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Effects of glucose and gibberellic acid on glucosinolate content and antioxidant properties of Chinese kale sprouts*

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Abstract: Glucosinolates, anthocyanins, total phenols, and vitamin C, as well as antioxidant capacity, were investigated in Chinese kale sprouts treated with both glucose and gibberellic acid (GA_3). The combination of 3% (0.03 g/ml) glucose and 5 μmol/L GA_3 treatment was effective in increasing glucosinolate content while glucose or GA_3 treatment alone did not influence significantly almost all individual glucosinolates or total glucosinolates. The total phenolic content and antioxidant activity of Chinese kale sprouts were enhanced by combined treatment with glucose and GA_3, which could be useful in improving the main health-promoting compounds and antioxidant activity in Chinese kale sprouts.

Key words: Glucosinolate; Antioxidant; Glucose; Gibberellic acid; Chinese kale sprouts
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1 Introduction

Chinese kale (*Brassica oleracea* var. *alboglabra* Bailey) is a native Chinese vegetable, which belongs to the Cruciferae family and has an extensive distribution in South China. People usually harvest stems for food when they are bolting. Nowadays, the sprouts are also consumed because of their high nutritional values and rich content of bioactive compounds, such as the anticarcinogenic glucosinolates (Cartea and Velasco, 2008; Dinkova-Kostova and Kostov, 2012; Kumar *et al.*, 2015; Mazumder *et al.*, 2016). The sprouts of Chinese kale and other crucifers contain at least 10 times more glucosinolates than the mature stages of the corresponding plants (Fahey *et al.*, 1997; Sun *et al.*, 2011; Yuan *et al.*, 2010b).

Glucosinolates are a group of sulfur- and nitrogen-containing secondary metabolites which exist mainly in cruciferous plants. The glucosinolate content varies depending on genetic and environmental factors, and is modulated by external signals such as sugar (Miao *et al.*, 2013) and phytohormones (Yuan *et al.*, 2009; Sun *et al.*, 2012; Wang *et al.*, 2012; Guo *et al.*, 2013a; 2013b; Huseby *et al.*, 2013; Kim *et al.*, 2013; Zang *et al.*, 2015). Glucose is not only the basal carbon and energy source but also a signaling molecule having regulatory effects throughout the life cycle of plant (Sheen, 2014). Our previous studies showed that glucose dramatically increases glucosinolate content in both *Arabidopsis* and crop brassicas (Wei *et al.*, 2011; Guo *et al.*, 2013c; Miao *et al.*, 2013). Gibberellins are plant hormones participating in the regulation of plant development, which are used extensively to elevate crop yield (Naeem *et al.*, 2001).

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It has been reported that gibberellic acid (GA_3) increases various secondary metabolites (Abbasi *et al*., 2012; Park *et al*., 2017). However, studies on the effect of GA_3 on glucosinolates are limited, the only report being an observation by Huang *et al*. (2014) that exogenous GA_3 enhanced glucosinolate content in the silique of oilseed rape remarkably. The role of GA_3 in glucosinolate accumulation in other cruciferous crops, such as Chinese kale, merits further investigation.

Previous studies have demonstrated that GA_3 inhibits sucrose-induced accumulation of anthocyanin in *Arabidopsis* (Loreti *et al.*, 2008) and negatively interferes with glucose signal transduction in the regulation of cell wall invertase and sucrose synthesis in grape berry (Zhang *et al.*, 2014). However, there are limited reports on the relationship between GA_3 and glucose in glucosinolate biosynthesis. In the current study, we analyzed the effects of glucose and GA_3 on glucosinolate biosynthesis in Chinese kale sprouts. We also investigated whether glucose and GA_3 together have an impact on the content of other antioxidants found in Chinese kale sprouts including anthocyanins, total phenols, and vitamin C. This antioxidant activity is thought to make a great contribution to the health benefits of cruciferous vegetables (Podsędek, 2007).

2 Materials and methods

2.1 Plant materials and cultivation conditions

Chinese kale seeds (*Brassica oleracea* var. *alboglabra* Bailey cv. Dasunchihua) were sterilized for 30 min in 7 ml/L sodium hypochlorite and washed with sterile water 4–5 times. They were then soaked in sterile-distilled water for 24 h at 25 °C. Sprouts were grown in petri dishes with three pieces of wet filter paper in a plant growth chamber at 23 °C with a photoperiod of 16 h light/8 h dark.

