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RESEARCH ARTICLE

Effect of jasmonic acid on glucosinolate metabolism in different organs of broccoli sprouts

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ABSTRACT

Edible sprouts, especially *Brassica* sprouts, contain high levels of health-promoting compounds. Exogenous elicitors have been used as strategies to improve the nutraceutical quality of *Brassica* sprouts. In this study, effects of jasmonic acid (JA) treatment on growth, the levels of glucosinolates and isothiocyanates, as well as myrosinase activity in different organs of broccoli sprouts were investigated. JA treatment markedly increased the contents of glucosinolates (GSLs), especially glucoraphanin, glucobrassicin and neoglucobrassicin in broccoli sprouts. However, gluconapin was not affected even decreased by JA treatment. Cotyledon, hypocotyl and root obtained the different results in induction of GSLs. Among these, neoglucobrassicin obtained the highest enhancement in three organs. Myrosinase activity in cotyledon of broccoli increased after JA treatment, while decreased in hypocotyl. Three concentrations of JA all significantly increased sulforaphane and isothiocyanates formation in cotyledon, hypocotyl and root of broccoli sprouts. Application of 100 μM JA led to the highest myrosinase activity, the least gluconapin and the most sulforaphane and isothiocyanates in cotyledon, as well as the most isothiocyanates in root. These results indicated that JA treatment could be an effective way to improve the cancer-prevention benefits of broccoli sprouts via enhancing sulforaphane and total isothiocyanates.

Keywords: Broccoli sprouts; Jasmonic acid; Glucosinolates; Isothiocyanates; Myrosinase

INTRODUCTION

Brassica vegetables are recognized as wellness and health-promoting foods because of their high levels of bioactive compounds, such as vitamin C, carotenoids, tocopherols, polyphenolics and glucosinolates (GSL_S) (Björkman et al., 2011). GSL_S are a major class of nitrogen- and sulfur-containing secondary metabolites involved in *Brassica* vegetable defense against pathogens (Bell and Wagstaff, 2017; Gu et al., 2012). When the plant tissue is disrupted, glucosinolates could be hydrolyzed into isothiocyanates (ITCs), thiocyanates, nitriles, epithionitriles and oxazolidines by the action of myrosinase (Bones and Rossiter, 2006; Gu et al., 2012). Among these compounds, ITCs, especially sulforaphane, displays diverse and important physiological activities including carcinogen detoxification, reducing the risk of cardiovascular diseases, anti-inflammatory and inhibition of pathogenic fungal growth as well as reducing blood glucose, etc. (Axelsson et al., 2017; Fahey et al., 2017; Guo et al., 2018).

Broccoli (*Brassica oleracea* var. *italica*) is widely consumed *Brassica* vegetable not just in China but all over the world. Moreover, broccoli sprouts contain much higher level of GSL_S than adult vegetables (Fahey et al., 1997). Hence, consumption of broccoli sprouts has become an popular way for people to enhance and keep their health status (Nguyen et al., 2016; Nguyen et al., 2017). Plant hormones have been used as elicitors to increase accumulation of bioactive compounds in plants (Guo et al., 2014c; Kim et al., 2006; Pérez-Balibrea et al., 2011). Jasmonic acid (JA) is generally acted as a growth regulator derived largely from linolenic acid through an octadecanoid pathway. In addition, exogenous JA also can activate plant defense gene either by acting directly on the genes or the octadecanoid pathway (Koo and Howe, 2009). JA has been used as a powerful inducer to enhance glucosinolate content in many plants (Baenas et al., 2014; Kastell et al., 2013). Addition of exogenous JA enhanced indole GSL_S content, particularly 1-methoxyindolyl-3-methyl glucosinolate in hairy root cultures of *Sinapis alba* and *Brassica rapa* (Kastell et al., 2013). Additionally, application of 150 μM JA also

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increased glucosinolate content in *Brassicaceae* sprouts, for instance glucobrassicin in turnip and rutabaga sprouts, glucoraphenin in radish sprouts and glucoraphanin in broccoli sprouts (Baenas et al., 2014). However, the effects of JA treatment on sulforaphane and ITCs formation as well as myrosinase activity in broccoli sprouts were not investigated in aforementioned studies.

