Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2019) 43: 138-150

doi:10.3906/tar-1807-28

Environmentally friendly fertilizers can enhance yield and bioactive compounds in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*)

Jeongyeo LEE[1], Young-Hee NOH[1], Kun-Hyang PARK[1], Dae-Soo KIM[1], Han Taek JEONG[2], Haeng-Soon LEE[1], Sung Ran MIN[1], HyeRan KIM[1,*]

[1]Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea
[2]Shin-an Grow, Jinju-si, Republic of Korea

Received: 06.07.2018 • **Accepted/Published Online:** 03.12.2018 • **Final Version:** 01.04.2019

Abstract: The objective of this study was to evaluate the performance of 3 environmentally friendly fertilizers and their effect on the growth of Chinese cabbage and its contents of bioactive compounds. The following fertilizers were applied: purslane extract, 5% sulfur, and 15% phosphoric acid with 5% calcium. A total of 9, 9, and 11 components were detected for fatty acids, carotenoids, and glucosinolates, respectively. The contents of *cis*-7 hexadecenoic acid, linoleic acid (C18:2n6c+t), and linolenic acid (C183n3) were generally increased, and also carotenoid amounts of violaxanthin, antheraxanthin, lutein, 13-*cis*-β-carotene, α-carotene, β-carotene, and 9-*cis*-β-carotene were significantly increased by fertilizer treatments. All fertilization treatments decreased the amounts of progoitrin, a strong goitrogenic, by 20.9% (H fertilization) to 53.8% (HJ fertilization). Expression analysis of fatty acid, carotenoid, and glucosinolate biosynthesis genes showed that fertilizer treatments affected the accumulation of bioactive components by changing gene expression levels. These environmentally friendly fertilizers can add value to Chinese cabbage by increasing its yield and nutritional value.

Key words: Environmentally friendly fertilizer, fatty acid, carotenoid, glucosinolate, Chinese cabbage

1. Introduction

Brassica species, including cabbage, canola, broccoli, kale, cauliflower, and Chinese cabbage play an important role in agriculture and horticulture. They also contribute to human health (Warwick and Francis 1994; Hanson et al., 2009). Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) is one of the most important vegetable crops in Korea. It is the major component of kimchi, a Korean everyday food. Chinese cabbage contains a high level of useful nutritional components, including dietary fiber, vitamins (A, C, and E), minerals (iron, potassium, sodium, and zinc), antioxidants (carotenoids, anthocyanins, and tocopherols), and other secondary metabolites (glucosinolates, terpenes, flavonoids, steroids, and waxes) (Oboh et al., 2008; Kliebenstein et al., 2001; Dekker et al., 2000; Subhasree et al., 2009).

Conventional fertilizer contains a chemical composition of essential minerals and elements to ensure a healthy and fast growth of plants. The most important factor in fertilizer is its effectiveness on nutritional quality of vegetables, productivity of crops, and soil fertility (Prasad, 2009; Hussain et al., 2002; Song et al., 2004; Dobermann and Cassman 2002; Camargo and Alonso, 2006). Fertilizers have been classified into 2 major types depending on their constituents, strength, and various other features. One is chemical fertilizer that is generally synthetic with manmade ingredients. The other is organic fertilizer derived from natural products. Chemical fertilizers are less expensive and their nutrients are more readily available to plants than organic fertilizers. Their effects are direct and fast because they have high amounts of soluble nutrients. However, long-term repeated use of chemical fertilizers may cause many negative effects, such as environmental pollution, destruction of beneficial soil organisms, reduction of soil fertility, and crops susceptible to diseases (Chen, 2006; Porazinska et al., 1999; Lopez-Perez et al., 1990; Abd-Alla et al., 1999). On the other hand, the nutrients in organic fertilizers exist in a variety of forms. Most organic fertilizers must be transformed into soluble forms to be absorbed by plants or soil organisms. The nutrient supply of organic fertilizer is more balanced, which keeps plants healthy. Organic fertilizers also contribute to the nutritional needs of plants. They also improve physical, chemical, and biological activities of soil for beneficial organisms (Chen, 2006; Logan et al., 1997). Several studies have investigated the effect of fertilizers

* Correspondence: kimhr@kribb.re.kr

on primary and secondary plant metabolites. Treatment with chemical fertilizer can reduce antioxidant levels, while organic fertilizer can enhance antioxidant contents in plants (Dumas et al., 2003). It has been reported that vegetables such as cabbage, spinach, onion, and green pepper generally have higher levels of flavonoids and antioxidant activities when fermented soybean broth fertilizer is used (Young et al., 2000). Vågen et al. (2007) have shown that broccoli yield and the contents of glucosinolates are significantly increased by nitrogen fertilizer. It has been reported that organic fertilization can result in significantly higher phenolic components in marionberry, strawberry, and corn compared to chemical fertilization (Asami et al., 2003). Weibel et al. (2000) have indicated that the flavonols of apple grown with organic fertilizer are 19% higher than those grown with chemical fertilizer. The nutritional content of tomatoes produced by organic fertilization is also richer in phenolic compounds and vitamin C compared to those produced by chemical fertilization (Akiyama et al., 2008).

Environmentally friendly fertilizer is made from natural sources. It is generally defined as an organic substance, such as the processing materials of crop residue or organic detritus after harvest crops. Environmentally friendly fertilizer has the potential to minimize environmental pollution compared to synthetic chemical fertilizers. In addition, it emphasizes the productivity of soil, crop quality, crop yield, environmental quality, and human health (Aldanondo-Ochoa et al., 2014; Läpple and Rensburg, 2011; Argyropoulos et al., 2013; Leifeld, 2012; Patil et al., 2014). In the past, agricultural production was focused on maximizing crop yield. Hence, chemical fertilizer has been used as a common agricultural practice. However, many consumers are interested in organically grown crops, regarded as having better quality with healthier and more nutritious products than conventionally grown crops. There has been an increasing popularity of organic or environmentally friendly agricultural products, as well as nutritional value added foods. Consequently, organic cultivation systems and advanced technologies for Chinese cabbage have become more demanding. However, very limited information is available on organic cultivation of Chinese cabbage. In the present study, we investigated the influence of 3 environmentally friendly fertilizers on Chinese cabbage yield and bioactive component contents to determine the best fertilizer or fertilizer combination for a better environmentally friendly growth of Chinse cabbage.

2. Materials and methods

2.1. Plant materials

Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) was cultivated in a greenhouse of Gyeongsangnam-do Agricultural Research and Extension Service in Jinju City, Gyoengnam Province, Republic of Korea (latitude 35°12′7″N, longitude 128°07′13″E). Chinese cabbage was sown in a seedbed on May 3, 2016. Then, 15-day-old seedlings were individually transplanted into plots. Fertilizer treatments were applied to 27-day-old plants on May 30, 2016. For Chinese cabbage fertilization, the following treatments were used: H, S, J, HS, HJ, SJ, HSJ, and no fertilizer (N). Compositions of H, S, and J were purslane (*Portulaca oleracea* or pigweed) extract (H: Hugwang fertilizer, Shin-an Grow Co, Ltd.), 5% sulfur (S: Seonsi fertilizer, Shin-an Grow Co, Ltd.), and 15% phosphoric acid with 5% calcium (J: Jeonbudae fertilizer, Shin-an Grow Co, Ltd.), respectively. For each treatment, 10 plants were selected and their aerial parts are sprayed with 200 mL of 0.001% diluted fertilizer solution. Fertilizer treated plants were cultivated in a greenhouse for 18 days. Plants were harvested on June 17, 2016. Plant materials were frozen in liquid nitrogen and stored at –70 °C for RNA isolation. They were freeze-dried for HPLC or GC analysis.

2.2. Methods

2.1.1. Fatty acid methyl ester (FAME) analysis by gas chromatography (GC)

Fatty acid profile was analyzed using the method of Rafael and Mancha (1993). Briefly, a total of 500 mg of freeze-dried sample was heated together with a reagent containing methanol: benzene:2,2-dimethoxypropane:H_2SO_4 at 39:20:5:2 (v/v). Simultaneous digestion and lipid transmethylation then took place in a single phase at 80 °C. After cooling at room temperature, the upper phase containing fatty acid methyl ester (FAME) underwent capillary gas chromatography (GC) analysis. FAMEs were analyzed by GC (YL-6100GC, Young Lin Science) with a flame ionized detector and INNOWAX capillary column (Agilent, 30 m × 0.32 mm × 0.5 μm). Each FAME component was identified and quantified using Supelco 37 Component FAME Mix (Sigma).

