JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Metabolomic Analysis of Phenolic Compounds in Buckwheat (*Fagopyrum esculentum* M.) Sprouts Treated with Methyl Jasmonate

Hyun-Jin Kim, Kee-Jai Park, and Jeong-Ho Lim*

Korea Food Research Institute, Seongnam, Gyeonggi 463-746, Republic of Korea

ABSTRACT: The effects of exogenous methyl jasmonate (MeJA) on phytochemical production in buckwheat sprouts cultivated under dark conditions (0, 1, 3, 5, and 7 d) were investigated by metabolomic analysis, using ultra performance liquid chromatography-quadrupole-time-of-flight (UPLC-Q-TOF) mass spectroscopy (MS) and partial least-squares-discriminant analysis (PLS-DA). MeJA-treated and control groups showed no differences in growth but were clearly discriminated from each other on PLS-DA score plots. The metabolites contributing to the discrimination were assigned as chlorogenic acid, catechin, isoorientin, orientin, rutin, vitexin, and quercitrin, which have various health effects. Moreover, isoorientin, orientin, rutin, and vitexin were assigned as the main phytochemicals of sprouts cultivated under dark conditions. The accumulation of these metabolites caused the phenolic compound content and antioxidant activity of the sprouts to increase. Further, this study revealed that their accumulation resulted from the stimulation of the phenylpropanoid pathway by MeJA treatment. Therefore, these metabolites may be useful for better understanding the effects of MeJA on buckwheat sprout phytochemicals and contribute to improving the functional quality of the sprouts.

KEYWORDS: Buckwheat sprout, *Fagopyrum esculentum*, methyl jasmonate, metabolomics, UPLC-Q-TOF, rutin, PAL, antioxidant

INTRODUCTION

With increasing clinical evidence suggesting that plant-derived foods have various potential health benefits, their consumption has been continuously growing at a rate of 5−10% per year.[1] Moreover, many health organizations worldwide recommend an increase in the intake of plant-derived foods to improve health status and to prevent chronic diseases.[2] The health benefits are, at least partially, associated with phytochemicals, including phenolic compounds, terpenoids, and alkanoids.[2,3] Hence, phytochemicals have become one of the major research topics in the field of functional food. In particular, recent research has focused on developing methods for increasing the content of useful phytochemicals in edible plants without gene modification or breeding. The simplest and most effective method is to employ plant stress response systems.

Under biotic and abiotic stress, plant physiology dramatically changes. The induction of defense systems, such as those involving proteinase inhibitors, produces a response that protects the plant from the stresses. As a part of this response, secondary metabolites, mainly phytochemicals with various health benefits, are accumulated.[4,5] However, in the absence of stress, healthy plants can also be induced by stress inducers to artificially produce secondary metabolites.

Among the various stress inducers, the plant hormones jasmonate and its methyl ester (methyl jasmonate, MeJA) play important roles in plant growth regulation and in endogenous and/or exogenous stress signaling. Both are accumulated through the octadecanoid pathway, which is stimulated by biotic and abiotic stresses and has been shown to be involved in a major wounding signal transduction pathway.[6] MeJA is used as an endogenous stress signal like jasmonate. In addition, when plants are attacked by insect herbivores, it is emitted with various organic, volatile compounds into the air and thereby transfers a stress signal to healthy, neighboring plants possessing MeJA receptors.[7] Although not subjected to any stress, the healthy plants respond to the exogenous MeJA and induce a self-defense system. Because of this particular role, exogenous MeJA is applied to stimulate the production of phytochemicals in various plants such as sweet basil,[8] raspberries,[9] and radish sprouts.[10]

Despite health concerns about foodborne illnesses from raw sprouts,[11] as well as antinutritional factors, raw seed sprouts such as those from 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. In particular, a number of these phytochemicals are associated with many health benefit effects.[12,13] In recent years, buckwheat sprouts have been under the spotlight in the international market because they contain high levels of phytochemicals, including rutin and proteins, which have a number of interesting pharmacological effects.[14,15] Recently, in a study of the effects of MeJA treatment on phytochemical synthesis, MeJA was shown to inhibit anthocyanin synthesis in buckwheat sprouts cultivated under light conditions.[16] However, the effects of MeJA treatment on the profiles of rutin and related compounds in sprouts cultivated under dark conditions have not been studied, despite the fact that the market for dark-grown sprouts is also economically important.

Therefore, in this study, we performed a metabolomic analysis by using ultra performance liquid chromatography-quadrupole-time-of-flight (UPLC-Q-TOF) mass spectroscopy (MS) to investigate the relationship between MeJA treatment and the phytochemical profiles of buckwheat sprouts grown in darkness. The data were applied to multivariate statistical analysis to find metabolites affected

Received: January 28, 2011
Accepted: March 18, 2011
Revised: March 16, 2011
Published: March 18, 2011

ACS Publications © 2011 American Chemical Society

by MeJA treatment. Additionally, the effect of MeJA treatment on the antioxidant activity of the sprouts was investigated.

MATERIALS AND METHODS

Buckwheat Sprout Cultivation and MeJA treatment. Buckwheat (*Fagopyrum esculentum* Moench) seeds (40 g) were washed and soaked in distilled water at 25 °C for 4 h, and then the seeds were placed in a tray (32 × 6 × 2.8 cm) with cheese cloth. Four separated trays were placed in a commercial sprout cultivator (MikroFarm; EasyGreen) with an autospraying system. The sprouts were cultivated in the dark at 18 °C (2 °C) for 7 d, with water automatically sprayed for 30 min every 12 h. For the application of MeJA to the sprouts, 0.1 mM MeJA (100 mL) dissolved in 0.25% ethanol was sprayed each day on the sprouts. As a control, 0.25% ethanol was sprayed on a different set of sprouts. No difference between samples treated with ethanol and water was observed. The sprouts were harvested at different cultivation times (0, 1, 3, 5, and 7 d). Samples on day 0 were the sprouts before MeJA treatment. Some of the harvested sprouts were immediately lyophilized for metabolomic analysis and antioxidant measurements, while others were stored at −70 °C for enzymatic assays.

