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Effect of Methyl Jasmonate on Phenolics, Isothiocyanate, and Metabolic Enzymes in Radish Sprout (*Raphanus sativus* L.)

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The effect of spraying exogenous plant hormone methyl jasmonate (MeJA) upon radish sprout (*Raphanus sativus* L.) was investigated in aspects of total phenolic content (TPC), isothiocyanate content, antioxidant activity of the radish extract, and enzymatic activities of phenylalanine ammonia lyase (PAL) and myrosinase. The MeJA treatment significantly increased the TPC that resulted in the increased DPPH• (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging capacity. In addition, the PAL activity also increased by 60% at 24 h after MeJA treatment. However, the same treatment decreased the amount of 4-methylthio-3-butenylisothiocyanate (MTBITC), a major isothiocyanate in radish sprout and the activity of myrosinase, an enzyme related to produce isothiocyanates.

KEYWORDS: Radish sprout; *Raphanus sativus*; methyl jasmonate; phenolics; 4-methylthio-3-butenyl-isothiocyanate; phenylalanine ammonia lyase; myrosinase; antioxidant activity

INTRODUCTION

Under biotic and abiotic stresses such as pathogen attack, physical wounding, and UV-light exposure, plants can elicit a defense response and increase secondary metabolites (*1*−*3*). Unlike the primary metabolites, secondary metabolites have been viewed in the past as the waste products from mistakes of the primary metabolism because they are not involved into the respiration, growth/development, and photosynthesis of plants (*4*−*5*). Nowadays, it is known through much investigation that these compounds, however, play important roles in various defensive mechanisms such as the induction of proteinase inhibitors, polyphenol oxidase, and phytoalexins under stresses (*1*, *2*, *5*−*7*). In addition, it has been found that many secondary metabolites such as phenolic compounds, terpenoids, and alkanoids may have various beneficial properties for humans (*8*−*10*). Therefore, secondary metabolites have attracted remarkable research interests, including investigations on how to improve their production via stress induction (*4*, *10*−*12*). In previous studies (*7*, *13*−*15*), elicitors such as chitosan, β-glucan, and yeast extracts, as well as the plant hormonal chemicals such as jasmonic acid (JA), methyl jasmonate (MeJA), and salicylic acid, have been considered as powerful inducers in many different plants. Among these inducers, MeJA and JA that are derived principally from linolenic acid through an octadecanoid pathway have been implicated as key components in wounding signal transduction (*16*, *17*). For example, exogenous MeJA has been found to be able to mimic the effects of wounding and pathogen attack, which resulted in the induction of not only phenolic compounds, terpenoids, and alkanoids but also proteinase inhibitors in various plants (*6*, *18*−*21*). However, to the best of our knowledge, the effect of MeJA on the induction of secondary metabolites in radish sprout has not been investigated. Since radish is an important vegetable in Asia, especially Korea, Japan, and China, and its intrinsic health benefits due to the bioactive compounds such as glucosinolates and isothiocyanates (*22*−*24*) recently have become known, it is of economic and medical significance to perform this study in terms of the effect of MeJA on the induction of bioactive phytochemicals in the radish sprout. Therefore, the relationship between the MeJA and the TPC, antioxidant capacity, and content of isothiocyanates of the radish sprout were investigated. Since PAL is the key regulatory enzyme for the phenylpropanoid pathway associated with the biosynthesis of phenolics and myrosinase for the production of isothiocyanates, both enzymatic activities of PAL and myrosinase were also evaluated.

MATERIALS AND METHODS

Chemicals. MeJA, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH•), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), L-phenylalanine, â-mercaptoethanol, cinnamic acid, bovine serum albumin, Bradford reagent, and Folin−Ciocalteu's reagent were purchased from Sigma Chemical Co. (St. Louis, MO). Dodecane was purchased from Aldrich Chemical Co. (Milwaukee, WI). All HPLC analytical grade solvents were from Fisher Scientific (Suwanee, GA).

Plant Culture and MeJA Treatment. Radish (*Raphanus sativus* L.) seeds purchased from Bay Farm Services, Inc. (Bay City, MI) were sown in 0.4 L plastic pots containing a commercial potting mixture (Fafard 3-B Mix, Fafard Inc., Anderson, SC) in the greenhouse located at Clemson University at Clemson, SC and watered every day without any fertilizer. Greenhouse cooling/heating set points were 25/27 °C

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under natural light condition. Radish sprouts (7 days old) were sprayed with 10 mL of 1 mM MeJA dissolved in 0.25% ethanol and immediately zipped with a vinyl pack (18 cm × 20 cm) with 10 holes (0.5 cm × 0.5 cm) for 30 min. After removal of the vinyl cover, the treated radish sprouts were set aside in the open air to completely remove the remaining MeJA. They are isolated to the control by at least 2 h. The control radish sprouts were only treated with 0.25% ethanol.

Extraction of Phenolic Compounds and Isothiocyanates from Radish Sprout. The radish sprouts were harvested at 0, 6, 12, 24, and 48 h after the treatment of MeJA and ground in liquid nitrogen into fine powder. Two grams of the radish sprout powder was mixed with 20 mL of 80% aqueous methanol to extract the phenolic compounds or with 20 mL of methyl *t*-butyl ether (MTBE) to extract isothiocyanates. The mixtures were shaken at room temperature for 12 h and then centrifuged at 2000*g* for 20 min. After centrifugation, the methanol and MTBE supernatants were used for the determination of phenolic compounds and isothiocyanates, respectively.

