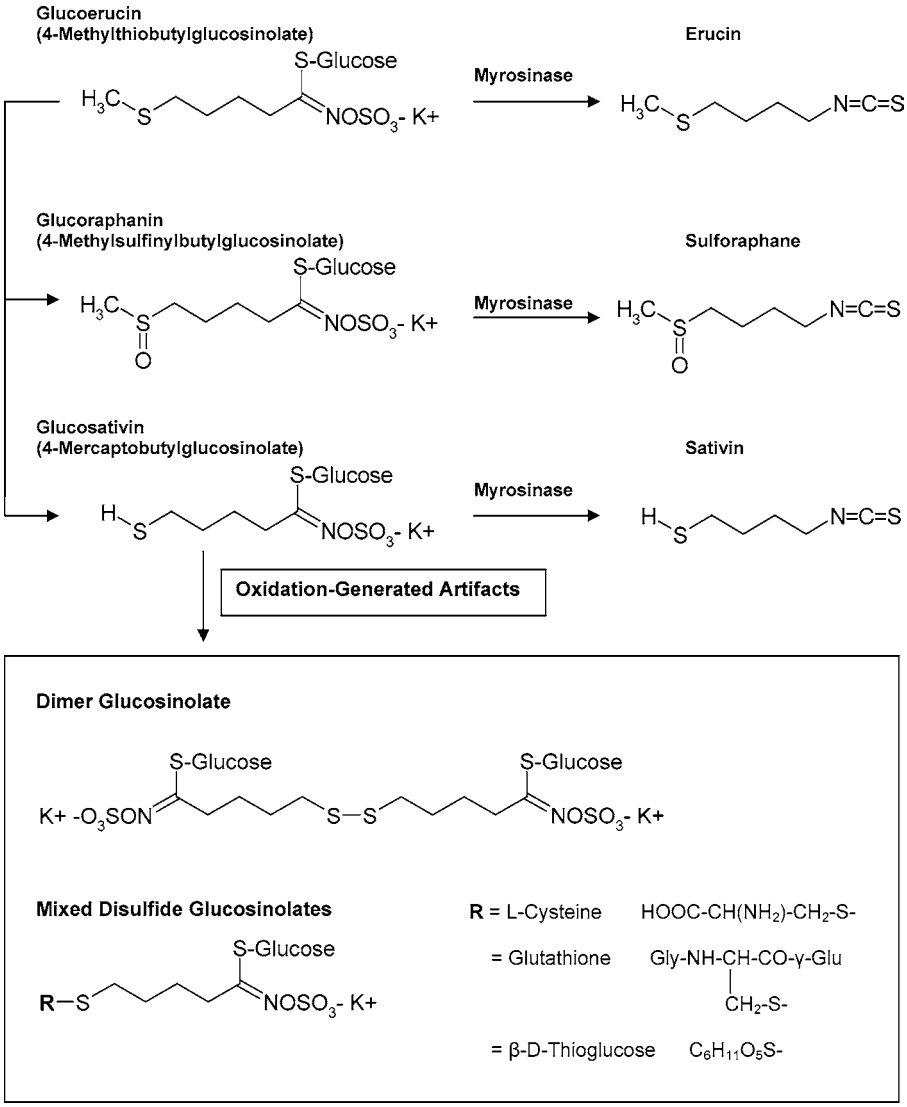

Figure 1. Structures of the major glucosinolates identified in *Eruca* and *Diplotaxis* species, the isothiocyanates derived from these glucosinolates, and the glucosinolate artifacts formed from oxidation of 4-mercaptobutylglucosinolate (glucosativin) during sample extraction.

glucopyranose moiety (**Figure 1**). About 120 glucosinolates have been identified from different Capparales species and are divided into three basic categories, aliphatic, aromatic, and indole, according to the type of side chain. Glucosinolates are sequestered in the plant vacuoles, and myrosinase, the glucosinolate hydrolytic enzyme, is localized in the cytosol and is also present at high levels in specialized cells called myrosin cells (*7*). When glucosinolates are exposed to myrosinase, during tissue damage, etc., glucose and an unstable intermediate are formed. This intermediate degrades to produce a sulfate ion, and a variety of products including isothiocyanates, thiocyanates, and nitriles. Formation of hydrolysis products is dependent on the glucosinolate itself and reaction conditions, for example, pH, or the presence of Fe^{2+} or epithiospecifier protein. It is only recently that non-*Brassica* species and different tissues of Capparales species have been evaluated for glucosinolate content. Previous data were predominantly for the major Brassicaceae crops only (*8*−*10*).

Seeds of *Eruca sativa* L. predominantly contain 4-methylthiobutylglucosinolate (glucoerucin) and low levels of 4-methylsulphinylbutylglucosinolate (glucoraphanin) (*11*, *12*). Recently, the major glucosinolate in leaves of rocket was identified as 4-mercaptobutylglucosinolate (glucosativin), probably derived from *S*-demethylation of 4-methylthiobutylglucosinolate (*13*). This glucosinolate upon hydrolysis produces 4-mercaptobutylisothiocyanate (sativin), a volatile and very pungent compound that may in part explain the distinct odor of salad rocket (*13*). Ontogenic LC/MS profiling of glucosinolates, flavonoids, and other secondary metabolites has recently been done for all tissues of plants grown from commercial seeds of *Eruca sativa* L. and various *Diplotaxis* species (*12*). **Figure 1** shows the structures of the major glucosinolates identified in rocket species, their myrosinase-derived isothiocyanates, and the oxidation artifacts that can be formed during sample extraction from 4-mercaptobutylglucosinolate.

