HortScience 41(6):1382–1385. 2006.

Genetic and Environmental Variation of Glucosinolate Content in Chinese Cabbage

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Additional index words. Brassica rapa var. *pekinensis*, gluconasturtiin, glucobrassicin indole-3-carbanol, phenethlyisothiocyanate, genotype × environment interaction

***Abstract*. Strong evidence exists to suggest that increased consumption of glucosinolates from *Brassica* vegetables is associated with reduced risk of cancer induction and development. Development of elite germplasm of these vegetables with enhanced levels of glucosinolates will putatively enhance health promotion among the consuming public. To evaluate levels of glucosinolate phenotypic variation in Chinese cabbage tissue and partition the total phenotypic variation into component sources (genotype, environment, and genotype-by-environment interaction), a set of 23 *Brassica rapa* L. var. *pekinensis* genotypes were grown in two different environments (field plots and greenhouse ground beds). Gluconasturtiin and glucobrassicin were found to account for ≈80% of total head glucosinolate content. Significant differences were found in glucosinolate concentrations between the lowest and highest genotypes for glucobrassicin (6-fold) and for gluconasturtiin (2.5-fold). Analysis of variance showed that for the three major glucosinolates (gluconasturtiin, glucobrassicin, and progoitrin), the genotypic effects described most of the phenotypic variation (62% averaged over the three compounds). The next most important factor was genotype × environment interaction (29%), whereas variation affiliated with the environment was found to be relatively minor (8%). These results suggest that genetic manipulation and selection can be conducted to increase glucosinolate content and the putative health promotion associated with consumption of Chinese cabbage.**

Epidemiological studies have suggested that an inverse relationship exists between dietary intake of phytochemicals called glucosinolates in *Brassica* vegetables and induction of cancer (Verhoeven et al., 1996). Glucosinolates are a group of sulfur-containing glucosides naturally occurring in cruciferous vegetables that are hydrolyzed by the endogenous enzyme myrosinase into isothiocyanates, thiocyanates, and nitriles (Fenwick et al., 1983). The primary forms of these phytochemicals include the aliphatic, indolyl, and aromatic glucosinolates derived from the amino acid precursors methionine, tryptophan, and phenylalanine respectively. Chinese cabbage (*Brassica rapa* L. var. *pekinensis*) is a relatively rich source of these chemicals, particularly gluconasturtiin (aromatic glucosinolate), glucobrassicin (indolyl glucosinolate), and progoitrin (aliphatic glucosinolate) (Daxenbichler et al., 1979). Gluconasturtiin and glucobrassicin are precursors to phenethlyisothiocyanate (PEITC) and indole-3-carbanol (I3C), strong inhibitors of carcinogenesis in mammalian systems (Bell et al., 2000; Hecht, 2000; Wong et al., 1997). In contrast hydrolyzed products of progoitrin have demonstrated goitrogenic activity in animals, but in most cases this appears to be limited to situations of iodine deficiency (Jongen, 1996).

Based on epidemiological, cell culture, and animal model studies, PEITC and I3C are currently undergoing clinical trials, and appear to slow or prevent several forms of cancer successfully (Bell et al., 2000; Wong et al., 1997). I3C, derived from glucobrassicin in cruciferous vegetables, has been shown to the lower incidence of hormone-dependent cancers by altering estrogen metabolism (Fowke et al., 2000; Keck and Finley, 2004). In the stomach, I3C is transformed into various bioactive compounds that can influence estrogen metabolism and promote hydroxylated metabolites at the C-2 position of the estrogen molecule and prevent hydroxylation at the C-16 position. Several investigations suggest that women who metabolize a large proportion of their estrogens in the form of 2-OH estrone as opposed to the 16-OH estrone have lower breast cancer risk (Badithe et al., 2001; Michnovicz et al., 1997; Wong et al., 1997). This research suggests that women whose diets are high in glucobrassicin content would be at less risk of developing breast cancer.