The sprouts in the treatment group were watered with 5 ml of 3% (0.03 g/ml) glucose and/or 5 μmol/L GA_3 5 d after sowing; those in the control group were watered with 5 ml sterile-distilled water. For each treatment, three replicates were taken for analysis. Three days after treatment, sprouts were harvested from the surface of the filter papers and immediately frozen in liquid nitrogen. Samples for vitamin C detection were kept in polyethylene bags at −80 °C, while the others were frozen by lyophilization with a freeze dryer (Vir Tis Inc., New York, USA), crushed into powder, and finally stored at −20 °C for further analyses of glucosinolates, anthocyanins, total phenolic content, and antioxidant capacity.

2.2 Glucosinolate assay

Glucosinolates were extracted and analyzed as previously described with minor modifications (Sun *et al*., 2012). In this study, freeze-dried samples (30 mg) were boiled in 2 ml water for 10 min. The supernatant was transferred to a new tube after centrifugation (5 min, 7000*g*), and the residues were boiled for 10 min with water (2 ml). High-performance liquid chromatography (HPLC) was performed with a Shimadzu HPLC (Shimadzu, Kyoto, Japan) with an SPD-M20A diode array detector. Data were given as nmol/mg fresh weight (FW).

2.3 Anthocyanin measurement

Anthocyanin content was detected according to the previous method (Teng *et al.*, 2005). The modification is that we used freeze-dried sprouts (200 mg) which were homogenized at 4 °C in 2 ml of 1% (v/v) hydrochloric acid in methanol.

2.4 Total phenolic content assay

Total phenolic content was determined as previously described with some modifications (Ainsworth and Gillespie, 2007). The total phenolic compounds of Chinese kale sprouts (200 mg) were extracted with 5 ml of 30% ethanol, which were then incubated at 37 °C for 1 h and finally centrifuged at 7000 r/min for 10 min at room temperature. The supernatant was collected. Phenolic compounds were determined using Folin-Ciocalteu reagent method by reading the absorbance at 765 nm. Gallic acid was used as a standard and the results were expressed as mg gallic acid equivalent (GAE)/g dry weight (DW).

2.5 Vitamin C content

The vitamin C content was determined as previously described with minor modifications (Yuan *et al.*, 2010a). Frozen sprouts (200 mg) were homogenized at 4 °C in 2 ml of 1.0% (0.01 g/ml) oxalic acid and the residues were washed twice with 1 ml 1.0% oxalic acid. The combined extract was centrifuged at

7000 r/min for 10 min. A 0.45-μm cellulose acetate filter was used to filter the supernatant. HPLC analysis was performed using the same system as in the glucosinolate assay, with a mobile phase of 0.1% oxalic acid at a flow rate of 1.0 ml/min. The vitamin C value was calculated from absorbance values at 243 nm, and mg/100 g FW was adopted to express the results.

2.6 Antioxidant capacity

Antioxidant capacity was evaluated using the ferric reducing antioxidant power (FRAP) method of Benzie and Strain (1996) with some modifications. FRAP working solution (2.7 ml) was mixed with 300 μl samples extracted from about 200 mg of freeze-dried sprouts. After 10 min incubation at 37 °C, we recorded the absorbance at 593 nm. $FeSO_4 \cdot 7H_2O$ standard curves were used to calculate FRAP values which were expressed as mmol/100 g DW.

2.7 Statistical analysis

Statistical analysis was carried out using the SPSS package program version 11.5 (SPSS Inc., Chicago, IL, USA). Differences in glucosinolate content for different treatments were tested using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test at a 95% confidence level ($P<0.05$). Differences in the contents of total anthocyanins, phenols and vitamin C, and antioxidant activity between the treatment and control groups were analyzed using the independent-samples *t*-test. The values are reported as means with standard error for all results.

