Plant Breeding **123**, 60—65 (2004)

ISSN 0179-9541

Genetic and environmental effects on glucosinolate content and chemoprotective potency of broccoli

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With 1 figure and 3 tables

Received February 28, 2003/Accepted July 11, 2003
Communicated by G. Wricke

Abstract

Broccoli is well recognized as a source of glucosinolates and their isothiocyanate breakdown products. Glucoraphanin is one of the most abundant glucosinolates present in broccoli and its cognate isothiocyanate is sulphoraphane, a potent inducer of mammalian detoxication (phase 2) enzyme activity and anti-cancer agent. This study was designed to measure: glucosinolate levels in broccoli florets from an array of genotypes grown in several environments; the elevation of a key phase 2 enzyme, quinone reductase, in mammalian cells exposed to floret extracts; and total broccoli head content. There were significant environmental and genotype-by-environment effects on levels of glucoraphanin and quinone reductase induction potential of broccoli heads; however, the effect of genotype was greater than that of environmental factors. The relative rankings among genotypes for glucoraphanin and quinone reductase induction potential changed, when expressed on a per head basis, rather than on a concentration basis. Correlations of trait means in one environment vs. means from a second were stronger for glucoraphanin and quinone reductase induction potential on a per head basis than on a fresh weight concentration basis. Results of this study indicate that development of a broccoli phenotype with a dense head and a high concentration of glucoraphanin to deliver maximum chemoprotective potential (high enzyme induction potential/glucoraphanin content) is a feasible goal.

Key words: *Brassica oleracea* — doubled haploids — glucoraphanin — isothiocyanates — Italica group — nutrition — sulphoraphane

Epidemiological evidence that relates broccoli vegetable consumption to a reduction in the risk of certain cancers in humans dates back 25 years. Such evidence has gained momentum and validity over the ensuing quarter-century, and it is particularly strong for cancers of the colon/rectum (Graham et al. 1978, Kohlmeier and Su 1997, Verhoeven et al. 1997) and prostate (Jain et al. 1999, Kolonel et al. 2000). This chemoprotective effect is thought to be due in large part to the glucosinolates present in these cruciferous vegetables (Beecher 1994, Zhang and Talalay 1994, Hecht 2000, Talalay and Fahey 2001).

Glucosinolates are β-thioglucoside *N*-hydroxysulphates with an aglycone (or R-group) that is an alkyl, alkenyl, thioalkyl, thioalkenyl, aryl, arylalkyl or indolyl moiety (reviewed by Rosa et al. 1997, Fahey et al. 2001). These compounds are hydrolysed by myrosinase to their cognate isothiocyanates when plant cells are damaged (e.g. chewed), or in the human gut as a result of the activity of its microflora (Shapiro et al. 1998, 2001). Certain isothiocyanates are known to inhibit tumour formation in mammals initiated by a variety of chemical carcinogens (Zhang and Talalay 1994). Sulphoraphane, the cognate isothiocyanate of glucoraphanin (4-methylsulphinylbutyl glucosinolate), has been the focus of numerous studies because it is a potent inducer of mammalian detoxication and antioxidant (phase 2) enzyme activity that protects against tumourigenesis in a rodent mammary tumour model (Zhang et al. 1994, Fahey et al. 1997). Robust evidence points to a pivotal role played by phase 2 enzymes in the detoxication of electrophiles and in the suppression of carcinogenesis and mutagenesis (Kensler 1997, Talalay and Fahey 2001).

Glucoraphanin is a relatively abundant aliphatic glucosinolate present in harvested florets of cultivated broccoli; however, other aliphatic glucosinolates, such as glucoiberin (3-methylsulphinylpropyl glucosinolate) and glucoerucin (4-methylthiobutyl glucosinolate), have also been identified in broccoli tissues. In general, glucoiberin, glucoerucin, and other aliphatic glucosinolates occur at levels $< 10\%$ of those of glucoraphanin (Carlson et al. 1987, Shelp et al. 1993, Kushad et al. 1999), and their cognate isothiocyanates typically account for $< 2\%$ of total glucosinolate-derived phase 2 induction potency of broccoli (Zhang et al. 1992, Prestera et al. 1993). Other prominent glucosinolates in broccoli florets are indole glucosinolates such as glucobrassicin (indole-3-ylmethyl glucosinolate), neoglucobrassicin (1-methoxyindole-3-ylmethyl glucosinolate), and 4-hydroxyglucobrassicin. Results of *in vitro* assays indicate that the primary hydrolysis products of these glucosinolates have very low phase 2 inducer potential which is estimated to be $< 5\%$ of the total for broccoli (Fahey et al. 1997, 1998).