Because the bioactivity of Chinese cabbage is putatively associated with concentration of glucobrassicin and gluconasturtiin, identification or development of genotypes with enhanced and stable levels of these compounds would provide a value-added opportunity for marketing this crop with superior health promotion to consumers. To assess the feasibility for genetic improvement in glucosinolate content, it is necessary to partition the phenotypic variability into its component sources [genotype, environment and genotype × environment (G×E) interaction]. When a high proportion of the phenotypic variance for a specific trait is the result of genotypic differences, genetic manipulation to improve trait performance is feasible. In contrast, if most of the phenotypic variance for the trait is associated with the environment, then cultural practices and crop management strategies might be used to create growing conditions that favor improved glucosinolate biosynthesis. When a high proportion of phenotypic variance is described by G×E interactions, then the most relevant approach would require a breeding program to create genotypes designed for specific locations or growing conditions. This study was conducted to evaluate levels of phenotypic variation in tissue content of these phytochemicals among Chinese cabbage cultivars and partition the variation into the genetic, environmental, and G×E interaction components of variance.

Materials and Methods

Plant material

Seeds of 25 genotypes, including 23 Chinese cabbage (*Brassica rapa* L. var. *pekinensis*) lines (18 land races and five commercial hybrids), one Chinese mustard (pak-choi; *Brassica rapa* L. var. *chinensis*) accession, and one radish (*Raphanus sativus* L.) × Chinese cabbage interspecific hybrid were obtained from the National Horticultural Research Institute, Suwon, Korea, and were germinated in flats in the greenhouse. Seedlings were transplanted into 1) a greenhouse ground bed maintained under a 25 °C/18 °C and 14-h/10-h day/night temperature and supplemental light regime, and 2) into field plots on the south farm of the University of Illinois in Champaign on 18 Aug. 2004 in a randomized complete-block design with three reps and with plots of six plants per genotype. The soil type was a Drummer silty clay loam (Typic Haplaquoll). At commercial maturity, four plants of each plot were harvested and separated into five portions; outer four to six leaves of the head, inner leaves of the head, and root tissue, which were then weighed, and bulked for each plot. Leaf subsamples of the inner and outer leaf bulks were then carefully dissected into stalk and

Received for publication 12 Apr. 2006. Accepted for publication 28 Apr. 2006.
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green leaf tissues. Subsamples of all five tissues for each genotype and replication were then weighed, frozen in liquid nitrogen, freeze-dried, reweighed, and stored at –20 °C for later analysis. In commercial production, the outer leaves of Chinese cabbage heads are removed and discarded. In this study, what we have termed the inner leaves of the head represent what is typically sold and consumed.

Glucosinolate analysis

Extraction and high pressure liquid chromatographic analysis of intact glucosinolates was conducted as described by Wathelet and colleauges (1991), with some modifications described by Brown and associates (2002) using a reverse-phase C^{18} column. The desulfated glucosinolates were eluted from a column with 3 mL deionized water and separated on a Dionex HPLC (Dionex Corporation, Sunnyvale, Calif.) system consisting of a variable ultraviolet detector set at 229 nm wavelength. Desulfoglucosinolates were eluted off the column in 40 min with a linear gradient of 0% to 20% acetonitrile in water at a flow of 1.0 mL·min^{-1}. Benzyl glucosinolate was used as an internal standard to calculate the concentrations of the other glucosinolates. The type and amount of glucosinolates in each sample were calculated in comparison with certified glucosinolate levels in a standard rapeseed reference material (BCR 367, Commission of the European Community Bureau of References, Brussels, Belgium). Using benzyl glucosinolate as an internal standard, the recovery of glucosinolates from the samples using this procedure was estimated at 95% to 97%.

Statistical analysis

Analysis of variance using PROC GLM (SAS Institute, 2000) was performed using the linear model

$$\chi_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \tau_{k(j)} + e_{ijk}$$

where χ_{ijk} is the kth replication of the phenotypic value of the ith genotype in environment j, μ is the overall mean, α_i is the fixed effect of genotype i, β_j is the random effect of environment j, $(\alpha\beta)_{ij}$ is the random interaction effect of genotype i in environment j, $\tau_{k(j)}$ is the nested effect of the kth block within the jth environment, and e_{ijk} is the experimental error associated with χ_{ijk}. F tests at $P < 0.05$ were used to determine the significance of the individual variance components. Fisher's smc analysis ($\alpha = 0.05$) was performed using PROC GLM (SAS Institute, 2000).

