Diversity Analysis of *Arabidopsis thaliana* (L.) Heynh Ecotypes for Glucosinolates in Shoots and Seeds

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Additional index words. *Brassica*, germplasm

Abstract. Glucosinolates (GS) are important secondary plant metabolites present in several plant species, including *Arabidopsis thaliana* (L.) Heynh. Although genotypic differences among a limited number of samples from a limited geographical range have been reported, there have been few studies exploring the variation from a wider genetic base. The objective of this study was to explore the genetic variation for GS in *A. thaliana* collected throughout the world. We screened 58 *A. thaliana* ecotypes collected from the geographic area of lat. 15° N to lat. 59° N and long. 137° E to long. 123° W. Elevation in these areas ranged from sea level to over 480 m. We believe that this study has covered a large geographical region and captured most of the available genetic variation in *A. thaliana* for GS. There was no geographical trend in *A. thaliana* shoot or seed tissue for GS concentration. Total shoot GS ranged from 1.1 to 52.8 $\mu\text{mol}\cdot\text{g}^{-1}$ dry weight (DW), averaging 9.3 $\mu\text{mol}\cdot\text{g}^{-1}$ DW among all ecotypes. Total seed GS ranged from 1.6 to 41.8 $\mu\text{mol}\cdot\text{g}^{-1}$ DW with an average of 16.8 $\mu\text{mol}\cdot\text{g}^{-1}$ DW among all ecotypes. Low and high GS-accumulating *A. thaliana* ecotypes identified in this study may provide a basis for further genetic analysis for GS metabolism. Information provided may also prove useful for improving concentrations of nutritionally beneficial GS in vegetable *Brassic*as.

Glucosinolates (GS) are important sulfur-containing secondary metabolites present in a number of plant species, including *Arabidopsis thaliana* (L.) Heynh (Halkier and Du, 1997). More than 100 types of GS have been reported in plant species with 23 different types present in *A. thaliana*. The family Brassicaceae, of which *A. thaliana* is a member, represents a diverse group of plant species commercially important in many parts of the world. The plants produce condiment mustard; leafy, stored, processed, and pickled vegetables; seed oils for margarine, salad oils, cooking oils, and industrial uses; animal fodders; and green manure crops (Williams and Hill, 1986). Isothiocyanate compounds produced in these plants through hydrolysis of GS by the enzyme myrosinase convey their unique flavor and volatile attributes, and they can also act as antioxidants and anticancer agents when consumed in the diet. The presence of GS in *A. thaliana* offers the opportunity to study the biochemical pathways of these compounds in a widely used model plant system. Information gained from *A. thaliana* may shed new

light on impact of GS and their hydrolysis products in chronic disease prevention.

Glucosinolates demonstrate anticancer properties through the production of detoxification enzymes formed during hydrolysis such as quinine reductase, glutathione-S-transferase, and glucuronosyl transferase (Fahey et al., 1997; Holst and Williamson, 2004). Sulforaphane, an important GS derivative, possesses the most potent anticancer properties (Zhang et al., 1992). Sulforaphane is derived from 4-methylsulfinylbutyl GS, found in abundance in broccoli (*Brassica oleracea* var. *Italica*) heads, and functions through disruption of mitotic cell division processes, hence suppressing tumor growth. Realizing the potential use of GS in cancer treatment, several studies have evaluated broccoli accession and found significant differences for aliphatic GS (possessing specific anticancer properties) indicating that there is a potential to improve broccoli for desirable GS (Brown et al., 2002; Jeffery et al., 2003). Such genetic variation has also been reported in *A. thaliana* (Kliebenstein et al., 2001) as well as other vegetable *Brassic*as (Castro et al., 2004; Schonhof et al., 2004). However, in all cases, the genetic base and geographical range of collection of material has been narrow. Therefore, our research objectives were to acquire *A. thaliana* germplasm from as wide a geographic region as possible and then screen both leaf and seed tissues for GS concentrations.

Materials and Methods

Plant materials. We acquired *A. thaliana* seeds of 58 different ecotypes from the

Arabidopsis Resource Center at The Ohio State University (Columbus, OH). The center maintains collections from 25 countries, detailed information on ecotypes, country of origin, altitude, and latitude and longitude of the original sample collection site (Table 1). Seed from all ecotypes were planted in superfine germinating mix (BM; Berger, Saint-Modeste, Quebec, Canada) in 12.5-cm diameter plastic pots arranged in a completely randomized design with three replications and grown in a controlled environment (Environmental Growth Chambers, Chagrin Falls, OH) at a constant air temperature of 20 °C under a 16-h photoperiod. Photosynthetically active radiation at the canopy height was 400 to 425 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (LI-188B; LI-COR, Inc., Lincoln, NE). After 2 weeks, seedlings were thinned to three plants per pot and fertigated with quarter-strength Arabidopsis nutrient solution (Arteca and Arteca, 2000) on a weekly basis. Shoots were harvested just before anthesis and triple-rinsed with deionized water to remove all possible soil and other materials, frozen in liquid nitrogen, and stored at –80 °C until high-performance liquid chromatography (HPLC) analysis.

For seed GS evaluation, the same 58 *A. thaliana* ecotypes were germinated and grown as previously described. To bulk seed samples for analysis, plants were randomly crossed within each ecotype and allowed to set seeds. Seeds were harvested along with whole plants, placed in a paper bag, and air-dried at room temperature for 7 d before they were cleaned for GS extraction.

Sample preparation. Both shoot and seed samples were freeze-dried at –40 °C for 48 h to minimize possible myrosinase hydrolysis during grinding. An aluminum bead was added to samples to facilitate grinding by vortexing in the sample tubes at 3000 rpm for 30 s conducted three consecutive times.