Therefore, the objective of the present work was to investigate the changes in glucosinolate content, myrosinase activity and the production of ITCs in cotyledon, hypocotyl and root of broccoli sprouts after treatment with JA.

MATERIAL AND METHODS

Plant material, seed germination and treatment

Broccoli seeds were supplied by Shouguang Wentian Seed Co. (Shandong, China). After sterilizing with 5 mL/L sodium hypochlorite, the seeds were placed in distilled water and soaked for 4 h at 30 °C. Then, they were weighed and spread evenly on trays filled with vermiculite as a substrate irrigated with distilled water. All sprouts with three replicates were grown in a growth chamber with a 16 h light/8 h dark cycle at 25 °C. JA was dissolved in 0.2% ethanol and sprayed on sprouts at 100 μM, 200 μM, 300 μM concentrations every 24 h after one day. The control sprouts were sprayed with 10 mL distilled water. Finally, the cotyledon, hypocotyl and root were rapidly and gently dissected from 5-days old sprouts and then frozen at -80°C for measurements.

Extraction and determination of GSL_s

A modified version of a previously reported procedure by Font et al. (2005) and Guo et al. (2014a) was used for the extraction and analysis of GSL_s. GSL_s were extracted twice with boiling 75 % methanol. After purification with DEAE-Sephadex A-25 column (acetic acid activated), desulpho-GSL_s were obtained by the addition of aryl sulfatase (Sigma-Aldrich, St. Louis, MO, USA) solution and incubation at 35 °C overnight. An Agilent 1200 HPLC system (Agilent Technologies Co. Ltd., USA) equipped with an Eclipse XDB-C18 column (5 μm particle size, 4.6 × 150 mm) was used to analyze GSL_s.

Myrosinase activity assay

The method of Guo et al. (2014b) was applied to assay myrosinase activity. The myrosinase activity corresponded to the amount of glucose formed per minute and milligram of protein. The previous procedure of Bradford (1976) was used to determine the protein content of the supernatant.

Sulforaphane determination

A previously described method for sulforaphane analysis was used in this study (Guo et al., 2014a). Broccoli sprouts (500 mg) were hydrolyzed at 37 °C for 3 h by endogenous myrosinase, then were extracted with dichloromethane. After concentrating on a rotary evaporator, the residue was dissolved in 10% acetonitrile and filtered through a 0.45 mm membrane before HPLC analysis. The sulforaphane production was expressed as mg/100g fresh weight of broccoli sprouts.

Total ITCs determination

The extraction method of ITCs was in accordance with that of sulforaphane extraction. The dichloromethane fraction containing ITCs was concentrated to about 1 mL. The determination of total ITCs was carried out following the previous procedure described by Tang et al. (2013) and Wang et al. (2015). The reaction mixture consisted of 1 mL of the dichloromethane fraction, 2 mL of methanol, 0.2 mL of 7 mmol/L 1,2-benzenedithiol and 1.8 mL of 50 mmol/L sodium borate buffer (pH 8.5). After reacting for 1 h at 65 °C, the mixture was centrifuged for 10 min at 5000 g. Analyses of ITCs were conducted on an Agilent 1200 HPLC system (Agilent Technologies Co. Ltd., USA) equipped with an Eclipse XDB-C18 column and a G1314B UV detector at 365 nm. Assay was performed at a flow-rate of 1.75 mL/min with 70% methanol and 30% H_2O. Production of ITCs was expressed as mg/100 g fresh weight of broccoli sprouts.

Statistical analyses

All statistical analyses were performed with SPSS 18.0 (SPSS Inc., Chicago, IL, USA) in our experiment. Data was analyzed by two-way ANOVA with treatment and organ as factors, followed by Duncan's multiple comparison test. Differences at $p<0.05$ were considered as statistically significant.