During the last two decades, consumption of fruits and vegetables has received increased recommendations based on epidemiological studies (*14*−*17*). In the past decade, there have been a considerable number of reviews on the bioavailability and effects of the hydrolysis products (*18*−*21*). Recently, there has been evidence suggesting that intact glucosinolates possess negative biological activities such as pro-oxidant activity and the induction of phase-I pro-carcinogenic cytochrome P450-type enzymes; however, the doses used in these studies were high, and in addition these studies were performed in rat models that are not always the best indicator of *in vivo* effects in humans (*22*, *23*) due to major differences in the gastrointestinal tracts of rats and humans. There is also evidence of direct antioxidant

activities of intact glucosinolates, but this is not thought to be a major contributor to the total antioxidant activities of food plants, as compared to other common antioxidants such as vitamin C, vitamin E, phenolics, and polyphenolics (*24*, *25*). However, there is good evidence to suggest that the isothiocyanates themselves can act as regulators of cellular redox status (*26*). Consumption of salad species, where there is minimal processing and no cooking to cause inactivation of myrosinase, is likely to lead to significant levels of health-promoting isothiocyanates being absorbed. Sulforaphane (4-methylsulfinylbutylisothiocyanate) derived from 4-methylsulfinylbutylglucosinolate (glucoraphanin) is one of the most promising natural anti-cancer compounds identified in Capparales species and is present in rocket species. There have been many *in vitro* and recently *in vivo* studies on the effects and absorption, disposition, metabolism, and excretion (ADME) of this isothiocyanate (*27*−*30*). There have been very few human intervention studies on rocket species.

Sprouts from the family Brassicaceae are increasingly consumed in salads or used in Chinese-style cooking, and there is increased interest in using sprouts as functional foods (*31*). More recently, sprouts of rocket species have also been promoted as new foods. With the previous studies on the various rocket species and subsequent identification of the major phytochemicals (*12*, *13*), it was therefore logical to profile phytochemicals in sprouts of these species, and also for the first time to evaluate different sources of these species to determine how geographical origin affected these profiles, that is, the influence of genetic factors on health-promoting phytochemicals. A further aim was to identify accessions, with different glucosinolate profiles, that could then be used for directed breeding programs and also for experiments to evaluate effects on human health in intervention studies.

MATERIALS AND METHODS

Plant Material. Seeds of wild rocket species were obtained from two gene bank centers: Institut für Pflanzengenetik and Kulturpflanzenforschung (IPK, Gatersleben, Germany) and Plant Genetic Resources-Centre for Genetic Resources (CGN-PGR, Wageningen, The Netherlands). Full details of accession origins can be found in the Supporting Information. All seeds were sown at 2 cm depth in PVC trays divided into 88 subsections (70 cm^3 each subsection) filled with a mixture (3:1) of peat (Agrofino Products NV, Arendonk-Belgium) and sand. They were kept in a Conviron E15 growth chamber under a 14 h photoperiod with 220 $\mu mol/m^2/s^1$ of PAR supplied by five fluorescent tubes (Osram Sylvania, Inc., Danvers, MA) and a regime of 22/15 °C, day/night temperature, 80/90% day/night relative humidity. For each accession, three separate subsamples were taken where possible. Because of limited seed numbers for some of the accessions, only duplicate subsamples were collected; these were commonly the samples for which the data presented gave the largest SD values for glucosinolate contents. Post-emergence development was carefully monitored, and on the day that the cotyledons had fully expanded the total aerial tissues were harvested. Seedlings were cut at the substrate level, and the fresh weight of tissue was recorded. Samples were flash-frozen and homogenized in liquid nitrogen within a few minutes of harvesting. All samples were freeze-dried, and the dry weight of each sample was recorded. Full details of each accession origin can be found in the Supporting Information.

Chemicals. All chemicals were of analytical grade and were obtained from Sigma/Aldrich. All solvents were of HPLC grade, and all water was ultrapure. All enzymes were obtained from Sigma. Benzylglucosinolate (glucotropaeolin) and glucoraphanin were gifts from Dr. Renato Iori (Istituto Sperimentale Colture Industriali, Bologna, Italy). Tris(2-carboxyethyl)phosphine hydrochloride (TCEP; >98% pure) was obtained from Fluka. All other glucosinolate standards, 4-mercaptobutylglucosinolate (glucosativin) and 4-methylthiobutylglucosinolate (glucoerucin), were purified from *Eruca sativa* leaves and seed, respectively, and glucobrassicin was purified from white cabbage heads, using methods previously described (*13*).