Glucosinolate high-performance liquid chromatography analysis. Glucosinolate extraction and analysis were performed as described by Charron et al. (2001). Briefly, 0.2 g of freeze-dried tissue was combined with 1 mL benzyl glucosinolate solution (1 mM) as an internal standard, 2.0 mL MeOH, and 0.1 mL barium—lead acetate (0.6 M) and centrifuged at 2000 g_n for 10 min. A 0.5-mL aliquot of supernatant was then added to a 1-mL column containing 0.3 mL DEAE Sephadex A-25 (Sigma-Aldrich, St. Louis, MO). The sample was desulfated by the procedure of Raney and McGregor (1990). Desulfoglucosinolates was eluted with 900 μL of water and collected into 2-mL vials. Extracted desulfoglucosinolates were separated with an Agilent 1100 HPLC unit (Agilent Technologies, Palo Alto, CA) with a photodiode array detector using a reverse-phase 250 mm $\times$ 4.6 mm i.d., 5- μm Luna C₁₈ column (Phenomenex, Inc., Torrance, CA) at a wavelength of 230 nm. The column temperature was set at 40 °C with a flow rate of 1 mL $\cdot\text{min}^{-1}$. The gradient elution parameters were 100% water for 1 min followed by a 15 min linear gradient to 75% water:25% acetonitrile. Solvent levels were then held constant

Received for publication 9 Feb. 2011. Accepted for publication 3 May 2011.

This research was funded through financial support of the Tennessee Agriculture Research Experiment.

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Table 1. List of *Arabidopsis thaliana* ecotypes accession, country of origin, altitude (m), and latitude (°), and longitude (°) of the site of original sample collection.

Ecotype accession	Location	Country	Altitude (minimum)	Altitude (maximum)	Latitude (minimum °)	Latitude (maximum °)	Longitude (minimum °)	Longitude (maximum °)
CS6603	Antwerpen	Belgium	0.3	30.5	N 51	N 52	E 4	E 5
CS1476	Point Grey, British Columbia	Canada	0.3	30.5	N 49	N 49	W 123	W 123
CS1492	Richmond, British Columbia	Canada	0.3	30.5	N 49	N 49	W 123	W 123
CS1096		Cape Verde	365.7	365.7	N 15	N 17	W 23	W 25
CS22591	Borky, Czech Republic (Brno area)	Czech Republic			N 49	N 49	E 15	E 15
CS1030	Bulhary	Czechoslovakia	51.8	51.8	N 48	N 48	E 19	E 19
CS22630	Argentat	France	30.5	61	N 45	N 45	E 1	E 2
CS935	Aua/Rhon	Germany	61	91.4	N 51	N 51	E 10	E 10
CS938	Achkarren/Freiburg	Germany	61	91.4	N 48	N 48	E 8	E 8
CS1264	Kashmir	India	481.6	481.6	N 34	N 34	E 74	E 74
CS22638	Kashmir	India	481.6	481.6	N 34	N 34	E 74	E 74
CS22656	Burren	Ireland			N 52	N 55	W 6	W 10
CS968	Bologna	Italy	30.5	61	N 44	N 45	E 11	E 12
CS969	Bologna	Italy	30.5	61	N 44	N 45	E 11	E 12
CS1640	Tsu	Japan	0.3	30.5	N 34	N 35	E 136	E 137
CS3963	Yamaoka-cho, Gifu Prefecture, Central of Honshu Island	Japan			N 38	N 38	E 136	E 137
CS3964	Kyoto city, western part of Honshu Island	Japan			N 38	N 38	E 136	E 137
CS22641	Tsu	Japan	0.3	30.5	N 34	N 35	E 136	E 137
CS22606	Karagandy	Kazakhstan			N 48	N 48	E 68	E 68
CS22642	Martuba/Cyrenaika	Libya	30.5	61	N 33	N 33	E 23	E 23
CS925		Lithuania			N 56	N 56	E 24	E 24
CS1286	Kaunas	Lithuania	0.3	30.5	N 54	N 55	E 23	E 24
CS22526	Ameland-Firehouse	The Netherlands			N 53.1	N 53.1	E 5.8	E 5.8
CS1436	Oystese	Norway	0.3	30.5	N 62	N 62	E 10	E 10
CS1336	Lipowiec/Chrzanow	Poland	152.4	152.4	N 50	N 50	E 19	E 19
CS1086	Coimbra	Portugal	30.5	61	N 40	N 41	W 8	W 9
CS22672	Orsova, Caras Severin County	Romania	16.7	16.7	N 44	N 44	E 22	E 22
CS1073	Chisdra	Russia	30.5	61	N 56	N 56	E 38	E 38
CS1076	Chisdra	Russia	30.5	61	N 54	N 54	E 35	E 35
CS1148	Estland	Russia	30.5	61	N 58	N 59	E 23	E 28
CS1594	Wilna/Litauen	Russia	30.5	61	N 55	N 55	E 25	E 25
CS970	Blanes/Gerona	Spain	0.3	30.5	N 41	N 42	E 3	E 3
CS1064	Canary Islands	Spain	384	384	N 28	N 28	W 15	W 15
CS1656	Alcala de Henares/Madrid	Spain			N 31	N 40	W 13	W 19
CS6622	Blanes/Gerona	Spain	0.3	30.5	N 41	N 42	E 3	E 3
CS22579	Billaberget, N Sweden (Harnosand area)	Sweden			N 62	N 62	E 15	E 15
CS22582	Spratteboda, S Sweden (Skane)	Sweden			N 62	N 62	E 15	E 15
CS997	Basel	Switzerland	91.4	400	N 47	N 48	E 7	E 8
CS6175		Tadjikistan			N 39	N 39	E 71	E 71
CS932	Kingswells, Aberdeen	United Kingdom			N 57	N 57	W 2	W 2
CS1062	Calver	United Kingdom			N 54	N 54	W 2	W 2
CS22550	Alston	United Kingdom			N 54.8	N 54.8	W 2.4	W 2.4
CS1210	Greenville, MI	United States			N 43	N 43	W 85	W 85
CS1622	Yosemite Nat. Park	United States	426.7	457.2	N 37	N 37	W 119	W 119
CS3885	Tacoma, WA	United States			N 38	N 38	W 97	W 97
CS6673	Columbia	United States	0.3	30.5	N 38	N 39	W 92	W 93
CS8068	Berkeley, CA	United States			N 37	N 37	W 122	W 122
CS8069	Santa Clara County, CA	United States			N 37	N 37	W 121	W 121
CS8070	Friedensville, PA	United States			N 40	N 40	W 74	W 74
CS8141	Hartford, CT	United States			N 41	N 41	W 72	W 72
CS22341	Seattle, WA	United States	0.3	45.7	N 47	N 47	W 122	W 122
CS22346	Seattle, WA	United States	0.3	45.7	N 47	N 47	W 122	W 122
CS22356	Harvard Square, Cambridge, MA	United States			N 42	N 42	W 71	W 71
CS22385	Ithaca, NY	United States			N 42	N 42	W 76	W 76
CS22401	Knox, IN	United States			N 38	N 38	W 87	W 87
CS22419	Cold Spring Harbor Laboratory, Long Island, NY	United States			N 40	N 40	W 73	W 73
CS22420	Cold Spring Harbor Laboratory, Long Island, NY	United States			N 40	N 40	W 73	W 73
CS22654	Kindalville, MI	United States			N 41	N 41	W 85	W 85

