Original article

Jasmonic acid regulating glucosinolates-myrosinase system of cabbage sprouts

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Summary

Isothiocyanates (ITCs) are a series components and have functions of anticancer, and antioxidant capacity. They are produced from glucosinolates (GLs) by the catalysis of myrosinase (MYR) when tissues or cells of *Brassica* vegetables are disrupted. In this study, the effects of jasmonic acid (JA) on GLs-MYR system in cabbage sprouts were investigated. The results showed that JA significantly inhibited the growth of cabbage sprouts and reduced their biomass. However, it effectively increased the GLs content, especially indole GLs glucobrassicin (GBC) and neoglucobrassicin (NGB). Under JA treatment, only 100 $\mu\text{mol L}^{-1}$ of JA increased the ITCs formation in cabbage sprouts through increasing the gene expression and activity of MYR. It was also found that cotyledon of cabbage sprouts contained the highest level of total GLs, aromatic GLs and indole GLs especially GBC and NGB, as well as the ITCs formation either in control or in JA treated sprouts. During the growth period, JA could effectively restrain the decrease of GLs content especially for the indole GLs including GBC and NGB, it also enhanced MYR activity and gene expression for GLs synthesis and ITC formation. JA treatment altered the GLs composition and increased the relative content of indole GLs including GBC and NGB. The 100 $\mu\text{mol L}^{-1}$ of JA was beneficial for increasing gene expression of GLs synthesis and ITCs formation so that it promoted the GLs-MYR system in cabbage sprouts.

Keywords

cabbage sprouts, glucosinolate, isothiocyanate, jasmonic acid, myrosinase

Significance of this study

What is already known on this subject?

- Jasmonic acid as a plant hormone could affect cabbage sprouts growth and metabolism. However, its effects on glucosinolates synthesis and degradation is unknown.

What are the new findings?

- Jasmonic acid increased the content of indole glucosinolates, enhanced myrosinase activity and induced isothiocyanates accumulation.

What is the expected impact on horticulture?

- Jasmonic acid treatment enhanced the functional quality of cabbage sprouts.

with myrosinase (MYR) and are converted to isothiocyanates (ITCs) (Lim et al., 2016).

The species and content of GLs have a direct influence on ITCs formation in addition to MYR activity. However, the contents of GLs in *Brassica* plants are mainly affected by plant species, genotype, environmental conditions (Gu et al., 2012; Wang et al., 2019) and inducing factors (Guo et al., 2011; Lu et al., 2016; Pérez-Balibrea et al., 2011). Reports showed that the biological metabolism of GLs can be improved by plant hormone as inducing factor (Guo, L. et al., 2014; Kim et al., 2006; Pérez-Balibrea et al., 2011). Jasmonic acid (JA) is a well characterized linolenic acid-derived cyclopentanone signal molecule and controls a broad range of plant developmental processes (Turner et al., 2002). In addition to its pivotal role in regulating plant growth, JA also plays a key role in defense gene expression either via the octadecanoid pathway or acting directly on the genes (Koo et al., 2009). Many reports have shown that JA has been applied successfully in many plant species to enhance the yield of GLs (Fritz et al., 2010; Kastell et al., 2013). In carrots, broccoli and other cruciferous *Brassica* sprouts, JA has been used as an effective inducer for improving the contents of GLs (Baenas et al., 2014; Kastell et al., 2013). It significantly increased the content of glucoraphanin in broccoli sprouts and glucobrassicin (GBC) in turnip sprouts by addition of a certain concentration of JA (Baenas et al., 2014). Kastell et al. (2013) found that exogenous JA can increase the content of neoglucobrassicin (NGB) in turnip and white mustard root. Wiesner et al. (2013) reported that JA can regulate the expression of genes related to the biosynthesis of GLs to improve the content of GLs, especially NGB when JA was applied to the young Chinese cabbage sprouts and mature leaves.

Introduction

Brassica vegetables and their sprouts have attracted the attention of researchers due to their high contents of glucosinolates (GLs), total phenolics and ascorbic acid, which could promote and maintain the health of the human body (Fahey et al., 1997; Nicola et al., 2013; Rangkadilok et al., 2002). As one of the widely consumed vegetables in the world, cabbage sprouts contain more GLs compared with the mature plant tissues (Gu et al., 2012; Rosa and Rodrigues, 1998). GLs are an important class of sulfur- and nitrogen-containing secondary metabolites in *Brassica* plants, and they participate in the process to resist microbial pathogens (Gu et al., 2011). After tissues are damaged or masticated, GLs contact

TABLE 1. The primers used for QRT-PCR.