Results and Discussion

Averaged across the 23 Chinese cabbage genotypes, the dominant glucosinolates found in Chinese cabbage were gluconasturtiin (phenethyl glucosinolate), glucobrassicin (3-indolylmethyl glucosinolate), progoitrin (2-hydroxy-3-butenyl glucosinolate), and neoglucobrassicin (N-methoxy-3-indolylmethyl glucosinolate) each of which displayed significant variation in concentration among plant parts (Table 1). Glucosinolate content in the inner leaves was found to be greater than concentrations found in the outer leaves of the head, whereas root tissues contained the highest levels of gluconasturtiin and neoglucobrassicin. Because the portion of the plant we have described as the inner leaves is what is typically sold and consumed, further observations will focus on these tissues. Averaged over the 23 genotypes, inner leaf concentrations of gluconasturtiin, glucobrassicin, progoitrin, and neoglucobrassicin were 4.81, 1.64, 1.27, and 0.57 μmol·g^{-1} dry weight, respectively, with gluconasturtiin and glucobrassicin accounting for ≈80% total leaf glucosinolate content (Fig. 1). These two compounds undergo hydrolysis to form PEITC and I3C, isothiocyanates with anticancer activity (Hecht, 2000).

Table 2 illustrates the significant variation in inner leaf and root glucosinolate content observed among the 25 tested genotypes. Significant differences were found in glucosinolate concentrations of inner leaf tissue between the lowest and highest genotypes for progoitrin (12-fold), glucobrassicin (6-fold), neoglucobrassicin (3.5-fold), and for gluconasturtiin (2.5-fold). Comparison of leaf glucosinolate concentrations harvested from the field and greenhouse plants indicated that the growing environment significantly impacts on the concentration of individual glucosinolates but not the total glucosinolate content. The growing environment did not exert as pronounced an effect on glucosinolate content as did genetic variation among the lines, except for neoglucobrassicin (Table 3).

Analysis of variance was applied to partition the total phenotypic variation of the different glucosinolates into components associated with genotype, environment, and G×E interaction (Table 3). For the three major glucosinolates (gluconasturtiin, glucobrassicin, and progoitrin), the genotypic effects described most of the variation in the model (62% averaged over the three compounds). The next most important factor contributing to the variation in the concentration of these three compounds was G×E interaction (29%), whereas variation affiliated with the environment and replication were found to be minor factors (8% and 1.4% respectively). In contrast, neoglucobrassicin displayed a unique pattern of variation in which the environment described the greatest portion of the variation (43%). Across the tested genotypes, both progoitrin and glucobrassicin were significantly different between the two growing environments in both head and root tissue, with the field environment generating the higher concentrations (Table 4). Gluconasturtiin and neoglucobrassicin were significantly different between environments for head tissue, but only gluconasturtiin was significantly different between environments for root tissue. Total glucosinolates were not different between environments in head tissue. The Pak-choi accession showed a similar glucosinolates profile to the Chinese cabbage genotypes. In contrast, Baemuchae (radish × Chinese cabbage

Table 1. Variation in glucosinolate concentrations among different plant parts of Chinese cabbage averaged over all genotypes and environments (μmol·g^{-1} dry weight).

Plant part	Progoitrin	Glucobrassicin	Neoglucobrassicin	Gluconasturtiin
Inner leaves[z]				
Green leaf tissue	1.55 a[y]	2.46 a	0.79 b	6.59 b
Leaf stalk/mid rib	1.04 b	0.86 c	0.39 c	3.29 c
Outer leaves				
Green leaf tissue	0.52 d	0.52 d	0.72 b	2.14 d
Leaf stalk/mid rib	0.67 c	0.25 e	0.23 c	1.06 e
Root	0.72 c	1.21 b	2.00 a	10.64 a

[z]Portion of plant typically consumed.
[y]Means followed by different letters within each column are significantly different at $P = 0.05$.