for 5 min and returned to 100% water for the final 5 min. Desulfoglucosinolates were identified by comparison with authentic standards and previously reported results

(Charron et al., 2001). Desulfonated forms of glucoiberin (3-methylsulfinylpropyl GS), glucobrassicin (indol-3-ylmethyl GS), gluconapin (3-butenyl GS), 4-methoxyglucobrassicin

(4-methoxyindol-3-ylmethyl GS), neoglucobrassicin, 1-methoxyindol-3-ylmethyl GS, progoitrin (2-hydroxybut-3-enyl GS), and sinigrin (2-propenyl GS) were provided by Sandro

Table 2. Average glucosinolate concentrations ($\mu\text{mol}\cdot\text{g}^{-1}$ dry weight) in shoots of *Arabidopsis thaliana* ecotypes.

Ecotype	Iberin	Progoitrin	Epiprogo	Glucoraph	Sinigrin	Sinalbin	Gluconapin	Hydroxy	Benzyl	Indolyl	4-Meth	Nasturtiin	1-Meth	Aliphatic	Aromatic	Indole	Total GS
Columbia	0.00	0.00	0.00	0.00	0.54	0.00	9.97	1.80	5.35	1.26	0.00	14.28	0.00	10.51	14.28	3.06	27.85
CS925	0.00	0.04	0.00	0.00	0.00	0.10	0.00	0.00	13.38	0.10	1.22	0.00	0.53	0.04	0.10	1.86	2.00
CS935	0.00	0.00	0.00	0.00	0.15	0.19	0.00	1.92	33.44	0.69	0.45	0.00	0.54	0.15	0.19	3.60	3.94
CS938	0.04	0.00	0.00	0.00	0.08	0.07	0.00	1.85	11.76	0.67	0.06	0.00	0.61	0.12	0.07	3.18	3.37
CS968	0.00	0.04	0.03	0.13	0.12	0.03	0.13	0.54	10.92	0.17	0.12	0.00	0.12	0.46	0.03	0.95	1.43
CS969	0.00	0.07	0.00	0.26	0.16	0.00	0.29	0.28	6.22	0.40	0.09	0.00	0.00	0.77	0.00	1.54	1.54
CS970	0.00	0.00	0.00	0.00	0.89	0.02	0.12	0.88	5.05	0.65	0.32	0.00	0.57	1.00	0.02	2.42	3.45
CS997	0.05	0.19	0.05	0.49	4.07	0.04	6.10	0.00	5.46	3.62	0.63	0.04	0.23	10.94	0.08	4.48	15.50
CS1030	1.21	0.00	0.06	0.02	0.04	0.02	0.04	0.04	5.10	1.43	0.51	0.00	0.06	1.37	0.02	2.05	3.43
CS1062	3.77	0.00	0.00	0.16	0.04	0.08	0.04	0.00	7.59	7.08	1.02	0.00	1.67	4.03	0.08	9.77	13.88
CS1064	0.04	0.00	0.05	0.00	5.16	0.04	0.03	0.30	6.86	2.04	1.81	0.00	0.96	5.28	0.04	5.12	10.43
CS1073	0.05	0.01	0.00	0.37	1.11	0.03	0.14	1.56	5.38	1.22	0.17	0.00	0.08	1.68	0.03	3.03	4.74
CS1076	0.06	0.00	0.00	0.00	0.02	0.00	0.00	1.96	5.57	0.44	0.06	0.00	0.03	0.08	0.00	2.50	2.58
CS1086	0.09	0.09	0.00	0.02	0.01	0.03	0.05	2.08	6.48	1.28	0.23	0.00	0.09	0.25	0.03	3.67	3.96
CS1096	0.15	0.00	0.07	0.00	0.04	0.01	0.01	1.53	5.43	3.23	0.24	0.00	0.09	0.27	0.01	5.09	5.37
CS1148	0.05	0.00	0.00	0.00	21.52	0.00	0.30	8.06	23.78	9.25	2.05	0.00	0.20	21.86	0.00	19.56	41.42
CS1210	0.12	0.00	0.00	0.11	0.07	0.00	0.00	6.37	11.15	8.71	1.07	0.00	1.31	0.30	0.00	17.47	17.77
CS1264	0.07	0.00	0.00	0.00	1.74	0.00	0.00	8.05	17.54	0.63	0.43	0.00	0.13	1.82	0.00	9.23	11.05
CS1286	0.15	0.00	0.13	0.00	0.11	0.00	0.00	0.47	10.19	7.35	2.07	0.00	1.54	0.39	0.00	11.43	11.82
CS1336	0.05	0.00	0.00	0.00	9.10	0.00	0.16	3.95	11.51	2.58	0.54	0.00	0.21	9.30	0.00	7.29	16.59
CS1436	0.00	0.00	0.18	0.57	0.24	0.00	0.47	4.60	8.99	0.64	0.22	0.00	0.00	1.46	0.00	5.47	6.93