Extraction of Phenolic Compounds from Buckwheat Sprouts. A ground buckwheat sprout sample (0.1 g) was mixed with 2 mL of 80% methanol, and the mixture was shaken at room temperature for 12 h. After centrifugation at 12 000 rpm for 10 min, the supernatant solution was used for metabolomic analysis and for the determination of phenolic compound levels and antioxidant activity.

Determination of Total Content of Phenolic Compounds. The total amount of phenolic compounds in the buckwheat sprouts was determined using Folin−Ciocalteu's reagent according to the method of Singleton and Rossi.[17] The methanolic extract (50 μL) was mixed with 450 μL of distilled water and 250 μL of 2 N Folin−Ciocalteu's reagent. The mixture was then added to 1.25 mL of 20% Na_2CO_3. The resultant solution was incubated at 25 °C for 20 min and then centrifuged at 5000 rpm for 10 min. The absorbance of the supernatant solution was measured at 735 nm. A standard curve was prepared using gallic acid (GA), and the absorbance was converted to phenolic content in terms of milligrams of GA equivalent (GAE) per gram of dry weight (DW).

Determination of Antioxidant Activity. The antioxidant activity of buckwheat sprouts was determined using the ferric reducing antioxidant power (FRAP) assay.[18] In this assay, the Fe(III)/tripyridyltriazine complex is reduced to the blue ferrous form (Fe(II)/tripyridyltriazine) by reductants (antioxidants) in the sample, with the color change monitored at 593 nm. For the assay, a methanol extract diluted with distilled water was mixed with 3.6 mL of FRAP reagent containing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine, and 20 mM $FeCl_3 \cdot 6H_2O$. After the reaction mixture was incubated at 37 °C for 30 min, its absorbance was measured at 593 nm. A standard curve was prepared using $FeSO_4 \cdot 7H_2O$, and the reducing power was reported as millimoles of Fe^{2+} per gram of dried sample.

Determination of Phenylalanine Ammonia Lyase (PAL) Activity. To determine the activity of PAL, a key regulatory enzyme in secondary metabolite production in plants, the crude enzyme was extracted from the sprouts by using 0.1 N sodium borate buffer (pH 8.8). After centrifugation at 12 000 rpm for 10 min, the supernatant solution was used as a source of the crude enzymes. The crude enzyme (0.5 mL) was mixed with 1 mL of 10 mM L-phenylalanine dissolved in 0.1 N sodium borate buffer (pH 8.8). The reactant was incubated at 37 °C for 6 h, and the cinnamic acid produced by the reaction was extracted using toluene. The amount of cinnamic acid recovered by toluene was measured at an absorbance of 290 nm with a spectrophotometer,[19] and the PAL activity was expressed in picokatals per milligram of protein. One katal was defined as the enzyme activity producing 1 mol of cinnamic acid equivalents per second. The amount of protein in the crude enzyme was determined by using the Bradford assay,[20] with bovine serum albumin as a standard.

UPLC-Q-TOF MS Analysis of Buckwheat Sprout Methanol Extract. A UPLC system (Waters, Milford, MA) equipped with a binary solvent delivery system and a photodiode array (PDA) detector was used to analyze methanol extracts of buckwheat sprout. The extract was injected into an Acquity UPLC BEH C_{18} column (2.1 × 50 mm, 1.7 μm; Waters) equipped to the UPLC system and equilibrated with water containing 0.1% trifluoroacetic acid (TFA). The sample was eluted in gradient mode with acetonitrile containing 0.1% TFA for 5 min at a flow rate of 0.3 mL/min, and the eluents separated by C_{18}-UPLC were continuously analyzed by two detectors, a PDA detector and a Q-TOF mass spectrometer (Waters). The Q-TOF was operated in ESI-negative mode. The capillary and sampling cone voltages were set at 3 kV and 45 V, respectively. The desolvation flow was set to 700 L/h at a temperature of 300 °C; the source temperature was set to 110 °C. The TOF MS data were collected in the range of m/z 50−1000, with a scan time of 0.2 s and an interscan delay time of 0.02 s. The MS/MS spectra of metabolites were obtained by a collision energy ramp from 10−30 eV. The accurate mass and composition for the precursor ions and the fragment ions were calculated and sequenced by a MassLynx (Waters) incorporated in the instrument. All information on MS data, including retention time, m/z, and ion intensity, was extracted by the MarkerLynx (Waters), and the resulting MS data were assembled into a data matrix. Assignment of metabolites contributing to the observed variance was performed by an elemental composition analysis software program, with calculated mass, mass tolerance (mDa and ppm), double bond equivalent (DBE), and i-Fit algorithm (the likelihood that an isotopic pattern of the elemental composition matches a cluster of peaks in the spectrum) implemented in the MassLynx and by the ChemSpider database (www.chemspider.com). Authentic standards were used to confirm the assignments and to quantitatively analyze the samples.

Data Processing. LC/MS data were aligned and normalized by using MarkerLynx (Waters). Peaks were collected using a peak width at 5% height of 1 s, a noise elimination of 6, and an intensity threshold of 50. Data were aligned with a mass tolerance of 0.04 Da and a retention time window of 0.15 min.