Determination of TPC of the Methanolic Extract of Radish Sprout. The total phenolic content (TPC) of the radish sprout extract was determined using the method described by Singleton and Rossi (*25*). Fifty microliters of methanolic extract was mixed with 450 μL of distilled water and 250 μL of 2 N Folin−Ciocalteu's reagent. The mixture added to 1.25 mL of 20% Na_2CO_3 was incubated at 25 °C for 20 min and then centrifuged at 2000*g* for 10 min. The absorbance of the supernatant was measured at 735 nm, and the standard curve was prepared using gallic acid. In this case, the absorbance was converted to the phenolic content in terms of milligrams of gallic acid equiv (GAE)/g of fresh weight (FW) of sample.

Separation of Antioxidant Phenolic Compounds. To separate antioxidant phenolic compounds in the radish sprout extract, reverse-phase C_{18} high-performance liquid chromatography (HPLC) was used. The Shimadzu LC-10AT HPLC system (Kyoto, Japan) consisted of a YMC ODS-AQ C_{18} column (250 mm × 4.6 mm, 5 μm; Waters, MA) and a Shimadzu SPD-M10V photodiode array detector (PDA). The column was equilibrated with HPLC water containing 0.05% trifluoroacetic acid (TFA). Fifty microliters of the methanolic extract was injected and eluted with HPLC water containing 0.05% TFA and acetonitrile at a flow rate of 1 mL/min. The absorbance of the eluant was scanned from 200 to 500 nm by the PDA.

Identification of Isothiocyanates. Isothiocyanates extracted from the radish sprout by MTBE were determined by GC/MS. A DB-5 capillary column (60 m × 0.25 mm, thickness = 0.25 μm; J&W Scientific, Folsom, CA) was installed in a Shimadzu GC-17A instrument that was also connected to a QP 5050 mass spectrometer (MS) detector (Kyoto, Japan). The GC oven temperature was programmed from 60 to 280 °C at a rate of 10 °C/min and held at 280 °C for 5 min. The injector and ion source temperatures were 220 and 290 °C, respectively. The detector voltage was set at 70 eV, and the MS spectra were obtained in the mass range of *m*/*z* 43−350. Helium was used as the carrier gas at a flow rate of 1.1 mL/min. Two microliters of the extract was injected in a split mode at a 1:5 ratio. Identification of the analytes was based on comparison with mass spectra of Wiley and NIST mass spectral databases, retention index (RI), and the purchased authentic compounds.

DPPH• Free Radical Scavenging Activity. The antioxidant capacity of the radish sprout extract was evaluated by the DPPH• free radical scavenging assay. The scavenging activity on DPPH• free radicals by the radish sprout extract was determined according to the method of Yamaguchi et al. (*26*) with slight modifications. The reaction mixture containing 0.1 mL of sample, 0.3 mL of 0.1 M Tris-HCl (pH 7.4), 0.1 mL of methanol, and 0.5 mL of 0.3 mM DPPH• was vigorously shaken and incubated in the dark at room temperature for 10 min. After incubation, the absorbance of the reaction mixture was spectrophotometrically measured at 517 nm, and the scavenging activity of the DPPH• free radical was calculated by using the following formula:

$$\text{scavenging activity (\%)} = \left(1 - \frac{\text{absorbance of sample at 517 nm}}{\text{absorbance of control at 517 nm}}\right) \times 100\ (\%) \quad (1)$$

The obtained DPPH• free radical scavenging activity was converted to the antioxidant activity in terms of mmol of Trolox equivalent (TE)/g of FW of sample.

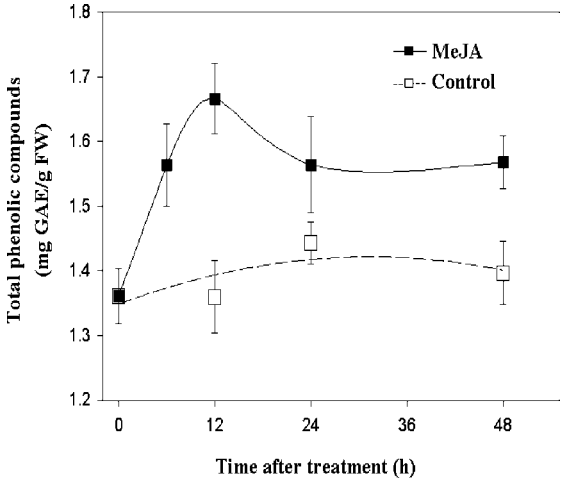

Figure 1. Time course of the total amount of phenolic compounds in the radish sprout treated with 1 mM MeJA. The amount of total phenolic compounds extracted by 80% methanol from radish sprout was spectrophotometrically determined at 735 nm. Gallic acid was used as a standard compound. Error bars in the figure were standard deviations of triplicate experiments.