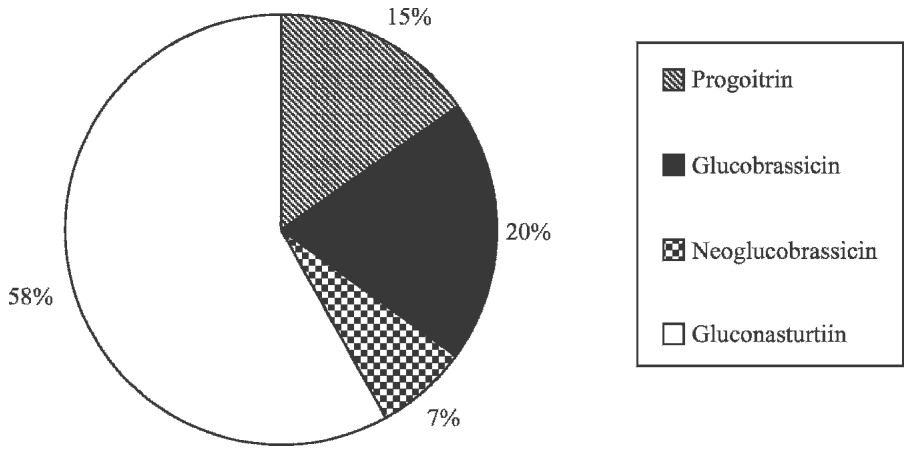

Fig. 1. Average relative percentage of glucosinolate composition in head tissue of 23 Chinese cabbage accessions.

Table 2. Mean and SD of glucosinolate concentrations in inner leaves (heads) and root tissue for all the tested genotypes from the field environment (μmol·g^{-1} dry weight).