Statistical Analysis. The mean-centered LC/MS data sets were analyzed by multivariate statistical analysis using SIMCA-P^+ version 12.0.1 (Umetrics, Umeå, Sweden). Partial least-squares discriminant analysis (PLS-DA) and orthogonal partial least-squares discriminant analysis (OPLS-DA) were used to visualize discrimination between the MeJA treated-sprouts and the control sprouts. Hotelling's $T2$ test was used to statistically analyze the difference between the groups. The outlying samples of the ellipse region, defined as the 95% confidence interval of the modeled variation, were excluded from further analysis. The quality of PLS-DA and OPLS-DA models was assessed by three parameters: R^2X, R^2Y, and Q^2Y. The goodness of fit was quantified by R^2X and R^2Y, and the predictive ability was indicated by Q^2Y. For validating models, a 7-fold validation was applied to the PLS-DA and OPLS-DA models, and the reliabilities of the models were further rigorously validated by a permutation test (n = 200).

For finding metabolites contributing to the discrimination between the MeJA treatment group and the control, differences in the metabolite intensities were tested by the independent t-test with the Mann−Whitney U-test, and the S-plot showing the combination of covariance $p(1)$ and correlation $p(\mathrm{corr})$ from the OPLS-DA model was used to better visualize the metabolites contributing to the discrimination.

In addition, statistical analysis of total phenolic content and PAL activity was performed by the independent t-test with the Mann−Whitney U-test.

RESULTS

Metabolomic Analysis of Methanol Extracts of Buckwheat Sprouts Treated with MeJA. Methanol extracts of buckwheat sprouts cultivated for different lengths of time (1, 3, 5, and 7 d) were analyzed by UPLC-Q-TOF MS. Four main peaks were

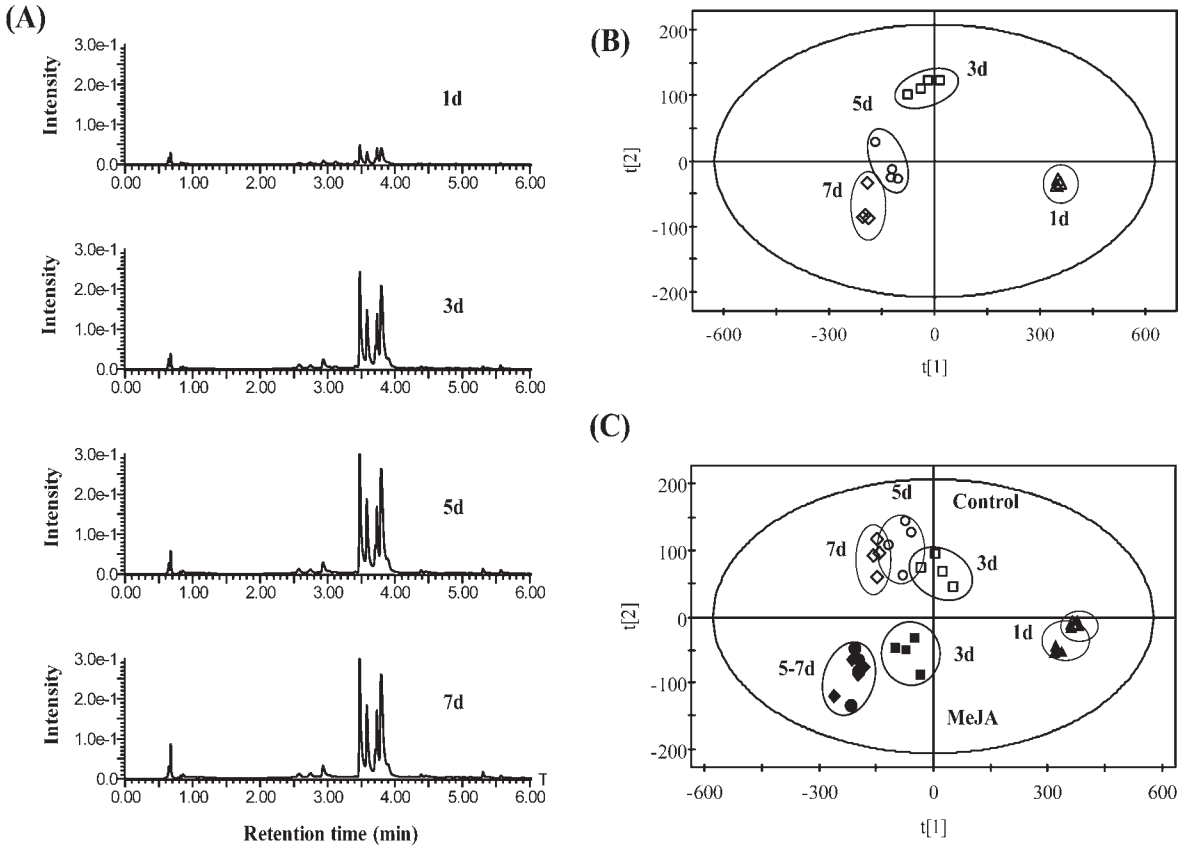

Figure 1. UPLC-Q-TOF-MS base peak intensity (BPI) profiles of methanol-soluble metabolites of buckwheat sprouts cultivated under dark conditions for 1, 3, 5, and 7 d (A), PLS-DA score plots of control sprouts according to cultivation time (B), and the score plots of control and MeJA-treated groups (C). The sprout metabolites were analyzed using an Acquity UPLC BEH C_{18} column (2.1 × 50 mm, 1.7 μm)-UPLC-Q-TOF with a gradient elution of acetonitrile containing 0.1% TFA. The Q-TOF was operated in ESI positive mode. All C_{18}-ULPC analyses were replicated five times.

observed from the UPLC spectra for sprouts cultivated for >3 d (Figure 1A). The MS data analyzed by Q-TOF MS were subjected to a PLS-DA score plot to visualize the differences among samples (Figure 1B and C). One-day-cultivated sprouts were clearly separated from the sprouts cultivated for >3 d by the primary component $t(1)$, and the separation gaps between samples on the PLS-DA score plot gradually narrowed with increasing cultivation time (Figure 1B). In addition, control groups were clearly discriminated from the MeJA-treated groups by the secondary component $t(2)$ regardless of the cultivation times, except for day 1 (Figure 1C). However, no separation between MeJA 5- and 7-d sprouts was observed.

