Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Variation in glucosinolates in pak choi cultivars and various organs at different stages of vegetative growth during the harvest period*

Biao ZHU[1], Jing YANG[2], Zhu-jun ZHU[†‡1,2]

([1]*Department of Horticulture, Zhejiang University, Hangzhou 310058, China*)

([2]*School of Agricultural and Food Science, Zhejiang Agricultural & Forestry University, Hangzhou 311300, China*)

[†]E-mail: zhjzhu@zju.edu.cn

Received Aug. 5, 2012; Revision accepted Dec. 4, 2012; Crosschecked Mar. 19, 2013

Abstract: Glucosinolates (GSs) play an important role in plant defense systems and human nutrition. We investigated the content and composition of GSs in the shoots and roots of seven cultivars of pak choi. We found that 'Si Yue Man' had the highest total and aliphatic GS contents in the shoots and the highest benzenic GS content in the roots, 'Shanghai Qing' contained the highest amounts of benzenic and total GS contents in the roots, while 'Nanjing Zhong Gan Bai' had the lowest benzenic, indole, and total GS contents in both the shoots and roots. Therefore, the 'Si Yue Man' cultivar appears to be a good candidate for future breeding. Variation between the shoots and roots was also examined, and a significant correlation among the total, aliphatic, and some individual GSs was found, which is of value in agricultural breeding. GS concentrations of the leaf, petiole, and root increased dramatically during the period of rapid growth of the dry matter of the plant 10 to 20 d after transplantation, reaching peak values on Day 20 and decreasing on Day 25. We conclude that the pak choi should be harvested and consumed from 20 to 25 d after transplantation to take advantages of the high GS content in the plant.

Key words: Glucosinolate, Pak choi, Cultivar, Organ, Growth stage
doi:10.1631/jzus.B1200213 **Document code:** A **CLC number:** S634.3

1 Introduction

Glucosinolates (GSs) are a group of nitrogen- and sulfur-containing secondary metabolites found mainly in plants of the Brassicaceae family (Fahey *et al*., 2001; Agerbirk and Olsen, 2012). They have a common basic structure comprising a β-D-thioglucose group, a sulfonated aldoxime moiety, and a variable side chain derived from amino acids, and are grouped into aliphatic, benzenic, and indole GSs by the structure of their side chains (Halkier and Gershenzon, 2006). GSs are chemically stable until being hydrolyzed into a variety of biologically active products catalyzed by endogenous or exogenous myrosinases (Halkier and Gershenzon, 2006). The breakdown products of GSs have lots of biological functions related to human health and nutrition, such as flavoring *Brassica* vegetables and preventing cancer, and related to plant defense systems, such as deterring herbivores and pathogens (Hecht, 2000; Mithen *et al*., 2000; Wittstock and Gershenzon, 2002; Padilla *et al*., 2007). GSs and their hydrolysis products have strong anticancer effects (Latte *et al*., 2011). For example, sulphoraphane, the isothiocyanate breakdown product of glucoraphanin, has been shown to inhibit Phase I carcinogenic activation enzymes and induce Phase II detoxification enzymes (Kim and Milner, 2005). The chemoprotective effects of isothiocyanates are involved in the modulation of multiple pathways of carcinogenesis such as: (a) protection against

‡ Corresponding author

* Project supported by the National Natural Science Foundation of China (Nos. 30871718 and 31201620), the Zhejiang Provincial Natural Science Foundation of China (No. R3080360), and the Fund for Zhejiang Higher School Innovative Research Team (No. T200916), China

environmental factors by targeting xenobiotic metabolism, (b) increasing the antioxidant capacity of cells, and (c) targeting tumour cell growth (Traka and Mithen, 2009). Similar effects are also produced by the hydrolysis products of indole GSs (Holst and Williamson, 2004). The 2-phenylethyl GS degradation product, 2-phenylethyl isothiocyanate, is highly toxic to a range of soil organisms, such as fungi and root-feeding nematodes (Potter *et al*., 2000; van Dam *et al*., 2009). However, some negative aspects of GSs have also been reported. For example, progoitrin is considered to be unsuitable for animal consumption because of its 'antinutritional' and goitrogenic properties (Griffiths *et al*., 1998). Thus, GSs have been investigated frequently in relation to their roles in human and animal food consumption and plant defense systems.

Previous studies have shown that, whether in *Brassica* crops such as *B. rapa*, *B. napus*, and *B. nigra*, or *Arabidopsis thaliana*, the composition and content of GSs vary considerably among and within species and are regulated both developmentally and environmentally in various organs and tissues (Rosa *et al*., 1996; Petersen *et al*., 2002; Brown *et al*., 2003; Bellostas *et al*., 2004; Velasco *et al*., 2007). Investigations of GS composition and content in *B. napus*, *A. thaliana*, and *Raphanus raphanistrum* during development showed that plants that produce GSs commonly accumulate them in all vegetative and reproductive parts throughout development (Clossais-Besnard and Larher, 1991; Brown *et al*., 2003; Malik *et al*., 2010). These studies have focused mostly on the variation in GSs from seed germination to silique formation during the whole life cycle and aimed to evaluate the synthesis, transport, and degradation of GSs, and their relationship to plant defense. However, as lots of *Brassica* crops are vegetables important in human consumption, a comprehensive understanding of GS composition and content is important, especially the variation in GSs in vegetative tissues during the long harvest period. Yet, the relevant research is still lacking.

