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Genotypic Variation of the Glucosinolate Profile in Pak Choi (*Brassica rapa* ssp. *chinensis*)

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Supporting Information

ABSTRACT: Thirteen different pak choi (*Brassica rapa* ssp. *chinensis*) cultivars were characterized regarding their glucosinolate profile analyzed by HPLC-DAD-MS. The identified glucosinolates were subjected to principal component analysis, and three distinct groups of pak choi sprouts were identified. Group differences were marked mainly by variations in the aliphatic glucosinolate profile such as differing levels of 3-butenyl glucosinolate and 2-hydroxy-3-butenyl glucosinolate as well as by their varying proportional ratios. In addition, the three groups of pak choi sprouts varied by the presence or absence of 2-hydroxy-4-pentenyl glucosinolate and in level and composition of butyl glucosinolates. This classification is reflected by relative mRNA expression level of 2-oxoacid-dependent dioxygenase. As in sprouts, the major glucosinolates in mature leaves were found to be the aliphatic glucosinolates. However, unlike in sprouts, an additional aliphatic glucosinolate, 5-methylsulfinylpentyl glucosinolate, was detected as characteristic ontogenetic variation in mature leaves in 12 of the 13 pak choi cultivars analyzed.

KEYWORDS: *Brassica rapa ssp. chinensis, secondary plant metabolites, alkenyl glucosinolate, 3-butenyl glucosinolate, 2-hydroxy-3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-oxoacid-dependent dioxygenase*

INTRODUCTION

Glucosinolates are a group of secondary plant metabolites found almost exclusively in plants of the order Brassicales, including horticulturally important *Brassica* crops.[1] Glucosinolates are composed of β-D-thioglucoside-*N*-hydroxysulfates containing a variable side chain and can be grouped into three chemical classes depending on the amino acid precursor of this side chain: (I) aliphatic from methionine, (II) indole from tryptophan, and (III) aromatic from phenylalanine or tyrosine. In intact cells, glucosinolates are kept separate from myrosinase, the β-thioglucosidase that hydrolyzes glucosinolates. Upon cell rupture, various biologically active compounds are formed, for example, isothiocyanates, nitriles, thiocyanates, epithionitriles, and oxazolidine-2-thione.[2] Recently, the breakdown products of glucosinolates have generated considerable pharmacological interest due to their beneficial biological activities in mammals.[3] Although about 120 glucosinolates are currently known,[4] only the breakdown products of certain glucosinolates have been found to have health-promoting effects. For example, breakdown products derived from alkenyl glucosinolates have been proposed to bear protective effects such as anticarcinogenic properties of 2-propenyl isothiocyanate[5] and 2-hydroxy-3-butenyl nitriles.[6] Furthermore, 3-butenyl isothiocyanate is known to exert a suppressive effect on postprandial hypertriglyceridemia as a risk factor related to atherosclerotic disease[7] as well as having antitumorigenic properties such as proliferation inhibition and apoptosis of cancer cells.[8] In addition, 4-pentenyl isothiocyanate was shown to have antimicrobial properties,[9] and the breakdown products of 3-indolylmethyl glucosinolate and its derivatives have been reported to be effective against the development of some types of cancer.[10−12]

Several epidemiological studies, for example, the meta study of Verhoeven and co-workers,[13] the Health Professionals' Study,[14] and the β-Carotene Retinol Efficiency Trial,[15] have revealed an inverse association between a high consumption of *Brassica* vegetables and a lower risk of cancer incidence. In addition, Sakauchi and co-workers[16] demonstrated that high intakes of pak choi are significantly inversely associated with the risk of urothelial cancer. Pak choi has traditionally been heavily consumed in eastern and southern China.[17] However, shifts in consumer trends toward healthier lifestyles over the past decade have resulted in increased consumption of sprouts in salads or in Asian-style cooking in Europe.[18,19] The glucosinolate profile of pak choi is dominated by alkenyl glucosinolates combined with relatively high levels of indole glucosinolates.[20,21] Consumption of sprouts that have not been processed or cooked is likely to lead to a significant absorption of health-promoting glucosinolate breakdown products because inhibition of myrosinase should not have taken place.[22]

Because the potential beneficial health effects of Brassicales species are directly related to their glucosinolate concentration and composition,[23] our aim was to determine the glucosinolate profile of different cultivars of pak choi sprouts as a first step to evaluating their potential as health-promoting agents. Previous studies have already categorized the morphological and genetic diversity of pak choi,[24] as well as determined the total glucosinolate concentrations in several cultivars[25] or individual glucosinolates of single cultivars[20,26] or have focused exclusively on

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Table 1. Oligonucleotide Primers Used for Gene Expression Analysis by Semiquantitative Real-Time RT-PCR

gene function (*gene name*)	oligonucleotide abbreviation	sequence	accession no.
actin2 (*BrACT2*)	Br-Af	ACGTGGACATCAGGAAGGAC	AC189447
	Br-Br	CTTGGTGCAAGTGCTGTGAT	
methylthioalkylmalate synthase 1 (*BrMAM1*)	Br-MAM1f	CTTAGGCTTCAACGACATCAT	FJ376038
	Br-MAM1r	GTTGATCCCTACCGTGTCCC	
methylthioalkylmalate synthase 3 (*BrMAM3*)	Br-MAM3f	CTTAGGCTTCAATGAAATCCA	FJ376040
	Br-MAM3r	GTTGATCCCTACCGTGTCTG	
methylthioalkylmalate synthase ($BrGS_{ELONG}$)	Br-ELONGf	TTTAGGCTTCGAAGACATCGA	EF611254
	Br-ELONGr	GTTGATCCCCACCGTGTCCG	
2-oxoglutarate-dependent dioxygenase 2 (*BrAOP2-1*)	Br-AOP2-1f	GATTGTTCTCGACTCCAAATAGA	FJ376073
	Br-AOP2-1r	TTTGAATACACGTGGATGCTGC	
2-oxoacid-dependent dioxygenase (*BrGSL-OH*)	Br-OH1f	AGGGAGTGATGAAGCTTGCC	FJ376074
	Br-OH1r	TAAGTCTGGCTCAGGACAGG	

the glucosinolate variation of fully developed pak choi plants[21,27] or a selection of *Brassica rapa* species.[28−30] Consequently, our study aimed at determining the glucosinolate profile of 13 different pak choi cultivars as well as ascertaining whether an ontogenetic shift in glucosinolate concentration and profile during vegetative plant development occurs.