3 Results

3.1 Effects of glucose and GA_3 treatments on glucosinolate accumulation

We detected 11 different kinds of glucosinolates in Chinese kale sprouts (Table 1). Seven kinds of aliphatic glucosinolate were identified, the most abundant being gluconapin at about 38%. Glucose or GA_3 treatment alone did not exert a remarkable influence on aliphatic glucosinolate content (Fig. 1), but the application of both together did. Progoitrin, sinigrin, gluconapin, gluconapoleiferin, and total aliphatic glucosinolates increased by 44%, 54%, 56%, 43%, and 45%, respectively, when compared with the control (Figs. 1b, 1c, 1e, 1g, and 1h).

Table 1 Common and chemical names of glucosinolates identified in Chinese kale sprouts

Trivial name	Chemical name (side chain)
Aliphatic glucosinolate	
3-Carbon chain length	
Glucoiberin	3-Methylsulfinylpropyl
Sinigrin	2-Propenyl
4-Carbon chain length	
Glucoerucin	4-Methylthiobutyl
Glucoraphanin	4-Methylsulfinylbutyl
Gluconapin	3-Butenyl
Progoitrin	2-Hydroxy-3-butenyl
5-Carbon chain length	
Gluconapoleiferin	2-Hydroxy-4-pentenyl
Indole glucosinolate	
Glucobrassicin	3-Indolylmethyl
Neoglucobrassicin	1-Methoxy-3-indolylmethyl
4-Hydroxyglucobrassicin	4-Hydroxy-3-indolylmethyl
4-Methoxyglucobrassicin	4-Methoxy-3-indolylmethyl

There were four kinds of indolic glucosinolates in Chinese kale sprouts, with 4-methoxyglucobrassicin being the predominant one (Fig. 2). Glucose or GA_3 treatment alone remarkably elevated the glucobrassicin level (Fig. 2a). When GA_3 was applied together with glucose, significant promoting effects on 4-methoxyglucobrassicin and neoglucobrassicin were observed, which were increased by 32% and 41%, respectively, when compared with the control (Figs. 2b and 2d).

As shown in Fig. 3, GA_3 or glucose treatment alone did not significantly influence total glucosinolate content, but combined treatment did, raising the content by 45% compared with the control.

3.2 Effects of glucose combined with GA_3 treatment on main antioxidant content and antioxidant capacity

As shown in Table 2, GA_3 combined with glucose treatment significantly increased total phenolic content and antioxidant capacity. The antioxidant capacity was increased by approximately 150% compared with the control. However, no remarkable change in the content of anthocyanins or vitamin C was observed.