Pak choi (*Brassica campestris* L. ssp. *chinensis* var. *communis*) is a very important *Brassica* vegetable in East, Northeast, and Southeast Asia. It accounts for 30%–40% of the vegetable production area in China and is widely consumed because of its nutritional bioactive components such as folate, vitamin C, carotenoids, polyphenols, and GSs (Tay and Toxopeus, 1993; Podsedek, 2007; Hanson *et al*., 2009; Verkerk *et al*., 2009). Pak choi is harvested and consumed over a period lasting several weeks from the young seedling to the big shoot stage. Though some studies have described the GS content of pak choi (He *et al*., 2000; Chen *et al*., 2008; Verkerk *et al*., 2009; Yang *et al*., 2009), little is known about variation in the GS content among cultivars and organs, especially the variation in GSs during the long harvest period. In the present study, we identified and examined the variation in GS composition and content in pak choi cultivars and organs. The variation in GS content in the leaf, petiole, and root of pak choi at different vegetative growth stages (during the harvest period) was further analyzed. This systematic study will help in selecting pak choi cultivars with high GS content for breeding and in guiding human vegetable consumption.

2 Materials and methods

2.1 Plant growth and sampling

Seven pak choi cultivars (*Brassica campestris* L. ssp. *chinensis* var. *communis*) with different representative characters and which are commonly grown and consumed in southern China were selected to investigate the total and individual GS contents of shoots and roots. They were 'Hangzhou You Dong Er' (HZYDE), 'Zhouye You Dong Er' (ZYYDE), 'Si Yue Man' (SYM), 'Shanghai Qing' (SHQ), 'Nanjing Zhong Gan Bai' (NJZGB), 'Chang Geng Bai' (CGB), and 'Ai Jiao Huang' (AJH). Seeds were germinated and grown in vermiculite for 3 weeks, and then the seedlings with 3–4 true leaves were transplanted into 10-L plastic containers containing aerated full-nutrient solution. The composition of the nutrient solution was as described by Yang *et al*. (2009), and the nutrient solutions were changed twice a week. The statistical design was a randomized complete block (RCB) with three replicates of 8 plants each. Plants were harvested after being grown for 2 weeks, and the shoots and roots were harvested separately, immediately frozen in liquid nitrogen, and freeze-dried. After being weighed, the samples were ground into fine powder and stored at −20 °C for analysis.

The 'HZYDE' cultivar was used to further

investigate the variation in GSs in various organs at different vegetative growth stages (during the harvest period). Plants at the six-leaf stage were used for the experiment and all plants were in the vegetative stage and did not start to bolt and flower. Plants were grown under the same conditions as above; the leaves, petioles, and roots were harvested separately at 10, 15, 20, and 25 d after being transplanted. Each treatment was replicated independently three times.

2.2 Glucosinolate analysis

The composition and content of GSs were determined as described by Yang *et al*. (2009), according to the method of Krumbein *et al*. (2005) with slight modification. Briefly, 0.25 g of sample powder was boiled with 10 ml 70% methanol, and then the supernatant was loaded onto a 1-ml mini-column (JT Baker, USA) containing 500 μl activated diethylaminoethyl (DEAE) Sephadex™ A25 (Amersham Biosciences, Sweden) to de-sulphate overnight with aryl sulfatase (Sigma-Aldrich Co., MO, USA). The resultant desulpho (ds)-glucosinolates were eluted with ultra pure water and identified and quantified by comparison of high performance liquid chromatography (HPLC) retention times, and coupled with an electrospray ionization ion trap mass detector system (Agilent 1100 series, Agilent Technologies, Palo Alto, CA, USA), as described by Krumbein *et al*. (2005) and Chen *et al*. (2008). The amount of each GS was calculated using sinigrin (Sigma-Aldrich Co., MO, USA) as an internal standard and based on the ultraviolet response factors of each compound relative to sinigrin (European Community, 1990; Chen *et al*., 2008). The GS concentration was expressed as μmol/g dry weight (DW) of samples.

2.3 Statistical analyses

Student's *t*-tests were used to analyze differences in GS levels between roots and shoots. Pearson correlations were used to analyze correlations of GS concentrations between roots and shoots. Differences in GS concentrations among cultivars were studied using analysis of variance (ANOVA). Data were compared using the least significant difference (LSD) at the 0.05 significance level. The variation in GS composition between the roots and shoots was analyzed by principal component analysis (PCA). All data were visualized using the principal components score and each point represented an individual sample.

3 Results

3.1 GS contents in pak choi cultivars

We detected three individual aliphatic GSs (glucoalyssin, gluconapin, and glucobrassicanapin), three indole GSs (4-methoxyglucobrassicin, glucobrassicin, and neoglucobrassicin), and one benzenic GS (gluconasturtiin) in both the shoots and roots of all the seven cultivars (Tables 1 and 2). Significant differences in GS concentrations were found among the seven cultivars (Fig. 1). 'SYM' had the highest aliphatic and total GS concentrations in the shoots and the highest benzenic GS concentration in the roots, and 'SHQ' contained the highest benzenic and total GS concentrations in the roots, while 'NJZGB' had the lowest benzenic, indole, and total GS concentrations in both the shoots and roots (Tables 1 and 2). These results may reflect genotypic differences

Table 1 Individual glucosinolate concentrations in shoots of seven pak choi cultivars five weeks after sowing

