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Increased antioxidant activity and polyphenol metabolites in methyl jasmonate treated mung bean (*Vigna radiata*) sprouts

Li LI¹, Yinmao DONG¹, Hankun REN¹, Yan XUE¹, Hong MENG¹, Minhui LI^{2*}

Abstract

Mung bean sprouts are a popular health food both in China and worldwide. We determined the optimal concentration of exogenous methyl jasmonate (MeJA) for the promotion of the sprouting in mung beans (*Vigna radiata*). The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging test showed that MeJA application resulted in significantly improved antioxidant capacity in the sprouts 72 h later. Measurement of total polyphenols in MeJA-treated beans from 0 to 168 h, using Folin–Ciocalteu colorimetry, showed that the polyphenols changing was significantly correlated with antioxidant activity. The main polyphenols isovitexin, kaempferol-3-O-rutinoside, daidzein, genistein, isoquercitrin, *p*-coumaric acid, and caffeic acid were quantified using high-performance liquid chromatography (HPLC/QqQ MS) and partial least squares discriminant analysis (PLS-DA). MeJA promoted the production of polyphenols, metabolites, and antioxidants in the sprouts; therefore, its use may allow sprouts to be prepared more quickly or increase their nutritional value.

Keywords: mung beans sprouts; methyl jasmonate; antioxidants; polyphenols; metabolite pathway.

Practical Application: We firstly studied the influences of MeJA on activities and metabolites of mung bean sprouts.

1 Introduction

The mung bean (*Vigna radiata*) is one of the most important short-season, summer-cultivated legumes. It is grown widely throughout the tropics and subtropics (Liu et al., 2011; Thomas et al., 2004). The seeds and sprouts are excellent sources of antioxidants in China, India, Bangladesh, and Southeast Asia (Fery et al., 2002). Mung bean sprouts are also used by western countries as a fresh salad vegetable.

Seed germination begins with water absorption, followed by significant chemical changes, including the interconversion of some compounds and the synthesis of new compounds (Tang et al., 2014a). Raw seed sprouts, such as those of broccoli, alfalfa, and beans, have been attracting attention as health foods because they are rich in various phytonutrients, such as minerals, amino acids, vitamins, proteins, and phytochemicals (Kavas & El, 1991). During sprouting, a large portion of the original nutritional value of the mung bean seeds is retained, and the amounts of some active substances increase significantly (Tang et al., 2014b).

Recent research has focused on developing methods of increasing the concentration of desirable phytochemicals present in edible plants, without using gene modification or breeding (Tang et al., 2014a). The simplest and most effective method is to take advantage of plants' stress response systems. Among various stress inducers, the plant hormone jasmonate and its methyl ester methyl jasmonate (MeJA) play important roles in the regulation of plant growth and in endogenous and/or exogenous stress signaling (Bennett & Wallsgrove, 1994). Both function as endogenous stress signal. When plants are

attacked by insect herbivores, they emit MeJA and various other organic, volatile compounds into the air and thereby transferring stress signals to healthy, neighboring plants possessing MeJA receptors (Farmer & Ryan, 1990). Although not under stress, the detection of exogenous MeJA triggers the induced defense responses of healthy plants. The detection of exogenous MeJA can stimulate the production of phytochemicals in a number of different plants, such as sweet basil, raspberries, and radish sprouts, thereby improving their antioxidant activity shelf life (Kim et al., 2011; Tang et al., 2014b). Plants cope with the vital stress conditions including biotic and abiotic (elicitor exposure) through a variety of defense responses, which are mediated by production of different secondary metabolites for instance phenolics, flavonoids, and alkaloids, etc (Saeed et al., 2017; Wang et al., 2017).