RESULTS

Effect of JA on sprout length and fresh weight of broccoli sprouts

The length and fresh weight of broccoli sprouts were both significantly decreased after all JA treatments ($p < 0.05$) (Fig. 1 A and B). The highest decrease in length and fresh weight of broccoli sprouts was observed under 300 μM JA treatment, in comparison with the control sample. No significant difference was found in fresh weight between 100 μM and 200 μM JA treatment.

Effect of JA on GSL_s contents in cotyledon, hypocotyl and root of broccoli sprouts

Seven kinds of GSL_s, including three aliphatic GSL_s and four indole GSL_s were detected in difference organs of broccoli sprouts (Fig. 2 and Fig. 3). The influence of JA treatment on individual GSL profile differed considerably. Application of JA significantly enhanced glucoraphanin

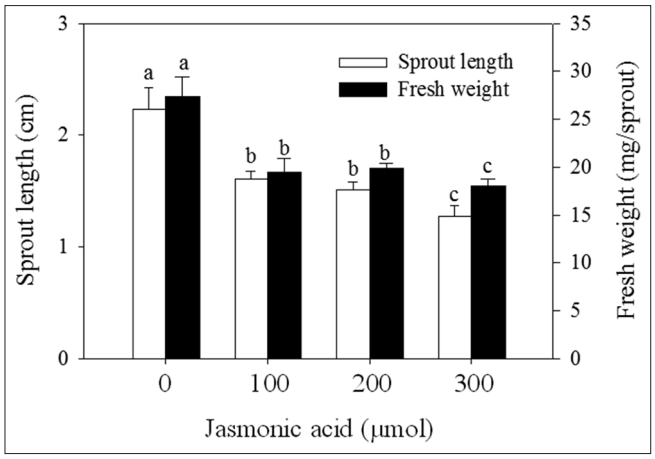

Fig 1. Sprout length, fresh weight of broccoli sprouts under JA treatment. Each point was expressed as mean ± SD (n=3). Values not sharing the same letter at the same index are significantly different at $p < 0.05$.

and glucoerucin content but did not affect even reduced gluconapin content in cotyledon (Fig. 2). Additionally, no significant difference was found in aliphatic GSL content in cotyledon among the three concentrations of JA.

On the other hand, JA treatment was found more effective for increasing indole GSL_S than aliphatic ones, especially glucobrassicin and neoglucobrassicin. The level of neoglucobrassicin in cotyledon, hypocotyl and root treated with 200 μM JA was 53.44, 49.67 and 13.12 times of that in the control samples, respectively. However, only glucobrassicin content in cotyledon was dramatically enhanced by JA treatment, which increased up to 7.67- to 8.91-fold of that in control cotyledon. Except neoglucobrassicin, other indole GSL_S in root was not affected by JA treatment.

Effect of JA on myrosinase activity in cotyledon, hypocotyl and root of broccoli sprouts

Compared to the control, JA treatment increased myrosinase activity in cotyledon but decreased it in hypocotyl and root. On the other hand, the myrosinase activity in hypocotyl and root continuously decreased with the increase of JA concentration. After treatment with 100 μM JA, compared to the control, myrosinase activity in cotyledon increased by 95.4%, but in hypocotyl and root decreased by 12.1% and 20.7%, respectively (Fig. 4).

Effect of JA on sulforaphane and ITCs formation in cotyledon, hypocotyl and root of broccoli sprouts

JA treatments dramatically increased the formation of sulforaphane and ITCs in broccoli sprouts ($p < 0.05$). While in cotyledon, there was no significant difference in sulforaphane formation among three concentrations (Fig. 5 A); however, 100 μM JA showed more ITCs formation than 200 μM and 300 μM JA (Fig. 5 B). In addition, no significant difference