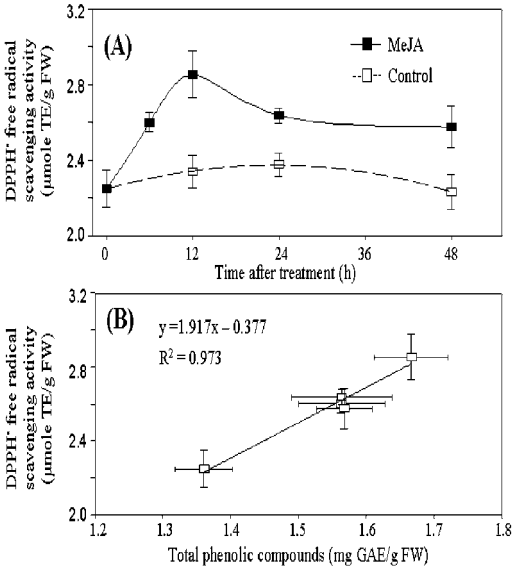

Figure 2. Time course of DPPH• free radical scavenging activity of the radish sprout treated with 1 mM MeJA (**A**) and its linear correlation (**B**). DPPH• free radical scavenging activity of the radish sprout extract was spectrophotometrically measured at 517 nm. Error bars in the figure were standard deviations of triplicate experiments.

Determination of Enzymatic Activities of PAL and Myrosinase. To determine the enzymatic activities of PAL and myrosinase, the crude enzymes were extracted from the radish sprout by 0.1 M sodium-borate buffer (pH 8.8) containing 15 mM β-mercaptoethanol. The extracts were saturated with ammonium sulfate at 80%, and the precipitates of the extracts were dissolved in 0.1 M sodium-borate buffer (pH 8.8) for PAL and in 0.1 M sodium-phosphate buffer (pH 6.5) for myrosinase. After centrifugation at 15 000g for 20 min, the supernatants were used as the crude enzymes. The amounts of proteins in the crude enzymes were measured by the Bradford assay (27) using bovine serum albumin as a standard.

PAL activity. Two-hundred microliters of the crude enzyme was added into 1 mL of 0.1 M sodium-borate buffer (pH 8.8) containing 10 mM L-phenylalanine. The reaction was incubated at 37 °C for 7 h, and then cinnamic acid, a product of the enzymatic reaction, was