Faulkner et al. (1998) have suggested that genetic factors which induce high levels of methylsulphinylalkyl glucosinolates in wild relatives of broccoli could be transferred to cultivated broccoli. This would require numerous generations of selection to improve the horticultural phenotype. Alternatively, the genetic diversity of glucoraphanin concentration extant in relatively elite broccoli germplasm could be more quickly captured by using the elite lines to breed new cultivars (Farnham et al. 2000).

Broccoli genotype has a significant effect on the glucosinolate profile in broccoli florets as well as on the plant levels of glucoraphanin (Giamoustaris and Mithen 1996, Faulkner et al. 1998, Kushad et al. 1999, Li et al. 2001). However, only a few studies (Farnham et al. 2000, Rosa and Rodrigues 2001, Brown et al. 2002) have examined and identified the interaction between genotype and environment on levels of glucoraphanin in broccoli tissues. Brown et al. (2002) estimated a moderately high broad sense heritability of 54% for this trait. Farnham et al. (2000) and Rosa and Rodrigues (2001) found high correlations between glucoraphanin expression in one environment and a second. Farnham et al. (2000) also showed that glucoraphanin concentration and phase 2 enzyme induction potential of genotypes were highly correlated.

In a previous study (Farnham et al. 2000), the focus was on genotypic differences for floret concentrations of glucoraphanin and phase 2 induction potential. In the current study, a subset of the previously examined elite broccoli genotypes grown in three environments was evaluated, and concentration of glucoraphanin and other glucosinolates and for phase 2 enzyme induction potential were estimated. Another goal was to examine the importance of broccoli head weight as a factor that governs the ultimate quantity of glucoraphanin (and hence the chemoprotective potential) of a harvested broccoli head. This is as critical in breeding for enhanced chemoprotective broccoli as is tissue concentration.

Materials and Methods

Plant materials: Plant materials evaluated in this study included 32 broccoli (*Brassica oleracea* L., Italica Group) entries (listed in Table 2). Twenty-three were doubled haploid lines developed at the US Vegetable Laboratory in Charleston, SC, and four were hybrids produced by crossing selected pairs of the above doubled haploid lines. Five commercial hybrid cultivars were also included: Everest (Syngenta Seeds, Gilroy, CA, USA), Futura, High Sierra, and Viking (Seminis Vegetable Seeds, Woodland, CA, USA), and Marathon (Sakata Seed, Salinas, CA, USA). All of the above entries were grown in replicated field trials in Charleston, SC during the fall seasons of 1997 and 1998. A subset of five doubled haploid lines USVL016, USVL032, USLVL042, USVL045, USVL070, and four cultivars 'Everest', 'High Sierra', 'Marathon' and 'Viking' were also replicated in a field trial conducted in 1996. No *a priori* information about glucoraphanin or enzyme induction potential of the subset of nine was available when they were chosen for replication in 1996. The additional 23 entries grown in the 1997 and 1998 trials were included based upon phase 2 enzyme induction potential, glucoraphanin concentration, and to maximize genetic diversity among the set of entries.

Field experiments and harvest: Field trials at the Charleston, SC site were carried out as previously described (Farnham et al. 2000). Entries were seeded in trays of a commercial potting mix (Metromix 360; Grace Sierra, Milpitas, CA, USA) in a greenhouse during the first week of August, and transplanted to the field the following September. Exact planting dates were 7, 4 and 11 September in 1996, 1997 and 1998, respectively. Spacing between rows was 102 cm, and spacing between plants within a row was 15 cm. All cultural practices (e.g. cultivation, fertilization and irrigation) for the trials were standard for local conditions. The soil type at the Charleston site is a Yonges loamy sand (fine loamy mixed, thermic Albaqualfs). Each study conducted in the 3 years (or environments) was designed as a randomized complete block, with two blocks in 1996 and three blocks in the remaining 2 years. Plots contained eight to 10 plants, depending on available seed supplies.