Cultivar	DW (g/plant)	Glucosinolate concentration (μmol/g DW)						
		Glucoalysin	Gluconapin	Glucobrassicanapin	Glucobrassicin	Gluconasturtiin	4-Methoxyglucobrassicin	Neoglucobrassicin
ZYYDE	0.620±0.060c[1]	0.100±0.011b	0.171±0.010d	0.414±0.091b	0.729±0.048a	0.618±0.036b	0.121±0.017ab	0.237±0.026b
HZYDE	0.755±0.018b	0.095±0.007b	0.679±0.095b	0.463±0.028b	0.602±0.035b	0.677±0.025b	0.099±0.003bc	0.303±0.005a
NJZGB	0.539±0.016d	0.097±0.012b	0.391±0.107cd	0.410±0.151b	0.354±0.022d	0.570±0.053b	0.076±0.002c	0.136±0.009c
CGB	0.620±0.040c	0.078±0.003b	0.461±0.058c	0.327±0.074b	0.472±0.040c	0.947±0.121a	0.104±0.010bc	0.152±0.012c
SHQ	0.755±0.016b	0.095±0.010b	0.806±0.021b	0.402±0.073b	0.492±0.016bc	0.835±0.055a	0.079±0.005c	0.123±0.026c
SYM	0.784±0.022b	0.171±0.023a	2.350±0.222a	1.176±0.268a	0.504±0.037bc	0.595±0.091b	0.129±0.020a	0.116±0.018c
AJH	0.930±0.040a	0.108±0.007b	0.362±0.086cd	0.468±0.078b	0.618±0.115b	0.661±0.057b	0.085±0.007c	0.109±0.021c
Average	0.715	0.106	0.746	0.523	0.539	0.708	0.099	0.168

[1]Data are means±standard deviation (SD). Means followed by different letters in each row are significantly different (P<0.05). DW: dry weight

Table 2 Individual glucosinolate concentrations in roots of seven pak choi cultivars five weeks after sowing

Cultivar	DW (g/plant)	Glucosinolate concentration (μmol/g DW)						
		Glucoalysin	Gluconapin	Glucobrassi-canapin	Glucobrassi-cin	Gluconasturtiin	4-Methoxygl-ucobrassicin	Neogluco-brassicin
ZYYDE	0.095±0.010bc[1]	0.036±0.01bc	0.042±0.014c	0.066±0.023bc	1.136±0.121bc	8.259±1.141bc	0.931±0.079b	1.063±0.057bc
HZYDE	0.095±0.003bc	0.051±0.005ab	0.134±0.012b	0.083±0.010b	1.519±0.301b	9.053±1.095b	1.098±0.223b	1.132±0.230bc
NJZGB	0.074±0.003c	0.027±0.009c	0.072±0.023bc	0.037±0.005c	0.884±0.295c	6.540±1.004c	0.523±0.097c	0.876±0.215c
CGB	0.076±0.017c	0.034±0.004bc	0.108±0.010bc	0.049±0.002bc	1.261±0.197bc	9.751±0.507b	1.020±0.160b	1.197±0.097bc
SHQ	0.111±0.006b	0.032±0.006c	0.085±0.017bc	0.042±0.010c	1.378±0.175bc	12.908±0.514a	0.848±0.013b	1.499±0.274ab
SYM	0.098±0.019bc	0.062±0.005a	0.382±0.063a	0.237±0.030a	1.619±0.275b	10.970±0.666ab	1.538±0.277a	1.879±0.414a
AJH	0.138±0.015a	0.035±0.004bc	0.043±0.021c	0.047±0.010bc	2.031±0.175a	9.822±0.509b	1.091±0.078b	1.116±0.031bc
Average	0.098	0.040	0.124	0.080	1.404	9.615	1.007	1.252

[1]Data are means±SD. Means followed by different letters in each row are significantly different (P<0.05)

among the pak choi cultivars and suggest that the 'SYM' cultivar may have the highest nutritional value and the strongest plant defense potential.

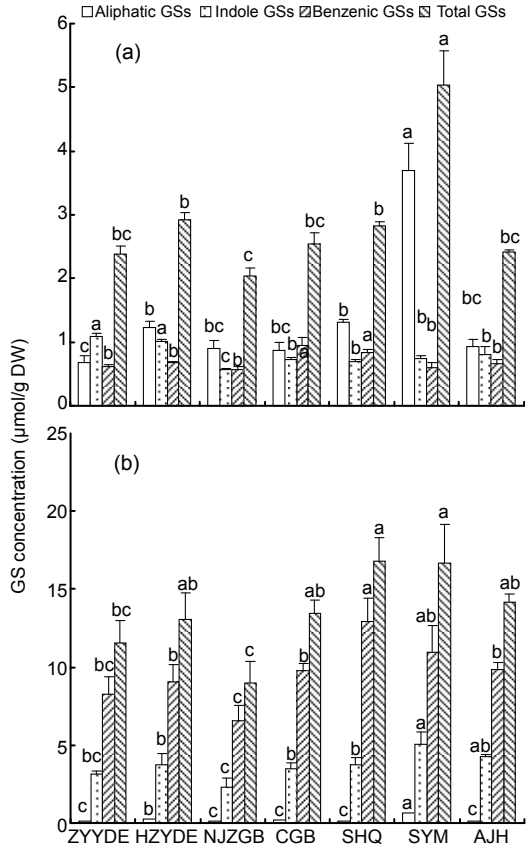

Fig. 1 Aliphatic, indole, benzenic, and total glucosinolates (GSs) in shoots (a) and roots (b) of seven pak choi cultivars

Bars with different letters indicate significant differences between cultivars (P<0.05)

Regarding the individual GSs, 'SYM' had the highest concentrations of gluconapin (2.350 μmol/g DW) and glucobrassicanapin (1.176 μmol/g DW) in the shoots, and neoglucobrassicin (1.879 μmol/g DW) and 4-methoxyglucobrassicin (1.538 μmol/g DW) in the roots. 'SHQ' and 'SYM' contained the highest root gluconasturtiin concentrations, 12.908 and 10.970 μmol/g DW, respectively.