Gene	Primer name	Primer sequences (5'→3')	Length (bp)
<i>Actin</i>	Sense	CTGTTCCAATCTACGAGGGTTTCT	132
	Anti-sense	GCTCGGCTGTGGTGGTGAA	
<i>MYB28</i>	Sense	AGACTGCGATGGACTAACTACCTAAA	112
	Anti-sense	CCGACCACTTGTTTCCACGA	
<i>CYP79F1</i>	Sense	GGAGTTAGACGAAGTGGTGGGA	185
	Ani-sense	TGTGGCTACCTTTGGGAATGA	
<i>CYP83A1</i>	Sense	CTGCGGCGGTTGTATGGG	134
	Anti-sense	AAGGTTCTTCACGTCGTCTTCAGT	
<i>UGT74B1</i>	Sense	CAAAGACGATAAAGGCTACGGC	119
	Anti-sense	TCCCAAAGGAACCAACGAA	
<i>ST5b</i>	Sense	CCGACACTACCTTACCGAACCA	102
	Anti-sense	CGTGAGGAAAAGAGGCGATG	
<i>FMO_{GS-OX1}</i>	Sense	GTGGACATTATACCGAACCTTACG	189
	Anti-sense	TGTGGACTTCTTTGGCGACCT	
<i>AOP2</i>	Sense	GAGTAACGGAAAGAAAGAAGACAAGG	196
	Anti-sense	ATAAGCGTGAAGAGTAGAACGAGGT	
<i>MYR</i>	Sense	AAGTCATCAGGGAGAAGGGTG	203
	Anti-sense	TGTTTGGCAGGGTTCTTAGTGG	

Get together, the previous researches mostly focus on the effect of exogenous JA on GLs content of *Brassica* sprouts. There are few reports on the research of the biological active substances such as ITCs formation and GLs content, as well as MYR gene expression and its activity in cabbage sprouts under JA treatment. In this study, the GLs-MYR system was investigated in cabbage sprouts with JA treatment under different concentrations. The aim was to reveal the mechanism of JA affecting GLs metabolism and ITCs formation in cabbage sprouts.

Materials and methods

Plant material and chemicals

Cabbage seeds (*B. oleracea* L. cv. Xialv 50) were purchased from Nanjing Jinshengda Seed Co. Ltd. (Jiangsu, China) and stored at -20°C for use. Sinigrin, sulforaphane, glucotropaeolin and JA were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other chemicals and reagents were of analytical grade (Shanghai Institute of Biochemistry, Shanghai, China).

Cultivation conditions and treatments

The seeds were selected and soaked with 5 mL L⁻¹ sodium hypochlorite for 15 min to sterilize and then washed with distilled water 3–4 times to reach neutral pH via monitoring pH of washing water approaching to 7.0. Then, the soaked seeds were immersed in distilled water for 3 h at 30°C. They were weighed and sown evenly on trays (15 cm in diameter) filled with vermiculite (1.5 cm in thickness under seeds). The sowed seeds were sprayed with distilled water for one day by an automatic spraying system provided a 5-sec mist every 10 min. Then they were manually sprayed with 10 mL JA (100, 200 and 300 µmol L⁻¹) two times per day during the whole germination progress. The control sprouts were sprayed with distilled water. All cabbage seeds germinated at 25°C constantly under a 16 h light/8 h dark photoperiod in a growth chamber. The 5-day germinated sprouts were rapidly and gently harvested for further measurements. For each subsample, three replicates were taken and weighed for

analysis. Sprouts and different organs including cotyledon, hypocotyl and root were also collected separately, and flash frozen in liquid nitrogen and then stored at -80°C for determination.

Sprouts length and weight measurement

The cabbage sprouts length and weight was measured using a vernier calipers and a precision electronic balance, respectively. The result was expressed as cm and g sprout⁻¹.

Glucosinolates content assay

The determination of GLs content was conducted according to Guo, L. et al. (2014) with minor modifications. Glucotropaeolin (1-benzyl GL) was used as an internal standard. The content was detected with an Agilent 1200 High Performance Liquid Chromatography (HPLC) system (Agilent Technologies Co. Ltd., Palo Alto, CA, USA) with an Eclipse XDB-C18 column (5 µm particle size, 4.6 × 150 mm; Agilent Technologies Co. Ltd.). The result was expressed as µmol g⁻¹ FW.