Heads were harvested when their diameter reached 10–12 cm (market stage). The mean number of days from transplant to harvest for each plot/entry was calculated based on the date plants in a plot produced a 10–12 cm size head. Two heads per plot were sampled at random for laboratory analysis, and subtending stalks were cut to a 15 cm length. Heads were immediately placed on ice in a cooler and within 30 min of field harvest, fresh weights (FWs) were recorded (excepting 1996), approximately half of the florets were cut from the stem, placed in individual sealable freezer bags, and frozen at −80°C. Boiling 80% methanol (aqueous) extracts were prepared from the fresh-frozen florets and stored at −20°C until needed for the bioassay of and for direct quantitation of quinone reductase induction potential and of glucosinolates by high-performance liquid chromatography (HPLC), as described in the previous work (Farnham et al. 2000).

Bioassay of quinone reductase induction potential: Bioassay of the representative phase 2 enzyme quinone reductase, was performed using Hepa 1c1c7 cells as described originally by Prochaska et al. (1992) and modified by Fahey et al. (1997). Excess myrosinase [0.0003 units/ml of cell culture medium; purified as described by Shikita et al. (1999)], and 500 μM ascorbate, was added at the time microtitre plates were dosed with broccoli extracts, to achieve complete hydrolysis of glucosinolates during a 48-h incubation at 37°C. With this method, conversion of extracted glucosinolates to their cognate isothiocyanates is essentially quantitative. One unit of inducer activity is the concentration that doubles quinone reductase activity in a microtitre well containing 150 μl of medium. Hence, a compound with a CD (the *Concentration* of a compound required to *Double* the quinone reductase specific activity in Hepa 1c1c7 murine hepatoma cells) of 1.0 μM has 6667 units of inducer activity per μmol. Inducer potency of extracts is expressed as units/g FW.

Paired-ion chromatography of glucosinolates: Plant extracts were chromatographed isocratically in acetonitrile/water (1 : 1, v/v) containing 5 mM tetradecylammonium bromide (TDAB) at a flow rate of 3 ml/min on a reverse-phase column (Whatman Partisil 10 ODS-2; 250 X 4 mm; Whatman, Inc., Clifton, NJ, USA) using a Waters (Waters Corp., Milford, MA, USA) HPLC system equipped with a photodiode array detector (Prestera et al. 1996). Sinigrin (allyl glucosinolate) was used as a standard. When compared with equimolar concentrations of sinigrin, relative integrated absorbance areas for alkyl glucosinolates, glucobrassicin, and neoglucobrassicin at 235 nm were 1.00-, 1.22- and 2.70-fold greater. The use of TDAB, an otherwise ideal paired-ion solvent system, does not permit resolution of glucoraphanin from glucoiberin, which is typically a minor component in most broccoli head samples. For the 1996 and 1997 data, a subset of representative germplasm from both years was subject to an alternative HPLC procedure (HILIC) that resolves glucoraphanin and glucoiberin to two separate chromatographic peaks (Troyer et al. 2001). Using this procedure, glucoiberin was not detected in over one-half of the 1996 and 1997 subset samples and on average, accounted for $< 5\%$ of the 'glucoraphanin/glucoiberin' peak obtained using TDAB as a paired ion. All 1998 samples were analysed by the paired ion method as well as the HILIC method. In this case, nearly two-thirds of all samples lacked glucoiberin and on average it was only 3.5% of the combined glucoraphanin/glucoiberin pool, so that the combined glucoraphanin/glucoiberin peak obtained from the paired ion method can be regarded as essentially 'glucoraphanin'.

Data analysis: Certain data (i.e. concentrations of glucoraphanin and quinone reductase induction potential) collected in 1996 and 1997 have already been presented (Farnham et al. 2000). The current analyses includes the results of our 1998 trial (not previously presented), used in combination with those from 1996 and 1997 to assess criteria not examined in the earlier work. Data for the nine entries grown in all 3 years were combined and analysed separately to estimate components of variance and to identify significant genetic, environmental, and genotype-by-environment interaction effects. Because no head weights were recorded in 1996, this analysis focused on glucosinolate concentrations.