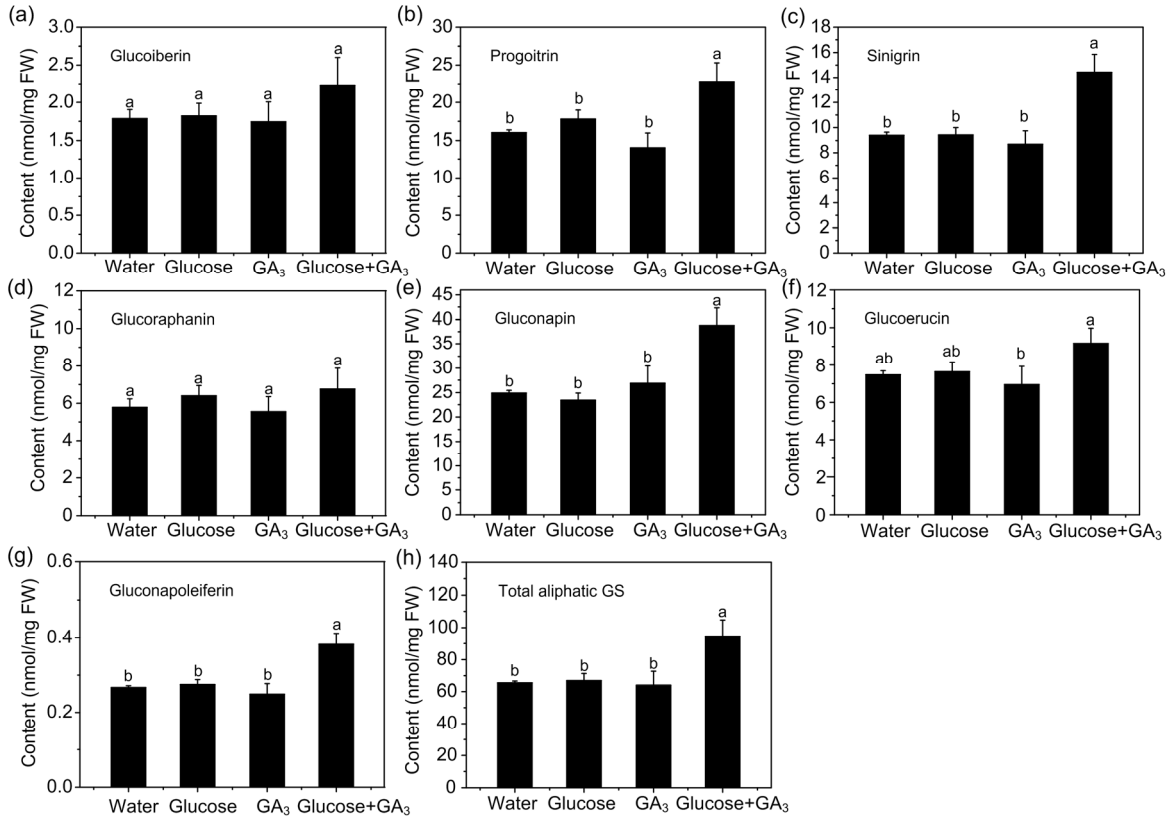

Fig. 1 Individual and total aliphatic glucosinolate content in Chinese kale sprouts under glucose and GA_3 treatments
(a) Glucoiberin; (b) Progoitrin; (c) Sinigrin; (d) Glucoraphanin; (e) Gluconapin; (f) Glucoerucin; (g) Gluconapoleiferin; (h) Total aliphatic glucosinolate. Each data point represents the mean of three independent biological replicates per treatment (mean±standard error (SE)). Values not sharing a common letter are significantly different at $P<0.05$

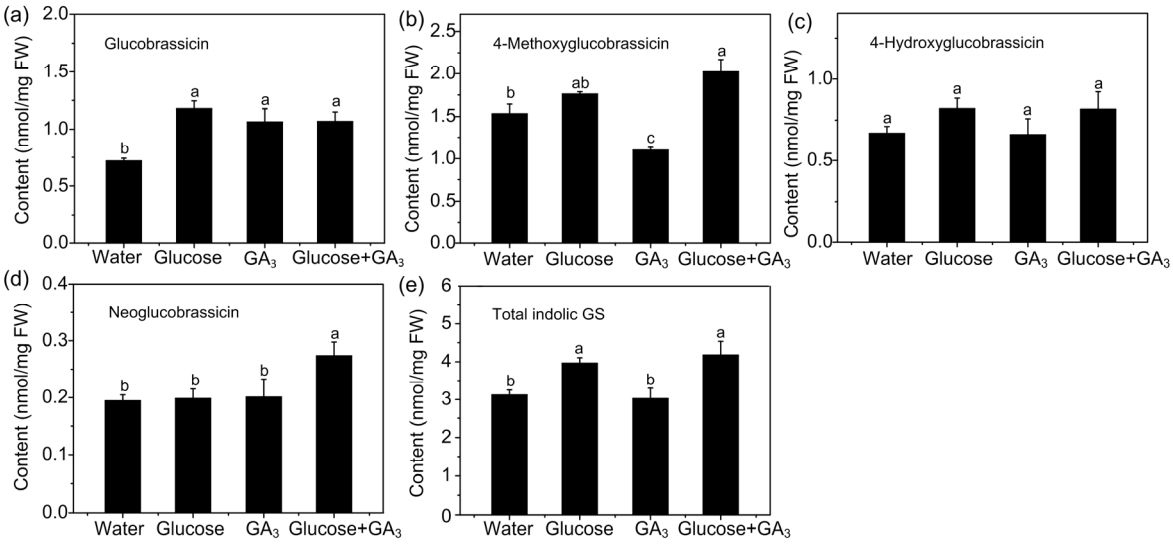

Fig. 2 Individual and total indolic glucosinolate content in Chinese kale sprouts under glucose and GA_3 treatments
(a) Glucobrassicin; (b) 4-Methoxyglucobrassicin; (c) 4-Hydroxyglucobrassicin; (d) Neoglucobrassicin; (e) Total indolic glucosinolate. Each data point represents the mean of three independent biological replicates per treatment (mean±SE). Values not sharing a common letter are significantly different at $P<0.05$