Glucosinolate Extraction and Analyses. Freeze-dried material from the *E. sativa* L. and *D. tenuifolia* L. accessions was blended to a fine powder using a commercial food processor, and a 200 mg subsample from each replicate sample was extracted by adding ca. 3 mL of boiling 90% methanol plus 200 μL of benzylglucosinolate (glucotropaeolin) as an internal standard. After being boiled for 2 min, the extracts were centrifuged (17 500*g*, 20 °C, 5 min), and the residue was re-extracted twice with boiling 70% methanol (3 mL). Extracts were combined to give a final volume of 10 mL, and a 2.5 mL aliquot was evaporated to dryness and taken up in 2.5 mL water. A 2 mL subsample of the aqueous sample was applied to a 1.5 mL bed volume DEAE Sephadex A-25 column prepared in a Pasteur pipet, and the adsorbed glucosinolates were desulfated using the method previously published by Heaney and Fenwick (*32*). Desulfo-glucosinolates were eluted with water and analyzed by HPLC, using a Phenomenex Luna C_{18} (*2*) (250 × 4.6, 5 μm) with a Securityguard precolumn, as described by Spinks et al. (*33*). Selected samples were also treated with TCEP to reduce any glucosinolate artifacts formed from 4-mercaptobutylglucosinolate during sample preparation to ensure accurate quantification of 4-mercaptobutylglucosinolate (*12*, *13*). Calibration curves, for conversion of peak areas to quantities, were produced using pure standards of the glucosinolates, de-sulfated in triplicate at five different concentrations. LC/MS analyses, using a Phenomenex Luna C_{18} (*2*) (250 × 4.6, 5 μm) with a Securityguard precolumn, for confirming glucosinolate identities, were conducted using a Micromass Quattro II triple quadrupole mass spectrometer (Waters, Manchester, UK) equipped with a Z-spray electrospray (ESI) ion source, which was coupled to a Hewlett-Packard 1050 quaternary pump HPLC system (Agilent Technologies, Stockport, UK). HPLC conditions were as follows: solvent A was ultrapure water (deionized, distilled); solvent B was 20% v/v HPLC grade acetonitrile in ultrapure water. The HPLC gradient was: 100% A at 0−5.0 min, 40% A at 18.0 min, and 100% A at 20−35 min. The HPLC column temperature was maintained at 25 °C and the autoinjector at 4 °C. The 1.25 mL/min mobile phase flow exiting the HPLC column was introduced directly into the APCI probe. The mass spectrometer APCI corona voltage was set to 3.5 kV, cone voltage to 29 V, source block temperature to 150 °C, and APCI probe temperature to 600 °C. Spectra were scanned between *m/z* 50 and 800 at a rate of 2 s/scan and with an interscan delay of 0.1 s. Data were processed using MassLynx 3.4 (Micromass UK Ltd., Manchester, UK) acquisition and processing software. All glucosinolate concentrations are expressed as μmol/g dry weight (DW) and for total glucosinolates as both DW and fresh weight (FW).

Statistics. Statistical analyses were performed using SuperANOVA v.1.11 (Abacus Concepts Inc., Berkeley, CA) software.

RESULTS AND DISCUSSION

Consumption of rocket species (*Eruca* and *Diplotaxis*) is increasing throughout the world, and they are especially popular in the Mediterranean and Middle-Eastern countries. These species have a variety of uses including salad ingredients, herbs, and as medicinal plants (*12*, *13*). Unlike the related *Brassica* crop species, there has been far less commercial breeding and utilization of the rocket species. Both *Eruca* and *Diplotaxis* species have a characteristic flavor and odor that is very different from other Capparales and Brassicaceae crop species; this is due to the presence of the thiol-containing glucosinolate, glucosativin (4-mercaptobutylglucosinolate), and the isothiocyanate derived from this glucosinolate (*12*, *13*). It is thought that glucoerucin (4-methylthiobutylglucosinolate) is the precursor for both glucosativin and glucoraphanin (4-methylsulfinylbutylglucosinolate); both are also found in *Eruca* and *Diplotaxis* species (**Figure 1**). Example chromatograms (227 nm) of various desulfo-glucosinolates are shown in **Figure 2**: standard mixture (**Figure 2A**), *E. sativa* seeds (**Figure 2B**), and a seedling sample of *E. sativa* without and with treatment of TCEP showing the