Quinone reductase induction potential and glucosinolate contents per head were calculated for each entry/plot combination in the 1997 and 1998 trials by multiplying measured head concentrations for a plot sample by mean head weight. All analyses of variance (ANOVA) were performed using Proc GLM of SAS (release 6.12; SAS Institute, Inc., Cary, NC, USA). The 1997 and 1998 trials were analysed both separately and in combination. Entry means were compared using Fisher's protected LSD. Pearson correlation coefficients were calculated for all pairs of induction potential, individual glucosinolate concentrations, head weight, days from transplant to harvest, glucosinolate contents per head, and quinone reductase induction potential per head.

Results

The percentage of total sums of squares variance attributed to environment, genotype, and genotype-by-environment interaction depended upon the trait evaluated in nine diverse broccoli genotypes tested in three environments (Table 1). Environment had a significant impact on days from transplant to harvest, glucosinolate concentrations, and quinone reductase induction potential expressed as a concentration. Only 6.8% of variance for days from transplant to harvest was explained by environment, while almost 75% of variance for concentration of the glucosinolate hydroxyglucobrassicin was due to environment. Variation in enzyme induction potential (42.5% of total variance) appeared to be under stronger environmental influence than was glucoraphanin (21.9% of total variance).

Genotypic effects were significant for days from transplant to harvest and for concentrations of induction potential and glucoraphanin, but not for the concentrations of the three indole glucosinolates (Table 1). The percentage of total sums of squares due to genotype was highest (82.9%) for days from transplant to harvest, while they were 33.8 and 52.8% for quinone reductase induction potential and glucoraphanin, respectively. A significant genotype-by-environment interaction was observed for all but one trait. With enzyme induction potential, glucoraphanin concentration, and days from transplant to maturity, the percentage of variance attributed to the interaction was significantly less than that attributed to genotype.

When evaluated on a 'per head' basis and averaged across 1997 and 1998, the range for quinone reductase induction potential represented a more than ninefold difference (Table 2). The top-ranked six entries with the highest quinone reductase induction potential per head were doubled haploids, and 'Marathon' was the seventh highest ranked genotype for this trait. The lowest ranked genotypes for enzyme induction potential per head were all doubled haploids except 'Everest', the second lowest individual. When evaluated on a FW (tissue concentration) basis, the induction potential differed about sevenfold from lowest to highest genotypes (Table 2). Lines with the highest concentration of induction potential were also doubled haploid lines. 'Marathon' was the highest-ranking hybrid and was ranked tenth overall. As observed with induction potential per head, 'Everest' was among a group of other entries, mostly doubled haploid lines, lowest for induction potential on a concentration basis.

Although certain genotypes ranked similarly overall when comparing quinone reductase induction potential on a concentration or per head basis, for other individuals there was a very significant change in relative rank for the different criteria (Table 2). For instance, the individual USVL069 with the highest concentration of induction potential dropped to sixth place when the potential was expressed on a per head basis. Contrary to this, USVL046 had a moderate induction potential on a concentration basis but was among the higher genotypes when potential was expressed on a per head basis.

Results for glucoraphanin revealed similar trends to those for induction potential, with some exceptions: there was approximately a 10-fold range in glucoraphanin per head, with about a sixfold range of glucoraphanin concentration (Table 2). 'Marathon' ranked sixth highest (after five doubled haploid lines) based on glucoraphanin per head and fifth highest based on glucoraphanin concentration. 'Everest' ranked third lowest for glucoraphanin per head and second lowest for concentration. When comparing results for glucoraphanin on a concentration and per head basis, observed changes in rank were similar to those that occurred for induction potential.