Determination of MYR activity and ITCs content

MYR activity was measured according to Guo, L. et al. (2014). The specific activity was expressed as nmol glucose formed per minute per mg protein.

Determination of ITCs content was performed as described by the previous report (Wang et al., 2015).

Gene expression assay (QRT-PCR, Quantitative Real-Time PCR)

The detection of related genes expression was following our published paper completely (Yang et al., 2017). The primers used in this study were shown in Table 1.

Statistical analysis

The data were expressed as the mean ± standard deviation (SD) with three replications ($n=3$). SPSS 18.0 (SPSS Inc., Chicago, IL, USA) software was used for statistical analysis and $p<0.05$ considered as significant difference by Duncan's multiple-range test.

Results

Effects of JA on the growth of cabbage sprouts

The growth of cabbage sprouts was obviously inhibited by JA, and the inhibition enhanced with the increase of JA

concentration (Figure 1A, B). Compared with the control, the sprouts length treated with 100, 200 and 300 $\mu\text{mol L}^{-1}$ JA decreased by 34%, 45% and 50%, respectively, and the weight of sprouts decreased by 60.37%, 55.32%, and 47.54%, respectively.

TABLE 2. Effect of JA on GLs contents ($\mu\text{mol g}^{-1}$ FW) in cabbage sprouts and different organs.

GLs	Parts	JA concentration ($\mu\text{mol L}^{-1}$)			
		0	100	200	300
GIB	Sprout	1.92±0.46b	2.46±0.62ab	3.00±0.17a	2.18±0.14b
	Cotyledon	5.46±0.47c	6.99±0.36b	7.01±0.84a	6.81±0.50b
	Hypocotyl	0.62±0.10b	1.43±0.31a	1.37±0.02a	0.70±0.30b
	Root	0.17±0.05c	0.27±0.04b	0.29±0.01b	0.45±0.01a
PRO	Sprout	2.91±0.18a	2.69±0.57a	1.27±0.10b	0.98±0.11c
	Cotyledon	2.73±0.12a	1.83±0.68b	1.17±0.03bc	0.78±0.18c
	Hypocotyl	3.18±0.11a	2.53±0.50b	1.36±0.44c	0.73±0.32c
	Root	7.62±2.32b	11.66±2.33a	7.71±1.81b	5.91±1.02b
SIN	Sprout	4.30±0.78a	4.52±0.45a	3.88±0.35ab	3.20±0.40b
	Cotyledon	10.61±0.95a	7.52±0.94b	7.31±0.30b	4.84±0.04c
	Hypocotyl	2.42±0.04a	2.86±0.54a	1.96±0.66ab	1.33±0.28b
	Root	1.95±0.24a	2.46±0.31a	2.14±0.15a	1.68±0.09b
GNA	Sprout	0.17±0.02a	0.39±0.02a	0.20±0.01a	0.22±0.08a
	Cotyledon	0.48±0.09a	0.51±0.09a	0.49±0.05a	0.58±0.10a
	Hypocotyl	0.09±0.01a	0.11±0.02a	0.10±0.03a	0.13±0.05a
	Root	0.20±0.01a	0.13±0.01b	0.16±0.03b	0.12±0.02b
GIV	Sprout	1.01±0.26a	1.51±0.20b	0.87±0.12c	0.57±0.07d
	Cotyledon	5.24±0.85a	2.50±0.02b	1.21±0.09c	0.70±0.07d
	Hypocotyl	1.49±0.03a	0.62±0.22b	0.39±0.17bc	0.30±0.04c
	Root	1.18±0.05a	0.74±0.02b	0.63±0.01c	0.48±0.05d
GNT	Sprout	1.84±0.11b	2.27±0.23a	1.95±0.21ab	1.03±0.03c
	Cotyledon	0.90±0.12c	3.41±1.07a	3.25±0.21a	1.99±0.62b
	Hypocotyl	0.24±0.01d	1.74±0.16a	1.24±0.37b	0.89±0.03c
	Root	0.11±0.02c	1.77±0.23a	1.45±0.17a	1.12±0.14b
4HGB	Sprout	0.50±0.13a	0.76±0.10a	0.72±0.08a	0.76±0.08a
	Cotyledon	1.20±0.31b	1.50±0.49a	1.57±0.29a	1.46±0.04a
	Hypocotyl	0.71±0.11a	0.27±0.04b	0.21±0.03b	0.13±0.02c
	Root	0.46±0.04a	0.12±0.02b	0.15±0.01b	0.12±0.03b
GBC	Sprout	0.40±0.14c	8.80±0.13b	8.67±0.71ab	9.10±0.10a
	Cotyledon	0.36±0.04b	16.38±2.32a	15.66±1.24a	17.23±2.54a
	Hypocotyl	0.18±0.03b	0.31±0.01a	0.23±0.07a	0.18±0.01b
	Root	0.26±0.02a	0.26±0.02a	0.20±0.01b	0.19±0.01b
4MGB	Sprout	0.28±0.01b	0.37±0.04a	0.42±0.04a	0.40±0.03a
	Cotyledon	0.26±0.01b	0.48±0.07a	0.53±0.07a	0.46±0.16a
	Hypocotyl	0.18±0.04b	0.42±0.07a	0.36±0.07a	0.31±0.05a
	Root	0.51±0.02a	0.29±0.01b	0.27±0.02b	0.28±0.02b
NGB	Sprout	0.22±0.09c	9.12±0.87a	10.29±1.23a	11.49±0.89a
	Cotyledon	0.07±0.01d	10.64±1.58c	15.35±1.67b	20.75±0.48a
	Hypocotyl	0.44±0.04c	8.72±0.35a	8.87±0.37a	6.40±0.41b
	Root	0.65±0.16c	3.68±0.32a	3.34±0.14a	1.82±0.16b
Total GLs	Sprout	13.55±2.18b	32.89±3.23a	31.27±3.02a	29.93±1.93a
	Cotyledon	27.31±2.97b	51.76±7.62a	53.55±4.79a	55.60±4.73a
	Hypocotyl	9.55±0.52b	19.01±2.22a	16.09±2.23a	11.10±2.01b
	Root	13.11±2.93ab	21.38±3.31a	16.34±2.36ab	12.17±1.55b