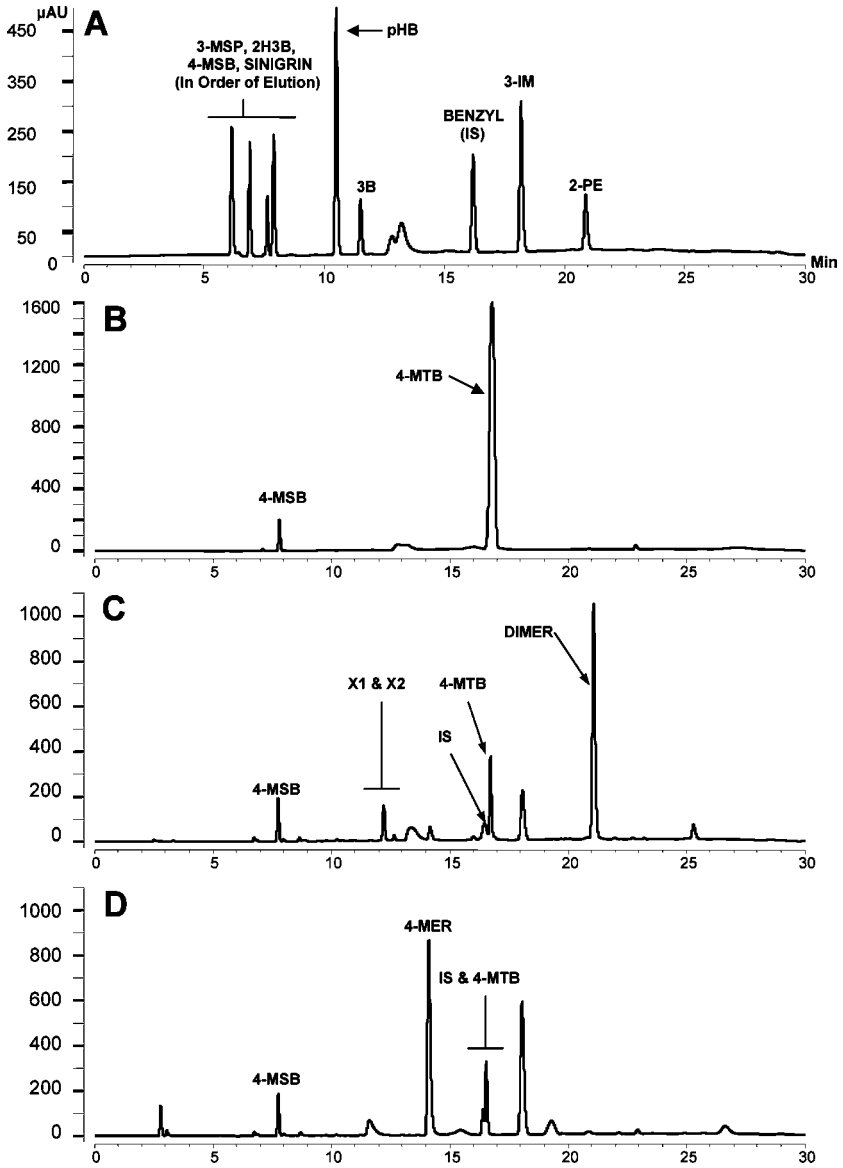

Figure 2. Example chromatograms of desulfo-glucosinolates. (**A**) Standard mixture, 10 μL injection of 0.1 mg/mL of each glucosinolate standard; (**B**) desulfo-glucosinolates from seeds of *Eruca sativa* L.; (**C**) non-TCEP treated desulfo-glucosinolate sample from a sprout sample of *Eruca sativa*; and (**D**) the same *Eruca sativa* sprout sample treated with 5 mg/mL TCEP to convert all artifact desulfo-glucosinolates (X1, X2, and dimer) back into 4-mercaptobutylglucosinolate. All unlabeled peaks are non-glucosinolates derived either from the sample or from the sulfatase. Glucosinolate peak ID: 3-MSP = 3-methylsulfinylpropyl (glucoiberin), 2H3B = (R) 2-hydroxy-3-butenyl (progoitrin), 4-MSB = 4-methylsulfinylbutyl (glucoraphanin), pHB = 4-hydroxybenzyl (sinalbin), 3B = 3-butenyl (gluconapin), 4-MER = 4-mercaptobutyl (glucosativin), 4-MTB = 4-methylthiobutyl (glucoerucin), 3-IM = 3-indolylmethyl (glucobrassicin), and 2-PE = 2-phenylethyl (gluconasturtiin).

disappearance of artifact peaks (X1, X2, and dimer) (**Figure 2C**) and their conversion back into glucosativin (**Figure 2D**).

Seeds of wild *E. sativa* and *D. tenuifolia* were obtained from two gene banks (IPK and CGN-PGR) that had been collected from a wide range of geographical locations. Sprouts were produced under controlled growth conditions, thus minimizing external environmental effects on phytochemical biosyntheses. Sprouts (aerial tissue only and not residual seed or roots) were harvested at the same physiological stage when the cotyledons had fully expanded, that is, at the stage when they would be harvested for consumption in salads, etc. This means that the accession/species harvest dates varied depending on the time that full cotyledon expansion was achieved; seedling age at harvest for all of the accessions is presented in **Tables 1**−**3**.

The same glucosinolates were found in all of the sprouts derived from seeds of *E. sativa* and *D. tenuifolia* accessions: glucoerucin, glucoraphanin, glucosativin, and glucobrassicin (3-indolylmethylglucosinolate) (**Tables 1**−**3**). There were none of the dimer or mixed-disulfide glucosinolates that have recently been reported, because these are extraction artifacts (*12*, *13*, *34*). Desulfo-glucosinolate identities in selected samples were confirmed by LC/APCI-MS and in comparison with standards (data not shown).