To further investigate the differences between the MeJA-treated and control groups at each harvest point, the discrimination between them was visualized on a PLS-DA score plot (Figure 2A−D) based on the model with R^2X (cum) and R^2Y (cum) values of 0.535−0.675 and 0.992−0.998, respectively, indicating the goodness of fit of the data, and with Q^2 (cum) values of 0.871−0.969, estimating the predictive ability of the model (Table 1). The PLS-DA models were validated by a permutation test. The R intercept values for all models were 0.885−0.906, and their Q intercept values were −0.062−0.288, indicating overfit of the model. In addition, the *P*-values obtained from 5-fold cross validation released that all groups fitted by different models were significantly different (Table 1). Although the reliability of the models was slightly decreased because of the model overfit, the PLS-DA score plots indicated that all control sprouts were clearly discriminated from the MeJA-treated sprouts by the primary component t(1) (Figure 2A−D). Moreover, the S-plots of $p(1)$ and $p(\mathrm{corr})(1)$ were generated using par scaling to identify the metabolites contributing to the discrimination (Figure 2E−H). The S-plots revealed that the metabolites at a far distance from the intersection of $p(1)$ and $p(\mathrm{corr})$ were the more relevant ions for explaining the discrimination between both groups. The levels of the metabolites with positive $p(\mathrm{corr})$ values were decreased by MeJA treatment, whereas those with negative values were increased. Inversely, in the model of 7-d samples (Figure 2H), the levels of the metabolites with positive $p(\mathrm{corr})$ values were increased by MeJA treatment, but those with negative values were decreased. Many of the number-marked metabolites in the S-plots were identified, and their fold changes were calculated (Table 2).

Qualitative and Quantitative Analysis of Buckwheat Sprout Metabolites. Although it was possible that some fragments of the mother peaks were recognized as new metabolites by MS, 420 metabolites were analyzed by UPLC-Q-TOF MS in this study. Of these metabolites, chlorogenic acid, catechin, isoorientin, orientin, rutin, vitexin, and quercitrin were the main metabolites affected by the MeJA treatment, and their quantitative changes were expressed by the fold changes versus control sprouts (Table 2). The levels of all major metabolites in the MeJA-treated sprouts increased 1.15−2.34-fold relative to those of the control sprouts during the 7 d, with the exceptions of catechin and vitexin for the 3- and 7-d cultivated