MATERIALS AND METHODS

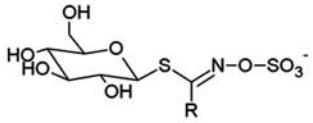
Plant Material. Different cultivars of *B. rapa* ssp. *chinensis* (pak choi) were obtained from seed companies in Taiwan (Known you Seed: cv. Sang Feng No. 2, Ky Show Jean, Speedy, Ky Late, Ky Early, Ally, New Burg, Ky All-In, Yu-Tsai-Sum, Green Master, Fun Jen), the Philippines (Allied Botanical: cv. Black Behi), and Germany (Enza Zaden Germany: cv. Joi Choi). Two of the used seed materials were signed by the company as hybrid (cv. Ky All-In and Ky Show Jean); all other cultivars were inbred. To determine only genotypic effects on glucosinolate biosynthesis, the putative environmental effects were standardized by pak choi cultivation under controlled conditions in a greenhouse. Each cultivar was sown on bars of fleece, containing 3 g of seeds per fleece (≈1100 seeds), placed in aluminum foil trays (33 × 10 cm) filled with perlite. Sprouts were kept in a greenhouse chamber arranged in a randomized complete block design with a 12 h photoperiod (220 μmol m^{-2} s^{-1} of photosynthetic active radiation), a temperature regimen of 24/20 °C (day/night)n and a relative humidity of 75%. The sprouts were watered as needed, and no fertilizer was added. The total aerial tissue of 10−12-day-old sprouts with fully expanded cotyledons was harvested. Sprouts were cut at the substrate level, and the fresh weight of tissue was recorded. From each accession, five replicates were taken. One tray with sprouts represents one replicate. Three grams of seeds produces about 1100 sprouts, and a pooled sample from sprouts of one tray was taken. Samples were frozen at −50 °C and lyophilized, and the dry weight (dw) was recorded. Dry plant material was ground to a fine powder and stored until analysis.

Additionally, each cultivar was sown in a sowing pan. After 10 days, seedlings were transplanted to 10 L pots and grown under greenhouse conditions (20 °C, photosynthetic active radiation 300 μmol m^{-2} s^{-1}, and relative humidity 60−75%). Fully developed plants (30 days old, 9−11 leaves) were harvested before flowering, and five replicates were taken of each cultivar. Plants were cut, and the fresh weight of leaves without midrib was recorded. A standardized plant material without midrib was used to compare several cultivars of pak choi that strongly differ in morphological leaf structure and portion of the midrib. A replicate comprises all leaves of two plants. Samples were frozen at −50 °C and lyophilized, and the dry weight was determined. Dry plant material was ground to a fine powder and stored until analysis.

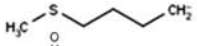
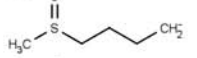
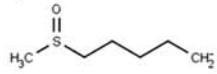
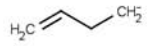
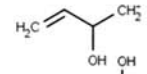
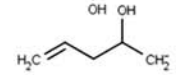
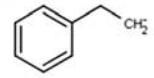
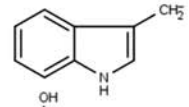
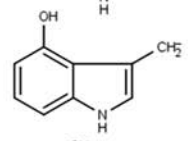
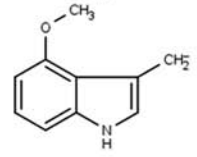
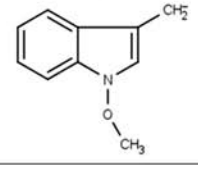
Sample Preparation and Desulfoglucosinolate Analysis. Glucosinolate concentration was determined as desulfoglucosinolates using a modified method according to DIN EN ISO 9167-1. Exactly 20 mg of powdered samples and 100 μL of 0.1 mM 2-propenyl glucosinolate (BCR-367R, Community Bureau of Reference, Brussels. Belgium) as internal standard were extracted with 750 μL of 70% (v/v) methanol heated at 70 °C, boiled for 10 min, and then centrifuged (2250*g*) for 5 min at room temperature. The supernatant was decanted and the residue re-extracted twice with 500 μL of hot 70% methanol each. The pooled extracts were loaded onto a mini column containing 500 μL of DEAD-Sephadex A-25 conditioned with 2 M acetic acid and washed with 6 M imidazole formate solution. After loading, the column was washed with 0.02 M sodium acetate buffer. Finally, 75 μL of aryl sulfatase solution (Sigma-Aldrich, Steinheim, Germany; activity > 0.5 U mL^{-1}) was added and incubated overnight. Desulfoglucosinolates were eluted with 1 mL of Milli-Q water and analyzed by HPLC using a Merck HPLC system (Merck-Hitachi, Darmstadt, Germany) with a Spherisorb ODS2 column (Bischoff, Leonberg Germany; particle size = 5 μm, 250 mm × 4 mm). HPLC conditions were as follows: solvent A, Milli-Q water; solvent B, 20% v/v acetonitrile in Milli-Q water; solvent C, 100% acetonitrile. The 60 min run consisted of 1% (v/v) B (2 min), 1−20% (v/v) B (34 min), a 6 min hold at 20% (v/v) B, 20% B to 100% (v/v) C (2 min), a 5 min hold at 100% (v/v) C, 100% (v/v) C to 1% (v/v) B (2 min), and finally a 10 min hold at 1% (v/v) B. Determination was conducted at a flow rate of 0.7 mL min^{-1} and a wavelength of 229 nm. Desulfoglucosinolates were identified on the basis of comparison of retention times and UV absorption spectra with those of known standards and were verified according to previous work.[31] Glucosinolate concentration was calculated using 2-propenyl glucosinolate as internal standard and the response factor of each compound relative to 2-propenyl glucosinolate.[32,33] Results are given as micromoles per gram dry weight (dw). Glucosinolate concentration was determined in five replicates per cultivar, and each replicate sample was measured in duplicate.

Gene Expression Analysis by Semiquantitative Real-Time RT-PCR. RNA was extracted from 100 mg of tissue using the NucleoSpin Plant Kit (Macherey-Nagel GmbH and Co. KG, Germany) including on-column DNase I digestion. RNA was quantified spectrophotometrically at 260 nm (Nanodrop ND1000, Technology Inc., USA), and the quality was checked using the ratio of the absorption at 260 and 280 nm with a ratio between 1.9 and 2.1 as acceptable. Single-stranded cDNA synthesis was carried out with total RNA using SuperScript III reverse transcriptase (Invitrogen GmbH, Germany) with oligo $d(T_{12-18})$ primers according to the manufacturer's instructions. Semiquantitative two-step real-time RT-PCR was performed using a SYBR Green 1 protocol in 96-well reaction plates on an Applied Biosystems 7500 Realtime PCR System. The following thermal profile was used for all reactions: 50 °C, 2 min, 95 °C, 10 min, 40 cycles of 95 °C, 30 s, and 60 °C, 1 min, followed by dsDNA melting curve analysis to ensure amplicon specificity. Each reaction

Table 2. Structural Formulas of the 11 Individual Glucosinolates Determined in 13 Different Pak Choi Cultivars in Sprouts and Mature Leaves



Basic structure of glucosinolates

Glucosinolate group	trivial name	chemical name	structure of R group
Aliphatic GS			
Alkyl GS	Glucoerucin	4-Methylthiobutyl	
	Glucoraphanin	4-Methylsulfinylbutyl	
	Glucoalyssin	5-Methylsulfinylpentyl	
Alkenyl GS	Gluconapin	3-Butenyl	
	Glucobrassicanapin	4-Pentenyl	
Hydroxy-Alkenyl GS	Progoitrin	2-Hydroxy-3-butenyl	
	Gluconapoleiferin	2-Hydroxy-4-pentenyl	
Aromatic GS	Gluconasturtiin	2-Phenylethyl	
Indole GS	Glucobrassicin	Indol-3-ylmethyl	
	4-Hydroxy-glucobrassicin	4-Hydroxy-indol-3-ylmethyl	
	4-Methoxy-glucobrassicin	4-Methoxy-indol-3-ylmethyl	
	Neoglucobrassicin	1-Methoxy-indol-3-ylmethyl	

GS – glucosinolates

was performed in a 10 μL volume containing 200 nM of each primer, 3 μL of cDNA (1:25 dilution in water), and 5 μL of Power SYBR Green Master Mix (Applied Biosystems, USA). Gene-specific primer sets are listed in Table 1. Data generated by semiquantitative real-time RT-PCR were collected and compiled using 7500 v2.0.1 software (Applied Biosystems). Data were exported to LinReg software[34] to determine the PCR amplification efficiency for each primer pair. Relative transcript levels were normalized on the basis of expression of an invariant control orthologous to *At3g18780*, *ACT2*, using the equation $2^{-\Delta CT}$.[35] The invariant control gene with highest homology to *ACT2* is localized on the *B. rapa* subsp. *pekinensis* BAC clone KBrB071H12 with Database GenBank Accession No. AC189447 from bp 67561 to 68935 in reverse orientation. Semiquantitative real-time RT-PCR was performed on three replicates measured in duplicate for each gene, and nontemplate controls were included.