CS1476	0.00	0.00	0.00	0.05	0.00	0.00	0.00	2.40	8.92	0.18	0.13	0.00	0.00	0.05	0.00	2.70	2.75
CS1492	0.17	0.00	0.00	1.33	0.08	0.01	0.02	1.23	5.98	1.28	0.30	0.00	0.15	1.59	0.01	2.96	4.56
CS1594	0.00	1.57	0.00	5.23	0.58	0.00	0.00	2.48	32.42	11.18	1.86	0.00	29.86	7.37	0.00	45.38	52.75
CS1622	0.43	0.00	2.97	0.00	0.08	0.00	0.03	4.67	6.52	0.88	0.32	0.00	0.08	3.50	0.00	5.95	9.45
CS1640	0.31	0.00	3.03	0.00	0.07	0.13	0.00	2.40	7.81	2.56	0.43	0.00	0.46	3.41	0.13	5.85	9.39
CS1656	0.24	0.38	0.00	0.00	0.07	0.02	0.27	1.89	9.47	5.48	0.38	0.00	0.12	0.96	0.02	7.87	8.85
CS3885	0.07	0.49	0.00	1.42	0.09	0.00	0.06	7.76	7.59	3.10	0.61	0.00	0.18	2.13	0.00	11.66	13.79
CS3963	0.05	0.00	0.43	1.18	0.32	0.04	0.03	2.01	8.49	1.87	2.05	0.00	0.24	2.02	0.04	6.17	8.23
CS3964	0.00	0.00	0.00	0.00	1.26	0.00	0.00	1.80	5.17	0.41	0.30	0.00	0.10	1.26	0.00	2.60	3.86
CS6175	0.00	0.00	0.00	0.00	0.25	0.00	0.00	1.48	5.52	0.20	0.16	0.00	0.15	0.25	0.00	1.98	2.23
CS6603	0.00	0.00	0.00	0.00	2.77	0.00	0.08	4.31	14.66	1.96	1.87	0.00	0.14	2.84	0.00	8.29	11.13
CS6622	0.00	0.38	0.00	1.05	0.34	0.00	0.00	4.75	41.15	2.29	1.28	0.00	0.30	1.96	0.00	8.63	10.59
CS6673	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.55	8.99	0.25	0.28	0.00	0.00	0.34	0.00	4.08	4.42
CS8068	0.00	0.00	0.00	0.00	0.29	0.00	0.00	4.11	12.16	0.41	0.39	0.00	0.24	0.29	0.00	5.15	5.44
CS8069	0.04	0.15	0.01	0.46	0.05	0.00	0.01	1.35	5.05	0.84	0.09	0.00	0.00	0.72	0.00	2.28	3.00
CS8070	0.00	0.00	0.00	0.00	1.18	0.00	0.00	1.64	10.49	1.58	2.50	0.00	0.33	1.18	0.00	6.06	7.25
CS8141	0.60	0.00	0.06	0.00	0.06	0.00	0.00	0.00	5.32	1.92	0.21	0.00	0.20	0.72	0.00	2.33	3.04
CS22341	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.29	14.27	0.47	0.00	0.00	1.00	0.00	0.00	12.76	12.76
CS22346	0.12	0.00	0.51	0.00	0.00	0.00	0.00	0.00	6.90	0.47	0.00	0.00	0.00	0.64	0.00	0.47	1.11
CS22356	0.00	0.00	0.00	0.00	2.20	0.00	0.07	0.00	12.89	1.93	1.02	0.00	1.42	2.26	0.00	4.36	6.62
CS22385	0.00	0.15	0.02	0.46	0.20	0.05	0.06	0.15	5.60	5.61	0.40	0.00	0.54	0.88	0.05	6.72	7.65
CS22401	0.00	0.00	0.00	0.00	5.80	0.00	9.17	0.00	7.09	2.81	0.46	0.00	0.15	14.97	0.00	3.42	18.39
CS22419	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.15	0.99	0.00	0.00	0.39	0.24	0.00	1.37	1.61
CS22420	0.00	0.36	0.00	0.90	0.43	0.00	0.58	0.57	35.67	1.95	1.04	0.00	0.25	2.28	0.00	3.82	6.09
CS22526	0.00	0.00	0.00	0.00	0.23	0.00	0.00	2.23	6.15	7.69	0.62	0.00	0.00	0.23	0.00	2.61	2.84
CS22550	0.47	0.00	0.16	0.00	0.09	0.00	0.00	0.00	19.81	0.81	0.15	0.00	1.36	0.71	0.00	9.67	10.38
CS22579	0.20	0.00	0.03	0.00	0.04	0.00	0.00	0.00	5.69	2.02	0.55	0.00	0.14	0.26	0.00	1.09	1.35
CS22581	2.20	0.00	0.00	0.00	0.00	1.40	0.00	3.65	8.70	0.81	0.15	0.00	0.14	0.26	0.00	6.35	9.95
CS22592	0.02	0.21	0.00	0.63	0.05	0.01	0.25	0.12	5.40	0.73	0.30	0.00	0.05	1.16	0.01	1.20	2.38
CS22606	0.00	0.00	0.00	0.00	0.00	0.63	0.82	7.89	10.19	4.44	2.25	0.00	0.31	0.82	0.63	14.88	16.33