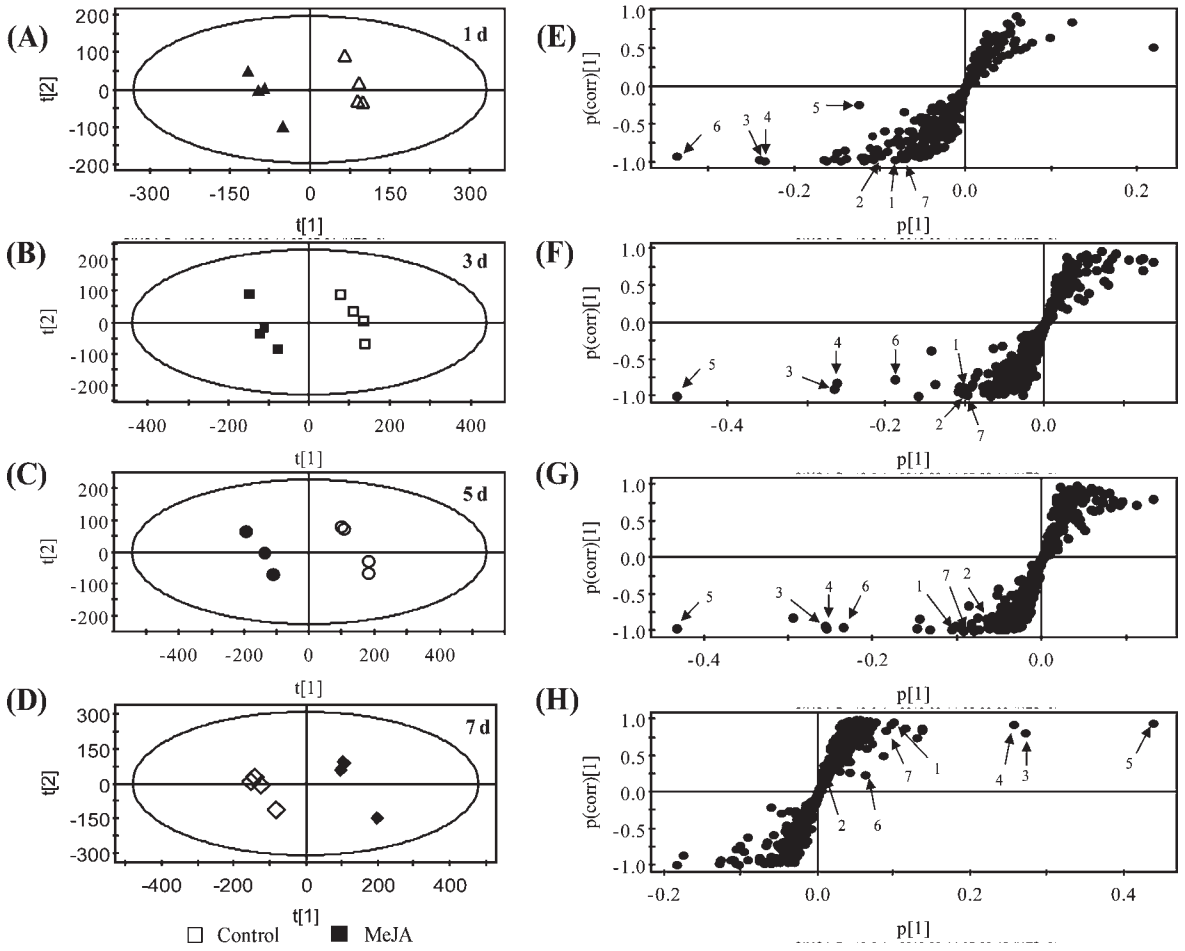

Figure 2. PLS-DA score plots of 1d (A), 3d (B), 5d (C), and 7d (D), and their S-plots (1d, E; 3d, F; 5d, G; 7d, H). The metabolite numbers correspond to those given in Table 2 (LC−MS). The PLS-DA score plots showed significant separation between the control and MeJA groups (A, $P < 0.045$; B, $P < 0.022$; C, $P < 0.015$; D, $P < 0.014$ by permutation test).

Table 1. Summary of Parameters Used for Assessing the Quality of PLS-DA Models

models	no.[a]	R^2X_{cum}[b]	R^2Y_{cum}[b]	Q^2Y_{cum}[b]	R intercept[c]	Q intercept[c]	P[d]
1d	2	0.535	0.993	0.871	0.906	−0.062	0.045
3d	2	0.568	0.992	0.925	0.952	0.288	0.022
5d	2	0.672	0.998	0.925	0.911	0.146	0.015
7d	2	0.675	0.995	0.969	0.885	0.104	0.014

[a] No. is the number of components. [b] R^2X_{cum} and R^2Y_{cum} are the cumulative modeled variation in the X and Y matrix, respectively, and Q^2Y_{cum} is the cumulative predicted variation in the Y matrix. [c] R and Q intercepts were obtained after the permutation test ($n = 200$). [d] P is the P-value obtained from cross-validation ANOVA of OPLS-DA.

sprouts. Although catechin and chlorogenic acid, occurring in low amounts in the sprouts, were remarkably affected by MeJA treatment, their VIP (variable importance in the projection) values were less than 1.00. However, of the seven identified metabolites, isoorientin, orientin, rutin, and vitexin had VIP values greater than 1.00, indicating high relevance to the difference between the sample groups. These results indicate that these four metabolites were the major metabolites contributing to the discrimination between the control and MeJA-treated sprouts on the PLS-DA score plot. The fold-change data and the UPLC profile (Figure 3A) showed that rutin was the most affected by MeJA treatment, with its content increased 1.68 times at 7 d.

Total Phenolic Content and Antioxidant Activity of Buckwheat Sprouts. The total amount of phenolic compounds rapidly increased after 1 d of cultivation, but a further increase was not observed in the control sprouts after 3 d (Figure 3C). However, the amount of phenolic compounds for the MeJA-treated sprouts continuously increased until 7 d. At 7 d, the amount (217 mg GAE/g of dried sample) of phenolic compounds was 60% higher than that of the control (136 mg GAE/g).

The pattern of antioxidant activity closely reflected the changes in total phenolic content (Figure 3D). After 3 d, a difference could be observed between the antioxidant activities of the control and MeJA-treated sprouts, with the biggest difference seen for the sprouts cultivated for 7 d. At this time point, the activity of the MeJA-treated sprouts (25 mM Fe^{2+}/g sample) was about twice that of the control.

DISCUSSION

In the present study, we investigated the effect of exogenous MeJA on both qualitative and quantitative changes in buckwheat sprout metabolites using UPLC-Q-TOF. At different cultivation time points (1, 3, 5, and 7 d), the metabolite profiles of MeJA-treated sprouts were compared to those of an untreated control

Table 2. Identification of Buckwheat Sprout Metabolites Analyzed by UPLC-Q-TOF MS and Their Fold-Change Analysis

no.[a]	identity	exact mass (M + H)	actual mass (M + H)	mass error (mDa)	MS fragments [ESI$^+$]	fold change (vs control)[b]				VIP[c]			
						1d	3d	5d	7d	1d	3d	5d	7d
1	chlorogenic acid	353.0873	353.0877	−0.4	191	1.72*	1.58*	1.81*	1.50*	0.33	0.54	0.62	0.50
2	catechin	289.0712	289.0653	5.9	245, 203, 125	1.86*	2.34*	2.07*	1.04	0.73	1.30	0.58	0.30
3	isoorientin	447.0927	447.0977	−5.0	357, 327, 297	1.51*	1.22*	1.25*	1.12*	2.88	3.44	1.33	5.12
4	orientin	447.0927	447.0977	−5.0	357, 327, 297	1.57*	1.27*	1.32*	1.24*	2.57	3.22	1.87	4.39
5	rutin	609.1456	609.1644	−18.8	300, 179	1.63*	1.47*	1.59*	1.68*	9.02	12.32	13.97	12.54
6	vitexin	431.0978	431.1027	−4.9	312, 311, 283	1.57*	1.08	1.15*	1.00	5.28	4.94	3.21	1.78
7	quercitrin	447.0927	447.0963	−3.6	285, 151	1.45*	1.44*	1.56*	1.21*	0.28	0.44	0.40	0.59

[a] No. was the number of metabolites marked in Figure 2. [b] Fold change was calculated by dividing the mean of normalized intensities of each metabolite from MeJA-treated sprouts by the mean intensity of the same metabolite from control sprouts. The asterisk (*) on the side of each fold-change value indicates a significant difference at $P < 0.05$. [c] VIP is the variable importance in the project, and its value of above 1.00 shows high relevance for explaining the differences of sample groups.