2.1.2. Determination of carotenoid composition

Carotenoids were extracted from Chinese cabbage samples (300 mg) with 3 mL of ethanol containing 0.1% ascorbic acid (w/v). This mixture was vortexed for 20 sec and then incubated at 85 °C in a water bath for 5 min. Then, 120 μL of KOH (80% w/v) were added to saponify any potentially interfering oils. After vortexing and incubating at 85 °C in a water bath for 10 min, samples were placed on ice. Then, 1.5 mL of cold deionized water and 1.5 mL of β-apo-8'-carotenol (12.5 μg/mL), an internal standard, were added. Next, carotenoids were extracted twice with 1.5 mL of hexane and centrifuged at 4000 rpm for 10 min at 4 °C to separate layers. Extracts were freeze-dried under a stream of nitrogen gas and resuspended in 50:50 (v/v) methanol/dichloromethane. For HPLC analysis, carotenoids were separated with an Agilent 1100 HPLC system by using YMC column (250 × 4.6 mm × 3 μm) and de-

tected with a photodiode array detector at the wavelength of 450 nm. Solvent A consisted of methanol/water (92:8 v/v) with 10 mM ammonium acetate. Solvent B consisted of 100% methyl tert-butyl ether. Flow rate was maintained at 1 mL/min. Samples were eluted with the following gradient: 0 min, 90% A/10% B; 20 min, 83% A/17% B; 29 min, 75% A/25% B; 35 min, 30% A/70% B; 40 min, 30% A/70% B; 42 min, 25% A/75% B; 45 min, 90% A/10% B; and 55 min, 90% A/10% B.

2.1.3. Determination of glucosinolate profile

Glucosinolate was extracted from 100 mg of freeze dried powder with 1.5 mL of 70% (v/v) MeOH at 70 °C in a water bath for 5 min. After centrifugation at 4000 rpm for 10 min at 4 °C, the supernatant was collected into a 15 mL tube and the residue was reextracted twice as described above. Supernatants were combined and taken as crude GSL extracts. These extracts were loaded into a minicolumn previously packed with DEAE-Sephadex A-25 and desulfated with 75 μL of aryl sulfatase solution. Desulfoglucosinolate (DS-GSL) was eluted into a 2 mL microcentrifuge tube with 1.5 mL of ultrapure water. Separation of DS-GSLs was carried out on a reversed phase Inertsil ODS-3 column (150 × 3.0 mm × 3 μm) with an E type cartridge guard column (10 × 2.0 mm × 5 μm) using an Agilent Technologies 1100 series HPLC system. Detection wavelength, column oven temperature, and flow rate were set at 227 nm, 40 °C, and 0.2 mL/min, respectively. The mobile phase consisted of water (solvent A) and acetonitrile (solvent B). DS-GSL samples were eluted with the following gradient: 0 min, 100% A/0% B; 2 min, 100% A/0% B; 7 min, 90% A/10% B; 16 min, 69% A/31% B; 19 min, 69% A/31% B; 21 min, 100% A/0% B; and 27 min, 100% A/0% B. Individual glucosinolates were identified based on their HPLC retention times and our database. They were quantified by including an external standard sinigrin (0.1 mg/mL for its desulfation) passed through the same extraction process together with sample preparation.

2.1.4. RNA isolation and quantitative real time PCR (qRT-PCR)

The total RNA was extracted from Chinese cabbage leaves using TriZol reagent (Invitrogen) following the manufacturer's instructions. Transcription levels were analyzed by quantitative real-time PCR (qRT-PCR). Gene specific primers were designed and primer sets are listed in Supplemental Table 1. Gene expression was normalized against the level of actin2 (*BrACT2*) as housekeeping gene. Real-time PCR reactions were performed in triplicates on an ABI7300 real-time PCR system using SYBR Green Master Mix (Applied Biosystems). The qRT-PCR protocol was as follows: initiated at 95 °C for 30 s, 40 cycles of 95 °C for 5 s, and 60 °C for 31 s followed by melting curve analysis. All samples were run in triplicates.

3. Results

3.1. The influence of fertilizer treatment on the growth of Chinese cabbage

The effects of environmentally friendly fertilizer treatments on the growth of Chinese cabbage were statistically analyzed in terms of leaf height, leaf diameter, leaf number, and fresh and dry weight. Results are shown in Table 1. Single fertilizer treatment with H, S, or J showed no significant difference in growth except for leaf height with S or J (14.0% and 15.4% increase, respectively), although fresh and dry weight of treated Chinese cabbage showed an increasing tendency. Double fertilizer treatments increased the growth of Chinese cabbage significantly, especially by SJ (with 18.9%, 14.6%, 16.9%, and 47.4% increase in leaf height, diameter, number, and fresh weight,

Table 1. Effect of different environmentally friendly fertilizer treatments on the growth of Chinese cabbage (N, no fertilizer; H, Hugwang fertilizer (purslane extract); S, Seonsi fertilizer (5% sulfur); J, Jeonbudae fertilizer (15% phosphoric acid and 5% calcium phosphate); HS, Hugwang + Seonsi fertilizers; HJ, Hugwang + Jeonbudae fertilizers; SJ, Seonsi + Jeonbudae fertilizers; HSJ, Hugwang + Seonsi + Jeonbudae fertilizers).

Fertilizer	Leaf height (cm)	Leaf diameter (cm)	Leaf number	Fresh weight (g)	Dry weight (mg/plant)
N	14.3 d	8.2 bcd	16.0 b	47.7 b	2.31 b
H	15.0 cd	7.2 d	17.7 ab	53.5 b	2.32 b
S	16.3 b	8.3 bc	18.0 ab	57.8 b	2.52 b
J	16.5 ab	8.3 bc	18.3 ab	57.3 b	2.88 b
HS	16.1 bc	7.7 cd	18.0 ab	52.9 b	2.77 b
HJ	16.2 bc	9.2 ab	17.7 ab	68.1 a	3.64 a
SJ	17.0 ab	9.4 a	18.7 a	70.3 a	3.71 a
HSJ	17.7 a	9.9 a	20.0 a	76.2 a	3.87 a

respectively). The HS treatment resulted in the least growth increase compared to HJ or SJ. The combination of 3 fertilizers (HSJ) showed the most dramatic effect on the growth of Chinese cabbage, resulting in 59.7% increase in fresh weight, with 23.8%, 20.7%, and 25.0% increase in leaf height, diameter, and number, respectively (Table 1).

3.2. Changes in contents of bioactive compounds by environmentally friendly fertilizations

Changes in contents of fatty acids, carotenoids, and glucosinolates were analyzed to understand the response of bioactive compounds to these environmentally friendly fertilizers. A total of 9 fatty acid compounds were identified from Chinese cabbage grown under different environmentally friendly fertilizer applications by GC-MS (Table 2). Linolenic acid (C18:3n3) was the major component, followed by palmitic acid (C16:0). Among the fatty acids detected, hexadecadienoic acid (C16:2n6), hexadecatrienoic acid (C16:3n3), stearic acid (C18:0), and *cis*-7 hexadecenoic acid (C16:ln9c) showed no significant change among different fertilization groups. The amount of palmitic acid (C16:0) was decreased while that of *cis*-7 hexadecenoic acid (C16:ln9c), trienoic acid (C16:3n6), linoleic acid (C18:2n6c+t), or linolenic acid (C18:3n3) was generally increased by these fertilization treatments. Palmitic acid showed the most dramatic change in terms of fatty acid content. It was significantly decreased by fertilization, ranging from 18.432 mg/g dry wt. to 23.546 mg/g dry wt. Most of the decrease in palmitic acid content (by 28.5%) was caused by HSJ treatment. It decreased more by double fertilizer treatment (HS, HJ, or SJ) compared to single fertilizer treatment (H, S, or J). Regarding the contents of trienoic acid and linoleic acid, the largest increase was obtained by HS and HJ treatments (8.1% and 9.0% increase, respectively) compared to the control. The contents of linolenic acid, the major fatty acid in Chinese cabbage, ranged from 45.045 mg/g dry wt. (no fertilizer) to 48.654 mg/g dry wt. (HSJ treatment). Treatment with H (4.6%), S (4.8%), J (6.3%), HS (6.0%), HJ (5.7%), SJ (6.9%), or HSJ (8.0%) fertilizer significantly increased linolenic acid contents. Fertilization had a tendency to increase total oil content. A significant increase resulted from SJ treatment (Table 2).