Statistical Analysis. Statistical analyses were conducted with the software Statistica 9 for Windows (version 9, Statsoft Inc.). The total glucosinolate level and the level of each desulfoglucosinolate were subjected to analysis of variance (ANOVA) and post hoc Tukey's HSD test. The relative amounts of individual glucosinolates were analyzed using principal component analysis (PCA) and were visualized by principal score and loading plots. Each value point in the score plot represents the mean of five samples, and each point in the loading plot represents the contribution of an individual glucosinolate to the score.

RESULTS AND DISCUSSION

Glucosinolate Profile of Pak Choi Sprouts. The glucosinolate profile of pak choi sprouts was analyzed in 13 different cultivars. Pak choi is an indigenous Asian species, and thus

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Table 3. Dry Matter and Glucosinolate Levels in Pak Choi Sprouts of 13 Different Cultivars[a]

(A) Dry Matter (%), Total and Individual Levels of Aliphatic Glucosinolates (μmol g^{-1} dry weight), and Ratio of 3-Butenyl to 2-Hydroxy-3-butenyl Glucosinolate

cultivar	dry matter	total GS	4MTB	4MSOB	3But	2OH3But	4Pent	2OH4Pent	total aliphatic	ratio 3But/ 2OH3But
group I										>3
Fun Jen	6.44	36.24 ± 4.94 b	nd	nd	24.17 ± 2.70 de	7.33 ± 1.60 ab	2.38 ± 0.27 abc	nd	33.89 ± 4.57 b	3.3
Green Master	6.23	63.30 ± 13.84 c	nd	0.65 ± 0.12 c	47.93 ± 10.08 f	7.85 ± 2.31 abc	4.60 ± 0.93 de	nd	61.03 ± 13.44 c	6.1
Joi Choi	8.37	26.67 ± 6.47 ab	nd	nd	22.10 ± 5.48 cde	2.29 ± 0.48 a	1.39 ± 0.37 ab	nd	25.78 ± 6.32 ab	9.7
group IIa										≈2
Ally	8.93	28.42 ± 6.79 ab	nd	nd	16.07 ± 3.41 abcd	10.28 ± 2.90 bcd	1.16 ± 0.25 a	0.12 ± 0.05 ab	27.63 ± 6.61 ab	1.6
Ky Show Jean	7.85	36.90 ± 4.85 b	nd	nd	24.49 ± 3.60 e	9.94 ± 0.87 bcd	1.64 ± 0.26 abc	0.13 ± 0.02 ab	36.20 ± 4.74 b	2.5
Sang Feng No. 2	6.05	36.52 ± 1.58 b	nd	nd	21.86 ± 0.53 bcde	10.57 ± 0.92 bcd	3.21 ± 0.07 cd	0.22 ± 0.01 bc	35.86 ± 1.53 b	2.1
group IIb										≈1
Ky All-In	8.61	37.48 ± 11.96 b	nd	nd	13.42 ± 2.55 a	21.50 ± 8.68 e	1.34 ± 0.28 ab	0.27 ± 0.08 bcd	36.53 ± 11.60 b	0.6
Ky Early	9.34	19.36 ± 3.90 a	nd	nd	9.45 ± 2.32 a	6.05 ± 0.78 ab	2.97 ± 0.65 bcd	0.20 ± 0.05 bc	18.68 ± 3.80 a	1.6
Ky Late	8.50	38.20 ± 5.46 b	nd	nd	14.72 ± 1.91 abc	13.92 ± 2.31 cd	7.82 ± 1.04 f	0.67 ± 0.12 ef	37.13 ± 5.38 b	1.1
Speedy	6.35	31.83 ± 3.80 ab	nd	nd	13.76 ± 1.45 ab	14.62 ± 2.04 d	2.07 ± 0.19 abc	0.32 ± 0.02 cd	30.78 ± 3.70 ab	0.9
Yu-Tsai-Sum	7.61	63.43 ± 11.71 c	nd	nd	26.16 ± 5.42 e	22.88 ± 3.58 e	11.85 ± 2.25 g	0.81 ± 0.12 f	61.69 ± 11.37 c	1.1
group III										≈1
Black Behi	6.86	27.54 ± 7.37 ab	0.48 ± 0.15 a	0.22 ± 0.06 a	9.82 ± 2.84 a	8.97 ± 2.45 bcd	4.67 ± 1.35 de	0.65 ± 0.17 e	24.85 ± 7.01 ab	1.1
New Burg	9.37	26.89 ± 4.53 ab	2.39 ± 0.42 b	0.52 ± 0.09 b	9.16 ± 1.46 a	7.38 ± 1.21 ab	4.99 ± 0.83 e	0.40 ± 0.06 d	24.81 ± 4.07 ab	1.2

(B) Total and Individual Levels of Indole and Aromatic Glucosinolates (μmol g^{-1} dry weight)

cultivar	I3M	4OHI13M	4MOI3M	1MOI3M	2PE	total indole
group I						
Fun Jen	0.53 ± 0.07 bc	0.09 ± 0.04 c	1.13 ± 0.13 d	0.60 ± 0.12 ef	nd	2.35 ± 0.36 c
Green Master	0.33 ± 0.06 ab	0.06 ± 0.02 bc	1.38 ± 0.23 e	0.50 ± 0.08 de	nd	2.27 ± 0.40 c
Joi Choi	0.31 ± 0.08 a	0.02 ± 0.01 a	0.37 ± 0.04 b	0.18 ± 0.03 a	nd	0.89 ± 0.15 a
group IIa						
Ally	0.34 ± 0.08 ab	0.02 ± 0.004 a	0.21 ± 0.05 ab	0.22 ± 0.04 ab	nd	0.79 ± 0.18 a
Ky Show Jean	0.32 ± 0.07 a	0.04 ± 0.005 ab	0.13 ± 0.01 a	0.22 ± 0.01 a	nd	0.70 ± 0.10 a
Sang Feng No. 2	0.22 ± 0.01 a	0.02 ± 0.001 a	0.19 ± 0.01 ab	0.23 ± 0.01 ab	nd	0.66 ± 0.04 a
group IIb						
Ky All-In	0.39 ± 0.14 ab	0.01 ± 0.005 a	0.28 ± 0.13 ab	0.25 ± 0.09 abc	nd	0.94 ± 0.36 a
Ky Early	0.25 ± 0.04 a	0.01 ± 0.005 a	0.21 ± 0.01 ab	0.21 ± 0.04 a	nd	0.68 ± 0.10 a
Ky Late	0.41 ± 0.04 ab	0.02 ± 0.01 a	0.27 ± 0.01 ab	0.37 ± 0.01 bcd	nd	1.07 ± 0.08 a
Speedy	0.42 ± 0.04 ab	0.02 ± 0.002 a	0.30 ± 0.04 ab	0.32 ± 0.02 abc	nd	1.05 ± 0.10 a
Yu-Tsai-Sum	0.67 ± 0.15 c	0.02 ± 0.005 a	0.31 ± 0.06 ab	0.74 ± 0.12 f	nd	1.73 ± 0.34 b