In order to improve the antioxidant activity and elucidate relevant metabolites levels for better usages of mung bean sprouts, the effect of different kinds of exogenous plant hormones on the sprouting process of mung beans was examined. The use of MeJA was further explored to determine the best conditions for its use, and the influence of MeJA on antioxidant activity and the total polyphenols in the sprouts was elucidated. In addition, the effect of exogenous MeJA on the polyphenol metabolite profile of the sprouts was tested using high-performance liquid chromatography (HPLC) coupled with a triple quadrupole mass spectrometry (QqQ MS) and partial least squares discriminant analysis (PLS-DA). MeJA-treated mung bean sprouts may be a food of high nutritional value, and is better for the production of cosmetics and medicinal products.

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2 Materials and methods

2.1 Chemicals and reagents

Mung beans (*V. radiata* Linn) (Beijing, China); 1,1-diphenyl-2-picrylhydrazyl radical (DPPH); Folin–Ciocalteu reagent (Biodee Biotechnology Co., Ltd, Beijing, China); MeJA; and gallic acid were purchased from Sigma Chemical Co. (St. Louis, MO). HPLC-grade methanol was purchased from Merck (Germany) and MS-grade formic acid from Sigma-Aldrich. Ultra-pure water (18.2 MΩ) was prepared with a Milli-Q water purification system (Millipore, Bedford, MA, USA).

The flavonoid references were purchased from Shanghai TAUTO Biotech. Co., Ltd. The phenolic acid references were purchased from Beijing Banxia Tech. Co., Ltd. All of the references were more than 98% pure by HPLC analysis. For HPLC/QqQ MS quantitative analysis, each standard contained isovitexin (0.56 mg/mL), kaempferol-3-O-rutinoside (0.70 mg/mL), daidzein (0.51 mg/mL), genistein (0.64 mg/mL), isoquercitrin (0.69 mg/mL), *p*-coumaric acid (0.72 mg/mL), and caffeic acid (0.61 mg/mL) and was dissolved in 1.5 mL of 75% (v/v) methanol. The solutions were stored in dark glass bottles at 4 °C. The working standard solutions were freshly prepared by diluting suitable amounts of the solutions mentioned with 75% (v/v) methanol before injection. The standard solutions and the sprouts extract were filtered through a 0.22 μm filter and a 5.0 μL volume of each was used for the HPLC/QqQ-MS analysis.

2.2 Screening of plant hormones species

The Folin–Ciocalteu reaction was used to evaluate the effect of different plant hormones on the concentrations of total polyphenols in mung bean sprouts. We screened the effects of 1.0 mmol/L each of auxin, gibberellin, kinetin, abscisic acid, ethylene, MeJA, and salicylic acid on the total polyphenols in 72 h sprouts.

2.3 Cultivation of sprouts and MeJA treatment

The mung bean seeds 10.0 g were germinated using previously published methods, with some modifications (Tang et al., 2014a). Different quantity of MeJA was dissolved in ethanol 5.0 mL, deionized water was added to 250 mL. In order to determine the appropriate concentration of MeJA to use, 1.0 μmol/L, 10.0 μmol/L, 100.0 μmol/L, 1.0 mmol/L, and 10.0 mmol/L of a 30 mL volume of MeJA, respectively, were sprayed onto the sprouts every 24 h for 72 h. The sprouts of MeJA-treated groups and control groups 1.0 g were put out every 24 h for 168 h, and germinating seeds were kept moist with sterile water and incubated in an incubator without light at 26 °C for chemical quantitative and biological analysis. There were 3 replicates of each treatment.

2.4 DPPH radical scavenging activity

The rates of DPPH radical scavenging in the extracts were measured by the DPPH method (Li et al., 2006). Fragments of seed (1.0 g, removed at various points from 0–168 h), along with 5.0 mL water, were extracted by ultrasound for 30 min and centrifuged at 5000 rpm for 5.0 min. The extracted solution was

diluted 10 times with methanol. For each sample, an aliquot of 0.5 mL at different concentrations was added to 1.0 mL DPPH solution (100 μM) for 30 min. Methanol was used as a blank solution. The decrease in absorbance was measured at 517 nm. DPPH radical scavenging activity was expressed as the percentage (%) of absorbance disappearance $[(A_{DPPH} - A_s)/A_{DPPH}] \times 100$, where A_s is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution.