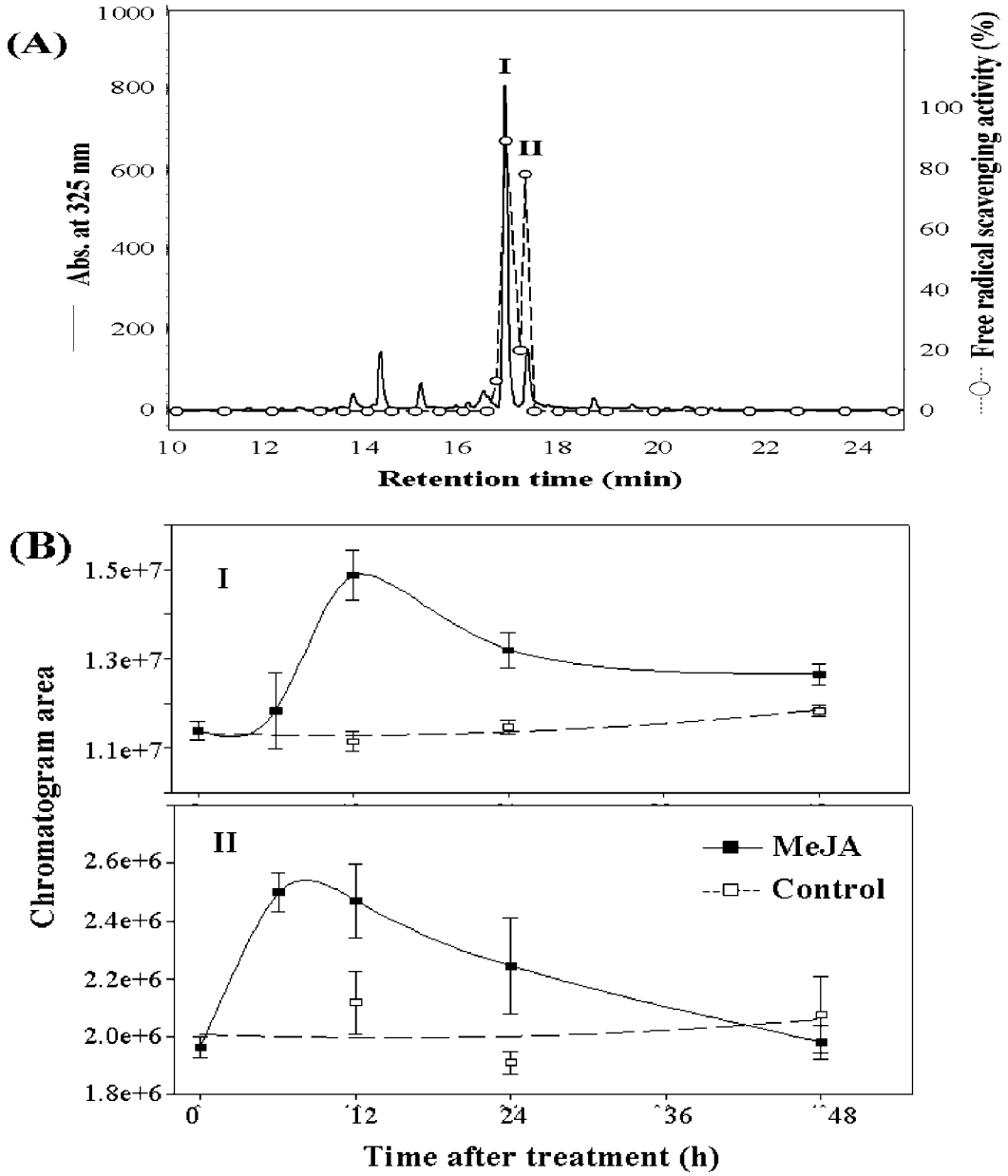

Figure 3. HPLC profile of the phenolic compounds of radish sprout and the DPPH• free radical scavenging activity of the collected fractions (**A**). Effect of MeJA on antioxidant phenolic compounds in radish sprout (**B**). Fifty microliters of the methanol extract was injected into the YMC ODS-AQ C_{18} column (250 mm × 4.6 mm, 5 μm) and eluted with HPLC water containing 0.05% TFA and acetonitrile at a flow rate of 1 mL/min. The absorbance of the eluant was scanned from 200 to 500 nm by PDA. Error bars in the figure were standard deviations of triplicate experiments.

extracted by toluene. The amount of cinnamic acid dissolved in toluene was measured spectrophotometrically at 290 nm (*28*). The PAL activity was expressed in ñkat. One katal was defined as the enzyme activity producing 1 mol of cinnamic acid equiv per second, and the specific activity was expressed as ñkat per milligram of protein.

Myrosinase Activity. Myrosinase activity in radish sprouts was determined according to the method of Pessina et al. (*29*) with slight modifications. The reaction mixture containing 950 μL of 33.3 mM sodium-phosphate buffer (pH 6.5) and 0.2 mM substrate sinigrin was preincubated at 37 °C for 5 min. The reaction was initiated by adding 50 μL of the crude enzyme and was incubated at 37 °C for 10 min. After incubation, the reaction mixture was diluted with methanol, and the concentration of the remaining sinigrin was determined spectrophotometrically at 227 nm. The sinigrin standard curve was preprepared. One myrosinase unit corresponded to 1 nM sinigrin transformed per minute. The specific activity is expressed as units per milligram of protein.

Experimental Design and Data Analysis. To investigate the effect of MeJA on radish sprouts, radish sprouts were cultivated at three randomly selected places in the greenhouse for adopting a randomized complete block design. Two plastic pots containing radish sprouts were in each place and for each harvest time. All experiments were in triplicate. All data were subjected to analysis of variance (SAS 9.1, SAS Institute Inc., Cary, NC) and also analyzed with nonlinear regressions. The least significant difference was used to find the difference among all sample means at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of MeJA on TPC and DPPH• Activity of the Radish Sprout Extract. Within 48 h after the 1 mM MeJA treatment, the TPC of the methanolic extract of radish sprouts significantly increased. Its highest amount was recorded at 12 h, which was 41% higher than that of the control at the same harvesting time. The phenomenon that the TPC of the radish sprout decreased after 12 h might be due to the consumption of some phenolic compounds for the biosynthesis of lignin involved in the plant