	Inner leaves (head)				Root			
Genotypes	Progoitrin	Glucobrassicin	Gluconasturtiin	Neoglucobrassicin	Progoitrin	Glucobrassicin	Gluconasturtiin	Neoglucobrassicin
Brassica rapa L. var. *pekinensis* (Chinese Cabbage)								
2[z]	1.1 ± 0.5	0.5 ± 0.3	3.3 ± 0.5	0.2 ± 0.1	0.5 ± 0.2	1.2 ± 0.7	5.0 ± 4.5	1.3 ± 1.4
26	4.5 ± 1.3	3.1 ± 1.0	3.4 ± 0.9	0.6 ± 0.0	3.4 ± 0.5	1.3 ± 0.3	11.8 ± 1.5	3.1 ± 0.3
32	0.5 ± 0.1	2.4 ± 1.0	1.8 ± 0.6	0.6 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	11.0 ± 1.1	2.9 ± 0.9
83	1.9 ± 1.1	7.5 ± 3.3	6.7 ± 2.6	0.8 ± 0.3	1.1 ± 0.4	1.5 ± 0.1	10.2 ± 2.8	1.0 ± 0.6
103	0.8 ± 0.4	1.8 ± 0.9	3.6 ± 1.3	0.4 ± 0.0	0.4 ± 0.1	1.5 ± 0.6	18.2 ± 1.0	3.2 ± 0.3
110	0.5 ± 0.4	1.3 ± 0.8	3.3 ± 1.1	0.2 ± 0.1	0.4 ± 0.2	1.3 ± 0.7	12.5 ± 4.3	3.5 ± 3.1
114	2.3 ± 0.5	3.3 ± 0.6	6.3 ± 0.4	0.4 ± 0.2	0.7 ± 0.6	2.8 ± 1.2	17.9 ± 2.2	0.0
118	2.1 ± 0.6	2.2 ± 0.3	5.9 ± 1.4	0.2 ± 0.1	1.0 ± 0.1	2.4 ± 0.1	17.2 ± 0.7	2.1 ± 0.4
122	0.4 ± 0.2	2.1 ± 0.6	4.9 ± 1.2	0.1 ± 0.1	0.2 ± 0.1	2.1 ± 0.4	12.7 ± 0.8	1.7 ± 0.2
124	1.7 ± 0.7	2.5 ± 1.3	3.9 ± 0.4	0.7 ± 0.5	0.5 ± 0.3	1.4 ± 0.7	7.3 ± .1.0	1.9 ± 1.0
128	1.7 ± 0.6	2.0 ± 0.6	3.4 ± 0.5	0.3 ± 0.1	0.6 ± 0.1	1.6 ± 0.3	12.2 ± 1.5	1.7 ± 1.1
145	2.3 ± 0.7	1.8 ± 0.1	6.1 ± 1.0	0.3 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	12.7 ± 0.5	2.3 ± 0.6
147	1.3 ± 0.5	3.2 ± 0.4	4.5 ± 0.9	0.3 ± 0.1	0.8 ± 0.3	2.3 ± 0.7	13.2 ± 0.5	1.6 ± 1.5
148	3.5 ± 0.6	1.1 ± 0.2	2.1 ± 0.6	0.5 ± 0.1	3.5 ± 1.9	1.0 ± 0.1	16.3 ± 0.2	1.7 ± 0.1
163	1.6 ± 0.6	3.9 ± 0.7	4.7 ± 0.9	0.2 ± 0.1	1.9 ± 0.4	3.1 ± 0.7	20.7 ± 0.9	3.4 ± 1.0
168	1.8 ± 0.3	1.6 ± 0.3	4.5 ± 0.6	0.2 ± 0.0	1.7 ± 0.2	1.3 ± 0.4	6.2 ± 1.1	1.4 ± 0.3
171	1.6 ± 1.1	1.4 ± 0.3	5.0 ± 0.8	0.2 ± 0.0	0.6 ± 0.2	0.7 ± 0.3	8.9 ± 6.5	1.8 ± 2.4
172	0.9 ± 0.2	0.7 ± 0.2	3.9 ± 0.0	0.2 ± 0.1	1.4 ± 0.2	1.2 ± 0.2	5.0 ± 2.9	1.4 ± 1.4
Bulam3	1.5 ± 0.1	1.4 ± 0.0	3.4 ± 0.3	0.1 ± 0.1	0.7 ± 0.0	4.8 ± 1.1	3.7 ± 1.1	0.2 ± 0.1
Chilsung	0.4 ± 0.1	0.8 ± 0.3	4.4 ± 0.8	0.3 ± 0.1	0.5 ± 0.3	1.3 ± 0.2	14.9 ± 3.7	2.8 ± 0.3
Maeryuk	0.7 ± 0.1	2.0 ± 0.8	6.3 ± 1.1	0.4 ± 0.1	0.6 ± 0.1	1.3 ± 0.2	12.8 ± 1.1	1.9 ± 0.4
Noranja	1.2 ± 0.3	1.0 ± 0.2	5.1 ± 1.1	0.3 ± 0.1	2.1 ± 0.8	2.3 ± 0.7	17.4 ± 2.3	3.9 ± 0.8
Noktop	0.4 ± 0.1	1.2 ± 0.3	4.0 ± 0.3	0.4 ± 0.2	0.5 ± 0.2	1.4 ± 0.2	14.3 ± 4.0	2.4 ± 0.9
Brassica rapa L. var. *chinensis* (Chinese mustard)								
73	1.8 ± 0.3	0.8 ± 0.4	2.1 ± 0.6	0.3 ± 0.2	1.7 ± 0.8	0.9 ± 0.3	10.0 ± 3.4	1.7 ± 1.3
Brassica rapa L. var. *pekinensis x Raphanus sativus* L. (interspecific hybrid)								
Baemuchae	1.1 ± 0.1	1.5 ± 0.2	5.9 ± 1.1	0.3 ± 0.0	0.5 ± 0.0	1.8 ± 0.1	25.1 ± 1.6	2.5 ± 0.5
LSD[y]	0.4	1.6	1.5	0.3	0.8	0.8	4.3	1.8

[z]Genotype number refers to accession number from Chinese cabbage germplasm collection from the National Horticultural Institute in Suwon, Korea. The last five Chinese cabbage accessions are commercial F_1 hybrids.
[y]LSD = least significant differences between genotype means at $\alpha = 0.05$.