A total of 9 carotenoid compounds were identified from Chinese cabbage leaves (Table 2). Total carotenoid contents ranged from 3351 μg/g dry wt. (no fertilizer) to 4380 μg/g dry wt. (HSJ treatment). The major carotenoid was β-carotene, ranging from 1525 μg/g dry wt. (H treatment) to 2198 μg/g dry wt. (HSJ treatment). Generally, the amounts of violaxanthin, antheraxanthin, lutein, 13-cis-β-carotene, α-carotene, β-carotene, and 9-*cis*-β-carotene significantly increased, whereas the contents of zeaxanthin and β-cryptoxanthin showed no change after these fertilizer treatments. The largest increase for each component resulted from double fertilization (increase of violaxanthin, antheraxanthin, and lutein by HJ, and increase of 9-*cis*-β-carotene by SJ) or triple fertilizer application (increase of 13-*cis*-β-carotene, α-carotene, and β-carotene by HSJ). Compared to control (no fertilizer), maximum increases for violaxanthin, antheraxanthin, lutein, 13-*cis*-β-carotene, α-carotene, β-carotene, and 9-*cis*-β-carotene were 20.2%, 34.0%, 21.5%, 58.5%, 49.6%, 39.7%, and 23.5%, respectively, resulting in total carotenoid increase of 30.7%.

As shown in Table 2, 11 glucosinolates (3 indole, 7 aliphatic, and 1 unknown) were identified. Most changes in total glucosinolate content resulted from SJ treatment (14.693μmol/g dry wt.) and HSJ treatment (10.361 μmol/g dry wt.) with significant increase and significant decrease, respectively, compared to no fertilizer treated sample. The major glucosinolate found in Chinese cabbage leaves was glucobrassicanapin (3.296 ~ 5.060 μmol/g dry wt.). It was increased by S (47.8%), HS (24.2%), HJ (20.0%), and SJ (53.5%) fertilization. There was no change in the components of gluconapin, glucoerucin, glucobrassicin, or unknown caused by these fertilization treatments. All fertilization treatments decreased the amount of progoitrin by 20.9% (H fertilization) to 53.8% (HJ fertilization). They also decreased the amount of 4-methoxyglucobrassicin by 19.5% (HJ fertilization) to 58.1% (J fertilization). Fertilization treatment with J, HS, SJ, or HSJ resulted in a significant decrease in the amount of neoglucobrassicin by 44.4%, 45.1%, 45.7%, or 51.2%, respectively. Sinigrin content tended to be decreased by these fertilization treatments. Such decrease was not detected after HSJ fertilizer treatment. Glucoalyssin was significantly induced by S, HJ, SJ, and HSJ fertilization treatments with an increase of 121.9%, 91.4%, 158.6%, and 159.5%, respectively. The amount of glucocochlearin was significantly increased only by HJ fertilization (1.117 μmol/g dry wt.) with a 69.2% increase (Table 2).

3.3. Expression analysis of fatty acid, carotenoid, and glucosinolate biosynthesis genes

Transcriptional levels of fatty acid, carotenoid, and glucosinolate biosynthesis genes in fertilizer treated Chinese cabbage samples were determined by qRT-PCR (Figures 1–3). Four fatty acids biosynthesis related genes displayed upregulated expression patterns by fertilizer treatments in general. However, they had different expression levels depending on fertilizer type (Figure 1b). The expression of *BrLACS1* was upregulated 1.19-fold and 1.23-fold by SJ and HSJ fertilization treatments, respectively. The maximum induction of *BrKCS1* was caused by HSJ fertilizer treatment with 1.35-fold higher expression. Fertilization with J, HS, HJ, and SJ also

Table 2. Comparison of bioactive compounds accumulation by different environmentally friendly fertilizations in Chinese cabbage (N, no fertilizer; H, Hugwang fertilizer (purslane extract); S, Seonsi fertilizer (5% sulfur); J, Jeonbudae fertilizer (15% phosphoric acid and 5% calcium phosphate); HS, Hugwang + Seonsi fertilizers; HJ, Hugwang + Jeonbudae fertilizers; SJ, Seonsi + Jeonbudae fertilizers; HSJ, Hugwang + Seonsi + Jeonbudae fertilizers).

Bioactive compound		N	H	S	J	HS	HJ	SJ	HSJ
Fatty acids	C16:0	25.766a	23.516b	23.546b	22.675b	20.534c	19.577cd	19.928c	18.432d
(mg/g dry wt.)	C16:1n9c	1.443b	1.805ab	1.747ab	1.805ab	1.802ab	1.979a	2.032a	2.022a
	C16:2n6	0.445a	0.460a	0.381a	0.367a	0.356a	0.304a	0.295a	0.308a
	C16:3n6	0.595ab	0.521ab	0.550ab	0.541ab	0.643a	0.584ab	0.499b	0.593ab
	C16:3n3	6.047a	8.058a	7.964a	7.973a	8.116a	7.810a	8.136a	7.878a
	C18:0	3.884a	3.230a	3.238a	3.416a	3.179a	3.604a	3.180a	3.636a
	C18:1n9c+t	6.600a	6.151a	6.539a	6.596a	6.097a	6.476a	6.440a	6.248a
	C18:2n6c+t	10.630c	11.848a	11.588a	10.847bc	11.326ab	11.591a	11.355ab	10.712c
	C18:3n3	45.045b	47.125a	47.212a	47.888a	47.736a	47.600a	48.141a	48.654a
	Total fatty acids	100.455ab	102.714ab	102.765a	102.108ab	99.789ab	99.525ab	100.006ab	98.483b
Oil contents (%)		9.981b	13.788ab	12.894ab	13.803ab	13.564ab	13.942ab	15.173a	14.345ab
Carotenoids	Violaxanthin	408.109ab	409.660ab	404.792b	460.571ab	482.012ab	490.702a	452.827ab	477.206ab
(ug/g dry wt.)	Antheraxanthin	91.153c	99.566bc	105.326abc	99.117bc	113.925ab	122.131a	118.790ab	116.437ab
	Lutein	1,043.315c	1,103.464bc	1,130.223abc	1,115.416abc	1,214.818ab	1,267.302a	1,255.040ab	1,262.775a
	Zeaxanthin	18.113a	26.304a	25.419a	25.362a	23.037a	30.662a	27.366a	21.152a
	β-cryptoxanthin	0.982a	0.894a	1.060a	1.034a	1.247a	1.149a	1.034a	1.212a
	13-*cis*-β-carotene	100.820c	122.583bc	140.962ab	124.617bc	148.316ab	129.776abc	138.948ab	159.785a
	α-carotene	14.610c	17.163abc	19.908abc	17.288abc	20.935ab	15.622bc	18.233abc	21.856a
	β-carotene	1,573.924c	1,525.268c	1590.896c	1,632.743bc	1,726.824bc	1,815.121abc	2,029.407ab	2,198.359a
	9-*cis*-β-carotene	100.464c	113.274abc	119.743ab	107.256bc	119.157ab	120.865ab	124.104a	121.439ab
	Total carotenoids	3,351.490e	3,418.176ed	3,538.329cde	3583.404cde	3,850.271bcd	3,993.330abc	4,165.749ab	4,380.221a
Glucosinolates	Progoitrin	0.839a	0.664b	0.594bc	0.445cd	0.436cd	0.388d	0.452cd	0.514bcd
(umol/g dry wt.)	Sinigrin	0.195a	0.193a	0.241a	0.143ab	0.082ab	0.162ab	0.106ab	NDb
	Glucoalyssin	0.370d	0.652bcd	0.821ab	0.474cd	0.612bcd	0.708abc	0.957a	0.960a
	Gluconapin	2.962a	3.284a	4.062a	3.182a	3.558a	3.588a	4.420a	3.036a
	Glucobrassicanapin	3.296c	3.568c	4.871a	3.395c	4.092b	3.969b	5.060a	3.492c
	Glucoerucin	0.947a	1.571a	0.953a	0.682a	0.876a	0.883a	0.953a	0.638a
	Glucocochlearin	0.660b	0.779ab	0.745ab	0.733ab	0.679ab	1.117a	0.738ab	0.544b
	Glucobrassicin	0.666a	1.482a	0.663a	0.603a	0.702a	1.104a	0.879a	0.619a
	Unknown	0.281a	0.502a	0.553a	0.462a	0.267a	0.231a	0.623a	0.107a
	4-methoxyglucobrassicin	0.697a	0.409bc	0.325bc	0.292c	0.465abc	0.561ab	0.417bc	0.372bc
	Neoglucobrassicin	0.162a	0.107ab	0.130ab	0.090b	0.089b	0.118ab	0.088b	0.079b
	Total glucosinolates	11.075ab	13.211ab	13.958ab	10.501ab	11.858ab	12.829ab	14.693a	10.361b