The data are the mean of three replicates per treatment and time point (SD). Different letters indicate statistical differences of the means of the same row at $p < 0.05$. GLs: glucosinolates; GIB: glucoiberin; PRO: progoitrin; SIN: sinigrin; GNA: gluconapin; GIV: glucoiberiverin; GNT: gluconasturtiin; 4HGB: 4-hydroxy glucobrassicin; GBC: glucobrassicin; 4MGB: methoxy glucobrassicin; NGB: neoglucobrassicin.

FIGURE 1. Effects of JA on growth of cabbage sprouts. Different letters in the same index indicate statistical differences at $p < 0.05$ among different JA concentrations.

Effects of JA on GLs contents in cabbage sprouts and different organs

The application of JA significantly increased the GLs contents in cabbage sprouts ($p < 0.05$) (Table 2). The content of total GLs in cabbage sprouts under $100 \mu\text{mol L}^{-1}$ JA treatment showed the highest value compared with the control. Aliphatic GLs were the major glucosinolates in cabbage sprouts, accounting for 76.09% of the total GLs, while indole GLs were the lowest and only accounted for 10.33%. The contents of aliphatic GLs in cabbage sprouts increased by 12.22% under $100 \mu\text{mol L}^{-1}$ JA treatment, while decreased by 10.57%, 30.65%, respectively, under 200 and $300 \mu\text{mol L}^{-1}$ JA treatments. The contents of aromatic GLs increased firstly and then decreased with the increase of JA concentration. This indicates that JA could significantly improve the contents of GLs, but it had concentration selection effect on aliphatic GLs and aromatic GLs. The content of GLs, especially GBC and NGB significantly increased except 4HGB. The application of JA significantly increased the content of total GLs in cotyledon and hypocotyl ($p < 0.05$), but there was no difference in different JA concentrations (Table 2). JA treatment significantly induced the content of aromatic GLs and indolic GLs of cotyledon, hypocotyl and root, but there was no significant effect on the content of aliphatic GLs. GBC and NGB were in-

duced by JA most significantly. Different JA concentrations increased the content of GBC and NGB in cotyledon mainly.