2.5 Determination of total amounts of polyphenols

The total amounts of polyphenols in MeJA treatments and the control group (at 24–168 h) were measured by Folin–Ciocalteu colorimetry (Kwok & Shetty, 1996). The Folin–Ciocalteu reagent was prepared by diluting the commercial reagent concentrate in a 1:2 ratio with water. From each batch, pieces of bean sprouts (1.0 g) was extracted with 5.0 mL methanol-water (75%) by ultrasonication for 30 min and centrifuged at 12,000 rpm for 5.0 min. The supernatant of the sample (0.1 mL) was added to 0.1 mL methanol-0.3% HCl (6:4) and the Folin–Ciocalteu reagent (0.1 mL) in order. 10% Na₂CO₃ (2.0 mL) was added to the mixture, which was then kept in the dark for 30 min. Then the samples were shaken thoroughly and the absorbance was measured at 750 nm using a microplate reader. The standard curve was constructed using gallic acid. Results were expressed as the mg of gallic acid equivalents per g of dry weight (mg GAE/g).

2.6 Quantitative analysis of the main polyphenols by HPLC-QqQ MS

The sample preparation for quantitative analysis of polyphenols was empirically optimized. The crushed samples, normalized to 1.0 g, were extracted by heating reflux and ultrasonication using 5.0 mL of 75% (v/v) methanol. The extracts (200 mg/mL) were filtered through a 0.22-μm filter, after which 5.0 μL volumes was injected into the HPLC-QqQ MS for analysis.

Chromatographic separation was carried out using an Agilent 1200LC series system (Agilent Technologies, Palo Alto, CA, USA) equipped with an online vacuum degasser, quaternary pump, autosampler, and thermostated column compartment. The Agilent 6410 QqQ MS system (Agilent Technologies, Palo Alto, CA, USA), equipped with an electrospray ionization source and operated in negative ion mode, was used for the quantitative analysis of the main chemical constituents in the MeJA-treated and control groups. The separation was performed on an Agilent ZORBAX Eclipse Plus C18 (3.0 × 150 mm, 3.5 μm) at a constant temperature of 35 °C. The mobile phase consisted of (A) water containing 0.1 g/kg acetic acid and (B) methanol. The linear gradient conditions were as follows: 0–15 min, 15–45% of B; 15–16 min, 45–100% of B. The flow rate was 0.4 mL/min. The electrospray ionization interface and mass spectrometer parameters were optimized for maximum sensitivity. During the MS analysis, the nebulization gas was set to 600 L/h at 350 °C, and the capillary voltage was maintained at 4,000 V. The detection was performed by negative ion electrospray ionization in multiple reactions monitoring mode, monitoring the transitions from molecular ion to dominant product ion

(Table 1). Data Acquisition was processed by Agilent Mass Hunter QqQ Quantitative. Authentic standards were used to confirm the assignments and to quantitatively analyze the samples.

2.7 Statistical analysis

Principal component analysis (PCA) and PLS-DA were performed using SIMCA-P software (v. 12.0.1, Umetrics, Umeå, Sweden). The data was analyzed by one-way analysis of variance (ANOVA) using SPSS17.0. Dunnett's t-test was used to analyze the significant difference among the groups, with significance level set as ($p < 0.05$).