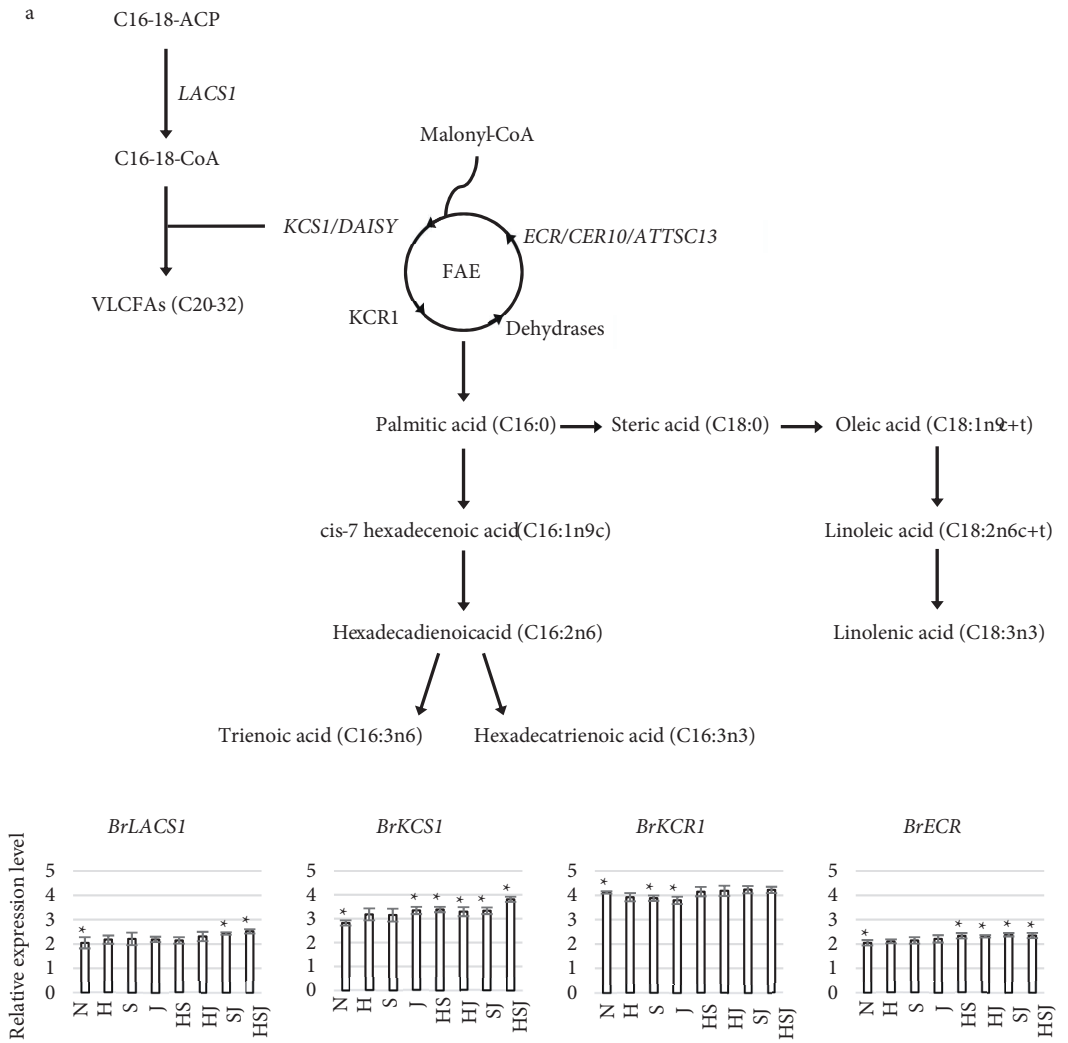


Figure 1. Effect of environmentally friendly fertilizer treatments on fatty acid biosynthesis gene expression in Chinese cabbage. (a) Simplified scheme of fatty acids biosynthetic pathways in plant. (b) Expression analysis of fatty acid biosynthesis genes. N, No fertilizer; H, Hugwang fertilizer (purslane extract); S, Seonsi fertilizer (5% sulfur); J, Jeonbudae fertilizer (15% phosphoric acid and 5% calcium phosphate); HS, Hugwang + Seonsi fertilizers; HJ, Hugwang + Jeonbudae fertilizers; SJ, Seonsi + Jeonbudae fertilizers; HSJ, Hugwang + Seonsi + Jeonbudae fertilizers. *, Significant differences between fertilizer treatments at $P < 0.05$.

significantly upregulated *BrKCS1* by 1.18-fold, 1.20-fold, 1.17-fold, and 1.18-fold, respectively. The expression level of *BrECR* was steadily increased by HS (1.14-fold), HJ (1.13-fold), SJ (1.16-fold), and HSJ (1.15-fold) fertilizer treatment compared to no fertilizer (N) treatment. The transcript level of *BrKCR1* was significantly reduced by S (1.06-fold) and J (1.09-fold) fertilizer treatments (Figures 1a and 1b).

The expression changes of carotenoid biosynthesis gene by our fertilizations are plotted (Figures 2a and 2b). The transcript level of *BrPSY*, an upstream gene of the carotenoid biosynthetic pathway, was not significantly changed by fertilization treatment. *BrPDS* and *BrZDS* showed abundant transcription levels. They were significantly upregulated by fertilizer containing S or J. Both genes were upregulated the most by HSJ treatment

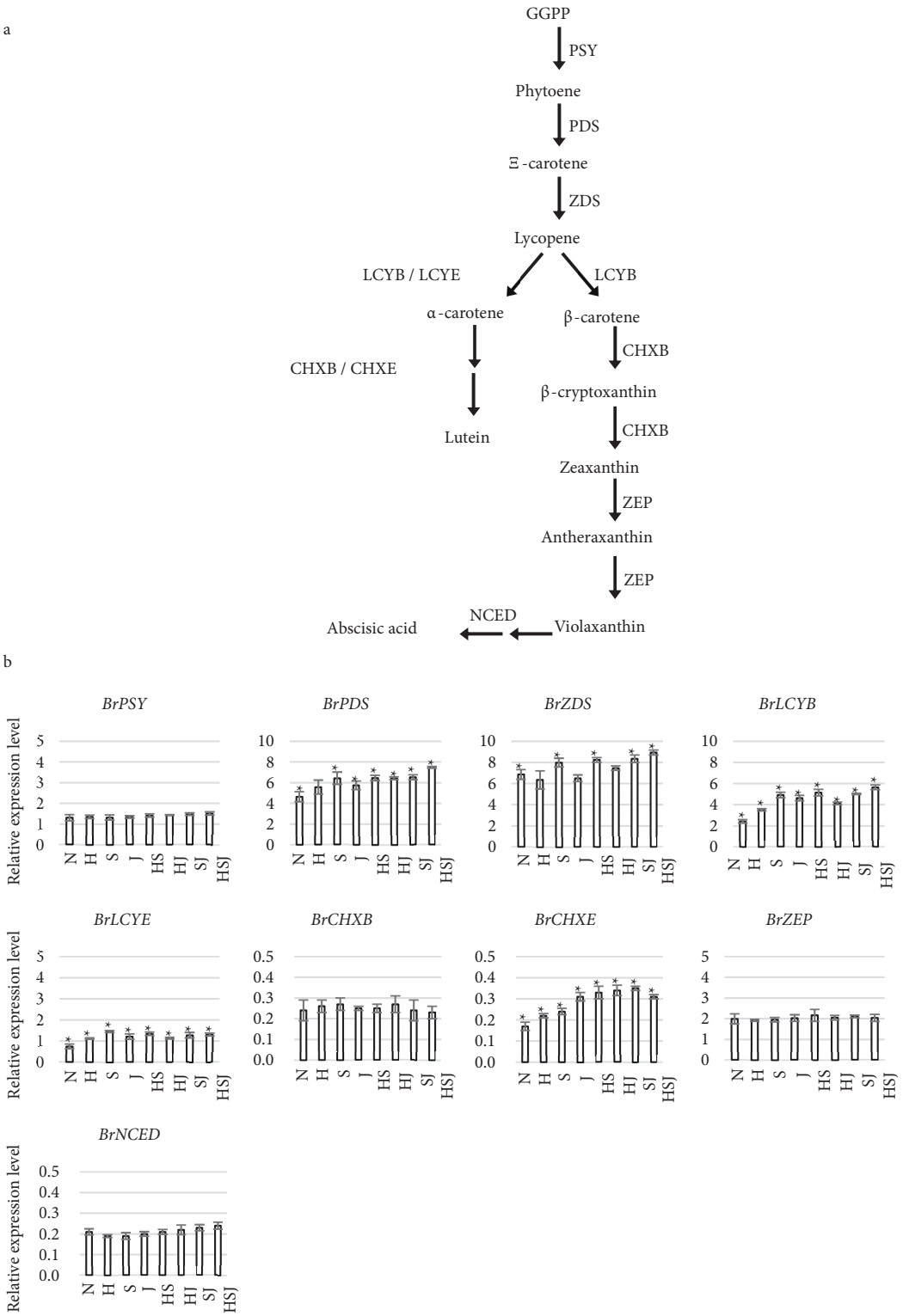


Figure 2. Analysis of carotenoid biosynthesis gene expression in Chinese cabbage cultivated with different environmentally friendly fertilizers. (a) Carotenoid biosynthesis pathway in plants. (b) Expression of carotenoid biosynthesis genes after different fertilizer treatments. N, No fertilizer; H, Hugwang fertilizer (purslane extract); S, Seonsi fertilizer (5% sulfur); J, Jeonbudae fertilizer (15% phosphoric acid and 5% calcium phosphate); HS, Hugwang + Seonsi fertilizers; HJ, Hugwang + Jeonbudae fertilizers; SJ, Seonsi + Jeonbudae fertilizers; HSJ, Hugwang + Seonsi + Jeonbudae fertilizers. *, Significant differences between fertilizer treatments at $P < 0.05$.

