

Analysis of gc-ms rice metabolomics data



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Integrated ion pathway-based and network-based analysis of GC-MS Rice metabolomics data under Diazinon Stress with Metaboanalyst and Metabolic Networks Reveals Reporter Reactions

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Abstract

Diazinon insecticide is widely applied throughout rice (*Oryza sativa L.*) fields in Iran. However, concerns are now being raised about its potential adverse impacts on rice fields. In this study, a time-course metabolic change in rice plants was investigated after diazinon treatment using gas chromatography–mass spectrometry (GC–MS) and subsequently, three different methods were used in order to link changes in metabolite levels to changes in biological pathways, namely metaboanalyst, metabonetwork, and analysis of reporter reactions with metabolic networks. statistical strategy of metaboanalyst, metabolic networks and reporter reactions was performed in order to find the stress-associated effects. According to the results, a wide range of metabolites were dynamically varied as a result of the plant response to diazinon such as biosynthesis and metabolism of sugars, amino acids, organic acids and phenylpropanoids, all correlating with the exposure time. Plant response was involved in multiple metabolic pathways, such as tricarboxylic acid cycle (TCA cycle), shikimate pathway, and biosynthesis of amino acids, most of which were correlated with the exposure time.

Key Words: Diazinon, Rice, Metabolomics, Metaboanalyst, Metabolic Networks, Reporter Reactions

1. Introduction

Rice (*Oryza sativa L.*) is grown all over the world and is cultivated in humid and temperate environments, which makes its production susceptible to fungi, insects and mites. More than 70 insect species have been recorded as rice pests, so that this would be considered as one of the major constraints on crop yields causing serious reduction in plant production. To address this problem, several kinds of insecticides, fungicides and herbicides are utilized in order to protect crops against pest damage. The application of pesticides in rice fields has become a popular approach towards controlling pest damages during early periods of rice cultivation in Asia [1]. Diazinon (O, O-diethyl O-(2-isopropyl-6-methylpyrimidin-4-yl) thiophosphate, Figure 1) is an organophosphorus pesticide (OPP) commercially introduced in 1952 [2]. Thanks to its inhibitory effects on acetyl cholinesterase enzyme in a vast majority of insects, Diazinon is universally utilized in agricultural sectors for plant protection against a variety of sucking and leaf-eating insects [3]. Nevertheless, several reports have demonstrated that diazinon is immunotoxic [4] cytotoxic [5] and genotoxic [6], hence it exhibits toxic properties and potential risk to human health. As one of the most popular crops worldwide, it seems essential to probe the influence of diazinon on metabolite profiling of rice. To this end, metabolomics is one of the most powerful tools for providing an overview of metabolite changes under various abiotic stresses, namely pesticide stress[7].

So far, No report regarding the influence of diazinon on metabolite profiling of rice has been recorded. Therefore, the present study was undertaken to investigate the effect of diazinon on rice metabolite profiling under sub-tropical climatic conditions.

Recently, Even though traditional data analysis methods like principal component analysis, clustering analysis and chemometrics have shown to be efficient for analysis of this kind of data (Raamsdonk et al, 2001; Allen et al, 2003), there are some limitations with these methods for uncovering the underlying biological principles (Weckwerth et al, 2004). Furthermore, there are still only few examples of studies on the use of metabolome data to understand regulatory principles in metabolism. The data processing challenges in metabolomics are quite unique and often require specialized (or expensive) data analysis software and a detailed knowledge of cheminformatics, bioinformatics and statistics.

Fundamentally, MetaboAnalyst is a web-based metabolomic data processing tool that accepts a variety of input data (NMR peak lists, binned spectra, MS peak lists, compound/concentration data) in a wide variety of formats. It also offers a number of options for metabolomic data processing, data normalization, multivariate statistical analysis, graphing, metabolite identification and pathway mapping.

MetaboNetworks is a tool to create custom sub-networks in Matlab using main reaction pairs as defined by the Kyoto Encyclopaedia of Genes and Genomes(KEGG) and can be calculate the shortest path between a set of

metabolites and plots the connectivity between metabolites as links in a network graph.

Reporter reaction analysis is an attempt to infer the differential reaction significance based on metabolite measurements, and hence provides a basis for understanding the underlying cellular processes responding to the perturbations.

In this study, for the first, we have explored metaboanalyst, metabolic networks and reporter reactions as a potential multivariate method for GC/MS analysis of metabolomics data of rice (*Oryza sativa L.*) plants under diazinon stress. More than 31 metabolites of sugars, amino acids and organic acids were quantitatively determined and time-course metabolic response of the plant during different post-treatment days were investigated and measured in before work.