3 Results and discussion

Plant hormones have different effects on the secondary metabolites and biological activities of mung beans sprouts. MeJA triggered the most significant increase ($p < 0.05$) in the total polyphenols. In the MeJA-treated groups, 1.75 ± 0.04 mg GAE/g of total polyphenols was recorded, 40% more than in the control group (1.25 ± 0.02 mg GAE/g). The addition of ethylene (1.03 ± 0.01 mg GAE/g) reduced the total polyphenols relative to that in the control group (1.25 ± 0.02 mg GAE/g). The influence of kinetin was not significant. Gibberellin, salicylic acid, and auxin increased the total polyphenols to 1.34 ± 0.01 , 1.31 ± 0.03 , and 1.57 ± 0.01 mg GAE/g, respectively. Gibberellin also significantly increased the sprouts' lengths. According to results reported in the literature, healthy plants respond to exogenous MeJA by activating their defense responses (Mandal, 2010; Xiao et al., 2009). MeJA may stimulate gene expression

and enzyme activity related to the synthesis of some secondary metabolites. The optimal conditions for the use of MeJA were clarified further in the following experiments.

3.1 Optimal concentration of MeJA

The effects of different concentrations of MeJA (1.0, 10.0, 100.0 $\mu\text{mol/L}$, 1.0, and 10.0 mmol/L) on the sprouting process were evaluated between 24 h and 72 h. First, 1.0 g sample of the sprouts was extracted with 5.0 mL methanol (75%, v/v) by ultrasonication. The total phenolic amounts were detected using Folin–Ciocalteu colorimetry. MeJA significantly increased total phenolic amounts after 72h. The optimal intervention solvent was determined to be 1.0 mmol/L MeJA, which produced a significant difference ($p < 0.01$) in the sprouts (Table 2).

3.2 Antioxidant activity and total phenolic amount in MeJA-treated sprouts

No obvious differences were observed in the appearances of MeJA-treated and control group sprouts, but MeJA treatment significantly affected the antioxidant activity and polyphenol metabolism of the sprouts. The variation in antioxidant activity during the sprouting process is summarized in Table 3. A significant increase in antioxidant activity occurred from 24 h to 72 h in the treatment groups. The DPPH clearance activity of the MeJA-treated sprouts reached 88% for sprouts at 72 h, about twice that of the control group. During the later stages of sprouting, from 96 h to 168 h, the influence of MeJA on antioxidant activity decreased. It is indicated that

Table 1. Characterization of 7 polyphenols and the optimized multiple reaction monitoring (MRM) parameters for the quantification of both MeJA-treated and control samples.

Compounds	Characterization of 7 polyphenols			MRM parameters		
	RT (min)	M-H/M (m/z)	Lost ions	Quantification transition(m/z)	Frag (V)	CE (V)
isovitexin	7.53	431	311;341	431→311	148	18
kaempferol-3-O-rutinoside	8.51	593	285;255	593→285	180	40
daidzein	12.26	253	223;132	253→223	150	33
genistein	15.50	269	133;159	269→133	150	35
isoquercitrin	7.91	463	300;271	463→300	170	30
p-coumaricacid	7.40	163	119;93	163→119	80	25
caffeic acid	5.00	179	134;89	179→134	90	35

Table 2. The content and the rate of change of total polyphenols at different levels of MeJA application (n = 3).

MeJA concentration	Total polyphenols (mg GAE/g)			variance ratio %
	24h	48h	72h	
1.0 $\mu\text{mol/L}$	$1.33 \pm 0.09\text{b}$	$1.61 \pm 0.06\text{bc}$	$2.10 \pm 0.26\text{ab}$	16
10.0 $\mu\text{mol/L}$	$1.46 \pm 0.05\text{b}$	$1.66 \pm 0.04\text{c}$	$2.04 \pm 0.09\text{ab}$	13
100.0 $\mu\text{mol/L}$	$1.30 \pm 0.07\text{b}$	$1.63 \pm 0.22\text{bc}$	$2.16 \pm 0.20\text{bc}$	27
1.0mmol/L	$1.45 \pm 0.06\text{b}$	$1.51 \pm 0.07\text{b}$	$2.92 \pm 0.22\text{d}$	62
10.0mmol/L	$1.43 \pm 0.35\text{b}$	$1.54 \pm 0.09\text{bc}$	$2.35 \pm 0.08\text{c}$	38
The control group	$1.10 \pm 0.11\text{a}$	$1.34 \pm 0.03\text{a}$	$1.80 \pm 0.01\text{a}$	—

Values are a mean \pm SD (n=3). Duncan's multiple range test was used to analyzed the differences of the groups. Different characters in the same line are $p < 0.05$.