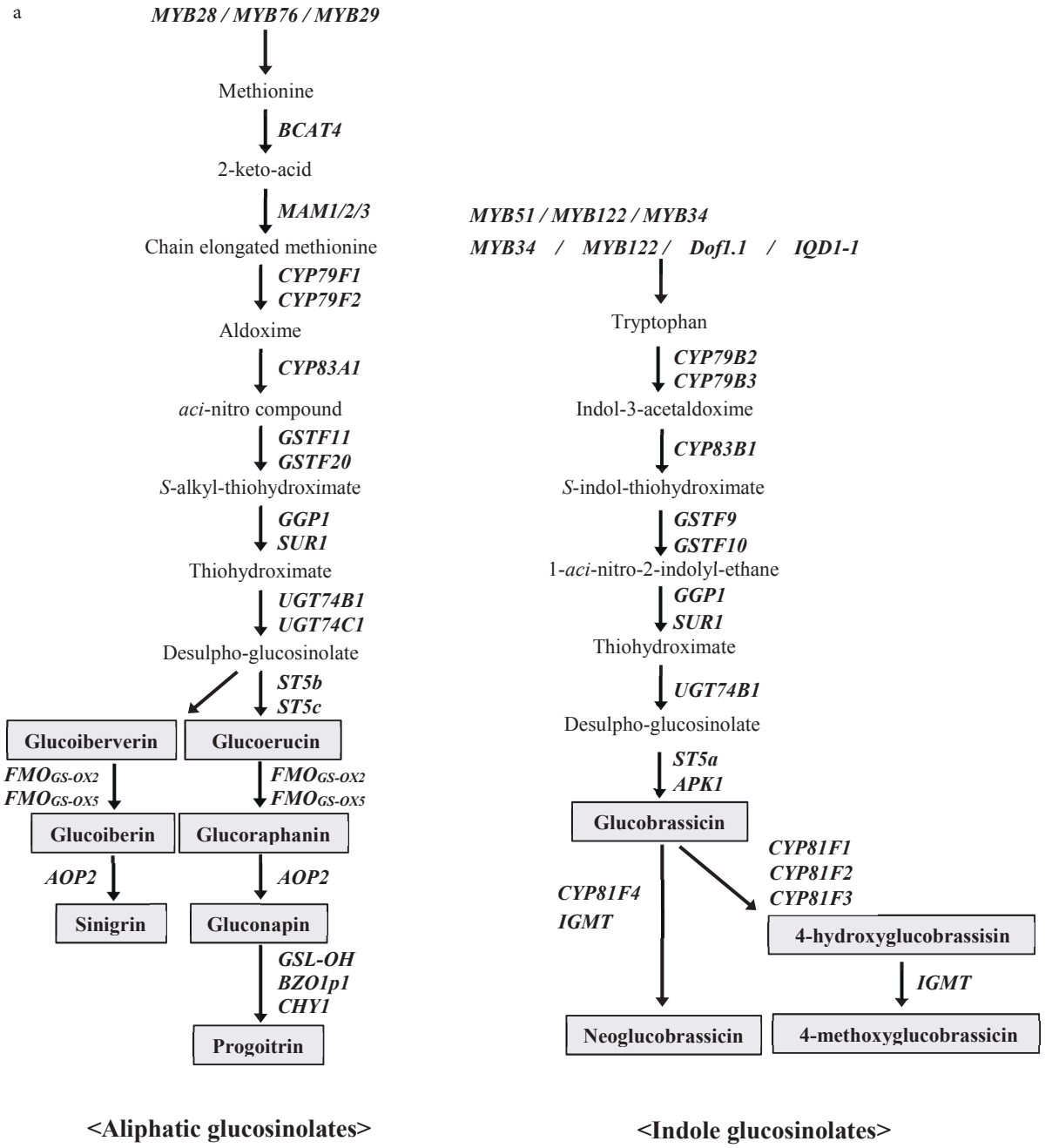
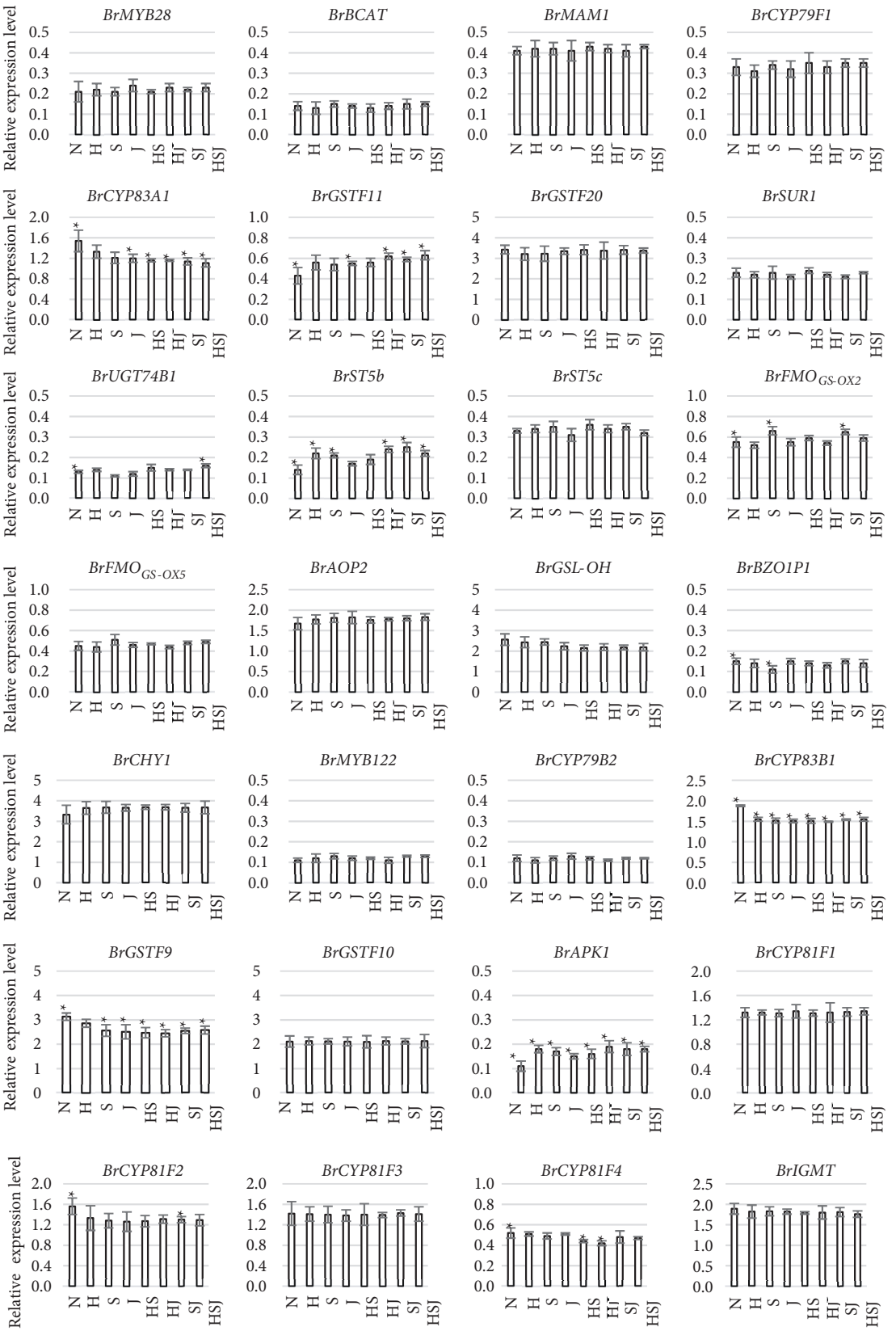


Figure 3. Effect of environmentally friendly fertilization on the expression of glucosinolate biosynthesis genes. (a) Schematic representation of glucosinolate biosynthesis. (b) Expression of glucosinolate biosynthesis genes. N, No fertilizer; H, Hugwang fertilizer (purslane extract); S, Seonsi fertilizer (5% sulfur); J, Jeonbudae fertilizer (15% phosphoric acid and 5% calcium phosphate); HS, Hugwang + Seonsi fertilizers; HJ, Hugwang + Jeonbudae fertilizers; SJ, Seonsi + Jeonbudae fertilizers; HSJ, Hugwang + Seonsi + Jeonbudae fertilizers. *, Significant differences between fertilizer treatments at $P < 0.05$.