With only a few exceptions, the sprouts produced from the Italian *Eruca sativa* seeds consistently had a high percentage of glucosativin (42−87%, mean = 64%) as compared to the other glucosinolates (**Table 1**). Although there were significant differences in the total glucosinolate content of the accessions, ranging from 326 to 5540 μmol/g DW, the percentage contents of the individual glucosinolates were very similar (**Table 1**). With a few exceptions, the contents of glucoerucin were also similar in the majority of Italian accessions (20−34%, mean = 23%) (**Table 1**). The percentage contents of glucoraphanin (5−26%, mean = 13%) and glucobrassicin (0.1−2.8%, mean =

Table 1. Geographical Region, Sprout Age at Harvest, Individual and Total Glucosinolates Content, and Percentage of Each Glucosinolate in Relation to the Total Glucosinolates in Italian Accessions of *Eruca Sativa* L.

accession number	Italian region	seedling age (days)[a]	glucosinolate[b] (μmol glucosinolate/g DW)					glucosinolate (% of total)			
			4-MTB	4-MSB	4-MER	3-IM	total	4-MTB	4-MSB	4-MER	3-IM
K5535	Puglia	4	5.5 ± 0.4	2.9 ± 0.5	22.2 ± 1.9	0.3 ± 0.08	30.9 ± 2.7	18	9.5	71.5	1
K5569	Puglia	4	2.2 ± 1.3	1.5 ± 0.5	21.5 ± 5.6	0.12 ± 0.01	25.3 ± 7.4	9	6	84.5	0.5
K5581	Puglia	4	10.6 ± 2.4	3.9 ± 0.7	21.5 ± 4.7	0.09 ± 0.03	35.9 ± 7.6	29	11	59.7	0.3
K5588	Puglia	5	2.2 ± 0.3	1.8 ± 0.1	3.4 ± 1.9	0.09 ± 0.01	7.5 ± 2.3	29	24	45.8	1.2
K7687	Puglia	5	0.9 ± 0.04	1.1 ± 0.09	1.9 ± 0.9	0.07 ± 0.01	4.1 ± 1.5	24	26	48.3	1.7
K5546	Campania	3	1.2 ± 0.2	0.8 ± 0.4	12.6 ± 3.5	0.06 ± 0.01	14.6 ± 3.1	8	5	86.6	0.4
K9345	Campania	5	3.3 ± 0.6	1.4 ± 0.2	11.3 ± 1.0	0.07 ± 0.01	16.0 ± 1.7	21	8	70.6	0.4
K5576	Basilicata	5	1.9 ± 0.3	0.7 ± 0.02	5.1 ± 0.9	0.05 ± 0.01	7.9 ± 1.3	25	9	65.4	0.6
K10108	Basilicata	5	2.6 ± 1.3	1.5 ± 0.5	8.6 ± 4.5	0.03 ± 0.01	12.8 ± 5.4	20	12	67.8	0.2
K10113	Basilicata	5	1.1 ± 0.2	0.7 ± 0.05	1.4 ± 0.4	0.09 ± 0.01	3.3 ± 0.7	34	21	42.2	2.8
K6071	Marche	5	1.5 ± 0.1	1.7 ± 0.03	10.2 ± 0.8	0.06 ± 0.01	13.6 ± 0.7	11	13	75.6	0.4
K8337	North	4	17.9 ± 1.6	6.1 ± 0.8	31.3 ± 2.3	0.06 ± 0.01	55.4 ± 3.6	32	11	56.9	0.1
K8636	North	5	11.0 ± 0.8	4.6 ± 1.9	27.4 ± 5.3	0.07 ± 0.01	43.1 ± 2.5	26	11	62.8	0.2
K9902	Sardinia	5	1.9 ± 0.5	0.7 ± 0.1	5.3 ± 2.9	0.09 ± 0.01	8.1 ± 3.5	24	9	65.9	1.1
K9918	Sardinia	5	7.2 ± 0.9	3.9 ± 0.4	14.8 ± 1.5	0.06 ± 0.03	25.9 ± 2.8	28	15	56.8	0.2

[a] Seedling age represents the age of the accession when the cotyledons had fully expanded. All seedlings were harvested at the same physiological stage. [b] Glucosinolate contents expressed as mean ± SD μmol glucosinolate/(gram dry weight seedling tissue). Glucosinolate abbreviations: 4-MTB = 4-methylthiobutylglucosinolate (glucoerucin); 4-MSB = 4-methylsulfinylbutylglucosinolate (glucoraphanin); 4-MER = 4-mercaptobutylglucosinolate (glucosativin); 3-Im = 3-indolylmethylglucosinolate (glucobrassicin). No other glucosinolates were detected in the samples.