Effects of JA on MYR activity in cabbage sprouts and different organs

As shown in Figure 2, JA significantly increased the MYR activity ($p < 0.05$), but there was no significant difference among different JA concentrations. The MYR activity of sprouts treated with 100, 200 and $300 \mu\text{mol L}^{-1}$ JA was 1.86-, 1.90- and 1.68-fold of the control, respectively. The results showed that the MYR activity in cotyledon was lower than that of hypocotyl and root. JA significantly increased the MYR activity in cotyledon and root ($p < 0.05$) and reduced the enzyme activity in hypocotyl ($p < 0.05$), there was no significant difference among different JA concentrations in cotyledon. The enzyme activity in hypocotyl decreased significantly with the increase of JA concentration, but increased in root.

Effects of JA on the formation of ITCs in cabbage sprouts and different organs

ITCs formation in sprouts, hypocotyl and root of cabbage was increased by spraying $100 \mu\text{mol L}^{-1}$ JA ($p < 0.05$), which was 1.46-, 1.42- and 2.05-fold of the control. However, there was no significant difference among 200, $300 \mu\text{mol L}^{-1}$ JA

FIGURE 2. Effects of JA on MYR activity of cabbage sprouts and different organs. Different letters in the same organ indicate statistical differences at $p < 0.05$ among different JA concentrations.

FIGURE 3. Effects of JA on ITCs content of cabbage sprouts and different organs. Different letters in the same organ indicate statistical differences at $p < 0.05$ among different JA concentrations.

FIGURE 4. Expression of GLs biosynthetic genes and MYR genes in cabbage sprouts under 100 $\mu\text{mol L}^{-1}$ of JA treatments.

treatments and the control (Figure 3). Under 100 $\mu\text{mol L}^{-1}$ JA, cotyledon had the highest ITCs formation, they were 8.13- and 14.99-fold of the hypocotyl and root, respectively.

Effects of JA on the expression of GLs biosynthetic genes and MYR genes in cabbage sprouts

The expression of GLs biosynthesis related genes in the cabbage sprouts treated with 100 $\mu\text{mol L}^{-1}$ JA was shown in Figure 4A. The results showed that there were significant differences in the expression level of related genes ($p < 0.05$). The expression levels of *MYB28*, *CYP79F1*, *CYP83A*, *UGT74B1*, *ST5b*, *FMO_{GS-OX1}* and *AOP2* were significantly upregulated, and they were 4.10-, 2.00-, 32.79-, 21.34-, 1.44-, 76.81-, 3.66-fold of the control, respectively. JA treatment also increased the MYR, significantly ($p < 0.05$), and it was 17.98-fold of the control (Figure 4B).

Effect of JA on GLs content in cabbage sprouts during germination

As shown in Table 3, the species of GLs in cabbage sprouts was not changed after JA treatment. During germination, total GLs and each GL content decreased except for GBC, 4MGB and NGB. The content of aliphatic GLs, aromatic GLs and indole GLs represented 78.07, 20.00 and 1.93% of the total GLs, respectively, in the first day of germination. After germinating for 5 days, their content changed to 75.56, 17.11 and 7.33%, respectively, indicating that germination increased

the percentage of indole GLs. Moreover, in JA-treated group, aliphatic GLs content decreased to 35.18%. However, indole GLs content increased to 57.92% and was 17.17 times of the control. In which, GBC and NGB were the major GLs, increased 51.76 and 33.78 times, respectively, compared with the control.

Effect of JA on MYR activity and ITCs formation in cabbage sprouts during germination

As shown in Figure 5A, germination could promote MYR activity of cabbage sprouts. Under 100 $\mu\text{mol L}^{-1}$ JA treatment, the MYR activity of cabbage sprouts increased with the increase of germination time, and the activity was higher than that of the control from the second day of germination. On the fifth day of germination, the activity was the highest, which was 5.57 U mg^{-1} higher than that of the control.

The formation of ITCs in cabbage sprouts decreased with the germination time (Figure 5B). There was no significant difference of the ITCs formation between the two groups during the first two days of germination. However, it was significantly higher than the control ($p < 0.05$) from day 3, and was 66.93% higher than the control at fifth day of germination.

Discussion

In the present study, SIN and PRO were the major GLs in aliphatic GLs of cabbage sprouts, accounting for 41.71

FIGURE 5. Effects of JA on MYR activity and ITCs content of cabbage sprouts.

TABLE 3. Effects of 100 $\mu\text{mol L}^{-1}$ of JA on GLs contents ($\mu\text{mol g}^{-1}$ FW) of cabbage sprouts at different germination stages.