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Table 3. continued

	(B) Total and Individual Levels of Indole and Aromatic Glucosinolates (μmol g^{-1} dry weight)											
cultivar	I3M		4OHI13M		4MOI3M		1MOI3M		2PE		total indole	
group III												
Black Behi	0.35 ± 0.08	ab	0.16 ± 0.05	d	0.79 ± 0.16	c	0.38 ± 0.06	cd	0.05 ± 0.01	a	1.70 ± 0.34	b
New Burg	0.64 ± 0.23	c	0.01 ± 0.01	a	0.34 ± 0.04	b	0.98 ± 0.16	g	0.08 ± 0.01	b	1.97 ± 0.44	b

[a]GS, glucosinolate; 4MTB, 4-methylthiobutyl; 4MSOB, 4-methylsulfinylbutyl; 3But, 3-butenyl; 2OH3But, 2-hydroxy-3-butenyl; 4Pent, 4-pentenyl; 2OH4Pent, 2-hydroxy-4-pentenyl; I3M, indol-3-ylmethyl; 4OHI3M, 4-hydroxyindol-3-ylmethyl; 4MOI3M, 4-methoxyindol-3-ylmethyl; 1MOI3M, 1-methoxyindol-3-ylmethyl; 2PE, 2-phenylethyl; nd, not detected. Means within columns followed by the same letter are not significantly different ($p \leq 0.05$, post hoc Tukey's HSD test). Each value represents the mean ± SD of five replicates.

almost all seeds of analyzed cultivars were obtained from seed companies in Asia. To standardize environmental effects on glucosinolate biosynthesis, sprouts and mature plants were cultivated under the same controlled conditions in a greenhouse chamber. In all cultivars 11 individual glucosinolates (Table 2) were quantitatively determined (Table 3). The aliphatic glucosinolate group contained two alkyl glucosinolates (4-methylthiobutyl, 4-methylsulfinylbutyl), two alkenyl glucosinolates (3-butenyl, 4-pentenyl), and two hydroxyalkenyl glucosinolates (2-hydroxy-3-butenyl, 2-hydroxy-4-pentenyl). The group of indole glucosinolates comprised indol-3-ylmethyl glucosinolate, 4-hydroxyindol-3-ylmethyl glucosinolate, 1-methoxyindol-3-ylmethyl glucosinolate, and 4-methoxyindol-3-ylmethyl glucosinolate, but the latter in only trace amounts. Furthermore, in two pak choi cultivars (Black Behi, New Burg) low levels of the aromatic 2-phenylethyl glucosinolate were found.

The aliphatic glucosinolates were the predominant glucosinolate group in sprouts of all pak choi cultivars, ranging from 92 to 98% of the total amount (Table 3A; Table S1 in the Supporting Information). However, there were pronounced differences in the total aliphatic glucosinolate concentrations ranging from 18.7 to 61.7 μmol g^{-1} dw (Table 3A). Indol-3-ylmethyl glucosinolate and its derivatives were found in all sprouts. They occurred at low levels varying in total from 0.68 to 2.35 μmol g^{-1} dw (Table 3B), representing 2−7% of the total glucosinolate profile.

The relative amount of the 11 identified glucosinolates of sprouts from the 13 pak choi cultivars were subjected to PCA to determine the genotypic differences in their glucosinolate profile. About 86% of the variance was accounted for by the first three principal components (PCs). The two highest ranking PCs obtained accounted for 49% (PC 1) and 22% (PC 2) of the total variance and were plotted in two-dimensional space (Figure 1). The PCA revealed three well-defined distinct groups of pak choi sprouts (Figure 1A) indicating key differences in the glucosinolate profiles between these cultivar groups. Both PCs play a significant role in discriminating between the pak choi sprouts of different cultivars as reflected by the corresponding loadings of each PC (Figure 1B). In particular, PC 1 was characterized by a high positive loading of 3-butenyl glucosinolate, whereas negative loadings of PC 1 corresponded to all subclasses of glucosinolates (aliphatic, alkyl, 4-methylthiobutyl, and 4-methylsulfinylbutyl; alkenyl, 4-pentenyl; hydroxyalkenyl, 2-hydroxy-4-pentenyl; indole, indol-3-ylmethyl, and 1-methoxyindol-3-ylmethyl; aromatic, 2-phenylethyl). PC 2 was also dominated by a high positive loading of 3-butenyl glucosinolate as well as 4-methoxyindol-3-ylmethyl glucosinolate and 2-hydroxy-3-butenyl glucosinolate by a negative loading. Thus, the group separation observed in PCA is explained in terms of the identified glucosinolates and their contribution to the total glucosinolate concentration.

Group I consisted of three pak choi cultivars (Fun Jen, Green Master, Joi Choi) that at the sprout stage had a glucosinolate profile dominated by 3-butenyl glucosinolate (67−83%). Together with 2-hydroxy-3-butenyl glucosinolate, the butenyl glucosinolates accounted for 87−92% of the total glucosinolate level. In contrast to butenyl glucosinolates, the pentenyl glucosinolate 4-pentenyl was found at far lower levels (5−7%), and 2-hydroxy-4-pentenyl was not detected at all (Table 3; Table S1 in the Supporting Information). These findings are in accordance with our expression studies of selected genes involved in aliphatic glucosinolate biosynthesis (Table 4 and Figure 2). In particular, this group showed a very low relative transcript level of *BrGSL-OH*. *BrGSL-OH* (Database GenBank accession no. FJ376074[36]) is a sequence in the *B. rapa* genome