Figure 3. Continued.



(1.61-fold and 1.30-fold, respectively). Transcript levels of *BrLCYB*, *BrLCYE*, and *BrCHXE* were induced by all fertilizer treatments. The expression of *BrLCYB* increased the most (2.33-fold) by HSJ fertilization. The expression levels of *BrLCYE* and *BrCHXE* increased the most by S fertilization and SJ fertilization (at 1.93-fold and 2.06-fold, respectively). However, transcriptional levels of *BrCHXB*, *BrZEP*, and *BrNCED* were not significantly changed by any fertilizer treatment in this study (Figure 2b).

The expression levels of glucosinolate biosynthetic genes were not dramatically altered by any fertilization treatment either (Figures 3a and 3b). The *BrCHY1* gene showed the highest expression in general. However, its transcript level was not significantly changed by any fertilization treatment. Only 11 of the glucosinolate biosynthesis genes (5 indole and 6 aliphatic) among a total of 28 examined were significantly changed in their expression levels by fertilizer treatment. Four aliphatic glucosinolate biosynthesis genes (*BrGSTF11*, *BrUGT74B1*, *BrST5b*, and $BrFMO_{GS\text{-}OX2}$) were upregulated while 2 (*BrCYP83A1* and *BrZO1P1*) were downregulated by fertilizer treatments. Changes in transcriptional level were highest for *BrCYP83A1*, *BrGSTF11*, and *BrUGT74B1* by HSJ treatment, *BrST5b* by SJ treatment, and $BrFMO_{GS\text{-}OX2}$ and *BrZO1P1* by S treatment. The expression levels of most indole glucosinolate biosynthesis genes (*BrCYP83B1*, *BrGSTF9*, *BrCYP81F2*, and *BrCYP81F4*) were downregulated by fertilization treatment, whereas *BrARK1* was upregulated by all fertilizer treatments (Figure 3b). The expression levels of *BrCYP83B1* and *BrARK1* were also affected by all fertilization treatments. The transcriptional level of *BrCYP81F2* was changed by SJ treatment only. H treatment showed no effect on the expression level of *BrGSTF9*.

4. Discussion

A proper supply of plant nutrients is required for cultivation of crops. The requirement of plant nutrients can be met by applying fertilizers to achieve the best yield. However, continuous use of chemical fertilizers, especially nitrogen, one of the most limiting nutrients in soil and essential for high crop yields, has not been helpful. Nitrogen fertilizer not only reduces crop yield by causing soil acidity and nutrient imbalance (Ayoola and Adeniyan, 2006), but also detracts the environment. The use of organic fertilizers or environmentally friendly fertilizers can minimize this problem. They can increase the productivity of soil, microbial biomass, crop quality, and crop yield with longer influence and more effectiveness (Tindall, 2000; Suresh et al., 1996; Suresh et al., 2004).

Extract from *Portulaca oleracea* (H fertilizer in this study) is known to have no cytotoxicity or genotoxicity. They have been certified safe for human consumption (Yen et al., 2001). They also have beneficial effects (protective against oxidative stress) (Madiha et al., 2012). They can also significantly alter the bacterial community without affecting intestinal pH (Zhao et al., 2013). It has been reported that purslane leaves and stems contain high levels of potassium, magnesium, and calcium, which are essential macronutrient components for protein synthesis, photosynthesis, fruit quality, and disease reduction (Teixeira and Carvalho, 2009; Karley and White, 2008). They also mediate a wide range of cellular responses (White, 2003; Sanders et al., 2002). S and J fertilizers are also environmentally friendly. The main ingredient of S is from nature. Both S and J fertilizers can be sprayed to leaves at a very low dose (1.22%), thus minimizing soil accumulation of fertilizers and acidification.

We carried out a field experiment to investigate the effect of these environmentally friendly fertilizers on the growth and bioactive components' contents of Chinese cabbage (Tables 1 and 2). Our results showed trends similar to those obtained for cabbage and broccoli (Selim Reza et al., 2016; Øvsthus et al., 2015; Naguib et al., 2012). The better nutrient uptake by cabbages resulting from organic fertilizer treatment compared to chemical fertilization treatment has suggested that vermicompost is a suitable substitute for urea, the most commonly used nitrogen source for the production of cabbage (Selim et al., 2016). The beneficial effect of organic fertilization on yield has been explained by slow release of nutrients, increased organic matter content, and decreased soil pH, leading to root growth and enhanced nutrients uptake (Selim et al., 2016).

Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) is a major vegetable crop in Korea. It is the principle ingredient of kimchi, a traditional Korean food and metabolite vegetable. It contains a range of essential vitamins, minerals, and phytochemicals (Bjӧrkman et al., 2011; Das et al., 2000). In our study, palmitic acid content significantly decreased while linolenic acid content significantly increased by all treatments with environmentally friendly fertilizer (Table 2). Palmitic acid is a saturated fatty acid commonly found in both animals and plants. It is a major component in palm tree oil known to be associated with an increased risk of coronary heart disease and some tumors in humans (Fattore and Fanelli, 2013). Linolenic acid, the major fatty acid in Chinese cabbage, is known to be able to prevent and treat heart disease and blood vessel disease (Lorgeril and Salen, 2004; Farvid et al., 2014). Our results suggest that these environmentally friendly fertilizers are effective in producing less deleterious, but more beneficial contents in Chinese cabbage for human health in terms of fatty acids. Several reports have also observed that mineral fertilization can result in higher levels of carotenoids (Brandt and Beeson, 1951; Eggert

and Kahrmann, 1984; Salomon, 1972). In our results all carotenoids, except zeaxanthin and β-cryptoxanthin, were significantly increased by our environmentally friendly fertilizers. Changes in total glucosinolate contents were meager compared to those in fatty acids or carotenoids (Table 2). The application of organic and bioorganic fertilizers has also significantly increased yield, total phenolics, flavonoids, and glucosinolates in 2 broccoli cultivars, demonstrating that organic fertilizers can enhance the yield of secondary metabolites (Selim et al., 2016; Vågen et al., 2007). Øvsthus et al. (2015) have reported that glucosinolate contents are significantly increased by extruded shrimp shell and mineral NPK fertilization compared to sheep manure or no fertilizer treated broccoli (Øvsthus et al., 2015). They also presented a positive correlation between total glucosinolate content in broccoli and sulfur content in fertilizer materials at the current fertilizer rate (Øvsthus et al., 2015). However, Rosa et al. have reported that nitrogen and sulfur fertilization does not change the total glucosinolate content in broccoli sprout (Rosa et al., 2006). In our results, total glucosinolates were increased significantly by SJ fertilization. However, whether the sulfur content only in fertilizer is correlated with glucosinolate content remained unclear because HSJ fertilization significantly decreased the total glucosinolate content. Progoitrin is known to be a strong goitrogenic that inhibits the synthesis of thyroid hormones, thyroxine, and tri-iodine-thyronine by selective binding to iodine, thus preventing iodine intake by the thyroid gland (Zukalova and Vasak, 2002). Breeding programs for *Brassica* crops, including broccoli, turnip, cabbage, cauliflower, kale, and Chinese cabbage are intensively focused on the production of *Brassica* vegetables without the use of progoitrin, or using low progoitrin concentrations for human and animal health (Ishida et al., 2014). Interestingly, progoitrin content was significantly decreased by the environmentally friendly fertilizers used in this study (Table 2), although some fertilizers (SJ) increased the total glucosinolate content. These results indicated that the fertilizers examined in this study might be beneficial for organic or environmentally friendly cultivation of Chinese cabbage by increasing its yields and nutritional value while minimizing damages to the environment and the soil.

In the present study, it was observed that the use of environmentally friendly fertilizers resulted in higher yields and higher bioactive component contents, as well as gene expression changes compared to control without treatment by these fertilizers. The HSJ treatment appeared to be the most promising fertilizer combination for higher biomass production, higher total fatty acid, and higher total carotenoid contents in Chinese cabbage. Furthermore, the contents of palmitic acid and progoitrin were decreased by using a combination of these fertilizers. Therefore, these environmentally friendly fertilizers might be useful for Chinese cabbage production. They can improve its nutritional value and contribute to the production of safer vegetables to promote human health with less destruction to the environment and soil.

Acknowledgements
This work was supported by grants from the Golden Seed Project (213007–05–1–SB610) funded by the Ministry of Agriculture, Food, and Rural Affairs, Republic of Korea. It was also supported by a grant from the Korea Research Institute of Bioscience and Biotechnology Research Initiative Program.