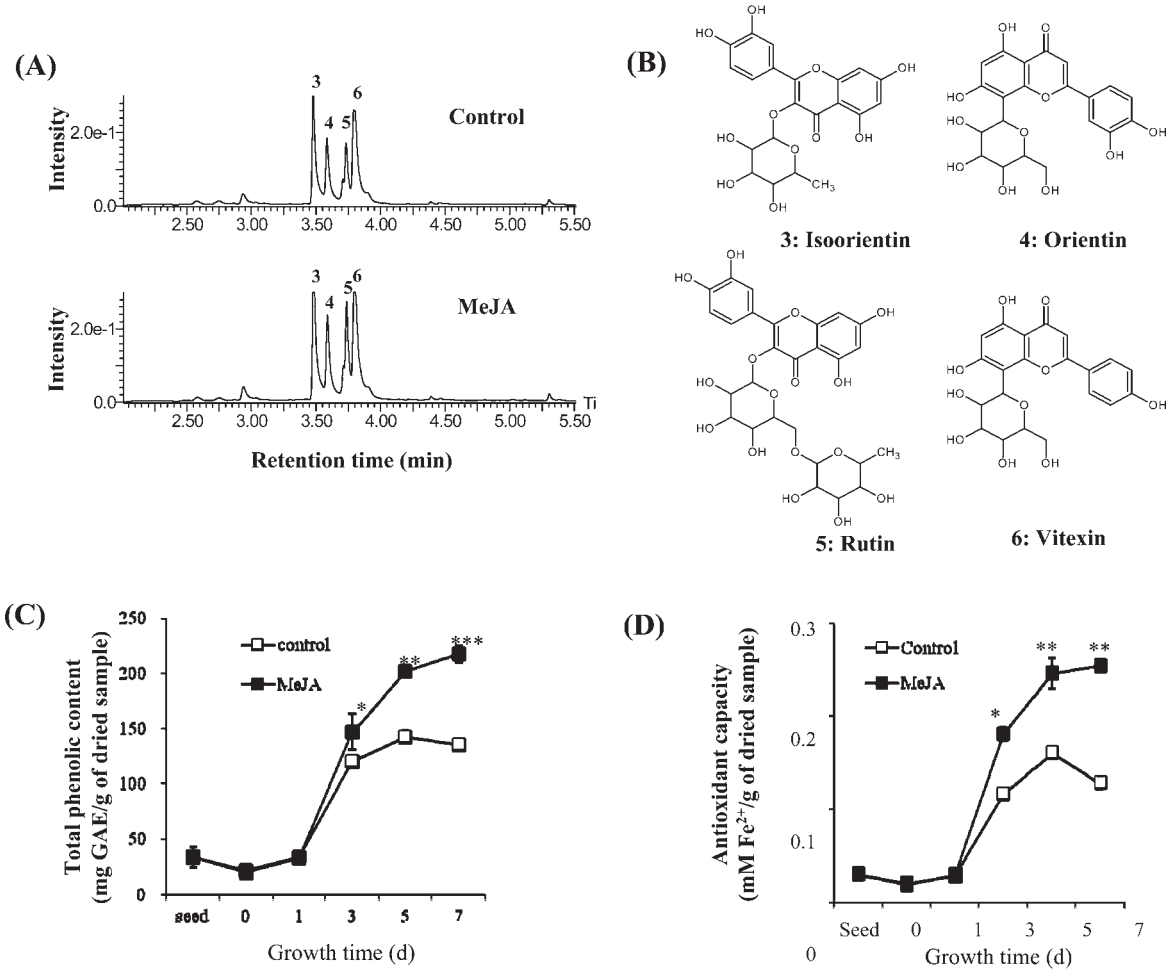

Figure 3. UPLC-Q-TOF-MS BPI profiles of control and MeJA-treated sprouts cultivated for 7 d (A), the chemical structures of the major metabolites isoorientin (3), orientin (4), rutin (5), and vitexin (6) (B), total phenolic contents of control and MeJA-treated groups (C), and antioxidant capacity of both groups (D). Total phenolic content and antioxidant activity were determined by Folin−Ciocalteu's reagent assay and the FRAP assay, respectively.

group by multivariate statistical analysis. No evident changes were observed in the growth of the buckwheat sprouts after exogenous MeJA application, in contrast to the sprouting inhibition seen for postharvested radishes.[21] Nonetheless, the metabolite profiles of the buckwheat were altered by the MeJA treatment (Figures 1 and 2). Of the many metabolites affected by MeJA treatment, seven metabolites (chlorogenic acid, catechin, isoorientin, orientin, rutin, vitexin, and quercitrin) were identified; four of these metabolites (isoorientin, orientin, rutin, and vitexin) were assigned as the main phytochemicals produced