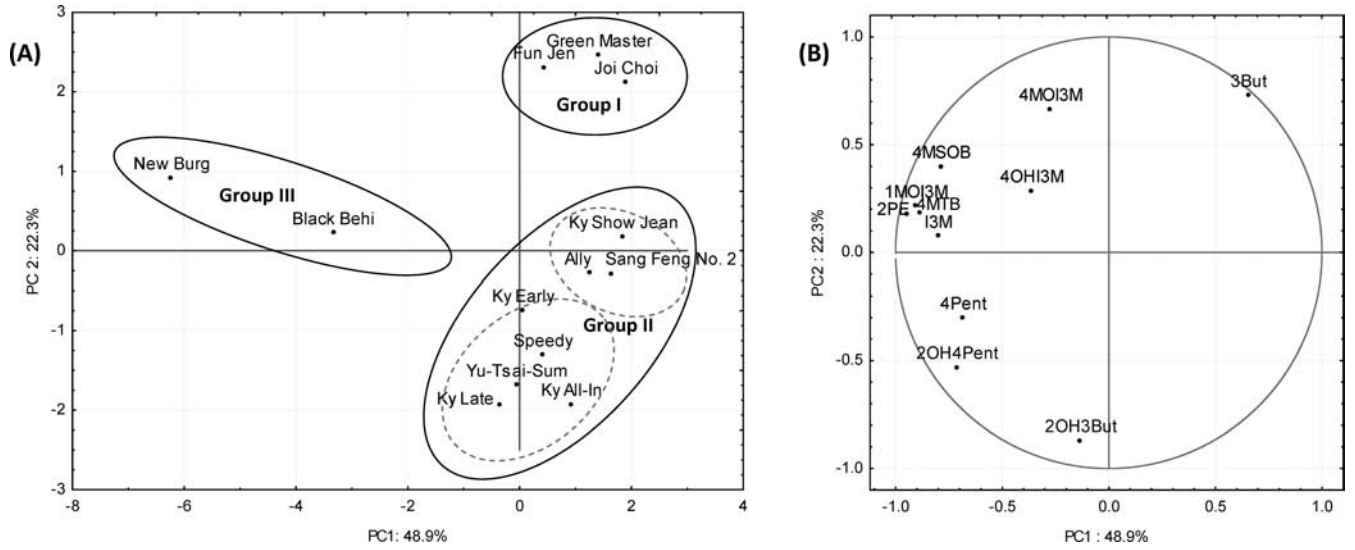

Figure 1. Score (A) and loading (B) plots of PCA results obtained by analyzing the relative amounts of 11 glucosinolates determined in sprouts of 13 different pak choi cultivars. 4MTB, 4-methylthiobutyl; 4MSOB, 4-methylsulfinylbutyl; 3But, 3-butenyl; 2OH3But, 2-hydroxy-3-butenyl; 4Pent, 4-pentenyl; 2OH4Pent, 2-hydroxy-4-pentenyl; I3M, indol-3-ylmethyl; 4OHI3M, 4-hydroxyindol-3-ylmethyl; 4MOI3M, 4-methoxyindol-3-ylmethyl; 1MOI3M, 1-methoxyindol-3-ylmethyl; 2PE, 2-phenylethyl.

with the closest sequence identity (85%) to the experimentally confirmed sequence At2g25450 of *Arabidopsis thaliana* encoding a hydroxylase catalyzing the conversion of alkenyl to hydroxyalkenyl glucosinolates. Thus, the low level of its expression may explain the low level of hydroxyalkenyl glucosinolates detected. The relative amount of indole glucosinolates varied from 3 to 6% with 4-methoxyindol-3-ylmethyl glucosinolate being the major indole glucosinolate present (Table 3B). The three pak choi cultivars of group I can be grown year-round and exhibit a strong tolerance to higher cultivation temperature, indicating that the origin of these pak choi cultivars is from tropical and subtropical areas.[25] These cultivars are morphologically characterized by a white midrib and white thickened petiole in mature plants, characteristics suggesting that these cultivars belong to the Chinese white pak type.[19]

Group II comprised pak choi cultivars having sprouts that also contained high levels of 3-butenyl glucosinolate and 2-hydroxy-3-butenyl glucosinolate; however, in contrast to group I, these two glucosinolates were found in different proportional ratios (Table 3A). In contrast to group I, group II contained 2-hydroxy-4-pentenyl glucosinolate. On the basis of the proportional ratio of 3-butenyl to 2-hydroxy-3-butenyl glucosinolate and the relative amounts of pentenyl glucosinolates, group II could be further split into two subgroups. Subgroup IIa contains pak choi cultivars that have a ratio of 3-butenyl to 2-hydroxy-3-butenyl glucosinolate of around 2 together with very low levels of 2-hydroxy-4-pentenyl glucosinolate (0.4−0.6% of total glucosinolate level) and also 4-pentenyl glucosinolate (4−6%), whereas subgroup IIb is characterized by a ratio of 3-butenyl to 2-hydroxy-3-butenyl glucosinolate of around 1 with increased levels of both pentenyl glucosinolates (0.6−1.6% 2-hydroxy-4-pentenyl and 4−20% 4-pentenyl glucosinolate, respectively) (Table 3A). The distinct difference in the glucosinolate profile between groups I and II, that is, with a shift to hydroxyalkenyl glucosinolates in group II, is emphasized by the increased relative transcript level of *BrGSL-OH* (Table 4). The *BrGSL-OH* expression levels are not sufficient for a distinct separation of subgroups IIa and IIb. Nevertheless, changes in glucosinolate accumulation and expression patterns of glucosinolate biosynthesis genes suggest that regulation is on transcriptional, translational, and post-translational levels. In addition, there are also morphological differences between the pak choi cultivars of groups I and II at the mature stage (except cv. Speedy and Sang Feng No. 2). In detail, the pak choi cultivars of group II grow straight and have an erect appearance with white to light green petioles, thin leaf stalks, and more slender leaves; hence, according to Larkcom,[19] these cultivars fall into the green leaf stalk category.

Group III comprised two cultivars: Black Behi and New Burg. Sprouts of this group were characterized by a diverse range of aliphatic glucosinolates, and in addition to the butenyl and pentenyl glucosinolates, butyl glucosinolates with a nonoxidized and oxidized thiolgroup (4-methylthiobutyl glucosinolate and 4-methylsulfinylbutyl glucosinolate) were also detected (Table 3A). Moreover, and in contrast to all other pak choi cultivars, the sprouts of these cultivars contained the aromatic 2-phenylethyl glucosinolate, although at a low level (Table 3B) when compared to previous studies on pak choi cultivars that had geographically originated from China.[26,37] Furthermore, cultivars of group III had a high relative abundance of indole glucosinolates of approximately 7% with 1-methoxyindol-3-ylmethyl glucosinolate as the major indole glucosinolate (Table 3B).

The differentiation of 13 pak choi cultivars into three groups is partially supported by the relative expression analysis of selected genes involved in the biosynthesis of aliphatic glucosinolates. Using semiquantitative real-time RT-PCR, we analyzed the expression of methylthioalkylmalate synthases (*BrMAM1*, *BrMAM3*, and $BrGS_{ELONG}$), 2-oxoglutatate-dependent dioxygenase 2 (*BrAOP2-1*), and 2-oxoacid-dependent dioxygenase (*BrGSL-OH*).[36] The genes *BrMAM1*, *BrMAM3*, and $BrGS_{ELONG}$ encode methylthioalkylmalate synthases responsible for the condensation of 2-oxoacid with acetyl-CoA as a first step of three for elongation of methionine by a single methylene group ($-CH_2-$) as product (Figure 2). *BrAOP2* and *BrGSL-OH* are genes encoding putative enzymes for side-chain modification of aliphatic glucosinolates in *B. rapa*. AOP2 catalyzes the conversion of S-oxygenated glucosinolates