References

Abd-Alla HM, Yan F, Schubert S (1999). Effects of sewage sludge application on nodulation, nitrogen fixation and plant growth of faba bean, soybean and lupin. J Appl Bot 73: 69-75.

Akiyama T, Shimo Y, Yanai H, Qin J, Ohshima D, Maruyama Y, Asaumi Y, Kitazawa J, Takayanagi H, Penninger JM, et al. (2008). The tumor necrosis factor family receptors RANK and CD40 cooperatively establish the thymic medullary microenvironment and self-tolerance. Immunity 29: 423-437.

Aldanondo-Ochoa AM, Casasnovas-Oliva VL, Arandia-Miura A (2014). Environmental efficiency and the impact of regulation in dryland organic vine production. Land Use Policy 36: 275-284.

Argyropoulos C, Tsiafouli MA, Sgardelis SP, Pantis JD (2013). Organic farming without organic products. Land Use Policy 32: 324-328.

Asami DK, Hong YJ, Barrett DM, Mitchell AE (2003). Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn using conventional, organic, and sustainable agricultural practices. J Agric Food Chem 51: 1237-1241.

Ayoola OT, Adeniyan ON (2006). Influence of poultry manure and NPK fertilizer on yield components of crops under different cropping systems in South West Nigeria. African J Biotechnol 5: 1386-1392.

Björkman M, Klingen I, Birch ANE, Bones AM, Bruce TJA, Johansen TJ, Meadow R, Mølmann J, Seljåsen R, Smart LE et al. (2011). Phytochemicals of Brassicaceae in plant protection and human health – influences of climate, environment and agronomic practice. Phytochemistry 72: 538-556.

Brandt CS, Beeson KC (1951). Influence of organic fertilization on certain nutritive constituents of crops. Soil Sci 71: 449-454.

Camargo JA, Alonso A (2006). Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. Environment Int 32: 831-849.

Chen JH (2006). The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. In: International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use, 16–20 October 2006; Bangkok, Thailand: Land Development Department, pp. 1-11.

Das S, Tyagi AK, Kaur H (2000). Cancer modulation by glucosinolates: A review. Curr Sci 79: 1665-1671.

Dekker M, Verkerk R, Jongen WMF (2000). Predictive modelling of health aspects in the food production chain: A case study on glucosinolates in cabbage. Trends Food Sci Tech 11: 174-181.

Dobermann A, Cassman KG (2002). Plant nutrient management for enhanced productivity in intensive grain production systems of the United States and Asia. Plant Soil 247: 153-175.

Dumas Y, Dadomo M, Di Lucca G, Grolier P (2003). Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. J Sci Food Agric 83: 369-382.

Eggert FP, Kahrmann CL (1984). Responses of three vegetable crops to organic and inorganic nutrient sources. In: Organic Farming: Current Technology and its Role in Sustainable Agriculture. Pub. No. 46. American Society of Agronomy, Madison, WI, USA, pp. 85-94.

Farvid MS, Ding M, Pan A, Sun Q, Chiuve SE, Steffen LM, Willett WC, Hu FB (2014). Dietary linoleic acid and risk of coronary heart disease: A systematic review and meta-analysis of prospective cohort studies. Circulation 130: 1568-1578.

Fattore E, Fanelli R (2013). Palm oil and palmitic acid: a review on cardiovascular effects and carcinogenicity. Int J Food Sci Nutr 64: 648-659.

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. *chinesis*) as affected by season and variety. J Sci Food Agric 89: 906-914.

Hussain MI, Shan SH, Sajjad H, Khalid I (2002). Growth, yield and quality response of three wheat (*Triticum aestivum* L.) varieties to different levels of N, P and K. Int J Agric Biol 4: 362-364.

Ishida M, Hara M, Fukino N, Kakizaki T, Morimitsu Y (2014). Glucosinolate metabolism, functionality and breeding for the improvement of Brassicaceae vegetables. Breed Sci 64: 48-59.

Karley AJ, White PJ (2008). Moving cationic minerals to edible tissues: potassium, magnesium, calcium. Curr Opin Plant Biol 12: 291-298.

Kliebenstein DJ, Kroymann J, Brown P, Figuth A, Pedersen D, Gershenzon J, Mitchell-Olds (2001). Genetic control of natural variation in *Arabidopsis* glucosinolate accumulation. Plant Physiol 126: 811-825.

Läpple D, Rensburg TV (2011). Adoption of organic farming: Are there differences between early and late adoption? Ecol Econ 70: 1406-1414.

Leifeld J (2012). How sustainable is organic farming? Agric Ecosyst Environ 150: 121-122.

Logan TJ, Lindsay BJ, Goins LE, Ryan JA (1997). Field assessment of sludge metal bioavailability to crops: Sludge rate response. J Environ Qual 26: 534-550.

Lopez-Perez A, Casanova E, Chacon LA, Paz PM, Guerrero JR (1990). Residual effect of three phosphate rocks from Tachina (Venezuela) in a greenhouse experiment with maize (*Zea mays* L.) as indicator plant. Revista Cientifica UNET 4: 29-48.

Lorgeril M, Salen P (2004). Alpha-linolenic acid and coronary heart disease. Nutr Metab Cardiovas 14: 162-169.

Madiha F, Hamdy T, Soliman MS, Uama F, Ahmed HB (2012). Subchronic feeding study of fenitrothion residues in maize and the protective action of purslane (*Portulaca oleracea*) extracts on rats. J Appl Sci Res 8: 3688-3696.

Naguib AEMM, El-Baz FK, Salama ZA, Hanaa HAEB, Ali HF et al. (2012). Enhancement of phenolics, flavonoids and glucosinolates of Broccoli (*Brassica olaracea*, var. *Italica*) as antioxidants in response to organic and bio-organic fertilizers. J Saudi Soc of Agr Sci 11: 135-142.

Oboh G, Raddatz H, Henle T (2008). Antioxidant properties of polar and non-polar extracts of some tropical green leafy vegetables. J Sci Food Agric 88: 2486-2492.

Øvsthus I, Breland TA, Hagen SF, Brandt K, Wold AB, Bengtsson GB, Seljåsen R (2015). Effects of organic and waste-derived fertilizers on yield, nitrogen and glucosinolate contents, and sensory quality of broccoli (*Brassica oleracea* L. var. *italica*). J Agric Food Chem 63: 10757-10767.

Patil S, Reidsma P, Shah P, Purushothaman S, Wolf J (2014). Comparing conventional and organic agriculture in Karnataka, India: Where and when can organic farming be sustainable? Land Use Policy 37: 40-51.

Porazinska DL, Duncanb LW, McSorley R, Graham JH (1999). Nematode communities as indicators of status and processes of a soil ecosystem influenced by agricultural management practices. Appl Soil Ecol 13: 69-86.

Prasad R (2009). Efficient fertilizer use: The key to food security and better environment. J Trop Agric 47: 1-17.

Rafael G, Mancha M (1993). One-step lipid extraction and fatty acid methyl ester preparation from fresh plant tissues. Anal Biochem 211: 139-143.

Reza MS, Islam AKMS, Rahman MA, Miah MY, Akhter S, Rahman MM (2016). Impact of organic fertilizers on yield and nutrient uptake of cabbage *(Brassica oleracea* var. *capitata*). Journal of Science, Technology and Environment Informatics 3: 231-244.

Rosa E, Rosa C, Alfredo A (2006). Effect of nitrogen and sulphur fertilization on glucosinolate in the leaves and roots of broccoli sprouts (*Brassica oleracea* var. *italica*). J Sci Food Agric 86: 1512-1516.

Salomon M (1972). Natural foods – myth or magic. Assoc Food Drug Offic Quart Bull 36: 131-137.

Sanders D, Pelloux J, Brownlee C, Harper JF (2002). Calcium at the crossroads of signaling. Plant Cell 14: 401-417.

Song JM, Liu AF, Wu XY, Liu JJ, Li HS, Zhao ZD (2004). Effects of N and K fertilizers on bread wheat quality of Jinan17. Sci Agric Sinica 37: 344-350.

Subhasree B, Baskar R, Laxmi KR, Lijina SR, Rajasekaran P (2009). Evaluation of antioxidant potential in selected green leafy vegetables. Food Chem 115: 1213-1220.

Suresh CF, Fang HC, Nan K, Hus KN (1996). Effect of continuous use of organic manures on the growth and yield of vegetable soybean and cabbage. Bull Taichung Dist Agric Improve Sta, Japan 46: 1-10.

Suresh KD, Sneh G, Krishn KK, Mool CM (2004). Microbial biomass carbon and microbial activities of soils receiving chemical fertilizers and organic amendments. Arch Agron Soil Sci 50: 641-647.

Teixeira M, Carvalho IS (2009). Effects of salt stress on purslane (*Portulaca oleracea*) nutrition. Ann Appl Biol 154: 77-86.