Table 2. Geographical Region, Sprout Age at Harvest, Individual and Total Glucosinolates Content, and Percentage of Each Glucosinolate in Relation to the Total Glucosinolates in Eurasian, North African, and Middle Eastern Accessions of *Eruca sativa* L.

accession num ber	country	seedling age (days)	glucosinolate[a] (μmol glucosinolate/g DW)					glucosinolate (% of total)			
			4-MTB	4-MSB	4-MER	3-IM	total	4-MTB	4-MSB	4-MER	3-IM
K8495	Spain	7	0.7 ± 0.1	0.4 ± 0.04	2.1 ± 0.8	0.06 ± 0.01	3.3 ± 0.9	21	13	64.2	1.8
CGN6982	The Netherlands	4	32.0 ± 1.4	5.4 ± 0.9	15.9 ± 3.7	0.09 ± 0.01	53.5 ± 2.4	60	10	29.8	0.2
K6121	Yugoslavia	4	4.6 ± 0.4	2.4 ± 0.4	4.3 ± 2.2	0.01 ± 0.001	11.3 ± 2.8	40	21	38.9	0.08
K6587	Croatia	4	13.7 ± 2.9	6.0 ± 1.2	5.6 ± 1.7	0.09 ± 0.01	25.4 ± 5.7	54	24	21.6	0.4
CGN7310	Czechoslovakia	5	2.4 ± 0.7	2.1 ± 0.2	1.5 ± 0.1	0	5.9 ± 0.8	40	35	25	0
K6281	Libya	4	21.2 ± 3.3	6.9 ± 0.5	4.1 ± 1.1	0.11 ± 0.01	32.3 ± 4.7	66	21	12.7	0.3
K6286	Libya	4	14.3 ± 5.8	5.1 ± 1.8	7.0 ± 1.7	0.20 ± 0.03	26.7 ± 8.7	54	19	26.3	0.7
K6288	Libya	4	10.7 ± 3.5	4.7 ± 0.6	3.3 ± 2.2	0.14 ± 0.01	18.9 ± 6.1	57	25	17.3	0.7
K9212	Tunisia	8	0	0.6 ± 0.2	0.5 ± 0.01	0.09 ± 0.01	1.1 ± 1.7	0	50	42	8
K4864	Egypt	4	8.2 ± 2.3	2.9 ± 0.8	3.3 ± 2.5	0.07 ± 0.04	14.5 ± 6.1	56	20	23.5	0.5
K2027	Iran	4	9.6 ± 1.2	5.3 ± 1.4	4.2 ± 0.9	0.18 ± 0.02	19.2 ± 3.3	50	27	22.1	0.9
K9832	Uzbekistan	4	8.5 ± 0.5	3.6 ± 0.2	1.8 ± 0.4	0.05 ± 0.01	13.9 ± 0.2	61	26	12.6	0.4
CGN6849	Pakistan	5	1.8 ± 0.2	0.7 ± 0.1	12.9 ± 2.8	0.04 ± 0.01	15.4 ± 2.8	12	4	83.7	0.3
CGN6852	Pakistan	5	12.7 ± 0.8	4.5 ± 0.5	3.6 ± 0.9	0.04 ± 0.01	20.9 ± 2.1	61	21	17.8	0.2
K7908	China	4	17.5 ± 1.7	5.2 ± 0.6	1.6 ± 0.4	0.12 ± 0.01	24.4 ± 2.0	72	21	6.5	0.5

[a] Glucosinolate contents expressed as mean ± SD μmol glucosinolate/(gram dry weight seedling tissue). Glucosinolate abbreviations are the same as for **Table 1**.

Table 3. Geographical Region, Sprout Age at Harvest, Individual and Total Glucosinolates Content, and Percentage of Each Glucosinolate in Relation to the Total Glucosinolates in *Eruca sativa* L. of Unknown Origins and in Three *Diplotaxis tenuifolia* L. Accessions

accession number	country	seedling age (days)	glucosinolate[a] (μmol glucosinolate/g DW)					glucosinolate (% of total)			
			4-MTB	4-MSB	4-MER	3-IM	total[b]	4-MTB	4-MSB	4-MER	3-IM
					E. sativa L.						
8/89	unknown	4	2.4 ± 0.5	2.5 ± 0.8	0.8 ± 0.2	0.2 ± 0.01	5.9 ± 1.5	41	42	13.6	3.4
K1458	unknown	4	3.4 ± 0.2	2.3 ± 0.3	16.5 ± 1.4	0.04 ± 0.01	22.3 ± 1.9	15	10	74.8	0.2
K8915	unknown	4	5.4 ± 0.3	3.6 ± 0.6	1.5 ± 0.3	0.11 ± 0.04	10.7 ± 1.2	51	34	14	1
K9399	unknown	4	8.2 ± 0.8	2.0 ± 1.7	10.3 ± 2.1	0.08 ± 0.01	20.6 ± 4.4	40	10	49.6	0.4
					D. tenuifolia L.						
DIPLO 1	unknown	7	0.7 ± 0.1	1.1 ± 0.2	22.8 ± 18.3	0	24.0 ± 18.6	0.3	4.6	95.1	0
D4866	Belgium	7	0.6 ± 0.1	1.1 ± 0.1	11.7 ± 1.0	0.05 ± 0.01	13.6 ± 1.3	5	8	86.6	0.4
D6552	Italy	7	1.2 ± 0.11	0.8 ± 0.03	36.6 ± 2.8	0.15 ± 0.06	38.7 ± 2.9	3	2	94.6	0.4

[a] Glucosinolate content expressed as mean ± SD μmol glucosinolate/(gram dry weight seedling tissue). Glucosinolate abbreviations are the same as for **Table 1**. [b] Low levels of 4-hydroxy-3-indolylmethylglucosinolate (4-hydroxyglucobrassicin) were also found in D6552.