Table 4. Relative Expression of Five Different Genes Involved in Aliphatic Glucosinolate Biosynthesis in Sprouts of 13 Different Pak Choi Cultivars[a]

cultivar	*BrMAM1*	*BrMAM3*	$BrGS_{ELONG}$	*BrAOP2-1*	*BrGSL-OH*
group I					
Fun Jen	0.005 ± 0.004	0.001 ± 0.000	0.001 ± 0.001	0.114 ± 0.009	0.090 ± 0.008
Green Master	0.008 ± 0.005	0.004 ± 0.002	0.002 ± 0.001	0.040 ± 0.013	0.000 ± 0.000
Joi Choi	0.009 ± 0.003	0.002 ± 0.001	0.005 ± 0.002	0.062 ± 0.005	0.010 ± 0.004
group IIa					
Ally	0.023 ± 0.009	0.011 ± 0.003	0.016 ± 0.001	0.041 ± 0.010	0.477 ± 0.067
Ky Show Jean	0.009 ± 0.002	0.003 ± 0.001	0.004 ± 0.001	0.032 ± 0.001	0.355 ± 0.040
Sang Feng No. 2	0.006 ± 0.003	0.002 ± 0.001	0.010 ± 0.004	0.074 ± 0.025	0.566 ± 0.043
group IIb					
Ky All-In	0.004 ± 0.003	0.005 ± 0.002	0.001 ± 0.000	0.017 ± 0.002	1.425 ± 0.375
Ky Early	0.009 ± 0.008	0.003 ± 0.003	0.008 ± 0.011	0.080 ± 0.027	0.381 ± 0.110
Ky Late	0.003 ± 0.001	0.000 ± 0.000	0.000 ± 0.000	0.090 ± 0.007	0.315 ± 0.035
Speedy	0.012 ± 0.005	0.003 ± 0.001	0.003 ± 0.001	0.057 ± 0.016	0.575 ± 0.046
Yu-Tsai-Sum	0.001 ± 0.001	0.001 ± 0.000	0.000 ± 0.000	0.182 ± 0.030	0.394 ± 0.039
group III					
Black Behi	0.024 ± 0.003	0.005 ± 0.004	0.008 ± 0.001	0.080 ± 0.009	0.469 ± 0.072
New Burg	0.018 ± 0.020	0.001 ± 0.000	0.005 ± 0.005	0.234 ± 0.125	0.224 ± 0.094

[a] *BrMAM1*, methylthioalkylmalate synthase 1; *Br-MAM3*, methylthioalkylmalate synthase 3; $BrGS_{ELONG}$, methylthioalkylmalate synthase; *BrAOP2-1*, 2-oxoglutarate-dependent dioxygenase 2; *BrGSL-OH*, 2-oxoacid-dependent dioxygenase. Each value represents the mean ± SD of three replicates.

to alkenyl glucosinolates. GSL-OH is responsible for the biosynthesis of hydroxylated alkenyl glucosinolates. Whereas relative expression levels of *BrMAM1*, *BrMAM3*, and $BrGS_{ELONG}$ did not show any correlation to the glucosinolate profile, the relative expression of *BrAOP2-1* was correlated with the relative amount of 4-pentenyl glucosinolate plus 2-hydroxy-4-pentenyl glucosinolate (R^2 = 0.54). Most strikingly, the relative expression of *BrGSL-OH* supports our group classification and correlates with the ratio of 3-butenyl glucosinolate to 2-hydroxy-3-butenyl glucosinolate (R^2 = 0.58). This analysis also demonstrates that cultivars of group I with the lowest levels of 2-hydroxy-3-butenyl glucosinolate and without 2-hydroxy-4-pentenyl glucosinolate have a very low expression of *BrGSL-OH*, whereas cultivars in group II with increasing relative amounts of 2-hydroxy-3-butenyl glucosinolate and 2-hydroxy-4-pentenyl glucosinolate show increasing relative expression of this gene as well (Table 4). Taken together, our study clearly shows that the pak choi cultivars were differentiated into three groups according to their glucosinolate profile of the sprout stage and that one determinant of this difference is the expression level of *BrGSL-OH*.

Ontogenetic Differences in the Glucosinolate Profile of Pak Choi. After grouping the 13 pak choi cultivars according to their glucosinolate profile obtained from their sprouts, we next analyzed whether this grouping was also reflected by the glucosinolate profile in leaves of mature pak choi plants. When compared to sprouts, the total glucosinolate levels of mature leaves were more than doubled in seven cultivars (mainly from group I and subgroup IIa), and this was predominately due to increasing levels of the aliphatic 4-pentenyl glucosinolate. In the six other cultivars, the concentration of 4-pentenyl glucosinolate showed no ontogenetic change and hence no increased total glucosinolate concentration in mature pak choi leaves (mainly from subgroup IIb and group III). Interestingly, an additional aliphatic glucosinolate, 5-methylsulfinylpentyl glucosinolate, was found in mature leaves of almost all pak choi cultivars (except cv. Ky All-In; Table 5; Table S2 in the Supporting Information). This ontogenetic variation is in contrast to other *Brassica* species (*B. carinata*, *B. nigra*, *B. juncea*, and *B. rapa* ssp. *pekinensis*) in which no additional glucosinolate occurred during plant development.[38]

Aliphatic glucosinolates were the major glucosinolate group in sprouts of all 13 tested pak choi cultivars and remained as such in mature leaves (Table 5), a finding that is consistent with various other *Brassica oleracea* varieties.[39] As the genome evolution of *B. rapa* is based on wild *B. oleracea*,[40] this genetic relationship between *B. oleracea* and *B. rapa* might explain the similar aliphatic glucosinolate responses during plant development. However, the relative amount of aliphatic glucosinolates decreased, whereas the relative amount of indole glucosinolates strongly increased in the mature leaves of all cultivars (Table 5). Furthermore, the increase in total indole glucosinolate level on a dry weight basis ranged from 2.5-fold (Yu-Tsai-Sum) to 25-fold (Sang Feng No. 2). The highest increase was always found for 1-methoxyindol-3-ylmethyl glucosinolate, making it the predominant indole glucosinolate of mature leaves in all cultivars tested (Table 5B). Thus, a change in the relative amounts of aliphatic and indole glucosinolates throughout growth showed opposite trends, that is, with the former decreasing and the latter increasing with growth. This ontogenetic-mediated shift in the glucosinolate profile with pronounced increases in indole glucosinolate levels might be caused by the reciprocal negative control of methionine- and tryptophan-derived glucosinolate pathways as demonstrated in *A. thaliana*.[41] In detail, overexpression of the positive regulator of indole glucosinolate genes (*IQD1*) leads to repression of the *CYP79F1* and *CYP79F2* genes involved in aliphatic glucosinolate biosynthesis. Conversely, positive regulators of aliphatic glucosinolate biosynthesis were shown to down-regulate the expression of regulators of indole glucosinolate (*ATR1/MYB34*, *HIG1/MYB51*, *HIG2/MYB122*).[42]

The relative amount of total aliphatic glucosinolates decreased in mature leaves compared to sprouts with a simultaneous increase of indole glucosinolates (Tables 3 and 5). However, the concentration of total aliphatic glucosinolates on a dry weight basis was significantly reduced only in the mature leaves of cv. Ky-All-In, Ky Late, and Yu-Tsai-Sum, all classified in subgroup IIb, that showed a decrease of the two butenyl glucosinolates, 3-butenyl and 2-hydroxy-3-butenyl (Table 5). Brown and co-workers[32] revealed during growth of *A. thaliana* that the rosette leaves showed a decreasing concentration of absolute aliphatic glucosinolates based especially on the predominant 4-methylsulfinylbutyl glucosinolate. Taken together,