MeJA promote polyphenol metabolism during the sprouting process of 24–96 h. Total phenolic content of the control group increased steadily during the period from 0 to 168 h. MeJA-treated sprouts from 24 h to 144 h showed higher content than that of the control group. Especially for sprouts at 72 h, the amount (2.93 ± 0.09 mg GAE g⁻¹) of total phenolics was 80% higher than that of the control group (1.63 ± 0.06 mg GAE g⁻¹). The variances of total phenolic content for MeJA-treatment groups decreased since 72 h (Table 3). Exogenous MeJA plays important roles in the regulation of plant growth and in endogenous and/or exogenous stress signaling, during which it induces the artificial production of metabolites such as polyphenols and ascorbic acid, which leads to improved antioxidant activity (Farmer & Ryan, 1990; Kim et al., 2006; Kim et al., 2007; Kim et al., 2011).

3.3 Quantitative analysis of the polyphenols using HPLC-QqQ MS

Seven main polyphenols were quantitatively analyzed by HPLC-QqQ MS in MeJA-treated and control groups, over the course of 24–168 h: isovitexin, kaempferol 3-O-rutinoside,

daidzein, genistein, isoquercitrin, *p*-coumaric acid, and caffeic acid. Although the total amount of polyphenols in the sprouts increased significantly at the 72h point, the concentration of one of isovitexin, one of the main flavonoids, decreased, whereas that of kaempferol 3-O-rutinoside, genistein, and *p*-coumaric acid increased. Of these chemicals, the concentration of genistein increased the most, showing an increase of 243% for sprouts at 72 h (Table 4).

3.4 Multivariate statistical analysis

To compare differences in the metabolite profiles of the MeJA-treated and control groups, PLS-DA scores plots were generated with the SIMCA-P software program. The quantitative results of the seven compounds were analyzed and subjected to a PLS-DA score plot, in order to visualize the differences among samples (Figure 1A, B). The results indicated that the MeJA-treated sprouts could be clearly discriminated from the control group sprouts after 72 h. MeJA had an obvious influence on the production of secondary metabolites in sprouts at 72 h, 120 h, and 168 h (Figure 1A). We also could see that from 24 h to 48 h, the differences

Table 3. The concentration of the total reducing substances and DPPH scavenging rate at 24–168 h, in both MeJA-treated and control samples.

sprouts(h)	DPPH scavenging rate (%)			total polyphenols (mg GAE/g)		
	Control groups	MeJA-treated groups	variance ratio (%)	Control groups	MeJA-treated groups	variance ratio (%)
24	35 ± 9 a	45 ± 2a	29	0.71 ± 0.22a	1.06 ± 0.02a	49
48	38 ± 2a	49 ± 4b	31	1.13 ± 0.01A	1.78 ± 0.05B	58
72	47 ± 3A	88 ± 2B	86	1.63 ± 0.06A	2.93 ± 0.09B	80
96	85 ± 4a	93 ± 0.3a	9	2.78 ± 0.10a	3.54 ± 0.10a	27
120	92 ± 0.02a	94 ± 0.2a	2	3.31 ± 0.15a	3.96 ± 0.12a	20
144	92 ± 0.20a	93 ± 0.2a	1	3.62 ± 0.13a	4.05 ± 0.12a	12
168	93 ± 0.37a	90 ± 2a	-3	3.90 ± 0.12a	2.99 ± 0.08a	-23

Values are a mean ± SD (n = 3). Different lowercases are $p < 0.05$; Different capitals are $p < 0.01$ (SPSS Independent-Samples T Test).

Table 4. Concentrations of the 7 polyphenols in different mung bean samples (µg/g).