Tindall M (2000). Mineral and organic fertilizing in cabbage. Residual effect for commercial cultivation on yield and quality performance with organic farming. Hort Bras 6: 15-20.

Vågen IM, Aamlid TS, Skjelvåg AO (2007). Nitrogen fertilization to broccoli cultivars at different planting times: Yield and nitrogen use. Acta Agr Scand, Section B – Soil Plant Sci 57: 35-44.

Warwick SI, Francis A (1994). Guide to the Wild Germplasm of *Brassica* and Allied Crops, Part V: Life History and Geographical Data for Wild Species in the Tribe Brassiceae (Cruciferae). Technical Bulletin, Centre for Land and Biological Resources Research. Ottawa, Canada: Agriculture and Agri-Food.

Weibel FP, Bickel R, Leuthold S, Alfoldi T (2000). Are organically grown apples tastier and healthier? A comparative field study using conventional and alternative methods to measure fruit quality. Acta Hort 517: 417-426.

White PJ, Broadley MR (2003). Calcium in plants. Ann Bot 92: 487-511.

Yen GC, Chen HY, Peng HH (2001). Evaluation of the cytotoxicity, mutagenicity and antimutagenicity of emerging edible plants. Food Chem Toxicol 39: 1045-1053.

Young JW, Mau JL, Ko PT, Huang LC (2000). Antioxidant properties of fermented soybean broth. Food Chem 71: 249-254.

Zhao XH, He X, Yang XF, Zhong XH (2013). Effect of *Portulaca oleracea* extract on growth performance and microbial populations in ceca of broilers. Poult Sci 92: 1343-1247.

Zukalova H, Vasak J (2002). The role and effect of glucosinolates of *Brassica* species – a review. Rost Vyroba 48: 175-180.

Supplemental Table 1. Real time RT-PCR primers used to determine the expression of genes involved in fatty acid, carotenoid, and glucosinolate biosynthesis in Chinese cabbage.

Primer name	Sequence (5' → 3')
Fatty acid synthesis genes	
BrLACS1 forward	GCAAATGTGCTAAACGGCTA
BrLACS1 reverse	GTTCGTGTCTTCCGGTTTCT
BrKCS1 forward	AGAGAGATTAACGGCGGAGA
BrKCS1 reverse	TCACGTTGCAAGAGTTGTGA
BrKCR1 forward	CTTTCGCCTTTCAGTTAGCC
BrKCR1 reverse	ATCCATCACAACGGTCAAGA
BrECR forward	GCGGTTTCCTCTTCAACATT
BrECR reverse	GATTAGAGCAGCAACAGCGA
Carotenoid synthesis genes	
BrPSY forward	GCTATCTACGTTTGGTGCAGAAGAA
BrPSY reverse	AAATGGCTGAATATCGACAGGGTAT
BrPDS forward	GAGCTCGAGGATGATGGTACTGTTA
BrPDS reverse	TAACTGGCACACCAACTAGCTTCTC
BrZDS forward	CCTTCTTGTCAAAGACCACACTCAT
BrZDS reverse	AGCTAGTGAGTTCCTCAGCTTGTCA
BrLCYB forward	AAGATATCCAAGAGAGGATGGTTGC
BrLCYB reverse	CCACCATGTAACCTGTAGAAGGATG
BrLCYE forward	ATGGATGAACAGTCTAAGCTCGTTG
BrLCYE reverse	ACACCGTAGTTGTTTGTGAAAGGAA
BrCHXB forward	CAGAGAAAACAAGCTCTCTGGACAC
BrCHXB reverse	CATCTGCCAAGAGAATCGGTAGTAA
BrCHXE forward	CCGATTGGCTCACATCACTC
BrCHXE reverse	AGCTTTTCCCTCCACTGCAT
BrZEP forward	AGACTTAAGCGCCATAAGAGGAGAA
BrZEP reverse	ACTTGACATACCAAGTGCCAGAGAC
BrNCED forward	CACATCCTCTGTTTTGTTCACGAC
BrNCED reverse	AAGAGTTTGTTCCTGGAGTTGTTCC
Glucosinolate synthesis genes	
BrMYB28 forward	ACCATACTGTCAACACGCCTCC
BrMYB28 reverse	CAGAAGTGACCTTAGCCGCAAC
BrBCAT4 forward	TGGGAAGAATTAGGATTCGG
BrBCAT4 reverse	GCCCTGGCCATAGTTAAGAA
BrMAM1 forward	CGGCTTGTATGTTCAACCAC
BrMAM1 reverse	TCAAGATTCCATCCTGGTGA
BrCYP79F1 forward	TTTCATTCCCAAAGGTAGCC
BrCYP79F1 reverse	TTCGACCAGAGAAAGCTCCT
BrCYP83A1 forward	ACCGTGGTCACGAGTTCATA
BrCYP83A1 reverse	GTGTGGGTGAGAACAAGTGG
BrGSTF11 forward	ATCTTCTTCGTCAGCCGTTT

Supplemental Table 1. Continued.

Primer name	Sequence (5' → 3')
BrSGTF11 reverse	CAAGGTCTTGCCCAATAGGT
BrGSTF20 forward	CCTCTGATCCTTACGGGAAA
BrGSTF20 reverse	GCTGCTTGTTCCTCACCTTT
BrSUR1 forward	CCAAACGCAAACATATTGCT
BrSUR1 reverse	AGAAGATCGAACTTGCGGAT
BrUGT74B1 forward	GCTGCTTTCTTCACCAACAA
BrUGT74B1 reverse	AAGGAAGGAAGCTCGTCGTA
BrST5b forward	TTCTGTCGAGGTTTGTCTGG
BrST5b reverse	CACATACGGCAAAGGATCAC
BrST5c forward	GGATCGTCCTGCTGTGTATG
BrST5c reverse	GTGATGAAGCAAGAAAGCCA
$BrFMO_{GS\text{-}OX2}$ forward	CTGCATGTGATGATGGTTCA
$BrFMO_{GS\text{-}OX2}$ reverse	TCAACGCGGTTATCATCAAT
Glucosinolate synthesis genes	
$BrFMO_{GS\text{-}OX5}$ forward	GTAGCAGCACGAGAGCTACG
$BrFMO_{GS\text{-}OX5}$ reverse	GAGTGGACTACGGTTCGGTT
BrAOP2 forward	GCATTGTTCTCGACTCCAAA
BrAOP2 reverse	CTCTACGACCAGCCTCAGTG
BrGSL-OH forward	ATATTCCATAACCCGCAAGC
BrGSL-OH reverse	CTCCATGGCGTCTTTAACCT
BrBZO1p1 forward	CTGCATGTTTATGGGCTCAC
BrBZO1p1 reverse	TACGTCAACGTCAGCTAGGG
BrCHY1 forward	GGCGCTTCCTACTTCTTGTC
BrCHY1 reverse	CTGCTTCCAATGCAGTCAAT
BrMYB122 forward	CGTGGTGAGTTTAGCCAAGA
BrMYB122 reverse	TCCAGTGGTTCTTGATCTCG
BrCYP79B2 forward	TGGTGAACAAACCGGAGATA
BrCYP79B2 reverse	GAAGGCTTCACGGAGGATAG
BrCYP83B1 forward	GAATGTGGTCGGTGACAAAG
BrCYP83B1 reverse	TATCTTCGCGTCTGCTATGG
BrGSTF9 forward	ATCTCGCCTTACAGCCTTTC
BrGSTF9 reverse	TCTGTCTTCGACGGTTATGC
BrGSTF10 forward	ATCTATGCGCCTTTATTCGC
BrGSTF10 reverse	ATACTCAGGCTGCCTCTGCT
BrAPK1 forward	TTGCTTCCTGAGGGAGATTT
BrAPK1 reverse	GGCTCGTAAGGGTCATCAAT
BrCYP81F1 forward	TCCCTCGCACGCCGACG
BrCYP81F1 reverse	AGGATGCGGCAGCGAGTTA
BrCYP81F2 forward	GATACTGCAGCCGTGACACT
BrCYP81F2 reverse	CCAAACGTTCATGTCCAATC
BrCYP81F3 forward	GCCGAGATCACCGATGGAA
BrCYP81F3 reverse	TGAACGTCTTCTCCTCCGC

Supplemental Table 1. Continued.

Primer name	Sequence (5' → 3')
BrCYP81F4 forward	TTAACGGAAGAGGACATCAAAG
BrCYP81F4 reverse	AAAGAGGGGAAGGAGACAAAGA
BrIGMT forward	GAACATTGCCTTTGACATGG
BrIGMT reverse	TCCAGCAGTGATAAGCTTGG
BrACT2 forward	TAGTGTTGTTGGTAGGCCAAGACAT
BrACT2 reverse	GGAGCTCGTTGTAGAAAGTGTGATG