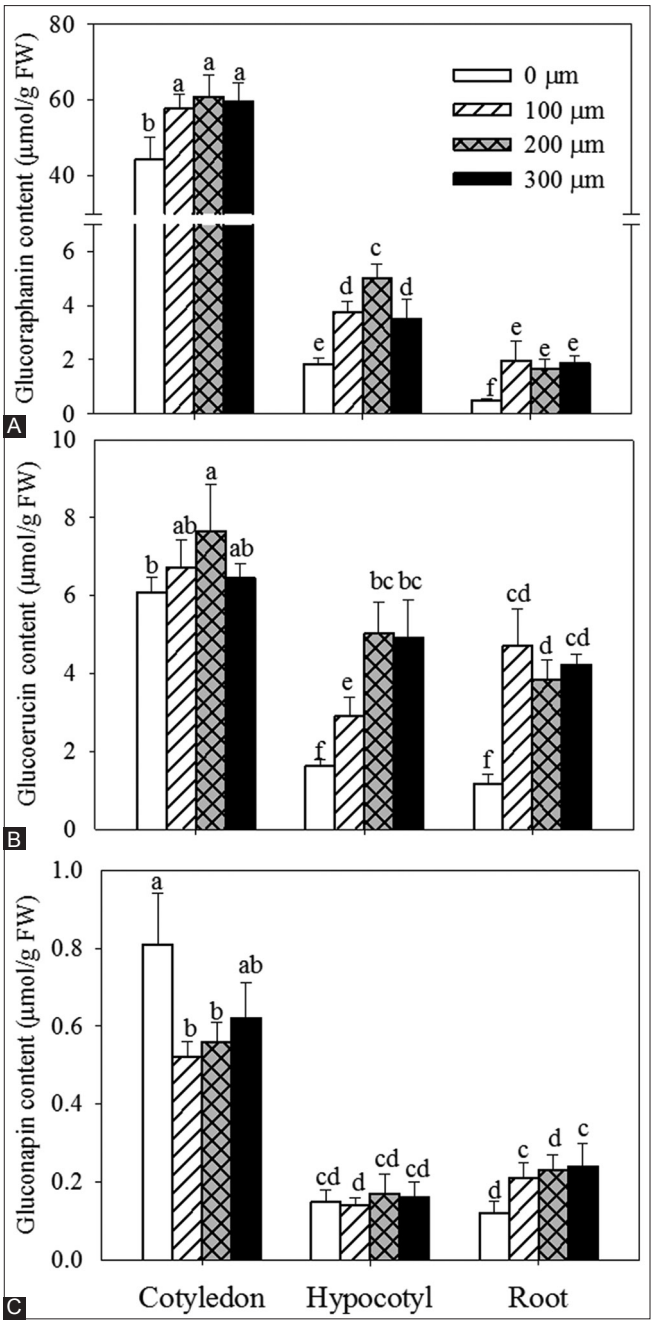

Fig 2. (A-C) Aliphatic glucosinolate content in different organs of broccoli sprouts under JA treatment. Each point was expressed as mean ± SD (n=3). Values not sharing the same letter are significantly different at $p < 0.05$.

was obtained in sulforaphane and ITCs formation in hypocotyl between 200 μM and 300 μM JA. Root contained the lowest sulforaphane and ITCs.

DISCUSSION

In the present study, the growth of broccoli sprouts measured as sprout length and fresh weight, which was markedly decreased by JA treatment when compared to