Glucoraphanin and quinone reductase induction potential concentrations were positively and significantly correlated with one another and also with days from transplant to harvest (Table 3, Fig. 1). Neither glucoraphanin nor induction potential concentrations were correlated with head weight. Glucoraphanin and induction potential per head were significantly correlated with days from transplant to harvest (Fig. 1), although the magnitude of the coefficients were lower than those associated with concentrations. Glucoraphain and induction potential per head were highly correlated with one

Table 1: Analysis of variance for nine genotypes tested in 1996, 1997 and 1998 showing mean squares for the main effects of environment (E), replications within environment (Rep/E), genotypes (G), and the genotype-by-environment (G × E) interaction and error

		Mean squares					
Source	df	QRIP[1]	GR	HGB	NGB	GB	DTH
E	2	12.50×10^9***	3.694**	0.1208***	0.123*	1.239***	445.50***
Rep/E	5	0.05×10^9	0.194	0.0009	0.017	0.014	5.42
G	8	2.48×10^9***	2.174***	0.0031	0.062	0.125	1242.98***
G × E	16	0.45×10^9**	0.225*	0.0013	0.036**	0.065**	54.47***
Error	40	0.16×10^9	0.099	0.0009	0.013	0.028	10.91
Total	71						

*, **, *** Significant at P = 0.05, P = 0.01, P = 0.001, respectively.
[1] Traits analysed include the concentrations of quinone reductase induction potential (QRIP), glucoraphanin (GR), hydroxyglucobrassicin (HGB), neoglucobrassicin (NGB), glucobrassicin (GB) all on a fresh weight basis, and days from transplant to harvest (DTH).

Table 2: Quinone reductase induction potential (QRIP) and glucoraphanin (GR) content expressed on a per head basis (QRIP and GR per head, respectively) and on a per fresh weight of florets basis (QRIP and GR concentration), averaged and ranked across 1997 and 1998 field environments

	QRIP per head		QRIP concentration		GR per head		GR concentration	
Genotype	Units/head $\times 10^6$	Rank	Units/g FW $\times 10^4$	Rank	μmol/head	Rank	μmol/g FW	Rank
USVL049	12.43	1	7.34	2	240.8	5	1.33	6
USVL073	12.32	2	6.61	3	265.6	1	1.30	8
USVL046	10.55	3	4.60	10	245.7	3	1.06	15
USVL036	9.24	4	6.04	4	209.0	9	1.27	9
USVL048	9.02	5	5.71	6	244.2	4	1.52	4
USVL069	8.33	6	8.28	1	194.6	13	1.85	1
Marathon	8.24	7	4.65	9	232.3	6	1.35	5
USVL013 × 073	8.11	8	3.14	19	262.5	2	1.00	16
USVL066	7.53	9	6.04	5	205.6	11	1.58	2
Viking	7.40	10	4.34	14	219.8	8	1.33	7
USVL032	7.36	11	5.00	8	223.1	7	1.53	3
USVL042	7.30	12	5.31	7	181.1	16	1.22	11
High Sierra	7.12	13	4.36	13	197.7	12	1.18	13
USVL018 × 039	7.08	14	3.58	17	175.6	17	0.87	18
USVL045	7.08	15	4.49	11	208.5	10	1.27	10
Futura	6.42	16	4.04	15	185.9	15	1.09	14
USVL047 × 036	6.06	17	3.00	21	189.5	14	0.93	17
USVL047	6.01	18	3.80	16	117.8	20	0.75	21
USVL022	4.91	19	3.10	20	128.7	18	0.80	20
USVL075	4.77	20	2.72	23	123.5	19	0.68	24
USVL039	4.08	21	3.28	18	107.0	21	0.86	19
USVL009	3.67	22	2.40	26	67.3	28	0.42	28
USVL018	3.53	23	2.68	24	66.5	29	0.45	27
USVL013 × 075	3.39	24	1.91	28	96.1	22	0.50	26
USVL067	3.19	25	2.28	27	78.4	26	0.55	25
USVL013	3.04	26	1.76	30	78.6	25	0.40	29
USVL029	2.92	27	4.37	12	81.7	23	1.18	12
USVL044	2.91	28	2.73	22	76.0	27	0.72	22
USVL028	2.65	29	2.58	25	80.0	24	0.70	23
USVL070	2.61	30	1.77	29	46.2	31	0.32	30
Everest	2.11	31	1.34	31	49.6	30	0.27	31
USVL016	1.34	32	1.24	32	24.7	32	0.24	32
$LSD_{0.05}$	2.31		1.33		61.8		0.36	