3.2 Differences in GS content between the roots and shoots

All seven individual GSs were present in both the shoots and roots of all seven pak choi cultivars, but in variable concentrations (Tables 1 and 2). The total GS concentration was 3.8-fold higher in the roots than in the shoots (13.523 vs. 2.881 μmol/g DW, respectively). PCA showed that root samples clustered far more loosely than shoot samples, indicating that variation in GSs was greater in the roots than in the shoots, suggesting variation in their survival abilities (Fig. 2).

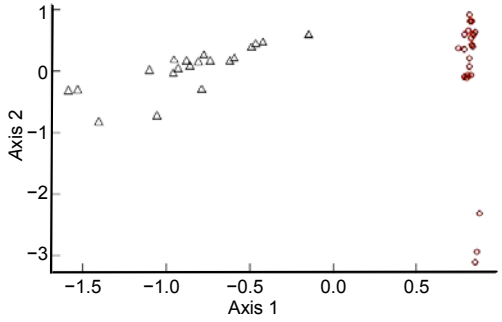

Fig. 2 Principal component analysis plot of shoot and root glucosinolate (GS) compositions of seven pak choi cultivars

Circles represent shoot samples, and triangles represent root samples

We also explored correlations in GS concentrations between shoots and roots in all the cultivars. Pearson correlation analysis showed that the concentrations of total GSs (Pearson's correlation coefficient r=0.584, P<0.01) and aliphatic GSs (r=0.927, P<0.01) of shoots and roots were quite strongly correlated, while the indole GSs (r=0.173) and benzenic GSs (r=0.395) were not. When analyzed at the level of individual GSs, glucoalyssin (r=0.683, P<0.01), gluconapin (r=0.927, P<0.01), glucobrassicanapin (r=0.851, P<0.01), and 4-methoxyglucobrassicin (r=0.660, P<0.01) contents showed significant correlations between the shoots and roots. Most of the correlated GSs were aliphatic, especially gluconapin, which constituted the majority of the aliphatic GSs.

3.3 GS contents at different stages of vegetative growth during the harvest period

The GS content and composition of leaves, petioles, roots, and whole plants of pak choi at different stages of vegetative growth were further analyzed in the 'HZYDE' cultivar. The GS concentrations of all the various organs varied a lot at different stages (Table 3, Fig. 3). The aliphatic, indole, benzenic, and total GSs of leaves, petioles, and roots increased rapidly from Days 10 to 20 after transplantation, and then started decreasing on Day 25 (Fig. 3). The total plant GS concentration increased from 5.009 (10 d) to 13.982 μmol/g DW (20 d) reaching a peak, and then decreased to 9.222 μmol/g DW on Day 25 (Fig. 3a). However, the concentrations of the indole GSs, 4-methoxyglucobrassicin and neoglucobrassicin, in both the leaves and roots increased continuously during the vegetative growth period (Table 3). In addition, the proportions of aliphatic, indole, and benzenic GSs relative to total GSs in the leaves, petioles, roots, and whole plant also changed a lot among the vegetative growth stages. In particular, the proportion of aliphatic GS relative to the total GS increased from 69.316% to 80.693% in the leaves and from 43.144% to 62.073% in the petioles when harvested on Days 10 and 25 respectively after transplantation. In contrast, the corresponding proportion of indole GSs relative to the total GS of leaves and petioles declined (data not shown). These results demonstrate that the total GS increased with the growth stage and reached a peak on Day 20 and the increment was due mainly to the desirable aliphatic GSs, suggesting an optimum harvest and consumption time.

4 Discussion

GSs are a group of metabolites, whose content and composition vary in different plants and organs. In the present study, we determined the contents of three individual aliphatic, three indole, and one benzenic GS in both the shoots and roots of seven local pak choi cultivars (Tables 1 and 2). Significant differences in GS concentrations were observed among the seven cultivars (Fig. 1). This genotypic variation