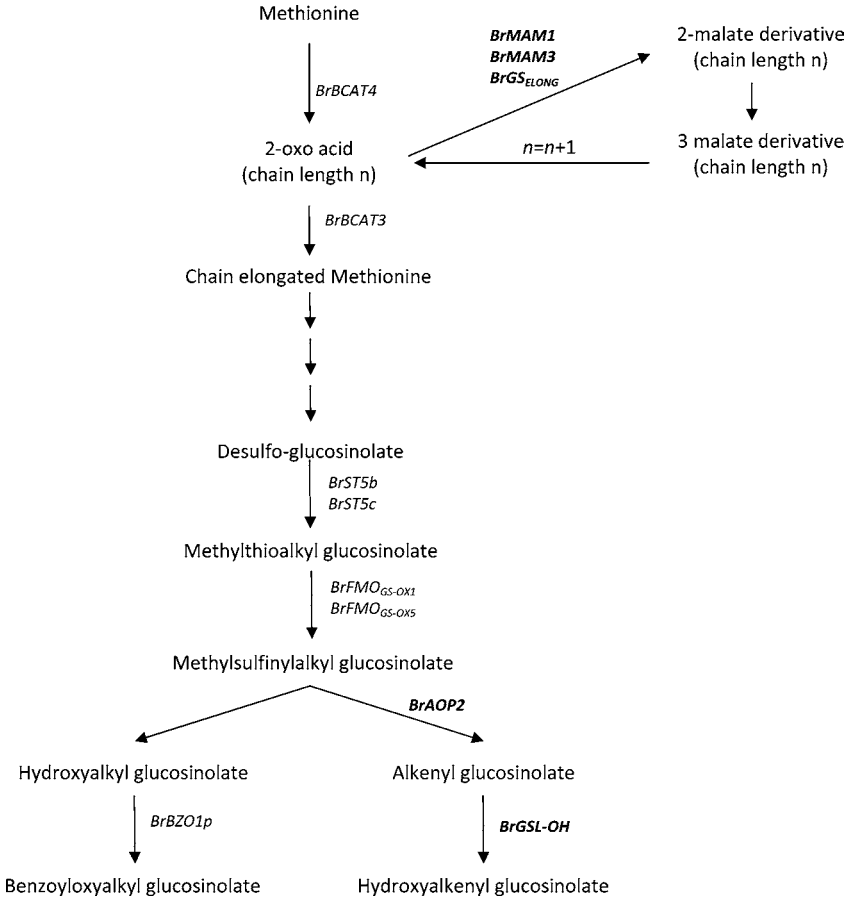

Figure 2. Biochemical pathway of aliphatic glucosinolates in *Brassica rapa*. Adapted from Figure 1 of Zang and co-workers.[36] Analyzed genes are given in bold. *BrBCAT3*, *BrBCAT4*, branched-chain aminotransferase; *BrMAM1*, *BrMAM3*, $BrGS_{ELONG}$, methylthioalkylmalate synthases; *BrST5b*, *BrST5c*, sulfotransferase; $BrFMO_{GS-OX1}$, $BrFMO_{GS-OX5}$, flavin-monooxygenase glucosinolate *S*-oxyenase; *BrAOP2*, 2-oxoglutarate-dependent dioxygenase; *BrBZO1p*, benzoate-CoA ligase; *BrGSL-OH*, 2-oxoacid-dependent dioxygenase.

these findings in pak choi and *A. thaliana* might indicate that during growth the changes in the composition of aliphatic glucosinolates in leaves are mainly caused by a decrease in butyl glucosinolates. Due to the ontogenetic modifications during plant development in mature leaves, the relative amounts of aliphatic and indole glucosinolates show only small variations between the different cultivars. Thus, it is not possible to use the glucosinolate profiles of mature pak choi plants for distinct differentiation in cultivar groups.

Brassica sprouts are preferentially consumed as a fresh product. Thus, prior to consumption, no loss of glucosinolates by preparation and domestic processing occurs due to tissue damage, leaching, or thermally and enzyme-catalyzed degradation.[43,44] Plant-based and bacteria-derived myrosinase catalyze hydrolysis of glucosinolates. This leads to the formation of functional breakdown products such as isothiocyanates and nitriles. Myrosinases are activated during the consumption and digestion of fresh products,[45] enabling a maximum of potential health-promoting glucosinolate breakdown products to be taken up. In this context, pak choi sprouts can also be described as potential natural functional food. In previous animal studies, for instance by Lakshmy et al.,[46] goitrogenic effects are sometimes described as caused only by the breakdown product of 2-hydroxy-3-butenyl glucosinolate, oxazolidine-2-thione. However, the concentration of 2-hydroxy-3-butenyl glucosinolate was remarkably low in pak choi sprouts. Thus, it could be assumed that pak choi sprouts are not associated with a putative health risk as proposed by Renko and co-workers.[47] Interestingly, they demonstrated that pak choi extract inhibits deiodinase and thyreoperoxidase activity in vitro but only at unphysiologically high concentrations ($\geq$100 μM). As a further harmful aspect, it was found that individual breakdown products of 1-methoxyindol-3-ylmethyl, indol-3-ylmethyl, and 2-propenyl glucosinolate given as purified glucosinolates or juice of broccoli floret heads are able to form DNA adducts and have mutagenic and genotoxic properties.[48−50] However, in pak choi sprouts only low levels of indol-3-ylmethyl and 1-methoxyindol-3-ylmethyl glucosinolate were detected. Additionally, individual analysis of feeding studies in humans showed that glucosinolates at such low levels are not able to form detectable protein adducts (Glatt and Barknowitz, unpublished data).

This study has shown that there are distinct differences in the glucosinolate profiles of pak choi sprouts from (I) different cultivars, (II) ontogenetic variation of the (III) glucosinolate composition, and (IV) individual glucosinolate concentration as revealed by the ratios of butyl/pentyl glucosinolate, 3-butenyl/2-hydroxy-3-butenyl glucosinolate, and aliphatic/indole glucosinolate. These differences could be used in future studies for in vitro and in vivo analyses of additive, synergistic, or suppressive functional effects of various glucosinolate compositions and their corresponding breakdown products with a relative uniform genetic background and within the same *Brassica* plant tissue.