Table 3: Correlation coefficients between means of days from transplant to harvest (DTH), head weight (HWT), concentrations of Quinone reductase induction potential (QRIP conc.) and glucoraphanin (GR conc.), and total QRIP and GR per head, for all genotypes grown in 1997 and 1998. Coefficients on the diagonal in italics result from correlations of genotype means for a trait in 1 year vs. means for that same trait in the second year

	Trait					
Trait	DTH	HWT	QRIP conc.	GR conc.	QRIP/head	GR/head
DTH	0.96***	−0.24	0.78***	0.77***	0.61***	0.57***
HWT		0.67***	−0.01	−0.02	0.48**	0.50**
QRIP conc.			0.72***	0.92***	0.84***	0.76***
GR conc.				0.78***	0.77***	0.83***
QRIP/head					0.85***	0.93***
GR/head						0.81***

, * Significant at P = 0.01 and P = 0.001, respectively.

another ($r = 0.93$), as were concentrations for these traits ($r = 0.92$).

Correlation of genotype trait means in one environment vs. the respective means from the second environment resulted in the highest correlation coefficients for days from transplant to harvest (Table 3). The smallest coefficients were observed for head weight. Higher coefficients were observed for quinone reductase induction potential and glucoraphanin on a per head basis than for the same characters on a concentration basis (Table 3).

Discussion

Few studies have contrasted the genetic vs. the environmental contribution to glucoraphanin concentration in broccoli florets. Shelp et al. (1993) observed significant effects of genotype and environment on glucoraphanin concentrations, but they limited their examination to two early maturing cultivars. Farnham et al. (2000) observed a positive correlation of glucoraphanin concentrations in heads from the same genotypes harvested in two different environments, concluding

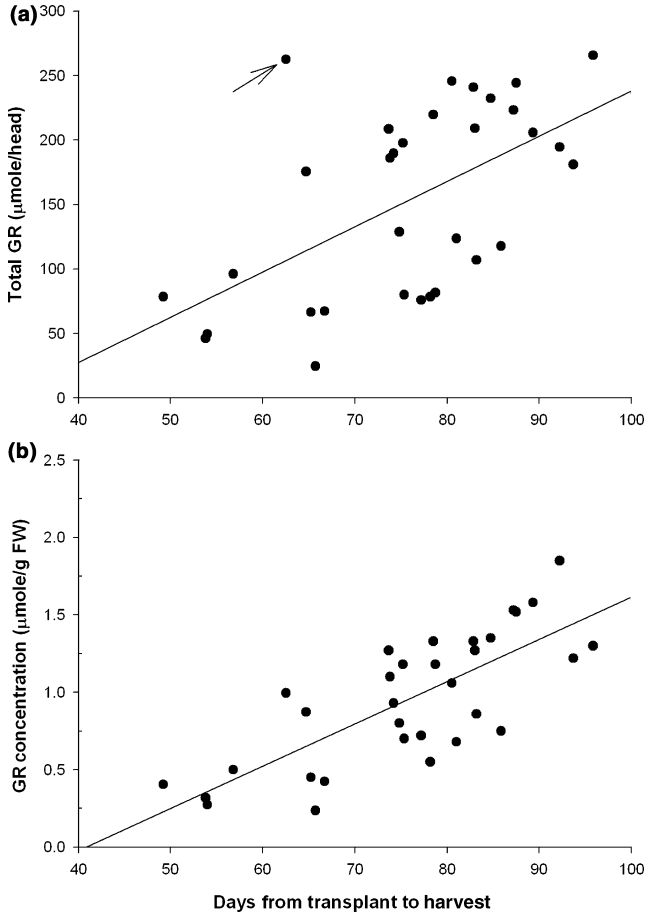

Fig. 1: Mean total glucoraphanin (GR) per broccoli head (a) and GR concentration in heads (b) regressed against mean days from transplant to harvest for 32 genotypes grown in 1997 and 1998. Note data point at approximately 63 days from transplant to harvest with total glucoraphain over 250 μmol/head, which represents a relatively early maturing genotype with a very high total glucoraphanin per head

that genotype plays a significant role in expression. Rosa and Rodrigues (2001) observed similar correlations between genotypes evaluated in two different environments, also indicating the key role of genotype for glucoraphanin level. Most recently, Brown et al. (2002) presented evidence, based on more environmental samples, that the effect of genotype on glucoraphanin level in broccoli is much greater than are environmental influences on this trait.