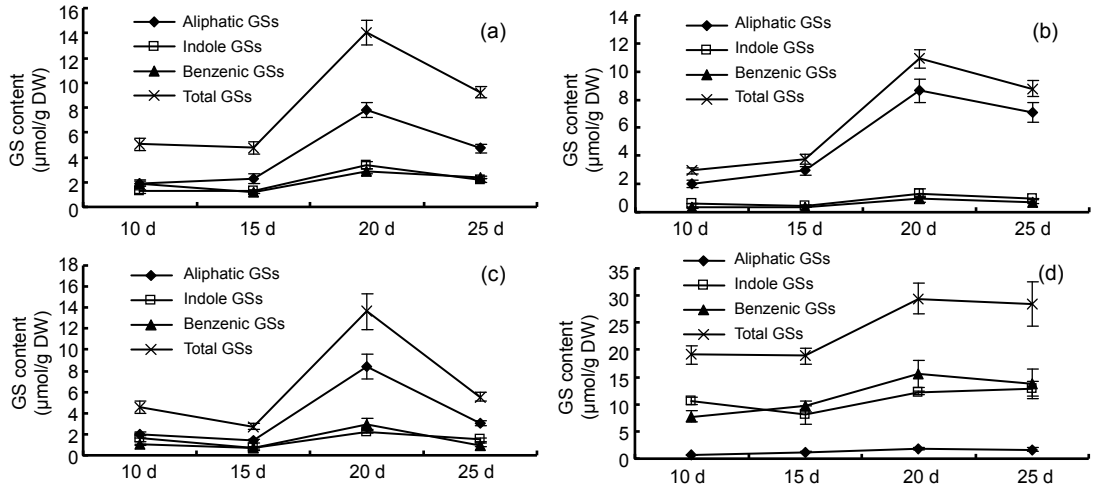

Fig. 3 Changes in glucosinolate (GS) content in the whole plant (a), leaf (b), petiole (c), and root (d) of 'Hangzhou You Dong Er' at different growth stages during the harvest period

Table 3 Individual glucosinolate concentrations in different organs of pak choi (Hangzhou You Dong Er) at different vegetative growth stages during the harvest period

Organ	Time (d)	Glucosinolate concentration (μmol/g DW)*						
		Glucoalysin	Gluconapin	Glucobrassi-canapin	Glucobrassicin	Gluconasturtiin	4-Methoxygl-ucobrassicin	Neogluco-brassicin
Root	10	0.134±0.014b	0.341±0.008b	0.233±0.014b	2.945±0.205b	7.771±1.021b	3.136±0.426a	4.547±0.279ab
	15	0.168±0.024b	0.720±0.142a	0.298±0.069b	2.847±0.291b	9.644±1.071b	2.914±0.443a	3.247±0.488b
	20	0.322±0.024a	0.960±0.083a	0.515±0.070a	4.617±0.113a	15.525±2.431a	3.241±0.452a	4.236±0.410ab
	25	0.127±0.013b	0.857±0.167a	0.669±0.134a	4.515±0.211a	13.791±2.708a	3.600±0.426a	4.804±0.938a
Petiole	10	0.100±0.018b	1.197±0.168c	0.676±0.109b	0.422±0.080b	1.016±0.073b	0.237±0.050a	0.925±0.172b
	15	0.055±0.005b	0.952±0.134c	0.348±0.037b	0.226±0.020c	0.678±0.152b	0.099±0.009b	0.383±0.083c
	20	0.528±0.064a	5.334±0.802a	2.600±0.515a	0.795±0.129a	2.969±0.522a	0.250±0.023a	1.155±0.094a
	25	0.128±0.024b	2.037±0.244b	0.914±0.163b	0.400±0.056b	0.979±0.159b	0.252±0.047a	0.810±0.111b
Leaf	10	0.060±0.012c	1.246±0.141b	0.710±0.087c	0.418±0.018b	0.318±0.042b	0.081±0.009c	0.118±0.023b
	15	0.070±0.014c	2.007±0.133b	0.871±0.178c	0.294±0.044b	0.393±0.064b	0.075±0.011c	0.062±0.010c
	20	0.261±0.031a	5.372±0.940a	2.989±0.131a	1.039±0.282a	0.926±0.194a	0.179±0.009b	0.137±0.029b
	25	0.110±0.015b	5.008±0.686a	1.955±0.137b	0.476±0.055b	0.740±0.109a	0.291±0.019a	0.191±0.038a

*Data are means±SD. Means followed by different letters in each row are significantly different ($P<0.05$)

in GS content in the pak choi cultivars is consistent with previous studies (He *et al*., 2000; Castro *et al*., 2004; Kim *et al*., 2010). The variation in GS content among the pak choi cultivars may be due to differences in their genetic backgrounds or developmental and environmental regulation (Rosa *et al*., 1996; Petersen *et al*., 2002; Brown *et al*., 2003; Bellostas *et al*., 2004; Velasco *et al*., 2007).

GSs play an important role in plant defense systems and human nutrition. The isothiocyanates of aliphatic GSs have beneficial effects on human health and the breakdown products of gluconasturtiin help plants to deter soil organisms (Hecht, 2000; Mithen *et al*., 2000; Potter *et al*., 2000; Malik *et al*., 2010). We discovered that the cultivar 'SYM' had high levels of shoot aliphatic GSs, especially gluconapin and glucobrassicanapin, and root benzenic and indole GSs. The dry weight data showed that the higher GS content of 'SYM' is not because of a lower dry matter yield (Table 1). Epidemiological studies have suggested that intake of GSs in cruciferous vegetables, including pak choi, reduces human cancer risks. For example, the gluconapin hydrolysis product, 3-butenyl isothiocyanate, is able to inhibit the growth of tumor cells in vitro by inhibiting proliferation and inducing apoptosis (Nastruzzi *et al*., 1996; Smith *et al*., 2004; 2005). Thus, high levels of beneficial GSs indicate that 'SYM' may be a good candidate for breeding new cultivars either for human consumption or plant defense.