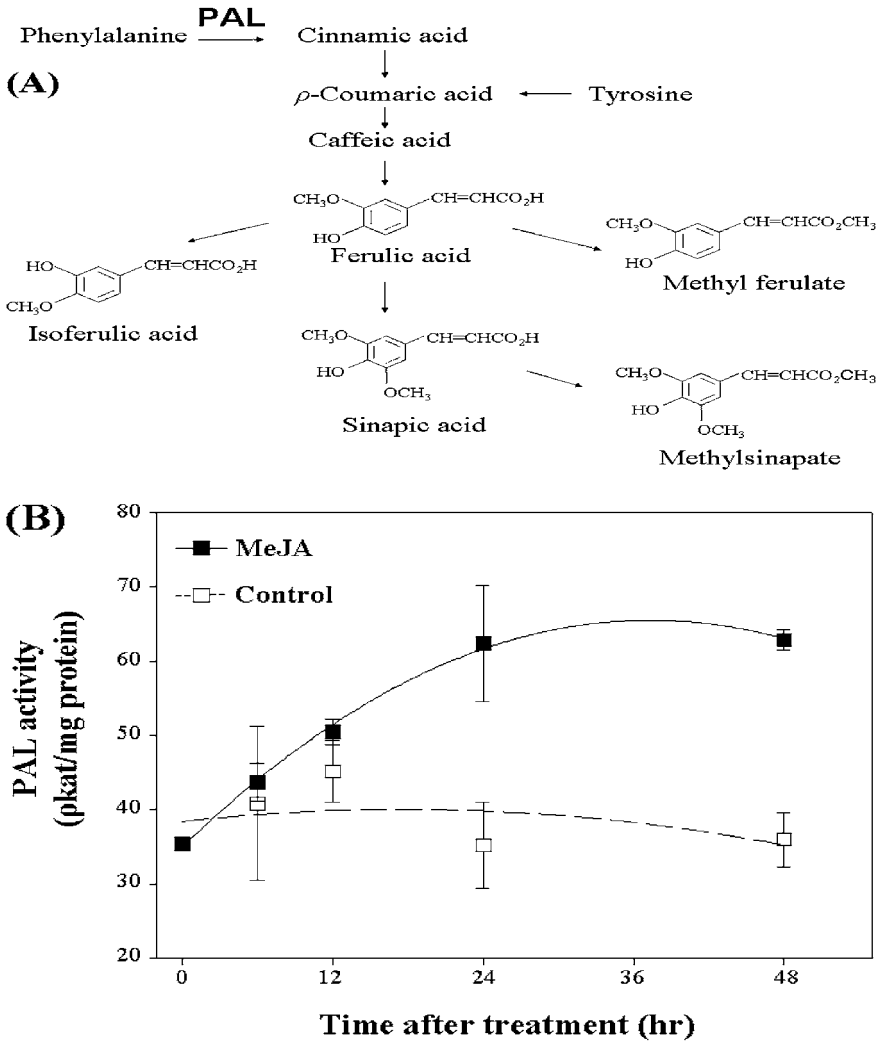

Figure 4. Phenylpropanoid pathway regulated by PAL and the structures of phenolic intermediates induced in the radish sprout by MeJA (**A**). Time course of PAL activity of the radish sprout treated with MeJA (**B**). The amount of cinnamic acid produced from phenylalanine by PAL was measured at 290 nm by a spectrophotometer, and the PAL activity was expressed in ρkat. Error bars in the figure were standard deviations of triplicate experiments.

defense systems. However, the TPCs of the radish spout after the treatment were all higher than those of the control. In other words, there was no significant difference in the TPC between the control (0.25% ethanol) and the sample before treatment (0 h) (**Figure 1**). This result was in agreement with the previous paper (*21*) that phenolic compounds could be significantly induced in some fruits after the MeJA treatment, although we used higher concentrations (1 mM) of MeJA on radish sprout in this research, while the other research groups used lower concentrations (0.01 and 0.1 mM) of MeJA on red and black raspberries. Nevertheless, it was confirmed that the phenolic compounds in radish sprouts could also be induced by the MeJA.

Following the evaluation of the antioxidant capacity of the radish sprout extract revealed that the treated samples had significantly higher DPPH• free radical scavenging activity than that of the control (**Figure 2A**). Its antioxidant activity (260−290 μmol of TE/100 g of FW) was quite comparable to those of peas (300 μmol of TE/100 g of FW) and red potato (350 μmol of TE/100 g of FW) (*30*).

The relationship between the phenolic compounds and the antioxidant activity was further investigated. A high correlation between the total amount of phenolic compounds and the DPPH• free radical scavenging activity of the radish sprout extract was quantitatively established in an equation of $y = 1.917x - 0.377$ ($R^2 = 0.973$) (**Figure 2B**). This result indicated that the increased free radical scavenging activity might be due to the increased phenolic compounds induced by MeJA treatment. A similar correlation was also found between the increased total phenolic content and the increased antioxidant capacity of potatoes by wounding stress (*31*). To further clarify which antioxidant phenolic compound(s) in radish sprout has (have) been affected by the MeJA treatment, the phenolic compounds in the radish sprout extract were separated and analyzed quantitatively and qualitatively by the C_{18} HPLC.

Effect of MeJA on Antioxidant Phenolic Compounds in Radish Sprout. One major peak and more than six small peaks were separated from the methanolic extract of the radish sprout by C_{18} HPLC. The antioxidant activity of all collected HPLC fractions was also measured (**Figure 3A**). Among these peaks, two peaks labeled as I and II that accounted for 50 and 14% of the total area of peaks, respectively, were found with the antioxidant activity. Further studies revealed that these two peaks contained five phenolic compounds (i.e., ferulic acid, isoferulic acid, sinapic acid, methyl ferulate, and methylsinapate) that were identified by GC/MS after acidic hydrolysis and chemical derivatization. Also, it was obvious that peak II possessed a stronger antioxidant activity than peak I regarding the activity/area ratio. Also, chromatographic quantification confirmed that peaks I and II were also significantly affected by the MeJA treatment (**Figure 3B**). Both peaks, which have been shown in

