SUPPLEMENTARY MATERIAL

LC-MS profiling of glucosinolates in the seeds of *Brassica elongata* Ehrh., and of the two stenoendemic *B. botteri* Vis and *B. cazzae* Ginzb. & Teyber

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Abstract

The glucosinolates (GLs) present in seed extracts of *Brassica elongata* Ehrh., *B. botteri* Vis and *B. cazzae* Ginzb. & Teyber from Croatia were identified by LC-MS. 4-Hydroxyindol-3-ylmethyl GL (**3**) was the major GL in the seeds of *B. elongata*, along with the four minor GLs 2-(*R*)-hydroxy-3-butenyl- (**1**), 3-butenyl- (**2**), 4-pentenyl- (**4**), and indol-3-ylmethyl (**5**) GLs. The seeds of *B. botteri* (Vis island) and *B. cazzae* (Sušac island) contained **2** as the major GL as well as **1**, **3**, **5** and 4-methoxyindol-3-ylmethyl GL (**6**). However, the GLs in *B. botteri* (Palagruža island) differed from other varieties having 2-propenyl GL (**7**) as the major GL in the seeds, and the four minor GLs **2**, **3**, **5**, and **6**. This first report of the GL content in the seeds of *B. elongata*, *B. botteri*, and *B. cazzae* indicates that the unique GL profiles could be specific to the geographical origin of the plant.

Keywords: *Brassica elongata*; *Brassica incana*; *Brassica botteri*; *Brassica cazzae*; glucosinolates; LC-MS

Experimental

General Experimental Procedures

All solvents were ACS grade and used as such. Formic acid was purchased from BDH (Toronto, ON, Canada). HPLC-grade MeOH and Et₃N (reagent grade) were purchased from Fisher Scientific (Whitby, ON, Canada). HPLC-grade H₂O was generated in the laboratory through a Nanopure Diamond Ultrapure water system provided by Barnstead (Dubuque, IA, USA). 2-(*R*)-hydroxy-3-butenyl GL and 3-butenyl GL were purchased from ChromaDex[®] Corporate (Irvine, CA, USA). 2-Propenyl GL was purchased from Fluka Sigma-Aldrich Canada (Oakville, ON, Canada). The intact 4-pentenyl-, indol-3-ylmethyl, and 4-methoxyindol-3-ylmethyl GLs were identified by comparison of the retention time, UV and mass spectra of authenticated standards (Blažević et al. 2013; Montaut et al. 2010).

Plant Material

Brassica elongata Ehrh. seeds were collected in July 2014, near Sinj, Croatia (315 meters above sea level (a.s.l.); Gauss-Kruger coordinates (GK): $X = 5633757$, $Y = 4842246$; voucher specimens number (v.s.n.) DBBE001). *B. botteri* Vis seeds were collected in July-August 2014, on Vis island (346 meters a.s.l.; GK: $X = 5591104$, $Y = 4767578$; v.s.n. DBBB001), and Palagruža island (31 meters a.s.l.; GK: $X = 5603924$, $Y = 4695048$; v.s.n. DBBB002), and *B. cazzae* on Sušac island (15 meters a.s.l.; GK: $X = 5622381$, $Y = 4735344$; v.s.n. DBBC001), open sea islands of central Adriatic Sea (Croatia). The plants were collected and identified by Dr. Mirko Rušćić at the Department of Biology, University of Split, Split, Croatia using Flora Europea (Tutin et al. 1968-1980). Voucher specimens are kept at the Department of Biology, University of Split, Split, Croatia.

LC-MS analysis of glucosinolates

Dried seeds (~ 500 mg) were frozen in liquid N₂, ground in a mortar, and immediately extracted for 5 min at 80 °C twice with EtOH/H₂O (5 mL, 7:3 v/v). The solution was filtered and concentrated to dryness. The extracts were dissolved in EtOH/H₂O (2 mL for *B. elongata* and 3 mL for *B. botteri* and *B. cazzae*, 7:3 v/v). LC-MS analysis was performed by injecting a 10 µL aliquot of the solution of crude extract into an Agilent Technologies HP 1100 (New Castle, DE, USA) high-performance liquid chromatograph equipped with a quaternary pump, automatic injector, diode-array detector (wavelength range 190-600 nm), degasser, and a Hypersil ODS column (5 µm, 4.6 × 200 mm). The two mobile phase solvents, MeOH and H₂O, were prepared with 0.15% Et₃N and 0.18% HCO₂H, added as ion-pairing reagents. Both solutions were filtered using 0.45 µm nylon membranes. The initial mobile phase was 100% HPLC-grade H₂O. At 10 min, the mobile phase was switched to a linear gradient of 100% H₂O to 100% MeOH over 60 min. After each run, the initial mobile phase conditions were set and the system was allowed to equilibrate. The flow rate was kept constant at 1 mL/min. The column temperature was held at room temperature (Zrybko et al. 1997). The HPLC was interfaced to an Agilent model 6120 mass spectrometer (Toronto, ON, Canada) with a Chemstation data system LC-MSD B.03.01. The electrospray interface was a standard ES source operating with a capillary voltage of 4 kV and temperature of 350 °C. The system was operated in the negative and positive ion electrospray modes. Nitrogen was used as nebulizing and drying gas at a flow rate of 10 L/min (35 psig). The mass spectrometer was programmed to perform full scans between *m/z* 100 and 1,000 amu.