We report herein, significant environmental effects on levels of all glucosinolates examined. However, glucoraphanin was unique in that it was the only broccoli glucosinolate evaluated in this study that was significantly affected by genotype. Significant genotype-by-environment interaction was observed for concentrations of most glucosinolates, but per cent total sums of squares attributable to genotype-by-environment for glucoraphanin was much lower than that attributable to genotype. It is useful to compare genotypic and environmental effects on glucosinolates with like effects on days from transplant to harvest (which is considered a highly heritable trait) (Borchers 1968, Hulbert and Orton 1984). Overall, results from the current study confirm the relative importance of genotype vs. environment in expression of glucoraphanin concentration, as well as chemoprotective potency (quinone reductase induction potential), of broccoli heads. The complementarity of these results to those described by others indicate that broccoli should respond well to selection for increased floret glucoraphanin concentration, which could be evaluated either directly, or by bioassay of enzyme potential (the ultimate biological response of interest).

In most markets broccoli is sold on a per head basis, not by weight. In studies to date, the focus has been on broccoli head glucoraphanin concentration, and the total delivery of glucoraphanin or enzyme induction potential per head has not been considered. Total glucoraphanin per head may be an essential criterion in considering enhancement of glucoraphanin content in certain broccoli genotypes. Genotypic comparisons based upon total glucoraphanin content per head are different from those derived from an evaluation of glucoraphanin concentration only. In particular, the range among genotypes increases for the total glucoraphanin content and relative rank of many genotypes changes. Interestingly, correlation coefficients between environments were higher for glucoraphanin and induction potential on a per head basis than on a concentration basis (Table 3). This might reflect some type of compensation by the plant to produce similar total amounts of glucoraphanin, regardless of the potential for increased or decreased head weight. It will be useful to evaluate this phenomenon in future studies testing genotypes in a larger sample of environments.

Our previous work (Farnham et al. 2000) and that described herein, indicate that there is no negative correlation between glucoraphanin concentration or quinone reductase induction potential and broccoli head weight (essentially a measure of density as all heads are harvested at same diameter). There has been no indication of a dilution effect such as that described by Rosa and Rodrigues (2001), whereby the accumulation of dry weight resulted in a reduction in the concentration of glucoraphanin. Based on current observations, it should be feasible to breed a very dense head with an elevated concentration of glucoraphanin to deliver maximum chemoprotective potential (high enzyme induction potential/glucoraphanin content). Positive correlations between glucoraphanin concentration and days from transplant to harvest indicate high concentration and late maturity can be combined in a single cultivar. Alternatively, focusing on total glucoraphanin per head as measured in this study, the presence of an early maturing outlier with high glucoraphanin (Fig. 1) indicates it will also be possible to develop early maturing cultivars that deliver high amounts of glucoraphanin and chemoprotective potency.

Acknowledgements

The authors acknowledge E. Gomperts for management and harvest of field plots. Funding was provided in part by a generous gift from the Lewis B. and Dorothy Cullman Foundation to Paul Talalay, whom we also thank for his intellectual support and encouragement and from Program Project Grant P01 CA 44530 from the National Cancer Institute, Department of Health and Human Services. Paula Wilson was supported by a Crop and Food Research Fellowship. Use of a company name or product by the U.S. Department of Agriculture does not imply approval or recommendation of the product to the exclusion of others that also may be suitable. One of the authors (JWF), as well as Johns Hopkins University, own stock in Brassica Protection Products (BPP), a company whose mission is to develop chemoprotective food products and which sells broccoli sprouts. JWF is a Board Member and scientific consultant to BPP and his stock is subject to

certain restrictions under University policy. The terms of this arrangement are being managed by Johns Hopkins University in accordance with its conflict of interest policies.

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