(Continued on next page)

Table 2. (Continued) Average glucosinolate concentrations ($\mu\text{mol}\cdot\text{g}^{-1}$ dry weight) in shoots of *Arabidopsis thaliana* ecotypes.

Ecotype	Iberin	Progoitrin	Epiprogo	Gluoraph	Simigrin	Sinalbin	Gluconapin	Hdroxy	4-	3-	4-	Nasturtiin	1-Meth	Aliphatic	Aromatic	Indole	Total GS
CS22638	0.00	0.00	0.00	0.00	0.23	0.00	0.00	0.88	0.88	6.52	0.30	0.17	0.00	0.14	0.00	1.49	1.72
CS22641	0.00	0.00	0.00	0.00	1.63	2.00	0.00	20.59	20.59	6.08	3.04	1.84	0.00	1.63	2.00	25.47	29.10
CS22642	0.00	0.00	0.00	0.00	0.17	0.00	0.20	5.37	5.37	8.63	0.11	0.00	0.00	0.37	0.00	5.69	6.06
CS22654	0.00	0.00	0.00	0.00	0.13	0.00	0.12	9.38	9.38	6.95	0.08	0.06	0.16	0.25	0.00	9.69	9.94
CS22656	0.12	0.00	0.07	0.00	0.00	0.00	0.00	4.22	4.22	5.12	2.59	0.38	0.00	0.19	0.00	7.55	7.74
CS22672	0.00	0.00	0.17	0.00	0.78	1.74	0.00	4.94	4.94	6.90	0.31	0.18	1.09	0.95	1.74	6.51	9.20
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.05	0.08	0.00	0.00	0.00	0.00	0.47	1.11
Maximum	3.77	1.57	3.03	5.23	21.52	2.00	9.97	20.59	20.59	41.15	11.18	2.50	29.86	21.86	14.28	45.38	52.75
Mean	0.20	0.07	0.14	0.26	1.14	0.12	0.52	2.90	2.90	10.48	2.22	0.63	0.86	2.33	0.37	6.62	9.32
LSD(0.05)	1.67	0.65	1.58	2.15	9.06	1.10	5.43	10.24	10.24	22.67	7.30	1.95	11.12	11.32	5.43	20.21	26.97

GS = glucosinolates; LSD = least significant difference.

Palmieri of the Instituto Sperimentale Industriale (Bologna, Italy). Gluconasturtiin (2-phenylethyl GS) was purchased from LKT Laboratories Inc. (St. Paul, MN). Response factors used were from quantification based on the International Organization for Standardization (Method 9167-1).

Statistical analysis. Data were subjected to one-way analysis of variance and Pearson correlation analysis was performed with SAS software (Version 9.1; SAS Institute, Cary, NC).

Results and Discussion

We received ecotypes collected from a wide range of geographic locations, evaluated them under controlled conditions, and present findings that establish genetic variations for GS present in the *A. thaliana* germplasm. Although many studies have assessed the genetic diversity for GS in vegetable *Brassicaceae* (Brown et al., 2002; Jeffery et al., 2003; Kushad et al., 1999; Schonhof et al., 2004), data are currently lacking on GS variations among available *A. thaliana* ecotypes.

Glucosinolates in *A. thaliana* shoots. The *A. thaliana* ecotypes differed ($P < 0.05$) for individual and total GS levels measured in shoot tissues (Table 2). Average total GS concentration among the ecotypes was $9.32 \mu\text{mol}\cdot\text{g}^{-1}$ DW, ranging from 1.11 to $52.75 \mu\text{mol}\cdot\text{g}^{-1}$ DW (Table 2). The relatively high SD (9.52) for this range of observation may indicate a large variation among the ecotypes for total GS concentration. Despite this large variation, there were only four ecotypes with greater than $25 \mu\text{mol}\cdot\text{g}^{-1}$ DW for total shoot

GS in this study with the majority of ecotypes (39) having less than $10 \mu\text{mol}\cdot\text{g}^{-1}$ DW total shoot GS (Table 2). The ecotypes also varied significantly for shoot tissue aliphatic, aromatic, and indole GS. Although all ecotypes contained at least trace amounts of indole GS, some of those were devoid of aliphatic and aromatic GS in shoot tissue (Table 2). Indole GS contributed at least 11% in some of the ecotypes to as high as 100% to the total GS concentration. However, aromatic and aliphatic GS contributed to total GS as high as 38% and 81%, respectively. Data presented provide a basis for genetic variation for individual and total GS in shoot tissues among available *A. thaliana* ecotypes. Geographical mapping of ecotypes on the basis of total GS did not show any particular pattern of distribution at a global level (Fig. 1).