Table 3. Percentage of variation in different glucosinolate concentrations associated with the genotype, environment, and genotype × environment interaction for 23 Chinese cabbage genotypes grown over two environments.

		Indolyl			
Source of variation	Aliphatic progoitrin	glucobrassicin	neoglucobrassicin	Aromatic gluconasturtiin	Head wt
Genotype (G)	76.8 (<0.0001)[z]	61.0 (<0.0001)	30.0 (<0.0013)	48.0 (<0.0001)	36.6 (<0.0001)
Environment (E)	5.4 (<0.0001)	9.1 (<0.0001)	43.0 (<0.0001)	9.7 (<0.0001)	42.7 (<0.0001)
G × E	16.8 (<0.0008)	29.0 (<0.0001)	23.3 (<0.0172)	40.0 (<0.0001)	11.4 (<0.0407)
Rep	1.0 NS	0.9 NS	3.7 NS	2.3 NS	9.3 (<0.0001)
R^2 described by the model	0.79	0.81	0.67	0.75	0.79

[z]Probability of significance.
[NS]Nonsignificant.

Table 4. Mean and LSD for different glucosinolate compounds in head and root tissue from field and greenhouse averaged over the 25 genotypes (μmol·g^{-1} dry weight).

	Inner leaves (head) tissue			Root tissue		
Compounds	Field	Greenhouse	LSD[z]	Field	Greenhouse	LSD
Progoitrin	1.5 a[y]	1.1 b	0.2	1.1 a	0.4 b	0.1
Glucobrassicin	2.0 a	1.3 b	0.3	1.7 a	0.8 b	0.1
Gluconasturtiin	4.3 b	5.3 a	0.4	12.7 a	8.5 b	0.7
Neoglucobrassicin	0.3 b	1.8 a	0.1	2.1 a	2.0 a	1.2
Total glucosinolates	8.2 a	8.4 a	0.7	17.5 a	11.5 b	1.0

[z]LSD = least significant difference between field and green house environments at $\alpha = 0.05$.
[y]Means followed by different letters between environments are significantly different at $P = 0.05$.

interspecific hybrid) has similar inner leaf glucosinolate content but, among all accessions, displayed the highest concentration of gluconasturtiin in root tissue.

In conclusion, our data indicate that significant phenotypic variation, which is primarily under genetic control, exists among commercial Chinese cabbage germplasm for glucosinolate content and suggest that genetic manipulation and selection can be conducted to increase glucosinolate content and the putative health promotion associated with consumption of these vegetables. Correlation analysis also revealed no strong positive or negative associations between concentrations of the various glucosinolates in the head tissue of Chinese cabbage (data not shown). This suggests that developing Chinese cabbage genotypes with improved anticancer activity is a feasible breeding objective. The observation that the greater proportion of the phenotypic variation is associated with differences among genotypes tends to agree with previous work concerning the regulation of aliphatic glucosinolates in broccoli (Brown et al., 2002; Farnham et al., 2005). Although the genetics and biosynthetic pathway for the aliphatic glucosinolates have been thoroughly investigated in *Arabidopsis,* and to a lesser extent in *Brassica napus,* very little information is available concerning the biosynthesis of the indolyl and aromatic glucosinolates (Mithen, 2001).

Although reports in the literature concerning Brussels sprouts (Heaney et al., 1983) and rape (Mailer, 1989) have indicated that the environment can play a major role in determining glucosinolate content in these *Brassica* species, our preliminary data, based on only two environments, suggests that environmental effects are not key in the regulation of glucosinolate content in Chinese cabbage. Although most of the observed variation in this study was described by the genotype, there was a significant effect of G×E interaction, suggesting that growing conditions impact the stability of individual genotype glucosinolate content. Our observations of neoglucobrassicin variation in Chinese cabbage are in agreement with

previous studies in cabbage (Verkerk et al., 2001) and oilseed rape (Loivamaki et al., 2004), in which concentrations of this compound were found to be upregulated by environmental stimuli. The relatively high content of glucosinolates (particularly gluconasturtiin) in root tissue of Chinese cabbage suggest that these tissues could serve as a tissue source for the extraction of these phytochemicals for use in the nutraceutical industry.

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