Industrial Crops & Products 121 (2018) 428–433

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Metabolomic elucidation of recovery of *Melissa officinalis* from UV-B irradiation stress

Sooah Kim[a], Hojoung Lee[b], Kyoung Heon Kim[a,*]

[a] Department of Biotechnology, Graduate School, Korea University, Seoul, 02841, South Korea
[b] College of Life Sciences and Biotechnology, Korea University, Seoul, 02841, South Korea

ARTICLE INFO

Keywords:
Metabolite profiling
UV-B
Gas chromatography-mass spectrometry
Melissa officinalis
Recovery

ABSTRACT

UV irradiation is a major stress and leads to the accumulation of secondary metabolites in plants as a protective mechanism. The altered metabolism caused by the stress will eventually return to basal conditions, however, the recovery mechanism after UV irradiation stress remains unknown. To understand how plant metabolism recovers following UV irradiation stress, global metabolite profiling of *Melissa officinalis* (lemon balm) was performed using gas chromatography/mass spectrometry (GC/MS). Principal component and hierarchical clustering analyses showed the significant discrimination of metabolite profiles between the control (non-irradiated), UV-irradiated *M. officinalis*, and *M. officinalis* allowed to recover from the UV stress. The glycolysis and phenylpropanoid pathway rapidly reverted to their original states. In contrast, the TCA cycle and amino acid biosynthesis returned slowly to their original states. This study determined that the metabolism and metabolite levels recover their original conditions after the removal of UV irradiation, and that the recovery time of each metabolic pathway differs.

1. Introduction

Metabolomics is the non-targeted or targeted analysis of entire metabolite profiles from living organisms (Fiehn, 2001). Since the concentrations of metabolites directly reflect current phenotypes, metabolite profiling can be useful for determining possible changes in the metabolism of living organisms in response to external stimuli or to genetic perturbations (Fiehn, 2002; Trethewey et al., 1999; Weckwerth, 2003).

Plants are known to produce vast and diverse metabolites. There are estimated to be over 200,000 metabolites produced by plants, including both primary and secondary metabolites (Fiehn, 2002). Regarding plant secondary metabolites in particular, flavonoids perform many physiological roles, providing antioxidative, antibacterial, antifungal, and antitumor effects (Ahmed et al., 2016; Cushnie and Lamb, 2005; Pietta, 2000). *Melissa officinalis* (lemon balm), which belongs to the *Lamiaceae* family, produces various flavonoids such as caffeic acid and rosmarinic acid (Miron et al., 2013; Shekarchi et al., 2012). Possibly due to its high flavonoid contents, *M. officinalis* has been reported to be effective in the treatment of headache, rheumatism, and Alzheimer's disease (Akhondzadeh et al., 2003; Geuenich et al., 2008; Lopez et al., 2009; Tagashira and Ohtake, 1998). Many studies have investigated methods for increasing the production of flavonoids by *M. officinalis* (Kim et al., 2010; Ondrejovic et al., 2012). UV-B irradiation was found to induce the up-regulation of genes for the phenylpropanoid synthesis pathway, and thus the accumulation of flavonoids in *M. officinalis*. This response was suggested to be a protective mechanism against UV irradiation (Kim et al., 2012; Manukyan, 2013).

Generally, any environmental stresses, such as UV irradiation, drought, and water excess, cause numerous phenotypic changes in plants (Jin et al., 2013; Lisar et al., 2012; Wijewardana et al., 2016). The cellular metabolism and regulatory functions of plants are disrupted by such stresses, and stressed plants can then recover from those stresses by altering their cellular metabolism. Previous studies have shown how plants recovered from stresses such as drought and water excess (Galle et al., 2007; Kirschbaum, 1988). However, plant metabolism related to the recovery from UV-B irradiation has not been understood.

In this study, metabolomics was used to reveal a possible recovery mechanism for UV-B-irradiated *M. officinalis*. Metabolites extracted from lemon balm were analyzed by gas chromatography/mass spectrometry (GC/MS), and the abundance of changes in metabolites was statistically assessed by principal component analysis (PCA) and hierarchical clustering analysis (HCA) to gain biological insights into the cellular metabolism associated with the recovery from UV irradiation in the plant.

* Corresponding author.
E-mail address: khekim@korea.ac.kr (K.H. Kim).

https://doi.org/10.1016/j.indcrop.2018.05.002
Received 3 February 2018; Received in revised form 29 April 2018; Accepted 1 May 2018

2. Materials and methods

2.1. Plant growth conditions and reagents

For this study, *M. officinalis* grown on decomposed soil was used. After two months, the plants of height of 10–15 cm were irradiated with UV-B at 0.3 w/m^2/s intensity for 2 h. The UV-irradiated plants were allowed to recover for 0 h (i.e., no recovery time), 3 h, or 12 h at room temperature. A control group was composed of non-irradiated plants. Each group had 5 replicates. The leaves of *M. officinalis* were then harvested and stored at −80 °C, prior to metabolite extraction.

Methanol was obtained from Merck (Darmstadt, Germany), and chloroform, sorbitol-$^{13}C_6$, and pyridine from Sigma-Aldrich (St. Louis, MO). Methoxyamine hydrochloride and *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) were obtained from Fluka (Buchs, Switzerland).