In the present study, we also found that the GS concentration was higher in the roots than in the shoots of pak choi, which is in accordance with previous studies of other species (Castro *et al*., 2004; Kabouw *et al*., 2010; Malik *et al*., 2010). The difference in GS concentration between the shoots and roots was not due to a general difference across all GSs but more to differences within some specific GS types. Aliphatic GSs were predominant in the shoots (Table 1) and their concentration was much higher in the shoots than in the roots, whereas the benzenic GS accounted for the majority of the GSs in the roots (Table 2), and its concentration, together with indole GSs, in the roots was much higher than that in the shoots. Similar results were observed in other *Brassica* species and in *Arabidopsis thaliana* (Vierheilig *et al*., 2000; Brown *et al*., 2003; Kabouw *et al*., 2010). The higher levels of GS and greater variation in GSs in the roots can be explained by the higher survival pressure that the roots endure in the soil environment where pathogens such as insects and microbes are abundant. The different GSs found in high amounts in the roots may act as defensive compounds to deter the soil organisms (Zangerl and Bazzaz, 1993; Potter *et al*., 2000; van Dam *et al*., 2009).

The correlation between GS concentrations in the shoots and roots may be due to the transport of GSs or their precursors through the phloem and also may be a part of the plant's defense system (Zangerl and Bazzaz, 1993; Merritt, 1996; Brudenell *et al*.,

1999; Chen and Andreasson, 2001; Chen *et al*., 2001; Grubb and Abel, 2006). The correlation and independence of GS compositions between the shoots and roots could be of value to breeding programs for agricultural practice. For example, plant breeders can increase the root aliphatic and 2-phenylethyl GSs to enhance the plant defenses against soil pathogens, and simultaneously increase the beneficial shoot aliphatic GSs to enhance the dietary and nutrition value for human health. Kabouw *et al*. (2010) reported that in white cabbage there were no significant correlations among total, aliphatic or indole GSs between the shoots and roots, and that the levels of only two individual GSs, 2-propenyl and 2-penylethyl, were significantly correlated between the shoots and roots. However, we believe that the difference between the two studies may be due to the species difference. White cabbage is a crop of *Brassica oleracea* var. *capitata*, while pak choi belongs to *Brassica campestris* ssp. *chinensis*, in which 2-propenyl GS is not found.

GS concentrations in the leaves, petioles, and roots of the cultivar 'HZYDE' increased during the period from Days 10 to 20 after transplantation, meaning that GS accumulation in this period was faster than dry matter accretion, although both were increasing dramatically (dry matter data not shown). GS accumulation reached its peak on Day 20 and declined on Day 25. The results are consistent with studies in other *Brassica* species and in *Arabidopsis thaliana* (Clossais-Besnard and Larher, 1991; Brown *et al*., 2003; Malik *et al*., 2010). While these previous studies also investigated the dynamic change in GSs in plants, they included the whole life cycle, whereas our study focused on the different vegetative growth stages. Dynamic changes in the proportions of different GSs in relation to the total GS concentration are regarded as part of plant development and defense systems (Halkier and Gershenzon, 2006). The aliphatic GSs have been reported to be related to the flavor and nutrition of *Brassica* vegetables (Schonhof *et al*., 2004; Padilla *et al*., 2007; Podsedek, 2007; Verkerk *et al*., 2009), and as people commonly consume the leaves and petioles of pak choi, increasing the aliphatic GS proportion should increase its nutritional value. Thus, we recommend harvesting and consumption of pak choi from Days 20 to 25 after transplantation, because during that period both the dry matter and the beneficial GS concentrations will reach their peaks.

5 Conclusions

The contents of natural compounds and nutrients in plants usually vary among cultivars and change dynamically in the course of development. In the current study, the variation in GS composition and content among pak choi cultivars was measured and the dynamic changes in GSs during the early growth period (from Days 10 to 25 after transplantation) were examined. The results indicated that the 'SYM' cultivar, which had a high level of beneficial GSs, may be a good candidate for future breeding programs for producing high quality cultivars containing high levels of GSs. We recommend that pak choi be harvested and consumed during the period from Days 20 to 25 after transplantation to take advantages of the highest levels of GSs in the plant.

Compliance with ethics guidelines

Biao ZHU, Jing YANG, and Zhu-jun ZHU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Agerbirk, N., Olsen, C.E., 2012. Glucosinolate structures in evolution. *Phytochemistry*, **77**:16-45. [doi:10.1016/j.phytochem.2012.02.005]

Bellostas, N., Sørensen, J.C., Sørensen, H., 2004. Qualitative and quantitative evaluation of glucosinolates in cruciferous plant during their life cycles. *Agroindustria*, **3**(3):5-10.

Brown, P.D., Tokuhisa, J.G., Reichelt, M., Gershenzon, J., 2003. Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. *Phytochemistry*, **62**(3):471-481. [doi:10.1016/S0031-9422(02) 00549-6]

Brudenell, A.J.P., Griffiths, H., Rossiter, J.T., Baker, D.A., 1999. The phloem mobility of glucosinolates. *J. Exp. Bot*., **50**(335):745-756. [doi:10.1093/jxb/50.335.745]

Castro, A., Aires, A., Rosa, E., Bloem, E., Stulen, I., Kok, L.D., 2004. Distribution of glucosinolates in *Brassica oleracea* cultivars. *Phyt. Ann. Rei Bot*., **44**(1):133-143.