Samples	Contents (µg/g)							total phenols(TP)	TP/Folin index (%)
	isovitexin	kaempferol 3-O-rutinoside	daidzein	genistein	isoquercitrin	<i>p</i> -coumaric acid	caffeic acid		
M-24	57.5	1.19	nd	nd	nd	1.71*	nd	60.4	5.7
M-48	56.9	3.29	nd	1.12	nd	3.49*	nd	64.8	3.6
M-72	26.4*	7.07*	4.31*	4.33*	nd	4.79*	nd	46.9	1.6
M-96	18.5*	14.9*	9.48*	8.09*	0.104*	14.9*	1.46*	67.4	1.9
M-120	4.65*	26.9*	7.26*	4.88*	0.248*	7.57*	1.08*	52.6	1.3
M-144	3.98*	43.9*	10.9*	8.27*	0.255*	7.26*	1.04*	75.6	1.9
M-168	1.49*	20.5*	5.36*	4.37*	nd*	5.26*	1.00*	38.0	1.3
C-24	58.2	1.63	nd	nd	nd	3.30	nd	63.1	8.9
C-48	59.1	4.91	nd	1.04	nd	4.45	nd	69.5	6.2
C-72	44.2	4.44	nd	1.26	nd	3.72	nd	53.6	3.3
C-96	25.1	22.3	3.47	1.89	0.123	7.02	nd	59.9	2.2
C-120	9.7	32.2	4.42	1.87	0.188	8.94	nd	57.3	1.7
C-144	11.9	36.8	5.60	3.53	0.132	8.09	nd	66.1	1.8
C-168	10.5	17.0	4.50	1.92	0.074	9.07	nd	43.1	1.1

M-24 indicated MeJA-treated group for 24h. C-24 indicated the control group for 24h. "nd" indicated it wasn't detected. *indicated significant difference between MeJA-treated group and the control group ($p < 0.05$). SPSS Dunnett-t test was used

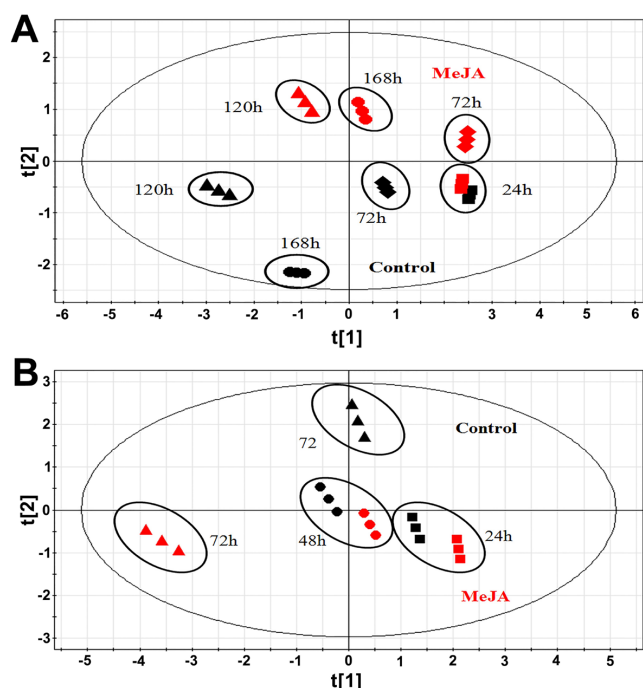


Figure 1. (A) Partial Least Squares-Discriminant Analysis (PLS-DA) score plots of control and MeJA-treated groups; (B) Partial Least Squares-Discriminant Analysis PLS-DA score plots of 24-72 h control and MeJA-treated groups.

between the secondary metabolites of the MeJA-treated and control groups were not obvious (Figure 1B). Using the PLS-DA scores plot, PCA was performed using SPSS 17.0 software, to identify the different metabolic compounds that account for the differences among groups. Isovitexin, kaempferol-3-O-rutinoside, genistein, and coumaric acid, all of which had relatively high loadings in the plot, are characteristic markers that can be used to identify the MeJA treatment groups with the most confidence.