Table 5. Dry Matter and Glucosinolate Levels in Pak Choi Leaves of 13 Different Cultivars[a]

(A) Dry Matter (%), Total and Individual Levels of Aliphatic Glucosinolates (μmol g^{-1} dry weight), and Ratio of 3-Butenyl to 2-Hydroxy-3-butenyl Glucosinolate

cultivar	total	dry matter	4MTB	4MSOB	5MSOP	3 But	2OH3But	4Pent	2OH4Pent	total aliphatic	ratio 3But/ 2OH3But
group I											
Fun Jen	106.84 ± 13.98 d	6.44	nd	nd	3.57 ± 1.60 abcd	70.67 ± 5.07 e	3.96 ± 1.47 ab	15.28 ± 3.29 cde	0.77 ± 0.22 ab	94.25 ± ± 11.43 c	17.8
Green Master	50.38 ± 6.75 a	6.21	0.30 ± 0.40 a	6.57 ± 1.36 c	4.66 ± 1.25 bcd	22.10 ± 0.97 c	3.23 ± 0.47 ab	3.09 ± 0.91 ab	nd	39.99 ± 5.35 a	6.8
Joi Choi	90.30 ± 22.16 bc	8.75	nd	nd	4.19 ± 0.55 bcd	44.37 ± 9.20 d	6.06 ± 1.40 abc	20.19 ± 7.29 e	nd	74.82 ± 18.44 c	7.3
group IIa											
Ally	86.73 ± 19.59 bc	9.18	nd	nd	8.38 ± 1.23 e	25.60 ± 6.65 c	21.82 ± 3.47 e	18.63 ± 3.18 e	3.75 ± 1.90 c	78.18 ± 14.52 bc	1.2
Ky Show Jean	86.24 ± 15.09 bc	7.75	0.74 ± 0.30 a	nd	8.39 ± 1.00 e	25.55 ± 4.28 c	15.04 ± 3.90 de	19.50 ± 2.85 e	1.14 ± 0.32 ab	70.36 ± 12.32 b	1.7
Sang Feng No. 2	87.69 ± 29.60 bc	5.70	0.70 ± 0.47 a	nd	2.22 ± 1.34 ab	11.47 ± 1.44 ab	41.51 ± 13.91 g	11.47 ± 4.57 bcde	3.66 ± 2.79 c	71.03 ± 21.72 bc	0.3
group IIb											
Ky All-In	35.63 ± 10.48 a	8.71	nd	2.18 ± 0.11 a	nd	4.36 ± 1.04 a	12.19 ± 2.81 cd	7.53 ± 3.71 abc	1.24 ± 0.59 ab	27.51 ± 7.67 a	0.4
Ky Early	83.47 ± 28.75 bc	9.57	nd	nd	6.77 ± 2.11 de	36.20 ± 9.98 d	9.90 ± 3.54 bcd	19.61 ± 8.54 e	nd	72.48 ± 24.17 bc	3.6
Ky Late	36.00 ± 9.32 a	8.49	nd	nd	2.79 ± 0.37 abc	5.82 ± 1.23 a	8.20 ± 1.11 a	8.51 ± 2.56 abcd	2.57 ± 1.50 abc	27.89 ± 5.26 a	0.7
Speedy	79.53 ± 32.83 b	6.27	0.30 ± 0.23 a	nd	1.33 ± 0.94 a	20.70 ± 8.80 bc	29.12 ± 5.30 f	16.24 ± 12.62 de	2.60 ± 2.59 bc	70.30 ± 27.89 b	0.7
Yu-Tsai-Sum	50.14 ± 23.63 a	7.60	nd	nd	5.57 ± 3.39 cd	18.23 ± 10.90 bc	9.63 ± 4.24 bcd	12.00 ± 3.56 cde	0.53 ± 0.39 ab	45.96 ± 22.09 a	1.9
group III											
Black Behi	38.10 ± 16.59 a	6.88	nd	nd	2.12 ± 0.82 ab	7.70 ± 2.06 a	8.09 ± 3.33 abcd	8.82 ± 4.41 abcd	1.32 ± 1.32 ab	28.06 ± 10.62 a	1.0
New Burg	38.91 ± 12.19 a	9.32	2.37 ± 1.29 b	4.99 ± 1.63 b	13.52 ± 3.60 f	4.91 ± 1.15 a	1.16 ± 0.27 a	2.52 ± 0.82 a	0.40 ± 0.35 a	29.88 ± 8.77 a	4.2

(B) Total and Individual Levels of Indole and Aromatic Glucosinolates (μmol g^{-1} dry weight)

cultivar	I3M	4OHI3M	4MOI3M	1MOI3M	2PE	total indole
group I						
Fun Jen	4.69 ± 0.93 f	nd	1.48 ± 0.37 b	6.42 ± 1.03 bcde	nd	12.59 ± 2.33 cd
Green Master	0.88 ± 0.29 a	0.27 ± 0.10 ab	0.43 ± 0.20 a	8.81 ± 0.81 efg	nd	10.39 ± 1.40 bc
Joi Choi	3.59 ± 1.11 def	0.20 ± 0.04 ab	0.41 ± 0.32 a	11.28 ± 2.26 g		15.48 ± 3.72 d
group IIa						
Ally	2.28 ± 0.50 bcd	1.33 ± 0.69 d	1.35 ± 0.34 b	3.60 ± 1.63 ab	nd	8.55 ± 3.16 b
Ky Show Jean	4.86 ± 0.40 f	0.65 ± 0.13 bc	0.40 ± 0.20 a	9.97 ± 1.72 fg	nd	15.88 ± 2.45 d
Sang Feng No. 2	3.71 ± 2.18 ef	0.42 ± 0.24 abc	0.89 ± 0.20 ab	11.64 ± 2.49 g	nd	16.66 ± 5.10 d
group IIb						
Ky All-In	3.11 ± 0.91 cde	0.01 ± 0.04 a	0.98 ± 0.11 ab	4.03 ± 1.16 abc	nd	8.12 ± 2.22 b
Ky Early	2.12 ± 0.81 abc	0.37 ± 0.26 abc	1.07 ± 0.22 ab	7.43 ± 3.28 def	nd	10.99 ± 4.58 bc
Ky Late	3.06 ± 1.01 cde	0.82 ± 0.07 cd	0.91 ± 0.13 ab	3.32 ± 1.35 ab	nd	8.12 ± 2.55 b
Speedy	1.44 ± 0.67 ab	0.82 ± 0.55 cd	0.47 ± 0.08 a	6.49 ± 1.06 cde	nd	9.23 ± 2.35 bc
Yu-Tsai-Sum	1.86 ± 0.33 abc	nd	0.61 ± 0.11 a	1.72 ± 0.72 a	nd	4.18 ± 1.15 a

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Table 5. continued

	(B) Total and Individual Levels of Indole and Aromatic Glucosinolates (μmol g^{-1} dry weight)											
cultivar	I3M		4OHI3M		4MOI3M		1MOI3M		2PE		total indole	
group III												
Black Behi	1.91 ± 0.74	abc	0.61 ± 0.47	bc	0.70 ± 0.07	ab	5.66 ± 3.14	bcd	1.18 ± 0.24		8.87 ± 4.41	bc
New Burg	0.88 ± 0.31	a	nd		3.45 ± 1.52	d	4.71 ± 1.24	bcd	nd		9.04 ± 3.07	bc

[a]4MTB, 4-methylthiobutyl; 4MSOB, 4-methylsulfinylbutyl; 5MSOP, 5-methylsulfinylpentyl; 3But, 3-butenyl; 2OH3But, 2-hydroxy-3-butenyl; 4Pent, 4-pentenyl; 2OH4Pent, 2-hydroxy-4-pentenyl; I3M, indol-3-ylmethyl; 4OHI3M, 4-hydroxyindol-3-ylmethyl; 4MOI3M, 4-methoxyindol-3-ylmethyl; 1MOI3M, 1-methoxyindol-3-ylmethyl; 2PE, 2-phenylethyl; nd, not detected. Means within columns followed by the same letter are not significantly different ($p \leq 0.05$, post hoc Tukey's HSD test). Each value represents the mean ± SD of five replicates.

■ ASSOCIATED CONTENT

Supporting Information

Additional tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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