2.2. Metabolite extraction

The harvested leaves were ground using a pre-chilled (−20 °C) mortar. Fifty milligrams of *M. officinalis* leaves were mixed with 1 mL of extraction solvent (methanol:chloroform:water = 2.5:1:1, v:v:v) at −20 °C, then 10 μL of 2 mg/mL sorbitol-$^{13}C_6$ was added as an internal standard. The mixture was vigorously vortexed for 30 min, and then centrifuged at 14,000*g* for 4 min at 4 °C. The supernatant was collected and vacuum-dried using a vacuum concentrator (NB-503CIR, N-BIOTEK, Bucheon, Korea). The dried metabolite samples were kept at −80 °C prior to derivatization and GC/MS analysis. The samples were derivatized with 50 μL of 20 mg/mL methoxyamine hydrochloride in pyridine for 90 min at 30 °C, and then subsequently mixed with 80 μL MSTFA at 37 °C for 30 min.

2.3. GC/MS analysis

GC/MS analysis was performed using an Agilent 7890A GC coupled to an Agilent 5975C MSD (Agilent Technologies, Wilmington, DE). A derivatized metabolite sample (1 μL) was injected into a DB-5MS column (30 m length, 0.25 mm internal diameter, and 0.25 μm film thickness; Agilent Technologies) at a split ratio of 1:10. Helium was used as a carrier gas in the GC with a flow rate of 1.0 mL/min. For GC/MS analysis, each metabolite sample was subjected to an initial temperature of 80 °C with holding for 2 min, and then ramped to 300 °C at 15 °C/min and held for 10 min. The injection and interface temperatures were 230 °C and 280 °C, respectively. Mass spectra of eluting compounds were collected using an electron impact positive ion source at 230 °C in a mass range of 50–600 *m/z*.

2.4. GC/MS data and statistical analysis

The spectra were preprocessed using the Automated Mass Spectral Deconvolution and Identification System (AMDIS; system version 3.2, National Institute of Standards and Technology, Gaithersburg, MD), with automated peak detection and mass spectral deconvolution (Stein, 1999). These spectra were further processed using the Golm Metabolome Database (GMD) and SpectConnect for automatic arraying of identified metabolite peaks, and for tracking of other unidentifiable metabolite peaks that were conserved across sample replicates and sample groups without the use of reference spectra (Kopka et al., 2005; Styczynski et al., 2007). All preprocessed data were normalized using the internal standard. The processed dataset was imported into Statistica for PCA of unsupervised multivariate data and univariate analysis (Fiehn et al., 2008). HCA was also performed to visualize and organize metabolite profiles using MultiExperiment Viewer (Saeed et al., 2006).

Table 1
Identified metabolites from *M. officinalis* using Golm Metabolome Database (GMD).

Identified metabolite			
Alcohols	eicosanol	Organic acids	caffeic acid
	glycerol		citric acid
Amines	hydroxylamine		fumaric acid
Amino acids	4-aminobutyric acid		galactonic acid
	alanine		glyceric acid
	asparagine		lactic acid
	aspartic acid		malic acid
	glutamic acid		quinic acid
	isoleucine		shikimic acid
	leucine		succinic acid
	phenylalanine		threonic acid
	proline	Sugars	2-ketogluconic acid
	serine		fructose
	suberyl glycine		galactinol
	threonine		glucose
	valine		myo-inositol
Fatty acids	hexadecanoic acid		raffinose
Inorganic acids	phosphoric acid		sucrose
			xylitol

3. Results and discussion

3.1. Identified metabolites in M. officinalis

The control, the UV-irradiated (*M. officinalis* treated with UV for 2 h without recovery time), and the recovered treatment groups (*M. officinalis* treated with UV irradiation for 2 h and then allowed to recover for 3 h or 12 h) were analyzed by GC/MS (Fig. S1). More than 100 peaks were detected from GC/MS mass spectral data and 37 metabolites were finally identified after the raw data were processed using AMDIS and SpectConnect. The identified metabolites were categorized into 7 chemical classes comprising alcohols, amines, amino acids, fatty acids, inorganic acids, organic acids, and sugars (Table 1). Amino acids accounted for 13 metabolites (35.1%), the highest proportion of identified metabolites. Organic acids and sugars accounted for 11 metabolites (29.7%) and 8 metabolites (21.6%), respectively. Alcohols, amines, fatty acids, and inorganic acids contained for only one or two identified metabolites in each of these classes. Although the number of metabolites identified was low, they were the key intermediates of the central metabolic pathways including the glycolysis, the TCA cycle, amino acid biosynthesis, and the phenylpropanoid pathway. For example, quinic acid is ubiquitous in plants and is a major intermediate in the phenylpropanoid pathway, being readily converted to shikimic acid, phenylalanine, and tyrosine (Hoffmann et al., 2004). Alanine is produced by alanine aminotransferases from pyruvate, and thus is closely associated with energy metabolism pathways such as the glycolysis and the TCA cycle. It has previously been reported that alanine accumulates in plants under anaerobic stress, and that it induces the biosynthesis of acidic amino acids such as glutamic acid and aspartic acid (Miyashita and Good, 2008; Ricoult et al., 2005).

3.2. PCA of metabolite profiles of M. officinalis irradiated with UV-B and allowed to recover

After the normalization of each metabolite, the dataset was statistically assessed by PCA using Statistica. PCA utilized to compare for the metabolite profiles of the control (non-irradiated), 2 h-irradiated *M. officinalis* without recovery (U2), 2 h-irradiated *M. officinalis* with recovery for 3 h (U2_R3), and 2 h-irradiated *M. officinalis* with recovery for 12 h (U2_R12) (Fig. 1). The generated PCA model showed a R^2X of 0.58 and a Q^2 of 0.61 based on cumulative values up to PC 2. The metabolite profiles of the control, U2, U2_R3, and U2_R12 were significantly discriminated by PC1 and PC2 (Fig. 1a). In particular, the