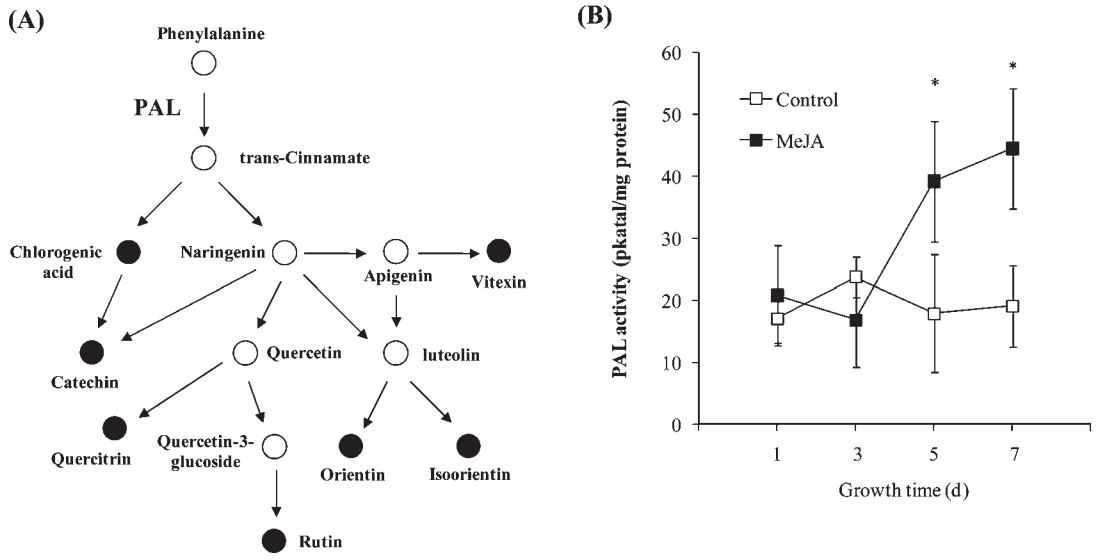

Figure 4. Schematic diagram of phenylpropanoid pathway associated with the production of buckwheat sprout phytochemicals (A) and PAL activity (B). The metabolites analyzed by LC/MS are colored; black is for increased metabolites, and the "○" is for no detected metabolites. One katal was defined as the enzyme activity producing 1 mol of cinnamic acid equivalents per second.

under dark conditions (Table 2). Anthocyanin, which is known as one of the major compounds in sprouts grown under light/dark conditions, has been reported to have a negative correlation with MeJA treatment in buckwheat seedlings.[16] In accordance with these results, anthocyanin was not detected in the sprouts grown under dark conditions. Consistent with the increase in phytochemical accumulation observed in many vegetables and fruits, including radish sprouts,[10] soybean seedlings,[22] lettuce,[23] and raspberries [9] following MeJA treatment, MeJA application resulted in a significant increase in the content of the main phytochemicals, regardless of the cultivation time, with the exception of day 1.

On the basis of the metabolites found in the study, it was postulated that the phenylpropanoid pathway associated with the production of buckwheat sprout metabolites was upregulated by MeJA (Figure 4A). Thus, the activity of PAL, the key regulatory enzyme of the pathway, was determined (Figure 4B). The activity of the MeJA-treated sprouts cultivated for 5 and 7 d was about twice as high as that of the control, similar to the previous results observed for MeJA-treated lettuce[23] and radish sprouts.[9] Therefore, our results indicate that the methanol-soluble metabolites released as phytochemicals in the buckwheat sprouts cultivated under dark conditions were produced through the phenylpropanoid pathway and that their contents were increased by the stimulation of the pathway by MeJA treatment. Hence, the results indicate that exogenous MeJA, as an airborne elicitor, may be taken up by receptors on the surface of the sprouts and used as a stress signal to induce the rapid synthesis of endogenous JA and MeJA, stimulating defense proteins and secondary metabolites associated with the stress response.[7,8] As seen with defense proteins, secondary metabolites induced by stresses or elicitors play essential roles in defense systems such as lignification, bitter taste, and antioxidants.[5,8,24] In this study, we also found that the antioxidant activity of the sprouts was increased by MeJA treatment, as the accumulation of antioxidant compounds showed a good correlation with the total amount of phenolic compounds.

The increased secondary metabolites are not only important for protecting plants from a number of stresses, but also for aiding in the prevention of human diseases and/or in health maintenance as a product of their diverse functional properties.[3] Although we did not investigate the relationship between metabolite accumulation and the functional properties of the sprouts beyond determining antioxidant activities, the functional properties of the sprouts may be increased by MeJA treatment. A number of clinical and animal studies have shown that the major metabolites that accumulated after MeJA treatment are positively related to health function. In particular, it has been reported that rutin, a strong antioxidant, has a number of interesting pharmacological effects such as antiplatelet aggregation and antiasthmatic.[25,26] Furthermore, the efficacy of buckwheat, which contains high amounts of rutin, has been shown in a clinical trial of patients with chronic venous insufficiency.[27] Additionally, many in vitro and in vivo studies have shown that other metabolites, as well as rutin, which we found in buckwheat sprouts, have antioxidant, antiobesity, and antithyroid effects.[28−30] Therefore, our data suggest that exogenous MeJA treatment may improve the functional quality of the sprouts.

In conclusion, a metabolomic study of methanol extracts of buckwheat sprouts provided metabolic information on the effects of MeJA treatment on the production phytochemicals. Without obvious change in the appearance of the sprouts, exogenous MeJA significantly increased the phenolic compound content and the antioxidant activity of the sprouts. Of the various metabolites affected by MeJA treatment, seven metabolites (i.e., chlorogenic acid, catechin, isoorientin, orientin, rutin, vitexin, and quercitrin) were identified; of these metabolites, isoorientin, orientin, rutin, and vitexin were assigned as the primary phytochemicals of sprouts cultivated under dark conditions. This study revealed that the accumulation of these compounds was caused by the stimulation of the phenylpropanoid pathway by MeJA treatment. Consequently, these metabolites could be used to better understand the MeJA effect on the production of buckwheat sprout phytochemicals and contribute to improving the functional quality of the sprouts.

AUTHOR INFORMATION

Corresponding Author

*Tel.: +82-31-780-9331. Fax: +82-31-709-9876. E-mail: jhlim@kfri.re.kr.

Funding Sources

This research was supported by a research grant from the Technology Development Program for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

REFERENCES

(1) Foley, C. M.; Kratz, A. M. Resources and guidelines on buying and using nutraceuticals. In *Nutraceuticals—The Complete Encyclopedia of Supplements, Herbs, Vitamins, and Healing Foods*; Roberts, A. J., O'Brien, M. E., Subak-Sharpe, G., Eds.; Perigree: New York, 2000; p 635.