Glucosinolates in *A. thaliana* seeds. There were also differences among *A. thaliana* ecotypes for total GS in seeds. Average total seed GS concentration ($16.75 \mu\text{mol}\cdot\text{g}^{-1}$ DW) was higher than in shoots, but the range for seed GS (1.63 to $41.79 \mu\text{mol}\cdot\text{g}^{-1}$ DW) was narrower (Table 3). There were seven ecotypes with greater than $25 \mu\text{mol}\cdot\text{g}^{-1}$ DW of total seed GS (Table 3). This indicated that there are not many lines with a high concentration of seed GS. The majority of ecotypes had less than $20 \mu\text{mol}\cdot\text{g}^{-1}$ DW total seed GS. Similar to the shoot tissue, indole-GS was the major component of total GS, contributing as high as 99% in some of the ecotypes. Contributions by aliphatic and aromatic components to total seed GS were 39% and 8%, respectively. Eucic GS was found in seeds of at least 33 ecotypes of *A. thaliana*, but it was not detected in shoots

Fig. 1. Geographical distribution pattern of total glucosinolate concentration ($\mu\text{mol}\cdot\text{g}^{-1}$ dry weight) in *Arabidopsis thaliana* shoot tissues.

Table 3. Average glucosinolates concentration ($\mu\text{mol}\cdot\text{g}^{-1}$ dry weight) in seeds of *Arabidopsis thaliana* ecotypes.

Ecotype	Iberin	Progoitrin	Epiprogo	Glucoraph	Sinigrin	Sinalbin	Glucanapin	4-Hydroxy	3-Indolyl	4-Meth	Nasturtiin	1-Meth	Aliphatic	Aromatic	Indole	Total GS
Columbia	0.00	0.00	0.00	0.00	0.05	0.03	0.00	1.96	0.00	0.00	0.00	0.00	3.37	0.05	0.03	5.34
CS925	0.07	0.03	0.01	0.00	0.03	0.03	0.02	2.84	0.50	2.50	0.04	0.04	3.40	0.67	0.06	8.78
CS932	0.11	0.13	0.10	0.27	0.02	0.04	0.08	2.95	0.14	0.98	0.00	0.05	0.05	0.84	0.08	3.98
CS935	0.00	0.25	0.03	0.00	0.03	0.36	0.03	3.09	1.38	1.23	0.07	0.20	0.92	1.71	0.56	5.30
CS938	0.07	0.57	0.24	0.01	0.03	0.14	0.08	2.27	13.38	4.17	3.67	0.95	1.68	5.92	1.09	11.79
CS968	0.15	0.15	0.02	0.00	0.03	0.26	0.01	3.47	11.76	2.83	7.52	0.03	7.19	0.67	0.28	21.01
CS969	0.17	0.00	0.00	0.00	0.03	0.20	0.00	5.29	13.90	2.51	0.03	0.00	18.77	0.43	0.20	26.60
CS970	0.00	0.04	0.25	0.19	0.04	0.08	0.55	1.80	9.39	3.49	0.00	0.00	0.53	1.99	0.08	5.82
CS997	0.04	0.46	0.04	0.37	0.03	0.55	0.07	2.76	12.02	1.31	0.03	0.34	3.59	2.31	0.89	8.33
CS1030	0.25	0.26	0.44	1.12	0.72	0.33	0.38	0.00	22.77	7.07	0.38	0.11	4.45	3.84	0.44	11.90
CS1064	0.31	0.49	0.00	0.00	0.23	0.00	0.07	0.00	6.37	0.71	2.92	0.00	1.35	1.23	0.00	4.98
CS1086	0.11	0.52	0.12	0.38	0.05	0.03	0.43	2.79	12.16	3.13	2.85	0.50	3.89	3.55	0.53	12.67
CS1210	0.31	0.15	0.00	0.04	0.40	0.00	0.09	4.29	8.43	0.18	0.04	0.00	5.38	1.11	0.00	9.88
CS1264	0.09	0.10	0.05	0.17	0.11	0.00	0.00	5.07	6.56	0.00	0.04	0.00	2.10	0.52	0.00	7.34
CS1336	0.27	0.03	0.02	0.00	0.12	0.06	0.01	2.05	12.16	0.13	7.81	0.00	13.97	0.69	0.06	26.26
CS1436	0.41	0.03	0.00	0.00	0.09	0.04	0.00	10.74	12.74	3.01	0.11	0.06	9.15	1.08	0.10	23.01
CS1492	0.00	0.00	0.00	0.02	0.08	0.03	0.00	2.37	11.89	0.06	0.00	0.00	1.84	0.09	0.03	4.28
CS1622	0.59	0.40	0.21	0.11	2.38	0.00	0.39	7.39	12.89	6.18	0.12	0.11	0.70	4.37	0.11	14.39
CS1656	0.06	0.02	0.01	0.02	2.10	1.21	4.01	2.77	8.77	2.49	0.03	0.02	5.68	6.31	1.23	10.96
CS3885	0.13	0.41	0.41	0.78	0.07	0.24	0.12	1.18	13.54	4.07	0.06	0.47	3.74	3.16	0.71	9.05
CS3963	0.50	0.58	0.00	0.05	0.58	0.00	0.00	8.64	9.73	3.63	0.04	0.05	7.47	1.88	0.05	19.79
CS3964	0.11	0.07	0.05	0.00	0.11	0.03	0.02	0.90	11.76	3.55	5.63	0.03	26.95	0.81	0.05	37.04
CS6175	0.08	0.05	0.02	0.00	0.03	0.02	0.00	1.29	13.38	1.79	0.11	0.02	22.81	0.50	0.04	26.00
CS6622	0.47	0.24	0.31	0.21	0.02	0.06	1.69	7.11	7.43	1.61	0.00	0.06	0.84	5.33	0.12	9.56
CS6673	0.32	0.08	0.00	0.01	2.36	0.02	0.03	2.95	11.63	2.82	0.06	0.04	13.34	2.94	0.06	19.17
CS8068	0.09	0.66	0.18	0.01	0.03	0.02	0.08	1.53	13.72	3.51	0.44	0.59	8.67	4.62	0.61	14.15
CS8069	0.06	0.66	0.11	0.01	0.04	0.02	0.06	1.44	13.54	3.66	9.83	0.93	10.51	4.35	0.95	25.45
CS8070	0.00	0.60	0.16	0.00	0.03	0.02	0.06	0.82	13.21	3.34	13.17	0.97	7.18	3.89	0.99	24.51
CS8141	0.18	0.62	0.16	0.01	0.03	0.02	0.08	1.27	12.16	3.10	6.09	0.78	5.03	4.68	0.80	15.48
CS22341	0.22	0.09	0.06	0.24	0.00	0.04	0.19	0.27	6.01	0.83	0.00	0.06	2.54	1.09	0.10	3.63
CS22346	0.23	0.02	0.03	0.13	0.00	0.02	0.56	0.07	4.12	0.18	0.63	0.00	1.97	0.96	0.02	2.84
CS22385	0.29	0.06	0.00	0.00	0.26	0.00	0.12	3.19	7.93	2.49	0.00	0.00	8.44	0.85	0.00	14.12
CS22420	0.43	0.25	0.00	0.04	0.51	0.00	0.11	7.43	14.27	3.78	0.00	0.00	6.51	1.35	0.00	17.71
CS22550	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.21	0.00	0.00	0.00	1.12	0.52	0.00	1.12
CS22591	0.45	0.69	0.00	0.03	0.00	0.08	0.00	8.36	13.90	3.10	0.00	0.00	0.37	1.33	0.08	11.83
CS22630	1.48	1.56	0.11	1.63	0.07	0.18	1.36	21.79	22.29	5.76	0.00	0.33	6.41	7.31	0.51	33.97
CS22638	0.32	0.17	0.23	0.59	0.73	0.38	3.21	0.40	11.89	2.30	1.92	0.00	3.37	5.25	0.38	7.99
CS22654	0.19	0.09	0.00	0.03	0.20	0.02	0.04	1.46	14.86	3.84	9.62	0.00	18.07	0.67	0.02	32.99
CS22656	0.00	0.00	0.00	0.00	0.04	0.02	0.00	1.47	152.86	0.29	0.31	0.00	3.51	0.04	0.02	5.57
CS22672	0.36	0.28	0.05	0.89	0.07	0.03	1.03	7.88	8.70	2.47	0.79	0.14	0.56	3.16	0.18	11.70
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.12	0.00	0.00	0.00	0.05	0.04	0.00	1.12
Maximum	1.48	1.56	0.44	1.63	2.38	1.21	4.01	21.79	152.86	4.91	13.17	0.97	26.95	7.31	1.23	37.04
Mean	0.24	0.27	0.09	0.18	0.29	0.11	0.37	3.58	15.47	2.53	1.86	0.17	6.19	2.30	0.29	14.16
LSD(0.05)	0.74	0.88	0.34	0.99	1.70	0.62	2.38	11.40	64.10	4.81	9.57	0.82	17.97	5.63	1.02	26.38