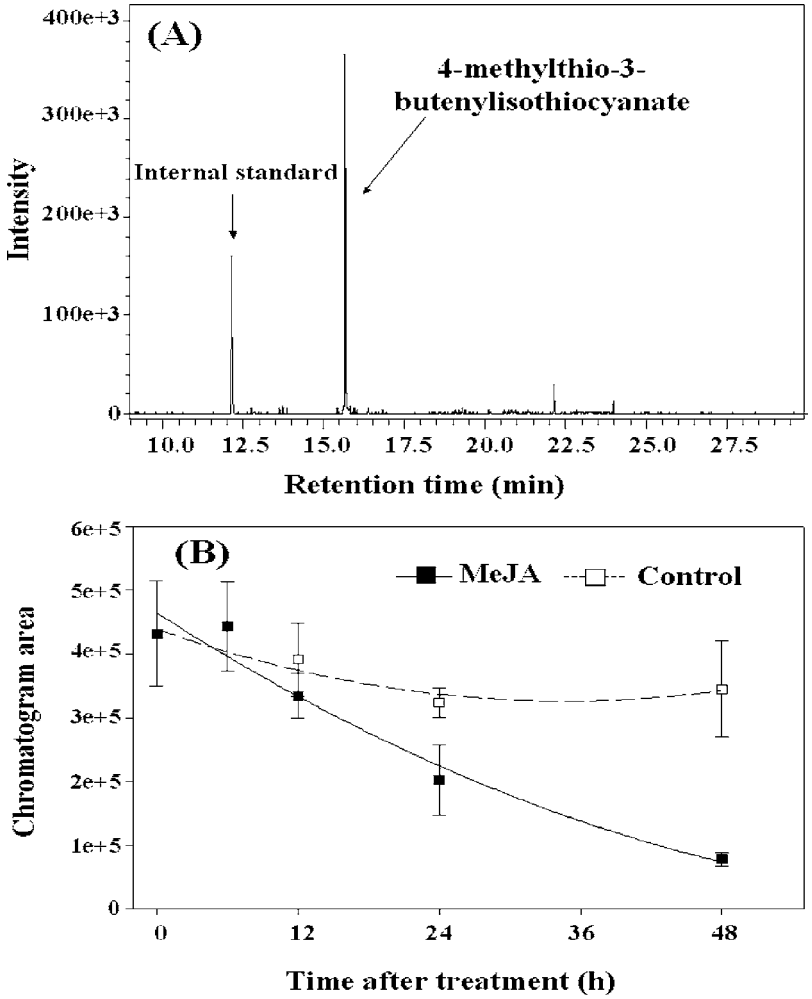

Figure 5. GC profile of isothiocyanates in radish sprout (**A**) and the effect of MeJA on the isothiocyanate content (**B**). Isothiocyanates were extracted by MTBE and determined by GC/MS equipped with a DB-5 capillary column. The GC oven temperature was programmed from 60 to 280 °C at a rate of 10 °C /min and held at 280 °C for 5 min. The injector and ion source temperatures were 220 and 290 °C, respectively. Error bars in the figure were standard deviations of triplicate experiments.

a time course change with very similar quantitative profiles to those of the total content of phenolics and antioxidant activity of the radish sprout extract with the MeJA treatment, approached their maximal amounts at 12 and 6 h, respectively, and then decreased gradually.

Determination of the PAL Activity. Many phenolic compounds in plants are produced through the phenylpropanoid pathway, which is initiated by the PAL (EC 4.3.1.5). The identified five free phenolics (i.e., ferulic acid, isoferulic acid, sinapic acid, methyl ferulate, and methylsinapate) in radish sprout can also be produced through this pathway (**Figure 4A**) (*32*). Therefore, there was a necessity to investigate the effect of MeJA on PAL and the relationship between the induction of phenolic compounds and the activity of PAL. As shown in **Figure 4B**, the PAL activity of the radish sprout significantly increased within 48 h after the MeJA treatment, while there was no significant change of the PAL activity of the control (about 36 ρkat/mg of protein). After the MeJA treatment, the highest PAL activity was achieved at 24 h, and its maximal level was kept at 62 ρkat/mg of protein for at least another 24 h. This induced activity was 72% higher than that of the control. This observation agreed with the previous report that MeJA at low concentrations could induce phenolic compounds and the PAL activity in *Coleus blumei* and tobacco cells (*33*, *34*). However, the appearance of the maximal value of the PAL activity lagged behind that of the TPC value after the MeJA treatment. This discrepancy indicated that the earlier increased TPC after the MeJA treatment might be attributed to the induced activities of other enzymes, instead of the PAL, that are related to the phenylpropanoid pathway.

Effect of MeJA on Isothiocyanates and Myrosinase in Radish Sprout. Isothiocyanates are produced from glucosinolates by myrosinase (EC 3.2.3.1) when the cruciferous vegetables are wounded. Isothiocyanates have been linked with several biological activities such as antimicrobial, antimutagenic, and anticarcinogenic activities (*35*−*38*). In this study, a major isothiocyanate (i.e., 4-(methylthio)butylisothiocyanate (MTBITC) known as an antimicrobial and antimutagenic agent (*24*)) was successfully extracted from the treated radish sprout by MTBE and identified by GC/MS (**Figure 5A**). Unlike the previous report that wounding could rapidly induce the content of MTBITC in radish (*23*, *24*), MTBITC significantly decreased in our study after the MeJA treatment. After 48 h of treatment, MTBITC in the radish sprout approached its lowest amount that was 4-fold less than that of the control (**Figure 5B**). Also, the myrosinase activity significantly decreased after the MeJA treatment (**Figure 6**). The enzyme's initial activity was determined at 69 U/mg of protein, but it decreased to 22 U/mg of protein within 12 h and was maintained without significant change up to 48 h, while there was no significant change of the