Fig. S1. HPLC chromatogram of *B. elongata* seed extract. **1:** 2-(*R*)-hydroxy-3-butenyl GL, **2:** 3-butenyl GL, **3:** 4-hydroxyindol-3-ylmethyl GL, **4:** 4-pentenyl GL, **5:** indol-3-ylmethyl GL.

Fig. S2. HPLC chromatograms of stenoendemic *Brassica* seed extracts: **a)** *B. botteri* from Vis island (Croatia), **b)** *B. botteri* from Palagruža island (Croatia), and **c)** *B. cazzae* from Sušac island (Croatia). **1:** 2-(*R*)-hydroxy-3-butenyl GL, **2:** 3-butenyl GL, **3:** 4-hydroxyindol-3-ylmethyl GL, **5:** indol-3-ylmethyl GL, **6:** 4-methoxyindol-3-ylmethyl GL, **7:** 2-propenyl GL.

2-(R)-hydroxy-3-butenyl GL (1)
 $C_{11}H_{18}NO_{10}S_2$

3-butenyl GL (2)
 $C_{11}H_{18}NO_9S_2$

4-hydroxyindol-3-ylmethyl GL (3)
 $C_{18}H_{19}N_2O_{10}S_2$

4-pentenyl GL (4)
 $C_{12}H_{20}NO_9S_2$

indol-3-ylmethyl GL (5)
 $C_{18}H_{19}N_2O_9S_2$

Fig. S3. ESI⁺ mass spectra of 2-(*R*)-hydroxy-3-butenyl GL (1), 3-butenyl GL (2), 4-hydroxyindol-3-ylmethyl GL (3), 4-pentenyl GL (4), indol-3-ylmethyl GL (5), 4-methoxyindol-3-ylmethyl GL (6), and 2-propenyl GL (7).

Table S1. ESI-LC/MS data of the chromatographic peaks depicted in Fig. S1, Fig. S2, and Fig. S3

Peaks no.	t_R (min)	$[M]^-$	Formula	Glucosinolate
1	7.6	388.0	$C_{11}H_{18}NO_{10}S_2$	2-(<i>R</i>)-hydroxy-3-butenyl GL
2	18.3	372.0	$C_{11}H_{18}NO_9S_2$	3-butenyl GL
3	20.9	463.0	$C_{16}H_{19}N_2O_{10}S_2$	4-hydroxyindol-3-ylmethyl GL
4	22.9	386.0	$C_{12}H_{20}NO_9S_2$	4-pentenyl GL
5	25.3	446.8	$C_{16}H_{19}N_2O_9S_2$	indol-3-ylmethyl GL
6	29.2	476.8	$C_{17}H_{21}N_2O_{10}S_2$	4-methoxyindol-3-ylmethyl GL
7	8.2	358.0	$C_{10}H_{16}NO_9S_2$	2-propenyl GL

Table S2. Distribution of glucosinolates in seeds of *Brassica botteri* Vis, *B. cazzea* Ginzb. & Teyber, and *B. elongata* Ehrh.

Plant	Glucosinolates* (%)						
	1	2	3	4	5	6	7
<i>B. botteri</i> (Vis island)	3.1	64.3	7.7	N.D.	4.8	20.1	N.D.
<i>B. botteri</i> (Palagruža island)	N.D.	27.0	25.1	N.D.	4.1	0.3	43.5
<i>B. cazzae</i> (Sušac island)	35.8	45.0	17.1	N.D.	1.3	0.8	N.D.
<i>B. elongata</i> (Sinj)	6.6	3.8	85	2.0	2.6	N.D.	N.D.

* Glucosinolates: **1** = 2-(*R*)-hydroxy-3-butenyl GL, **2** = 3-butenyl GL, **3** = 4-hydroxyindol-3-ylmethyl GL, **4** = 4-pentenyl GL, **5** = indol-3-ylmethyl GL, **6** = 4-methoxyindol-3-ylmethyl GL, **7** = 2-propenyl GL. N.D. = not detected.

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