GS = glucosinolates; LSD = least significant difference.

(Tables 2 and 3). The maximum concentration of eucin found in the seeds was $4.91 \mu\text{mol}\cdot\text{g}^{-1}$ DW. This indicates that some of the GS may possibly be synthesized at a later stage of plant development.

In the case of seed GS, there was no specific pattern of geographical distribution in terms of total GS (Fig. 2). It is possible that soil type, fertility status of the soil as well as climatic conditions of the location from where samples were collected may have an influence on the levels of GS in seeds of *A. thaliana*. Every attempt was made to minimize any possible environmental impacts in the current evaluation; however, the variation in seed GS observed in this study may not entirely be from genetic contributions.

Correlations among glucosinolates for shoot and seed tissues in *A. thaliana*. There were strong positive correlations for total shoot GS with the individual shoot GS of gluconapin, nasturtiin, aliphatic, total aromatic, and total indole-GS (Table 4). Similar trends in GS were observed with the seed tissues (Table 4). This has advantages and disadvantages in any improvement efforts of a plant species for a trait of interest, because it provides an opportunity to improve a trait independently. Although indole-GS had a positive correlation with total GS in seeds, aliphatic and aromatic GS were weakly correlated. Other strongly correlated GS with total GS in seeds were 3-indolyl and 4-meth, whereas others showed a mixed pattern (Table 4).

Contrary to the present findings, higher concentrations of GS are usually present in seed tissues of *A. thaliana* (Brown et al., 2003). Although the overall average total GS concentration was higher in *A. thaliana* seeds

in the present study, the highest concentration of total GS for any ecotype was found in the shoot tissue. It is possible that the conversion of one type of GS into another during changes in plant developmental stages differed among the *A. thaliana* ecotypes. Brown et al. (2003) reported concentrations of GS from inflorescence and silique tissues in *A. thaliana* plants to range from 23 to $30 \mu\text{mol}\cdot\text{g}^{-1}$ DW, and the average GS shoot tissue concentration among *A. thaliana* ecotypes in the current evaluation of $27.85 \mu\text{mol}\cdot\text{g}^{-1}$ DW is in agreement. This also indicates the precision of the analyses between two independent studies.