0.7%) were low for all of the Italian accessions (**Table 1**). The two accessions from Northern Italy had the highest total glucosinolate, more than 4000 μmol/g DW. Other characteristics identified were that, with two exceptions, the *Eruca* from Puglia in Southern Italy consistently had high total glucosinolate contents (**Table 1**).

Eruca accessions from Central and Eastern Europe had much higher percentage contents of glucoerucin (40−60%, mean =

49%), and lower glucosativin contents (22−39%, mean = 29%), as compared to the Italian accessions (**Table 2**). The percentage glucoraphanin contents were higher in European accessions (10−35%, mean = 23%) as compared to the Italian accessions. The one *Eruca* accession from Spain, although having relatively low total glucosinolate content, had percentages of individual glucosinolates similar to those found in the Italian accessions.

With one exception, that of the Tunisian *Eruca* accession, all of the North African *Eruca* had high total glucosinolate contents and high percentages of glucoerucin (54−66%, mean = 58%) (**Table 2**). The percentage contents of glucoraphanin in the North African accessions (19−25%, mean = 21%) were similar to European accessions, but the North African accessions had low glucosativin contents (13−26%, mean = 20%). Although the Tunisian accession had a low total glucosinolate content, the ratio of glucosinolates was unusual as compared to all other *Eruca* accessions; it had no detectable glucoerucin but much higher percentages of glucoraphanin (50%) and glucosativin (42%). The one accession from Iran had total glucosinolate content and percentages of individual glucosinolates comparable to those of the accessions from Libya and Egypt.

Eruca accessions from Uzbekistan and Pakistan had high total glucosinolate contents (**Table 2**). With one exception, the accessions from Uzbekistan and from Pakistan had high percentages of glucoerucin (61%), average contents of glucoraphanin (21 and 26%, respectively), and low percentage contents of glucosativin (13 and 18%, respectively). The single accession from China had high total glucosinolate content; a high percentage of this was glucoerucin, and the overall glucosinolate profile was similar to the Uzbekistan and Pakistan accessions, suggestive of an imported origin of the Chinese *Eruca*.

In addition, there were four *E. sativa* accessions stored at IPK of unknown origin (**Table 3**). By comparison with the known origin samples, it is possible that accession K1458 is of Italian origin due to its very high glucosativin contents (75%) as compared to the other glucosinolates in this sample. Accessions 8/89 and K8915 have high percentages of glucoerucin (41% and 51%, respectively) and low percentages of glucosativin (14% for both), and thus could be of European origin. Accession K9399 has a profile (glucoerucin = 40%, glucoraphanin = 10%, glucosativin = 50%) similar to the Yugoslavian accession K6121 (glucoerucin = 40%, glucoraphanin = 21%, glucosativin = 39%).

The glucobrassicin content in the majority of *E. sativa* accessions was low (0−2.8% of total; Italian mean = 0.7%, European and Spanish = 0.5%, North African [not including Tunisia] = 0.6%, Uzbekistan and Pakistan = 0.3%, and China 0.5%). The exception was the Tunisian sample, which, in addition to the unusual aliphatic glucosinolate profile, had a high glucobrassicin percentage content (8%).

The three accessions of *D. tenuifolia* that were analyzed all had consistently high total glucosinolate contents and a very high glucosativin percentage (87−95%, mean = 92%) that was higher than for all of the *E. sativa* accessions (**Table 3**).

Total glucosinolate data for all of the accessions, expressed as mean ± SD μmol glucosinolates/(g fresh weight), are shown in **Figure 3A−C**. The data are presented in the same order as the tables for easy comparison. The concentrations of glucoerucin in some of the *Eruca* accessions are comparable to those previously found for broccoli sprouts (*35*). However, in the case of some accessions of *Eruca* and *Diplotaxis*, the sprouts also contain high levels of other glucosinolates that are precursors