Table 2 Contents of total anthocyanins, phenols and vitamin C, and antioxidant activity in Chinese kale sprouts upon GA_3 combined with glucose treatment

Treatment	Total anthocyanins (U/g DW)	Total phenols (mg GAE/g DW)	Vitamin C (mg/100 g FW)	Antioxidant activity (mmol Fe^{2+}/100 g DW)
Water	8.52±0.38	22.94±0.42	118.25±6.12	13.97±0.02
Glucose+GA_3	9.60±0.49	25.89±0.60*	121.50±11.80	34.92±0.01*

Each data point represents the mean of three independent biological replicates per treatment (mean±SE). * Values are significantly different for combined treatment of glucose with GA_3 when compared with water treatment (P<0.05)

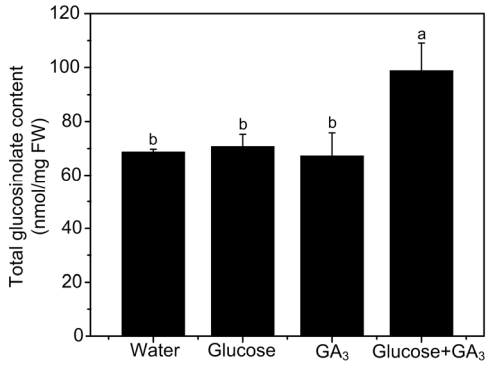

Fig. 3 Total glucosinolate content of Chinese kale sprouts under glucose and GA_3 treatments

Each data point represents the mean of three independent biological replicates per treatment (mean±SE). Values not sharing a common letter are significantly different at P<0.05

4 Discussion

As a native Chinese vegetable, Chinese kale is widely consumed, and the relatively low seed price makes it feasible to produce sprouts commercially. Low concentrations of glucose (3%) and GA_3 (5 μmol/L), being economical and safe, were used in this study to search for a practicable way of enhancing glucosinolate and antioxidant attributes of Chinese kale sprouts.

Glucosinolate has anticarcinogenic properties as well as being involved in the flavor and aroma of cruciferous crops (Dinkova-Kostova and Kostov, 2012). A number of studies have been conducted with the aim of improving these properties. Chemical regulation was one of the most studied methods, especially with phytohormones, which have been reported to play important roles in the regulation of glucosinolate biosynthesis (Yuan *et al.*, 2010a; Guo *et al.*, 2013a; Kim *et al.*, 2013; Schweizer *et al.*, 2013; Frerigmann and Gigolashvili, 2014; Zang *et al.*, 2015). Our previous research indicated that 5% (0.05 g/ml) glucose elevated glucosinolate in Chinese kale and pak choi sprouts (Wei *et al.*, 2011) and the study by Huang *et al.* (2014) showed a promoting effect of 100 μmol/L GA_3 on glucosinolate content in the siliques of oilseed rape. In our present study we observed that neither glucose nor GA_3 alone affected the content or composition of individual aliphatic glucosinolates nor most of the indolic glucosinolates. This could be due to different plant species or the concentrations of glucose and GA_3 used. However, the combined treatment of glucose and GA_3 markedly boosted the accumulation of many aliphatic glucosinolate compositions and two kinds of indolic glucosinolates (4-methoxyglucobrassicin, neoglucobrassicin) (Figs. 1 and 2), exerting a synergistic effect on glucosinolate accumulation, although the mechanism involved remains to be elucidated. This kind of synergistic effect between glucose and a phytohormone on glucosinolate biosynthesis is not unique. We previously reported that jasmonic acid could remarkably enhance the effect of glucose on glucosinolate biosynthesis in *Arabidopsis thaliana* (Guo *et al.*, 2013c). GA_3 is much cheaper than jasmonic acid, and easy to handle making it a useful tool for improving glucosinolates in cruciferous plants. Our observation is quite different from the counteractive impact of GA_3 on sucrose-induced anthocyanin accumulation in *Arabidopsis* (Loreti *et al.*, 2008), indicating that the same phytohormone may function diversely in different signaling pathways.