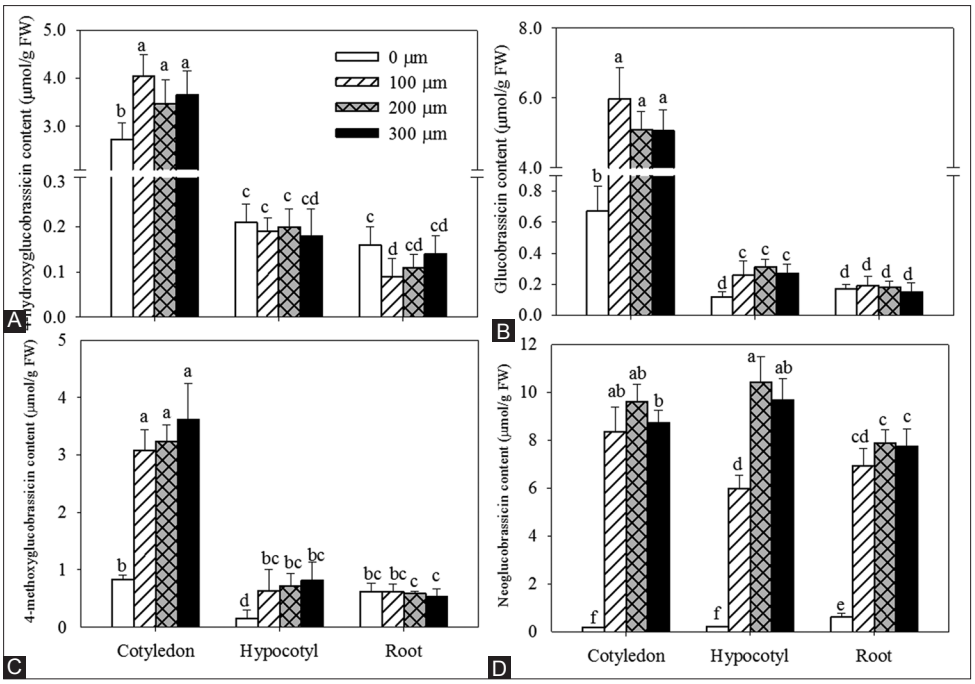

Fig 3. (A-D) Indole glucosinolate content in different organs of broccoli sprouts under JA treatment. Each point was expressed as mean ± SD (n=3). Values not sharing the same letter are significantly different at $p < 0.05$.

the control. This result was in line with the findings by Kastell et al. (2013) and Baenas et al. (2014), which showed that addition of JA significantly decreased the fresh weight and biomass in hairy root and *Brassicaceae* sprouts such as broccoli, rutabaga, turnip and radish.

Broccoli sprouts are rich in glucoraphanin, which can be hydrolyzed into the corresponding ITC, sulforaphane by the action of myrosinase (Guo et al., 2018). In our study, the most substantial glucosinolate in broccoli sprouts was glucoraphanin, accounting for 72% of total glucosinolate content, which was in consistent with the results of Guo et al. (2011) and Pérez-Balibrea et al. (2011). However, it was different from the findings of Guo et al. (2014c) and Sun et al. (2015), who found that the glucoerucin, not glucoraphanin was the most abundant one. Glucoerucin is the precursor of glucoraphanin and can be converted to glucoraphanin by the hydrolysis of the enzyme encoding of GSL-OX (Li et al., 2008). Hence, the different phenomenon might be attribute to the different activities of the enzyme GSL-OX (Guo et al., 2014c). Additionally, previous studies found that glucosinolate composition and concentration varied considerably among different organs within the same plant and a plant species (Brown et al., 2003; Pérez-Balibrea et al., 2008). The results of this study indicated that the cotyledon of broccoli sprouts contained the highest glucosinolate content except neoglucobrassicin.

Many factors can dramatically induced biosynthesis of glucosinolates, such as wounding, phytohormone,

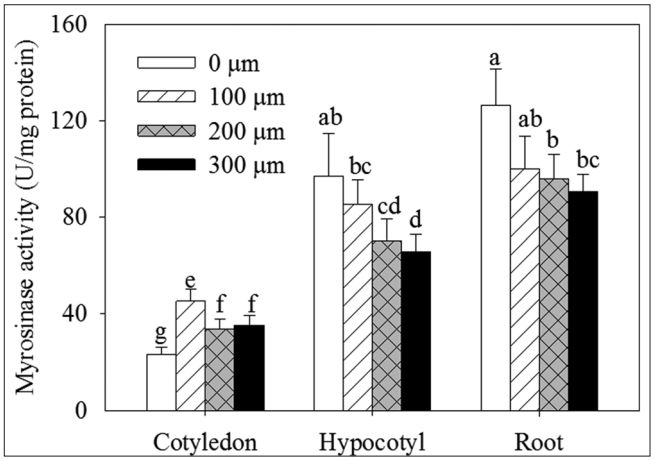

Fig 4. Myrosinase activity in different organs of broccoli sprouts under JA treatment. Each point was expressed as mean ± SD (n=3). Values not sharing the same letter are significantly different at $p < 0.05$.