Chen, S., Andreasson, E., 2001. Update on glucosinolate

metabolism and transport. *Plant Physiol. Biochem*., **39**(9):743-758. [doi:10.1016/S0981-9428(01)01301-8]

Chen, S.X., Petersen, B.L., Olsen, C.E., Schulz, A., Halkier, B.A., 2001. Long-distance phloem transport of glucosinolates in *Arabidopsis*. *Plant Physiol*., **127**(1):194-201. [doi:10.1104/pp.127.1.194]

Chen, X.J., Zhu, Z.J., Gerendas, J., Zimmermann, N., 2008. Glucosinolates in Chinese *Brassica campestris* vegetables: Chinese cabbage, purple cai-tai, choysum, pakchoi, and turnip. *Hortscience*, **43**(2):571-574.

Clossais-Besnard, N., Larher, F., 1991. Physiological role of glucosinolates in *Brassica napus*. Concentration and distribution pattern of glucosinolate among plant organs during a complete life cycle. *J. Sci. Food Agric*., **56**(1): 25-38. [doi:10.1002/jsfa.2740560104]

European Community, 1990. Determination of the oilseed glucosinolate content by HPLC. *Off. J. Eur. Commun.*, **170**:27-34.

Fahey, J.W., Zalcmann, A.T., Talalay, P., 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*, **56**(1):5-51. [doi:10.1016/S0031-9422(00)00316-2]

Griffiths, D.W., Birch, A.N.E., Hillman, J.R., 1998. Antinutritional compounds in the *Brassicaceae*: analysis, biosynthesis, chemistry and dietary effects. *J. Hort. Sci. Biotechnol*., **73**(1):1-18.

Grubb, C.D., Abel, S., 2006. Glucosinolate metabolism and its control. *Trends Plant Sci*., **11**(2):89-100. [doi:10.1016/j.tplants.2005.12.006]

Halkier, B.A., Gershenzon, J., 2006. Biology and biochemistry of glucosinolates. *Ann. Rev. Plant Biol*., **57**(1):303-333. [doi:10.1146/annurev.arplant.57.032905.105228]

Hanson, P., Yang, R., Chang, L., Ledesma, L., Ledesma, D., 2009. Contents of carotenoids, ascorbic acid, minerals and total glucosinolates in leafy brassica pakchoi (*Brassica rapa* L. *chinensis*) as affected by season and variety. *J. Sci. Food Agric*., **89**(5):906-914. [doi:10.1002/jsfa.3533]

He, H., Fingerling, G., Schnitzler, W.H., 2000. Glucosinolate contents and patterns in different organs of Chinese cabbages, Chinese kale (*Brassica alboglabra bailey*) and choy sum (*Brassica campestris* L. ssp *chinensis* var. *utilis* Tsen et Lee). *Angewandte Botanik*, **74**(1-2):21-25.

Hecht, S.S., 2000. Inhibition of carcinogenesis by isothiocyanates. *Drug Metab. Rev*., **32**(3-4):395-411. [doi:10.1081/DMR-100102342]

Holst, B., Williamson, G., 2004. A critical review of the bioavailability of glucosinolates and related compounds. *Nat. Prod. Rep.*, **21**(3):425-447. [doi:10.1039/b204039p]

Kabouw, P., Biere, A., Putten, W.H., van Dam, N.M., 2010. Intra-specific differences in root and shoot glucosinolate profiles among white cabbage (*Brassica oleracea* var. *capitata*) cultivars. *J. Agric. Food Chem*., **58**(1):411-417. [doi:10.1021/jf902835k]

Kim, J.K., Sang, M.C., Kim, S.J., Lee, D.J., Lee, S.Y., Lim, S.H., Sun, H.H., Kweon, S.J., Cho, S.H., 2010. Variation of glucosinolates in vegetable crops of *Brassica rapa* L. ssp. *Pekinensis*. *Food Chem.*, **119**(1):423-428. [doi:10.1016/j.foodchem.2009.08.051]

Kim, Y.S., Milner, J.A., 2005. Targets for indole-3-carbinol in cancer prevention. *J. Nutr. Biochem*., **16**(2):65-73. [doi:10.1016/j.jnutbio.2004.10.007]

Krumbein, A., Schonhof, I., Schreiner, M., 2005. Composition and contents of phytochemicals (glucosinolates, carotenoids and chlorophylls) and ascorbic acid in selected Brassica species (*B. juncea*, *B. rapa* subsp. *nipposinica* var. *chinoleifera*, *B. rapa* subsp. *Chinensis* and *B. rapa* subsp. *rapa*). *J. Appl. Bot. Food Qual.*, **79**(3):168-174.

Latte, K.P., Appel, K.E., Lampen, A., 2011. Health benefits and possible risks of broccoli—an overview. *Food Chem. Toxicol.*, **49**(12):3287-3309. [doi:10.1016/j.fct.2011.08.019]

Malik, M.S., Riley, M.B., Norsworthy, J.K., Bridges, W.J., 2010. Glucosinolate profile variation of growth stages of wild radish (*Raphanus raphanistrum*). *J. Agric. Food Chem*., **58**(6):3309-3315. [doi:10.1021/jf100258c]

Merritt, S.Z., 1996. Within-plant variation in concentrations of amino acids, sugar, and sinigrin in phloem sap of black mustard, *Brassica nigra* (L) Koch (*Cruciferae*). *J. Chem. Ecol*., **22**(6):1133-1145. [doi:10.1007/BF02027950]

Mithen, R.F., Dekker, M., Verkerk, R., Rabot, S., Johnson, I.T., 2000. The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. *J. Sci. Food Agric*., **80**(7):967-984. [doi:10.1002/(SICI)1097-0010(20000515)80:7<967::AID-JSFA597>3.3.CO;2-M]