Phenolic compounds and vitamin C are important bioactive compounds in cruciferous vegetables for their high content and antioxidant activity, which defend cells against oxidative damage and prevent chronic disease (Podsędek, 2007). We therefore analyzed the effects of treatment with glucose and GA_3 combined on the content of these antioxidants. Anthocyanins, a vital kind of natural pigments belonging to the phenolic compounds, are known to protect

against neuronal and cardiovascular illnesses, as well as cancer and diabetes (Castañeda-Ovando *et al.*, 2009). Liang *et al*. (2013) reported that GA_3 is effective in inducing phenolic production in salvia miltiorrhiza bunge hairy roots, and Loreti *et al.* (2008) demonstrated that GA_3 has a counteractive effect on sucrose-induced anthocyanin accumulation in *Arabidopsis*. In this study, we found that the total phenolic content of Chinese kale is elevated when treated by glucose and GA_3 together, while no remarkable difference in anthocyanin content was observed (Table 2).

Our former studies showed that Chinese kale is rich in antioxidant vitamin C (Sun *et al.*, 2012; Wei *et al.*, 2011). A similar result was observed in this survey. The content of vitamin C in Chinese kale sprout amounted to more than 100 mg per 100 g FW (Table 2). It was previously reported that GA_3 has a positive effect on maintaining vitamin C in Chinese jujube (*Zizyphus jujuba* M) (Jiang *et al.*, 2004). In the current study, the combined application of glucose and GA_3 exerted little effect on vitamin C content in Chinese kale spouts. Our former study found that vitamin C content is dramatically reduced by 5% glucose treatment in Chinese kale sprouts (Wei *et al.*, 2011), and hence it is possible that glucose is counteracting the promoting effect of GA_3 on vitamin C accumulation.

Treatment with glucose and GA_3 combined increased antioxidant activity by 150% compared with the control (Table 2). A high correlation had previously been found between antioxidant activity and the content of phenolic compounds in radish sprouts (Kim *et al.*, 2006), and a similar result was observed in the current study. The antioxidant activity of Chinese kale sprouts was boosted when the total phenolic content was increased under the combined treatment of glucose and GA_3.

5 Conclusions

The combination of 3% glucose and 5 μmol/L GA_3 treatment dramatically enhanced the accumulation of glucosinolates in Chinese kale sprouts as well as other health-promoting compounds, such as total phenols. This treatment also enhanced antioxidant activity. Our results indicate that the combined application of glucose and GA_3 is potential in improving health-promoting compounds and antioxidant attributes of Chinese kale sprouts.

Compliance with ethics guidelines

Hui-ying MIAO, Meng-yu WANG, Jia-qi CHANG, Han TAO, Bo SUN, and Qiao-mei WANG 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.

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中文概要

题　目：葡萄糖和赤霉酸对芥蓝芽菜中芥子油苷含量及其抗氧化能力的影响

目　的：探究外源施加葡萄糖和赤霉酸对芥蓝芽菜中芥子油苷的积累，花青素、多酚和维生素 C 等抗氧化物的含量，以及总抗氧化活性的影响。

创新点：首次发现葡萄糖和赤霉酸可以协同促进芥蓝芽菜中几乎所有种类芥子油苷以及总芥子油苷的积累，并且可以大幅度提升总多酚的含量以及抗氧化能力。

方　法：以芥蓝芽菜为材料，使用 3%（0.03 g/ml）葡萄糖和 5 μmo/L 赤霉酸进行外源处理，以水处理作为对照组。用高效液相色谱法分析芥子油苷和维生素 C 的含量；采用分光光度法检测花青素的含量；用 Folin-Ciocalteu 试剂法测定总多酚的含量；利用亚铁还原能力实验（FRAP）法进行总抗氧化能力的评估。

结　论：葡萄糖和赤霉酸共同处理可以有效提升芥蓝芽菜中有益健康的植物化学物质含量以及抗氧化能力。

关键词：芥子油苷；抗氧化物；葡萄糖；赤霉酸；芥蓝芽菜