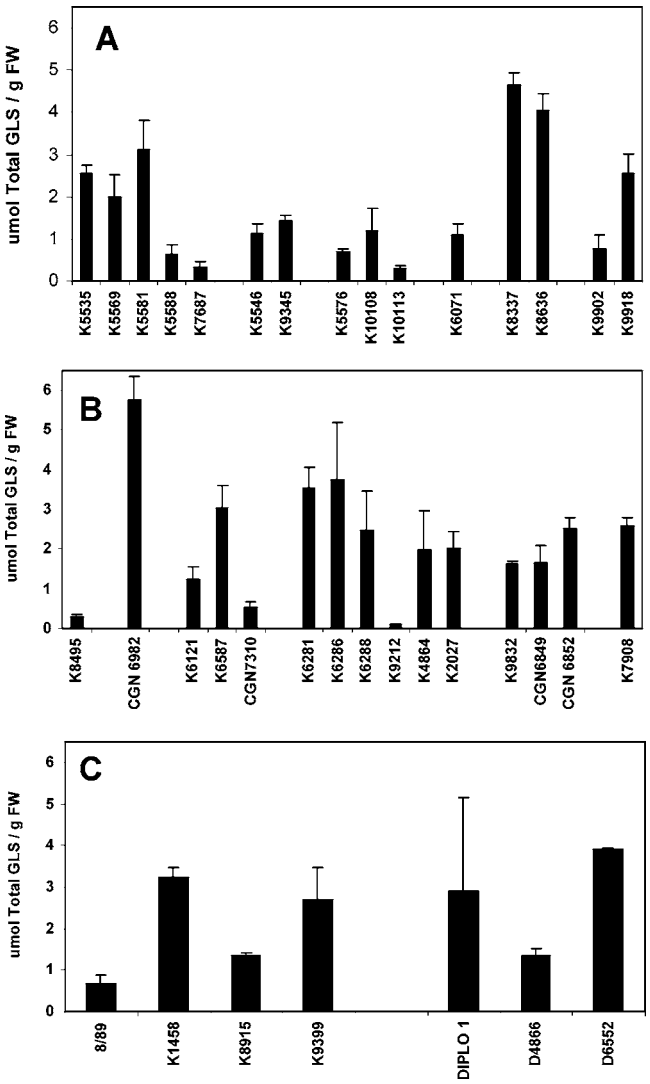

Figure 3. Bar charts showing the total glucosinolate contents of the *Eruca sativa* L. and *Diplotaxis tenuifolia* L. accessions expressed as mean ± SD μmol glucosinolates/g fresh weight of seedling tissues. (**A**) Italian accessions; (**B**) Eurasian, North African, and Middle Eastern accessions; and (**C**) unknown origin *Eruca sativa* L. accessions, and *Diplotaxis tenuifolia* L. accessions.

of isothiocyanates that could also be significantly biologically active, that is, erucin and sativin.

Geographical origin clearly has a significant effect on the profiles and concentrations of glucosinolates in rocket species. This variation can subsequently have an effect on the beneficial effects gained from consumption of these species, due to the variation in the profiles and concentrations of the glucosinolate-derived isothiocyanates. There are considerable data on the ADME, biological activities, and effects of sulforaphane (4-methylsulfinylbutyl-isothiocyanate) in both *in vitro* cancer cell models and *in vivo* with mouse, rat, and human intervention studies (*18*, *19*, *26*−*31*, *36*−*42*). Although there have been some data from a rat study suggesting that low dietary doses of Brassicaceae have no beneficial effects and there is no phytochemical synergism, the data from long-term epidemiological studies in humans and recent human intervention studies suggest the opposite, especially for gastrointestinal and hormone-dependent cancers (breast and prostate) (*18*, *19*, *26*−*31*, *43*). There are much less data on erucin (4-methylthiobutylisothiocyanate), but the information available shows that erucin is both bioavailable in humans and a good inducer of beneficial phase

II detoxification enzymes and of apoptosis in cancer cells (*31*, *36*, *37*, *44*−*47*). There are currently no data available on the *in vitro* or *in vivo* effects of sativin (4-mercaptobutylisothiocyanate). It is clear that in *Brassica*, *Eruca*, and *Diplotaxis* sprouts the high aliphatic glucosinolates contents can lead to a high release of the corresponding isothiocyanates when these foods are consumed. There have been very few studies on the synergistic effects of isothiocyanates and especially in dietary matrices, that is, as foods. In the case of rocket species, based on the limited structure activity data, it is obvious that a combination of sulforaphane, erucin, and the novel bifunctional sativin could have a greater health effect than that found with broccoli seedlings. Clearly, both *in vitro* and *in vivo* studies need to be performed with rocket seedlings (*Eruca* and *Diplotaxis* species), especially because these species are used as human foods with increasing popularity.

Analysis of genetic variation effects on phytochemical profiles can be difficult due to the availability of seed and the amount of seed that can be provided for any one project. Gene banks are vital sources of seed material, but can only provide a limited amount of seed in most cases. Thus, in the data presented, certain accessions had glucosinolate values with large SD. This is due to variation between the replicates and is indicative of greater potential genetic variation in these samples than found in other accessions where the SD values were low. All of the *Eruca* and *Diplotaxis* accessions analyzed are either wild or partially cultivated species; that is, in their region of origin they may have been grown for flavor qualities, and thus some genetic selection has occurred on the basis of this desired property. It is clear from these analyses that further research with more accessions from the same countries and also from countries not evaluated would generate valuable data for the selection of species with different glucosinolate profiles and concentrations that could then be exploited for breeding programs and optimizing health-beneficial properties. In addition, more seeds need to be produced for those accessions with different glucosinolate profiles so they can be evaluated more carefully for genetic variation. In addition, these differences in sprout profiles, with the different ratios of glucoerucin:glucoraphanin: glucosativin, could be used in human intervention studies to evaluate synergistic effects of isothiocyanates in different ratios with essentially the same genetic background and the same food matrices.

Supporting Information Available: Details of accession origins. This material is available free of charge via the Internet at http://pubs.acs.org.

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