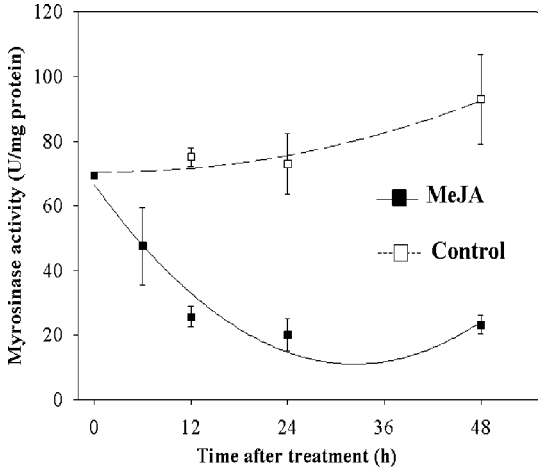

Figure 6. Time course of myrosinase activity of radish sprout treated with MeJA. One myrosinase unit corresponded to 1 nM sinigrin transformed per minute. The specific activity is expressed as units per milligram of protein. Error bars in the figure were standard deviations of triplicate experiments.

enzyme activity in the control plants. Even though it was previously reported that isothiocyantes could be accumulated by wounding and phototropic stimulation in radish (*23*, *24*) and MeJA could induced myrosinase in hairy root cultures of *Tropaeolum majus* (*39*), our study, however, observed a quantitative decrease of MTBITC and deceased enzyme activity of myrosinase in the radish sprouts after the MeJA treatment. Such a discrepancy in tests may need more investigation.

Conclusion. To increase the nutritional value of radish sprouts, exogenous MeJA, which is well-known as a plant hormone and one key component in wounding/stress signal transduction, was sprayed on the 7 day old radish sprout (*R. sativus* L.). After the treatment, it was found that MeJA could significantly increase the total amount of phenolic compounds and resulted in a higher DPPH• free radical scavenging activity. In addition, the activity of PAL, a key regulatory enzyme responsible for the phenylpropanoid metabolism associated with the production of phenolic compounds, also increased in the radish sprout after the MeJA treatment. However, our investigation also observed a decreased myrosinase activity and a parallel trend of decreased isothiocyanate content. Even though more detailed research is needed to explore the complex relationship between the MeJA and the secondary metabolites in radish sprout, this study has demonstrated to a certain extent that MeJA can significantly affect the nutritional value (antioxidant capacity) of radish sprout and may be one useful tool for the induction of health-benefiting chemicals in our plant diet.

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LITERATURE CITED

(1) Kessler, A.; Baldwin, I. T. Plant responses to insect herbivory: the emerging molecular analysis. *Annu. Rev. Plant Biol.* **2002**, *53*, 299−328.

(2) de Bruxelles, G. L.; Roberts, M. R. Signals regulating multiple responses to wounding and herbivores. *Crit. Rev. Plant Sci*. **2001**, *20*, 487−521.

(3) Frohnmeyer, H.; Staiger, D. Ultraviolet B radiation-mediated responses in plants. Balancing damage and protection. *Plant Physiol*. **2003**, *133*, 1420−1428.

(4) Yeoman, M. M.; Yeoman, C. L. Manipulating secondary metabolism in cultured plant cells. *New Phytol*. **1996**, *134*, 553−569.

(5) Bennett, R. N.; Wallsgrove, R. M. Secondary metabolites in plant defense mechanisms. *New Phytol.* **1994**, *127*, 617−633.

(6) Farmer, E. E.; Ryan, C. A. Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 7713−7716.

(7) Radman, R.; Saez, T.; Bucke, C.; Keshavarz, T. Elicitation of plants and microbial cell systems. *Biotechnol. Appl. Biochem*. **2003**, *37*, 91−102.

(8) Kris-Etherton, P. M.; Hecker, K. D.; Bonanome, A.; Coval, S. M.; Binkoski, A. E.; Hilpert, K. F.; Griel, A. E.; Etherton, T. D. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am. J. Med*. **2002**, *113* (Suppl. 2), 71−88.

(9) Reddy, L.; Odhav, B.; Bhoola, K. D. Natural products for cancer prevention: a global perspective. *Pharmacol. Ther*. **2003**, *99*, 1−13.

(10) Oksman-Caldentey, K.-M.; Inzé, D. Plant cell factories in the post-genomic era: new ways to produce designer secondary metabolites. *Trends Plant Sci.* **2004**, *9*, 433−440.

(11) Lavola, A. Accumulation of flavonoids and related compounds in birch induced by UV B irradiance. *Tree Physiol.* **1998**, *18*, 53−58.

(12) Dörnenburg, H.; Knorr, D. Strategies for the improvement of secondary metabolite production in plant cell cultures. *Enzyme Microb. Technol*. **1995**, *17*, 674−684.

(13) Zhao, J.; Davis, L. C.; Verpoorte, R. Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnol. Adv.* **2005**, *23*, 283−333.

(14) Kim, H.-J.; Chen, F.; Wang, X.; Rajapakse, N. C. Effect of chitosan on the biological properties of sweet basil (*Ocimum basilicum* L.) *J. Agric. Food Chem*. **2005**, *53*, 3696−3701.

(15) Loivamäki, M.; Holopainen, J. K.; Nerg, A.-M. Chemical changes induced by methyl jasmonate in oilseed rape grown in the laboratory and in the field. *J. Agric. Food Chem*. **2004**, *52*, 7607−7613.

(16) Creelman, R.; Mullet, J. E. Biosynthesis and action of jasmonates in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1997**, *48*, 355−381.