Treatment	Time (day)	Aliphatic GLs			Aromatic GLs			Indolic GLs			Total GLs	
		GIB	PRO	SIN	GNA	GIV	GNT	4OH-GB	GBC	4MGB		NGB
Control (0 μmol L ⁻¹)	1	13.03±0.76a	22.31±1.63a	30.61±1.90a	3.22±0.68a	5.86±0.27a	19.24±3.77a	2.33±0.66a	0.14±0.05d	0.12±0.03c	0.23±0.02c	96.10±4.37a
	2	10.87±0.90b	15.1±0.70b	19.9±0.00b	1.37±0.10c	4.32±0.26b	13.39±1.25b	1.53±0.03b	0.24±0.00c	0.1±0.02c	0.43±0.02b	67.25±0.75b
	3	5.33±0.13c	8.84±0.56c	10.93±0.45c	1.92±0.06b	2.26±0.35c	8.37±2.21c	1.11±0.23c	0.38±0.01b	0.17±0.01b	0.43±0.02b	39.74±3.71c
	4	2.89±0.58d	5.52±0.38d	6.47±1.08d	0.39±0.01e	1.29±0.25d	5.75±0.00d	0.87±0.20d	0.46±0.01a	0.17±0.01b	0.59±0.11a	24.39±2.21d
	5	2.14±0.83d	2.91±0.35e	4.28±1.09e	0.43±0.00d	0.66±0.34e	1.84±0.22e	0.34±0.03e	0.17±0.04d	0.23±0.01a	0.27±0.03c	13.79±2.96e
JA (100 μmol L ⁻¹)	1	13.03±0.76a	22.31±1.63a	30.61±1.90a	3.22±0.68a	5.86±0.27a	19.24±3.77a	2.33±0.66a	0.14±0.05e	0.12±0.03d	0.23±0.02d	96.10±4.37a
	2	10.71±0.64b	13.98±0.21b	18.69±0.91b	1.06±0.20b	3.89±0.20b	14.49±0.12b	1.58±0.02b	1.15±0.01e	0.25±0.01c	2.29±0.02c	68.09±1.43b
	3	6.18±0.04c	10.72±1.84c	10.18±0.29c	0.87±0.19b	2.08±0.21c	9.71±2.28c	1.04±0.37c	3.92±0.00c	0.23±0.01c	5.58±0.02b	50.81±4.57c
	4	3.23±0.83d	7.10±0.10d	6.63±1.67d	0.45±0.03c	1.16±0.22d	6.39±0.21d	0.92±0.02d	5.81±0.04b	0.31±0.00b	9.05±0.29a	41.05±2.37d
	5	2.46±0.62e	2.69±0.57e	4.52±0.45e	0.39±0.02d	1.51±0.20d	2.27±0.23e	0.76±0.10e	8.80±0.13a	0.37±0.04a	9.12±0.87a	32.89±3.23e

The data are the mean of three replicates per treatment and time point (SD). The comparison GLs for control and JA treatment was carried out respectively. Different letters in the same column for the control and JA treatment, respectively, indicate statistical differences at $p < 0.05$. GLs: glucosinolates; GIB: glucobrassicin; PRO: proglutinin; SIN: sinigrin; GNA: gluconapin; GIV: glucoiberin; GNT: gluconasturtiin; 4HGB: 4-hydroxy glucobrassicin; GBC: glucobrassicin; 4MGB: methoxy glucobrassicin; NGB: neoglucobrassicin.

and 28.13% of the total GLs, respectively. Kusznierevicz et al. (2008) and Martinez-Villaluenga et al. (2009) found the main varieties of GLs in white cabbage were GBC and SIN. However, in summer and winter white cabbage, GIB, SIN and GBC were the main GLs (Martinez-Villaluenga et al., 2009). This difference may be due to different cabbage species and different growth stages. The contents of GLs in cabbage cotyledon, hypocotyl and root were obviously different. The content of GLs was highest in cabbage cotyledon, and then was hypocotyl and root, the content of PRO and NGB was the highest in the root, while the other GLs was highest in cabbage cotyledon. These results indicated that even the same species in the same growth stage, the content of GLs in different tissues were also different (Brown et al., 2003; Pérez-Balibrea et al., 2008).