3.5 Metabolomic analysis of mung bean sprouts treated with MeJA

The amount of isovitexin in the sprouts steadily decreased after 24 h, as MeJA accelerated the metabolism of isovitexin. The concentrations in the MeJA-treated groups were 59%, 74%, 48%, 33%, and 14% of the amount in the control group at 72 h, 96 h, 120 h, 144 h, and 168 h, respectively. At the start of metabolism, the main flavone C-glycosides in the seeds might be hydrolyzed to flavone (apigenin) by the flavone glucoside hydrolase (FGH). MeJA intervention potentially stimulated the activity of hydrolase, which transformed isovitexin into apigenin, after which other kinds of secondary metabolites were generated.

Examination of the synthesis pathway of the quantitative chemicals suggested that aglycon hydrolyzed from isovitexin might be added with hydroxyl groups and glycosides at position C-3 and thus transformed to flavone-3-O-glucosides. The amount of kaempferol 3-O-rutinoside in MeJA-treated groups was higher

than that in the control group from 144 h to 168 h, an effect that was also detected for isoquercitrin. The amount of isoquercitrin in the MeJA-treated group (at 120 h and 144 h) increased more than 30% compared with the control group. The results indicated that MeJA might activate the enzymes of flavonoid glycosyltransferase (FGT) or flavanone 3-hydroxylase (F3H) during a later stage of the sprouting process, increasing the synthesis of some flavone-3-O-glycosides. A previous study on anthocyanin accumulation in radish sprouts reported that the activities of phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H), CoA ligase (4CL), chalcone synthase (CHS), chalcone isomerase (CHI), F3H, dihydroflavanone reductase (DFR), anthocyanin synthase (ANS), Myb transcription factors (MYB) could be significantly increased by the application of MeJA (Kelley et al., 2010).

The concentration of the isoflavone genistein was also significantly increased by MeJA application, as from 72 h to 168 h, the concentrations in MeJA-treated groups were 2 to 4 times higher than in the control group. The activity levels of isoflavone synthase (IFS) and 2-hydroxyisoflavanone dehydratase (IFD) might be increased under these circumstances, which accelerated the transformation of the flavonone naringenin to some isoflavones. A similar change was observed for the daidzein concentration. In the literature, the amount of soy isoflavones increased to 960 $\mu\text{g/g}$ after exogenous MeJA was sprayed onto soybeans in the field (Junlan & Zhao, 2011). Soy isoflavones not only play an important role in protection against environmental damage to the plant itself but are also often used as food additives and in pharmaceutical and chemical raw materials. Soy isoflavones have significant antioxidant, anticancer, and estrogen-like effects, help regulate blood lipids, and are important to human health.

Coumaric acid is a key substance in the phenylpropanoid pathway that is transformed from phenylalanine by the catalysis of ammonia-lyase (PAL). Biosynthesis of phenolics and flavonoids in plants initiates through the deamination of L-phenylalanine to trans-cinnamic acid and ammonia by a strategic enzyme phenylalanine PAL. (Khan et al., 2015; Saeed et al., 2017). In the present study, coumaric acid increased 211% from 72 h to 96 h, while caffeic acid was detected from the sprouts at 96 h. The buds' length and appearance from 72 h to 96 h also showed significant changes. It could be concluded that the stage from 72 h to 96 h was the most important metabolic stage for the sprouts. MeJA promoted the creation of metabolites, and therefore may be used in production of mung beans sprouts to speed their development or increase their nutritional value. The rate-limiting enzyme PAL in the phenylpropanoid pathway might be activated by MeJA intervention, leading to an increase in *p*-coumaric acid and derivative caffeic acid. The influence of MeJA on the polyphenol metabolic pathway is expressed in Figure 2. MeJA-treated sprouts, especially during the 72 h to 96 h stage, have good potential for use in food and cosmetic products and may be considered a new antioxidant that is natural and environmentally friendly with a relatively simple production process.