pathogen, herbivore and sugar, etc. (Gu et al., 2012). JA as an elicitor and signal molecule has been used successfully in different plants to improve production of GSL_S (Baenas et al., 2014; Kastell et al., 2013). In the present study, application of JA increased the concentration of GSL, such as glucoraphanin, glucoerucin, glucobrassicin and neoglucobrassicin in three organs. Particularly, neoglucobrassicin obtained the maximum increase in sprouts, which was in accordance with the finding in Hairy Root of *Brassica rapa* by Kastell et al. (2013), who found that neoglucobrassicin content enhanced by about 16 times on day 14 after application of 100 μM JA. Interestingly, JA had no effect or even decreased the concentration

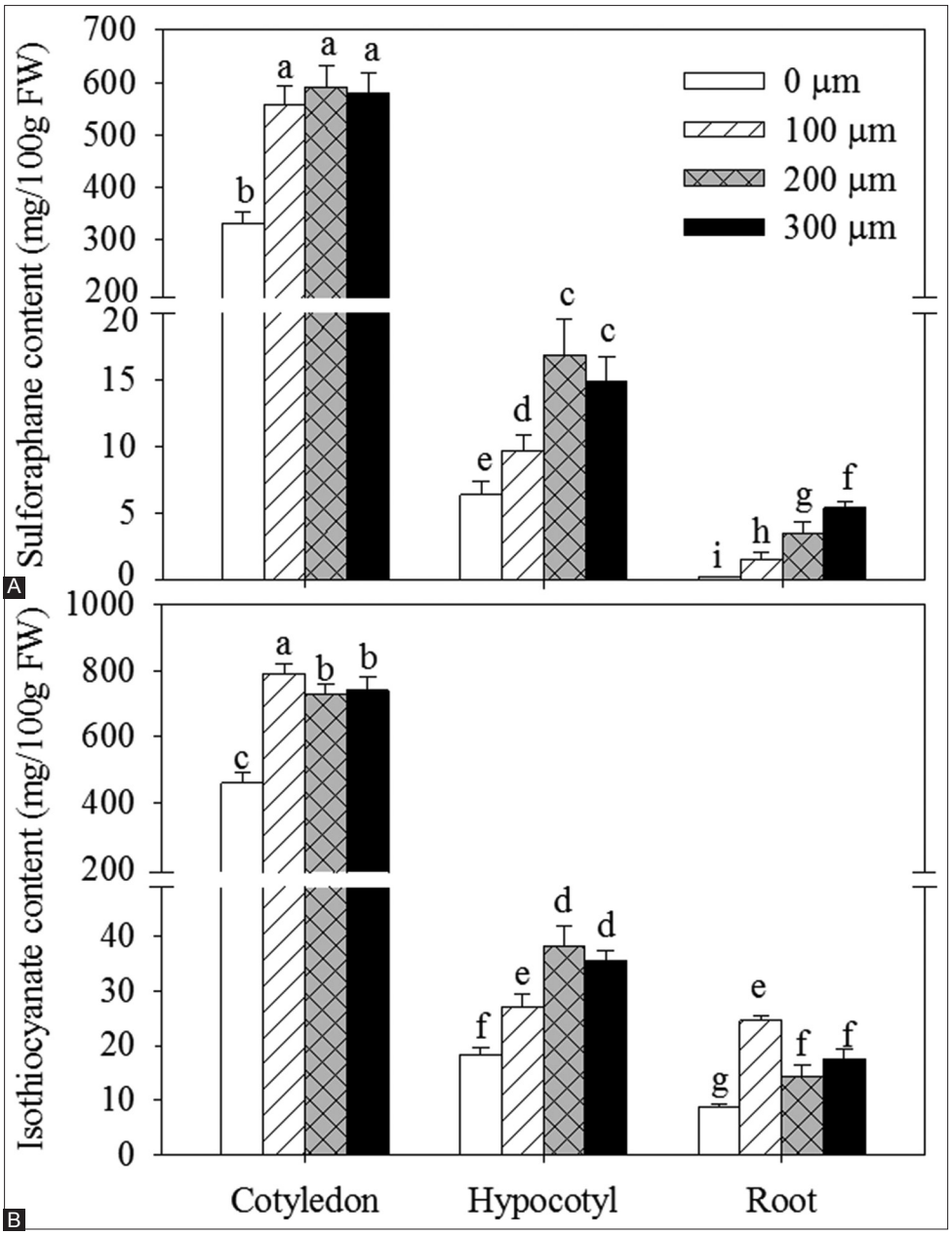

Fig 5. The formation sulforaphane (A) and ITCs (B) in different organs of broccoli sprouts under JA treatment. Each point was expressed as mean ± SD (n=3). Values not sharing the same letter are significantly different at $p < 0.05$.

of gluconapin. A substantial increase of glucobrassicin content was only found in cotyledon of broccoli sprouts after JA treatments. Additional researches are needed to expound the underlying mechanism of different effects of JA on different GSL_S and organs.

Different effects of JA treatment on myrosinase activity in different organs were also detected in the present study. Myrosinase activity in hypocotyl and root reduced after JA treatments. The result was similar to the result of Kim et al. (2006), who reported that jasmonates hormone (methyl jasmonate) decreased myrosinase activity in radish sprouts. However, myrosinase activity in cotyledon significantly enhanced after JA treatments. ITCs, hydrolyzed from aliphatic and aromatic GSLs, are thought to be accountable for decreasing the risk of in the development of various types of cancer (Traka and Mithen, 2009; Zhang, 2004). In addition, the ITCs formed from indole GSLs are unstable, but the hydrolysis products of indole GSLs, in particular indole-3-carbinol, have the potential anticancer activity (Fahey et al., 2001). Sulforaphane, an isothiocyanate hydrolyzed from glucoraphanin, has received the most attention. In addition, the enzymolytic ITCs from gluconapin and glucoerucin are 1-butene-4-isothiocyanat and erucin, which also presented anticancer effects in *vitro* and *in vivo* (Melchini and Traka, 2010; Morroni et al., 2018).