The total GLs content was significantly increased by JA treatment at the certain germination time (Tables 2 and 3). On the one hand, this might be related to the inhibition of growth caused by JA (Figure 1), so that inhibited the bio-availability of sulphur. On the other hand, JA treatment increased the genes expression related with GLs biosynthesis (Figure 4A). The content of indole GLs was much lower than aliphatic GLs in untreated sprouts. However, the content of indole GLs especially GBC and NGB increased significantly after treatment with JA compared with the control (Tables 2 and 3). Moreover, the content of NGB was the highest in root, but lowest in cotyledon in the control. However, its content was the highest in cotyledon but lowest in root when treated with JA. GBC content increased significantly only in cabbage cotyledon (Tables 2 and 3). These indicate that JA modified the composition of GLs and induced indole GLs biosynthesis mainly in cotyledon.

In addition to biosynthesis of GLs, JA also affected MYR activity (Figures 2 and 5A). In the present study, MYR activity in cabbage sprouts and cotyledon was lower than that in hypocotyl and root. This was related to the expression of specific activity (U mg^{-1} protein). In sprouts, protein content was higher in cotyledon and the whole sprouts than hypocotyl (data not shown). JA treatment significantly increased the activity of MYR in cabbage sprouts, cotyledon and root, and there was no significant difference of MYR activity between the whole sprouts and cotyledon after treating with different JA. During the entire process of germination, the MYR activity in the control group showed an upward trend and reached the highest, which was similar to the previous researches (Guo et al., 2017; Guo, R. et al., 2014; Williams et al., 2008). This showed that JA, as a plant hormone, can enhance the activity of MYR in cabbage sprouts via causing a stress environment.

In the present study, the formation of ITCs in cabbage cotyledon was the highest, which was consistent with the distribution of precursor GLs in cabbage sprouts (Table 2). The content of ITCs in cotyledon, hypocotyl and root treated with 100 $\mu\text{mol L}^{-1}$ JA significantly increased compared with the control, while that of cabbage treated with 200 $\mu\text{mol L}^{-1}$ and 300 $\mu\text{mol L}^{-1}$ JA had no change. The possible reason is that although the content of precursor GLs such as SIN, GIB, GNA and GIV was not increased by 100 $\mu\text{mol L}^{-1}$ JA, the MYR activity significantly increased by 200 and 300 $\mu\text{mol L}^{-1}$ JA compared with the control, while the GLs content significantly decreased, so the formation of ITCs was consistent with the control (Figure 3). JA treatment significantly increased the content of GLs, especially GBC and NGB in cabbage sprouts and cotyledon. However, as the enzymatic hydrolysis of GBC and NGB, ITCs was not stable and formed the

indole-3-carbinol and 2-diindolylmethane eventually, which also had anticancer and antioxidant activity (Fuentes et al., 2015). During germination, the content of GLs in the control group decreased gradually, which caused the content of ITCs decreased gradually, and the content of ITCs decreased with the rise of water content by dilution effect (Pérez-Balibrea et al., 2008).

Interestingly, under 100 $\mu\text{mol L}^{-1}$ JA treatment, MYR activity increased significantly in cotyledon (Figure 2), but ITCs formation did not change in this organ (Figure 3). This might be related to the procedure of ITCs formation. In plants, GLs are converted into an aglycone moiety, glucose and sulphate under MYR catalysis. The aglycone moiety is unstable and simultaneously rearranges to form ITCs and other components including thiocyanates, nitriles and epithionitriles (Gu et al., 2012). The formation of hydrolyzed products relies on the structure of GLs and the reaction condition, which mainly includes pH, the presence of epithiospecifier protein (ESP) and Fe^{2+} . Hence, although MYR activity was increased by JA treatment, GLs was possibly converted to nitriles and epithionitriles. In this study, 200 and 300 $\mu\text{mol L}^{-1}$ of JA were not beneficial for improving ITCs formation. Maybe higher concentration altered the pH of reaction conditions (Bones and Rossiter, 2006).

Conclusions

In conclusion, SIN and PRO were the major GLs in aliphatic GLs of cabbage sprouts. JA treatment significantly inhibited the growth of cabbage sprouts and increased the percentage of indole GLs content. One hundred $\mu\text{mol L}^{-1}$ of JA increased the formation of ITCs mainly in hypocotyl and root of cabbage sprouts by improving GLs synthesis, MYR gene expression and its activity.

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Conflict of interest

The authors declare no competing financial interest.

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