Nastruzzi, C., Cortesi, R., Esposito E., Menegatti, E., Leoni, O., Iori, R., Palmieri, S., 1996. In vitro cytotoxic activity of some glucosinolate-derived products generated by myrosinase hydrolysis. *J. Agric. Food Chem.*, **44**(4):1014-1021. [doi:10.1021/jf9503523]

Padilla, G., Cartea, M.E., Velasco, P., Haro, A., Ordás, A., 2007. Variation of glucosinolates in vegetable crops of *Brassica rapa*. *Phytochemistry*, **68**(4):536-545. [doi:10.1016/j.phytochem.2006.11.017]

Petersen, B.L., Chen, S., Hansen, C.H., Olsen, C.E., Halkier, B.A., 2002. Composition and content of glucosinolates in developing *Arabidopsis thaliana*. *Planta*, **214**(4):562-571. [doi:10.1007/s004250100659]

Podsedek, A., 2007. Natural antioxidants and antioxidant capacity of *Brassica* vegetables: a review. *LWT-Food Sci. Technol*., **40**(1):1-11. [doi:10.1016/j.lwt. 2005.07.023]

Potter, M.J., Vanstone, V.A., Davies, K.A., Rathjen, A.J., 2000. Breeding to increase the concentration of 2-phenylethyl glucosinolate in the roots of *Brassica napus*. *J. Chem. Ecol*., **26**(8):1811-1820. [doi:10.1023/A:1005588405774]

Rosa, E.A.S., Heaney, R.K., Portas, C.A.M., Fenwick, G.R., 1996. Changes in glucosinolate concentrations in *Brassica* crops (*B. oleracea* and *B. napus*) throughout growing seasons. *J. Sci. Food Agric*., **71**(2):237-244. [doi:10.1002/(SICI)1097-0010(199606)71:2<237::AID-JSFA574>3.0.CO;2-P]

Schonhof, I., Krumbein, A., Brückner, B., 2004. Genotypic effects on glucosinolates and sensory properties of broccoli and cauliflower. *Food*, **48**(1):25-33. [doi:10.1002/food.200300329]

Smith, T.K., Lund, E.K., Parker, M.L., Clarke, R.G., Johnson, I.T., 2004. Allyl-isothiocyanate causes mitotic block, loss of cell adhesion and disrupted cytoskeletal structure in HT29 cells. *Carcinogenesis*, **25**(8):1409-1415. [doi:10.1093/carcin/bgh149]

Smith, T.K., Lund, E.K., Clarke, R.G., Bennett, R.N., Johnson, I.T., 2005. Effects of Brussels sprout juice on the cell cycle and adhesion of human colorectal carcinoma cells (HT29) in vitro. *J. Agric. Food Chem*., **53**(10):3895-3901. [doi:10.1021/jf048025v]

Tay, D.C.S., Toxopeus, H., 1993. *Brassica rapa* L. cv. Group Pak Choi in Plant Resources South-East Asia. Number 8: Vegetables. Pudoc Scientific Publishers, Wageningen, p.130-134.

Traka, M., Mithen, R., 2009. Glucosinolates, isothiocyanates and human health. *Phytochem. Rev.*, **8**(1):269-282. [doi:10.1007/s11101-008-9103-7]

van Dam, N.M., Tytgat, T.O.G., Kirkegaard, J.A., 2009. Root and shoot glucosinolates: a comparison of their diversity, function and interactions in natural and managed ecosystems. *Phytochem. Rev.*, **8**(1):171-186. [doi:10.1007/s11101-008-9101-9]

Velasco, P., Cartea, M.E., Gonzalez, C., Vilar, M., Ordas, A., 2007. Factors affecting the glucosinolate content of kale (*Brassica oleracea acephala* group). *J. Agric. Food Chem*., **55**(3):955-962. [doi:10.1021/jf0624897]

Verkerk, R., Schreiner, M., Krumbein, A., Ciska, E., Holst, B., Rowland, I., de Schrijver, R., Hansen, M., Gerhauser, C., Mithen, R., *et al*., 2009. Glucosinolates in *Brassica* vegetables: the influence of the food supply chain on intake, bioavailability and human health. *Mol. Nutr. Food Res*., **53**(2):S219-S265. [doi:10.1002/mnfr.200800065]

Vierheilig, H., Bennett, R., Kiddle, G., Kaldorf, M., Ludwig-Muller, J., 2000. Differences in glucosinolate patterns and arbuscular mycorrhizal status of glucosinolate-containing plant species. *New Phytol*., **146**(2):343-352. [doi:10.1046/j.1469-8137.2000.00642.x]

Wittstock, U., Gershenzon, J., 2002. Constitutive plant toxins and their role in defense against herbivores and pathogens. *Curr. Opin. Plant Biol*., **5**(4):300-307. [doi:10.1016/S1369-5266(02)00264-9]

Yang, J., Zhu, Z.J., Gerendás, J., 2009. Interactive effects of phosphorus supply and light intensity on glucosinolates in pakchoi (*Brassica campestris* L. ssp. *chinensis* var. *communis*). *Plant Soil*, **323**(1-2):323-333. [doi:10.1007/s11104-009-9940-1]

Zangerl, A.R., Bazzaz, F.A., 1993. Theory and Pattern in Plant Defense Allocation. *In*: Fritz, R.S., Simms, E.L. (Eds.), Plant Resistance to Herbivores and Pathogens. University of Chicago Press, Chicago, p.363-391.