Brown et al. (2003) found that aliphatic GS contributed more than 80% of the total GS in *A. thaliana* leaf tissues. Contributions of this level were only in a few *A. thaliana* ecotypes in the present study with the majority of ecotypes showing less aliphatic GS percentages of total GS. Indole GS was the major contributor to total GS among the ecotypes in the present study. However, the declining trend of contribution of aliphatic GS to the total GS observed by Brown et al. (2003) is in agreement with the present study when we compare shoots and seeds. Brown et al. (2003) also reported additional GS in seeds that were not present in the shoots at vegetative growth stages for *A. thaliana*. Kliebenstein et al. (2001) identified a single locus (*GS-AOP*), which accounted for over 60% of the variation for shoot tissue aliphatic GS among 39 *A. thaliana* ecotypes. However, the authors found that the same locus exhibited no significant association with aliphatic GS in seed tissues. Such conclusions would indicate that accumulations of GS in shoot and seed

tissues may be under different genetic controls (Kliebenstein et al., 2001). The present study was useful to screen the ecotypes to identify the high and low genotypes, but it would be interesting to study the variation among the same ecotypes at distinct growth stages. Furthermore, identification of GS gene expression at each growth stage would be possible and could lead to the determination if the same gene(s) act at different stages or there are a different set of genes.

We found a much higher concentration of GS in both shoots and seeds in some of the ecotypes than reported earlier by Brown et al. (2003). The identification of higher GS concentrations among *A. thaliana* ecotypes may be because of wider geographic coverage of the ecotypes in the present study. What was interesting to note is that shoots with high GS concentrations were not the ecotypes that had the highest GS concentrations in the seed tissues, indicating that GS concentrations may vary in different plant parts in different ecotypes (Brown et al., 2003).

The influence of climatic conditions on GS concentrations has been reported (Charron et al., 2005; Schonhof et al., 2004) for several *Brassica* genotypes. Among *A. thaliana* ecotypes, variation in GS side-chain modifications was attributed to a limited number of loci having strong impacts on GS contents (Kliebenstein et al., 2001). Therefore, it is would be difficult to conclude how much of the variation at the phenotypic level reported here is the result of environmental and genetic variables.

We did not find any particular geographic distribution pattern among ecotypes for GS concentration. This may indicate that there was not a single center of origin for higher concentration ecotypes of *A. thaliana*. Through the process of evolution of the *A. thaliana* genome, genetic changes controlling GS biosynthesis may have taken place independently at various locations throughout the worldwide geographic distribution of ecotypes.

More than 100 types of GS have been identified in *A. thaliana*. We report only 19 GS to be present in the shoot tissues and 20 GS present in the seed tissues of the *A. thaliana* ecotypes evaluated in the present study. Evaluations were conducted at the seed stage and in shoots just before anthesis in the present study, indicating the number of GS may vary according to different growth stages. Furthermore, negative or no correlations between different GS from seeds and shoots indicated that GS metabolism may be independent from one growth stage to another. Correlation observations made in the present study were also in agreement with previous observations (Kliebenstein et al., 2001). Low and high GS-containing ecotypes identified in shoots and seeds from this study may provide a basis for further investigations aimed at identifying genes associated with GS biosynthesis. These ecotypes can also be used for other research such as microarray analysis and genetic transformation of *A. thaliana* to manipulating the GS levels.

Fig. 2. Geographical distribution pattern of total glucosinolate concentration ($\mu\text{mol}\cdot\text{g}^{-1}$ dry weight) in *Arabidopsis thaliana* seed tissues.

Table 4. Pearson's correlation coefficients among various glucosinolates in shoots and seeds of *Arabidopsis thaliana* ecotypes.²

	Shoot GS	Iberin	Progoitrin	Epiprogo	Glucoraph	Sinigrin	Sinalbin	Glucanapin	4-Hdroxy	Benzyl	3-Indolyl	4-Meth	Nasturtiin	1-Meth	Aliphatic	Aromatic	Indole	Total
Seed GS																		
Iberin																		
Progoitrin	0.08																	
Epiprogo	-0.02	0.35																
Glucoraph	0.10	0.10	0.35															
Sinigrin	0.33	-0.07	0.10	0.64														
Sinalbin	-0.25	-0.08	-0.13	0.05	0.03													
Glucanapin	0.07	-0.13	0.22	0.11	0.20	0.36												
4-Hdroxy	0.55	0.11	0.22	0.22	0.27	0.42	0.70											
Benzyl	-0.22	-0.15	-0.08	-0.20	-0.06	0.15	-0.10	-0.02										
3-Indolyl	0.19	0.43	0.57	0.08	0.35	0.34	0.10	0.01	0.16									
4-Meth	-0.24	0.30	0.04	0.04	-0.20	-0.19	-0.13	-0.13	-0.31	-0.06								
Nasturtiin	-0.34	0.73	0.42	0.42	0.03	-0.18	-0.03	-0.14	-0.22	-0.07	0.24							
1-Meth	-0.13	-0.22	-0.25	-0.25	-0.28	0.01	-0.09	-0.18	-0.11	-0.04	0.34	0.52						
Aliphatic	0.06	0.55	0.65	0.65	0.32	0.39	0.41	0.58	-0.02	-0.19	0.50	0.14	0.59	-0.24				
Aromatic	-0.43	0.54	0.41	0.41	0.15	0.07	0.59	0.32	-0.24	-0.09	0.33	0.34	0.79	-0.11	0.73			
Indole	0.02	0.07	-0.13	-0.13	-0.23	0.05	-0.13	-0.19	0.15	-0.12	0.47	0.59	0.15	0.87	-0.03	0.04	-0.09	
Total		0.20	0.02	0.02	-0.16	0.13	-0.02	-0.05	0.13	-0.16	0.58	0.62	0.30	0.79	0.21	0.23	0.97	0.62

²Correlation coefficients above the diagonal are correlation for glucosinolates from shoots, whereas below the diagonal correlations are from the seeds. GS = glucosinolates.

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