In the present study, sulforaphane concentration accounted for 62.59% of total ITCs in broccoli sprouts. As expected, three concentrations of JA all enhanced the formation of sulforaphane and ITCs in cotyledon, hypocotyl and root of broccoli sprouts, which was in accordance with changing trend of the content of precursor GSLs. Besides, there was no significant difference in the level of total aliphatic GSLs in cotyledon of broccoli sprouts among the three concentrations of JA, but 100 μM JA treatment led to more ITCs formation in cotyledon than 200 μM and 300 μM JA treatment. The probable explanation for this phenomenon was the fact that myrosinase activity in cotyledon treated with 100 μM JA was higher than that of 200 μM and 300 μM JA treatment. Further research will be carried out to investigate the induction of hydrolysis products of indole GSLs by JA treatment and the molecular mechanisms involved.

CONCLUSIONS

In conclusion, except gluconapin, the content of other GSLs in broccoli sprouts increased after JA treatment. However, the different effects were found in different organs and different GSLs as well as JA concentrations. No significant difference was found in all individual GSL content in cotyledon among 100 μM, 200 μM and 300 μM JA. In particular, JA treatment resulted in the highest enhancement of neoglucobrassicin content in cotyledon, hypocotyl and root. Treatment with JA enhanced myrosinase activity in cotyledon but reduced it in hypocotyl and root. Besides, the yield of ITCs and sulforaphane in three organs also increased after JA treatment, which was similar to changing trend of the content of glucoraphanin and aliphatic GSLs. Cotyledon contained the majority of GSLs and ITCs both in control sample and in JA treated sprouts. In summary, treatment with JA is an effective strategy for selective increase the bioactive compounds GSLs and ITCs in broccoli sprouts and is potential for the production and commercialization of functional sprouts.

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Authors' contributions

Y.Z. and L.G. conceived designed the study; Y.Z., F.W. and L.G. carried out the experiments; F.W. analyzed the data; Y.Z. and L.G. wrote the manuscript. All authors read and approved the final manuscript.

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