(17) Liechti, R.; Farmer, E. E. The jasmonate pathway. *Science* **2002**, *296*, 1649−1650.

(18) Doughty, K.; Kiddle, G. A.; Pye, B. J.; Wallsgrove, R. M.; Pickett, J. A. Selective induction of glucosinolates in oilseed rape leaves by methyl jasmonate. *Phytochemistry* **1995**, *38*, 347−350.

(19) Aerts, R. J.; Gisi, D.; De Carolis, E.; De Luca, V. Methyl jasmonate vapor increases the developmentally controlled synthesis of alkaloids in *Catharanthus* and *Cinchona* seedlings. *Plant J.* **1994**, *5*, 635−643.

(20) Singh, G.; Gavrieli, J.; Oakey, J. S.; Curtis, W. R. Interaction of methyl jasmonate, wounding, and fungal elicitation during sesquiterpene induction in *Hyoscyamus muticus* in root cultures. *Plant Cell Rep.* **1998**, *17*, 391−395.

(21) Wang, S. Y.; Zheng, W. Preharvest application of methyl jasmonate increases fruit quality and antioxidant capacity in raspberries. *Int. J. Food Sci. Technol*. **2005**, *40*, 187−195.

(22) Li, X.; Kushad, M. M. Correlation of glucosinolate content to myrosinase activity in horseradish (*Armoracia rusticana*). *J. Agric. Food Chem.* **2004**, *52*, 6950−6955.

(23) Hasegawa, T.; Yamada, K.; Kosemura, S.; Yamamura, S.; Hasegawa, K. Phototropic stimulation induces the conversion of glucosinolate to phototropism-regulating substances of radish hypocotyls. *Phytochemistry* **2000**, *54*, 275−279.

(24) Nakamura, Y.; Iwahashi, T.; Tanaka, A.; Koutani, J.; Matsuo, T.; Okamoto, S.; Kenji Sato, K.; Ohtsuki, K. 4-(Methylthio)-3-butenyl isothiocyanate, a principal antimutagen in daikon (*Raphanus sativus*; Japanese white radish). *J. Agric. Food Chem.* **2001**, *49*, 5755−5760.
(25) Singleton, V. L.; Rossi, I. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult.* **1965**, *16*, 144−158.
(26) Yamaguchi, T.; Takamura, H.; Matoba, T.; Terao, J. HPLC method for evaluation of the free radical scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 1201−1204.
(27) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein−dye binding. *Anal. Biochem.* **1976**, *72*, 248−255.
(28) Hao, Z.; Charles, D. J.; Yu, L.; Simon, J. E. Purification and characterization of a phenylalanine ammonia−lyase from *Ocimum basilicum*. *Phytochemistry* **1996**, *43*, 735−739.
(29) Pessina, A.; Thomas, R. M.; Palmieri, S.; Luisi, R. An improved method for the purification of myrosinase and its physiochemical characterization. *Arch. Biochem. Biophys.* **1990**, *280*, 383−389.
(30) Miller, H. E.; Rigelhof, F.; Marquart, L.; Prakash, A.; Kanter, M. Antioxidant content of whole grain breakfast cereals, fruits, and vegetables. *J. Am. Coll. Nutr.* **2002**, *19*, 312S−319S.
(31) Reyes, L. F.; Cisneros-Zevallos, L. Wounding stress increases the phenolic content and antioxidant capacity of purple-flesh potatoes (*Solanum tuberosum* L.). *J. Agric. Food Chem.* **2003**, *51*, 5296−5300.
(32) Dixon, R. A.; Paiva, N. L. Stress-induced phenylpropanoid metabolism. *Plant Cell* **1995**, *7*, 1085−1097.
(33) Szabo, E.; Thelen, A.; Petersen, M. Fungal elicitor preparations and methyl jasmonate enhance rosmarinic acid accumulation in suspension cultures of *Coleus blumei*. *Plant Cell Rep.* **1999**, *18*, 485−489.
(34) Sharan, M.; Taguchi, G.; Gonda, K.; Jouke, T.; Shimosaka, M.; Hayashida, N.; Okazaki, M. Effects of methyl jasmonate and elicitor on the activation of phenylalanine ammonia−lyase and the accumulation of scopoletin and scopolin in tobacco cell cultures. *Plant Sci.* **1998**, *132*, 13−19.
(35) Hashem, F. A.; Saleh, M. M. Antimicrobial components of some *Cruciferae* plants. *Phytother. Res.* **1999**, *13*, 329−332.
(36) Hamilton, S. M.; Teel, R. W. Effects of isothiocyanates on cytochrome P-450 1A1 and 1A2 activity and on the mutagenicity of heterocyclic amines. *Anticancer Res.* **1996**, *16*, 3597−3602.
(37) Hecht, S. S. Chemoprevention of cancer by isothiocyanates, modifiers of carcinogen metabolism. *J. Nutr.* **1999**, *129*, 768S−774S.
(38) Fahey, J. W.; Zalcmann, A. T.; Talalay, P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* **2001**, *56*, 5−51.
(39) Wielanek, M.; Urbanek, H. Glucotropaeolin and myrosinase production in hairy root cultures of *Tropaeolum majus*. *Plant Cell, Tissue Organ Cult.* **1999**, *57*, 39−45.

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