(2) Espín, J. C.; García-Conesa, M. T.; Tomás-Barberán, F. A. Nutraceuticals: Facts and fiction. *Phytochemistry* **2007**, *68*, 2986–3008.

(3) Briskin, D. P. Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. *Plant Physiol.* **2000**, *124*, 507–514.

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

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

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

(7) 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.

(8) Kim, H.-J.; Chen, F.; Wang, X.; Rajapakse, N. C. Effect of methyl jasmonate on secondary metabolites of sweet basil (*Ocimum basilicum* L.). *J. Agric. Food Chem.* **2006**, *54*, 2327–2332.

(9) 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.

(10) Kim, H.-J.; Chen, F.; Wang, X.; Choi, J.-H. Effect of methyl jasmonate on phenolics, isothiocyanate, and metabolic enzymes in radish sprout (*Raphanus sativus* L.). *J. Agric. Food Chem.* **2006**, *54*, 7263–7269.

(11) Taormina, P. J.; Beuchat, L. R.; Slutsker, L. Infections associated with eating seed sprouts: an international concern. *Emerging Infect. Dis.* **1999**, *5*, 626–634.

(12) Nestle, M. Broccoli sprouts in cancer prevention. *Nutr. Rev.* **1998**, *56*, 127–130.

(13) Hirayama, F.; Lee, A. H.; Binns, C. W.; Zhao, Y.; Hiramatsu, T.; Tanikawa, Y.; Nishimura, K.; Taniguchi, H. Soy consumption and risk of COPD and respiratory symptoms: a case-control study in Japan. *Respir. Res.* **2009**, *10*, 56–62.

(14) Li, S. Q.; Zhang, Q. H. Advances in the development of functional foods from buckwheat. *Crit. Rev. Food Sci. Nutr.* **2001**, *41*, 451–464.

(15) Chen, Z. Y.; Jiao, R.; Ma, K. Y. Cholesterol-lowering nutraceuticals and functional foods. *J. Agric. Food Chem.* **2008**, *56*, 8761–8773.

(16) Horbowiczi, M.; Grzesiuk, A.; Dębski, H.; Koczkodaj, D.; Saniewski, M. Methyl jasmonate inhibits anthocyanins synthesis in seedlings of common buckwheat (*Fagopyrum esculentum* Moench). *Acta Biol. Cracov., Ser. Bot.* **2008**, *50*, 71–78.

(17) Singleton, V. L.; Rossi, I. A. Colorimetry of total phenolics with phospho- molybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.

(18) Benzie, I. F. F.; Strain, J. J. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In *Methods in Enzymology*; Lester, P., Ed.; Academic Press: New York, 1999; Vol. 299, p 15.

(19) 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.

(20) 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.

(21) Wang, C. Y. Methyl jasmonate inhibits postharvest sprouting and improves storage quality of radishes. *Postharvest Biol. Technol.* **1998**, *14*, 179–183.

(22) Franceschi, V. R.; Grimes, H. D. Induction of soybean vegetative storage proteins and anthocyanins by low-level atmospheric methyl jasmonate. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6745–6749.

(23) Kim, H.-J.; Fonseca, J. M.; Choi, J.-H.; Kubota, C. Effect of methyl jasmonate on phenolic compounds and carotenoids of romaine lettuce (*Lactuca sativa* L.). *J. Agric. Food Chem.* **2007**, *55*, 10366–10372.

(24) Wink, M. Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. *Theor. Appl. Genet.* **1988**, *75*, 225–233.

(25) Sheu, J. R.; Hsiao, G.; Chou, P. H.; Shen, M. Y.; Chou, D. S. Mechanisms involved in the antiplatelet activity of rutin, a glycoside of the flavonol quercetin, in human platelets. *J. Agric. Food Chem.* **2004**, *52*, 4414–4418.

(26) Jung, C. H.; Lee, J. Y.; Cho, C. H.; Kim, C. J. Anti-asthmatic action of quercetin and rutin in conscious guinea-pigs challenged with aerosolized ovalbumin. *Arch. Pharm. Res.* **2007**, *30*, 1599–1607.

(27) Ihme, N.; Kiesewetter, H.; Jung, F.; Hoffmann, K. H.; Birk, A.; Muller, A.; Grutzner, K. I. Leg oedema protection from a buckwheat herb tea in patients with chronic venous insufficiency: a single-centre, randomised, double-blind, placebo-controlled clinical trial. *Eur. J. Clin. Pharmacol.* **1996**, *50*, 443–447.

(28) Liu, C. L.; Chen, Y. S.; Yang, J. H.; Chiang, B. H. Antioxidant activity of tartary (*Fagopyrum tataricum* (L.) Gaertn.) and common (*Fagopyrum esculentum* moench) buckwheat sprouts. *J. Agric. Food Chem.* **2008**, *56*, 173–178.

(29) Kim, J.; Lee, I.; Seo, J.; Jung, M.; Kim, Y.; Yim, N.; Bae, K. Vitexin, orientin and other flavonoids from *Spirodela polyrhiza* inhibit adipogenesis in 3T3-L1 cells. *Phytother. Res.* **2010**, *24*, 1543–1548.

(30) Gaitan, E.; Lindsay, R. H.; Reichert, R. D.; Ingbar, S. H.; Cooksey, R. C.; Legan, J.; Meydrech, E. F.; Hill, J.; Kubota, K. Antithyroid and goitrogenic effects of millet: role of C-glycosylflavones. *J. Clin. Endocrinol. Metab.* **1989**, *68*, 707–714.

NOTE ADDED AFTER ASAP PUBLICATION

This manuscript was originally published on the web on April 1, 2011, with errors to the Results Section and Figure 3. The corrected version was reposted April 22, 2011.