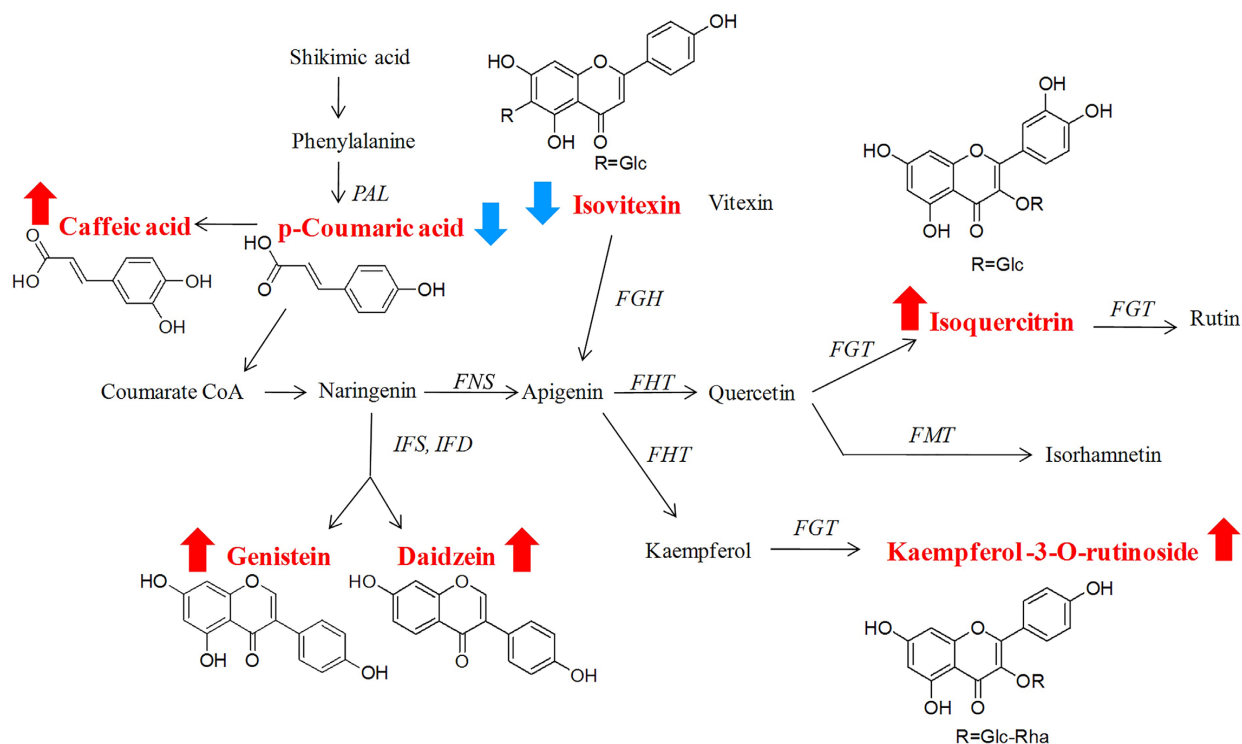


Figure 2. Schematic diagram of the phenylpropanoid pathway associated with the production of mung bean sprout phytochemicals.

4 Conclusions

Plant hormones stimulate the accumulation of metabolites that are crucial to the plants' own defensive capabilities. The increased amount of metabolites and the increase in related biological activities are important in disease prevention and treatment in humans. After mung bean sprouts were treated with MeJA, they produced greater amounts of phenolic acids, glycosides, and soy isoflavones that are excellent antioxidants and have other beneficial properties, including anti-platelet cohesion and antibacterial effects. MeJA is an excellent antioxidant synergist, and its application is a promising technique for improving mung bean sprout crop quality.

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