2. Materials and Methods

2. 1. Materials

Seeds of Shiroodi variety (*Oryza sativa ssp. indica*) were obtained from Rice Research Institute of Iran and were subsequently cultivated in the same open field to avoid the influence of growing location. Diazinon was employed to prevent the plant from insect damage, while its applied concentration was based on the recommended permitted dosage from Plant Protection Organization (PPO). Plants were subjected to 10% granular formulation of diazinon ($15 \text{ kg hectare}^{-1}$) during heading and flowering time. Untreated plants were planted under the same experimental conditions. Rice leaves

were taken from control and treated plants at 24, 48, 96 and 120 h after treatment. Seven replicates at each time point were collected and immediately chilled in liquid nitrogen. Frozen leaves were manually ground in a mortar, using liquid nitrogen in order to keep samples at cryogenic temperature and eventually all samples were stored at $-80\text{ }^{\circ}\text{C}$ until metabolite analysis[8].

2. 2. Chemicals

HPLC-grade methanol was purchased from Merck company (Darmstadt, Germany). Ultrapure water was prepared by a Milli-Q system (Millipore, MA, USA). N-Methy-N-(trimethylsilyl) trifluoroacetamide (MSTFA), methoxyamine hydrochloride, sorbitol, trimethylchlorosilan (TMCS) and pyridine were obtained from Sigma-Aldrich Company (Steinheim, Germany).

2. 3. Sample Preparation

Extraction procedure and derivatization of the metabolites were carried out using a modified method described by Roessner et al. [9]. Approximately 50 mg of powdered rice leaves were homogenized in 1400 μL of 100% methanol ($4\text{ }^{\circ}\text{C}$), and 50 μL of internal standard (2 mg sorbitol ml^{-1} water) was added. The mixture was then vortexed for 15 min at $70\text{ }^{\circ}\text{C}$. The extract was vigorously mixed with 1400 μL water ($4\text{ }^{\circ}\text{C}$) and subsequently centrifuged for 10 min at 2200 g. The polar metabolite fraction containing aliquots of the methanol/water (1000 μL) was transferred to an eppendorf tube and dried in vacuum for 6-16 h. A combination of oxidation and silylation reactions was conducted as derivatization procedure. First, dried residue was dissolved and

derivatized via adding 80 μL of methoxyamine (20 mgml^{-1} in pyridine) solution and incubated in 30 $^{\circ}\text{C}$ water bath for 90 min. Then, 80 μL of MSTFA and 0.8 μL TMCS (1% MSTFA) were added for trimethylsilylation (37 $^{\circ}\text{C}$ for 30 min).

2. 4. GC–MS Analysis

A Varian ion trap MS 4000 and electron impact ionization detection was coupled with a Varian cp-3800 gas chromatograph. MS transfer line and ion trap temperatures were set at 270 and 200 $^{\circ}\text{C}$, respectively. One μL of derivatized sample was injected into gas chromatography (GC) and metabolites were separated on a 30 m \times 0.25 mm \times 0.25 μm DB-5 MS column. Injection temperature was set at 250 $^{\circ}\text{C}$ and the ion source temperature was adjusted to 200 $^{\circ}\text{C}$. Helium was used as carrier gas at a flow rate of 1 mLmin^{-1} . Analysis was performed under the following temperature program: 5 min of isothermal heating at 70 $^{\circ}\text{C}$, followed by a 5 $^{\circ}\text{Cmin}^{-1}$ ramp for oven temperature to 310 $^{\circ}\text{C}$ and a final 10-min heating at 310 $^{\circ}\text{C}$. Ion trap was operated in full scan mode at mass range of 50 – 650 m/z . Both chromatograms and mass spectra were evaluated using the GC-MS Postrun Analysis (Varian).

2. 5. DataProcessing

Metabolomic data processing and statistical analysis was performed for the GC-MS chromatograms by following the workflow of MetaboAnalyst 2.0, a web-based comprehensive tool suite for metabolomic data analysis (<http://www.metaboanalyst.ca>).

References:

- [1] G. Matthews, R. Bateman, and P. Miller, Pesticide application methods, John Wiley & Sons, 2014.
- [2] P. Li, E. Swanson, and F. Gobas, Diazinon and its degradation products in agricultural water courses in British Columbia, Canada. *Bulletin of environmental contamination and toxicology* 69 (2002) 59-65.
- [3] Y. Zhang, W. Zhang, X. Liao, J. Zhang, Y. Hou, Z. Xiao, F. Chen, and X. Hu, Degradation of diazinon in apple juice by ultrasonic treatment. *Ultrasonics sonochemistry* 17 (2010) 662-668.
- [4] E. Z. Neishabouri, Z. Hassan, E. Azizi, and S. Ostad, Evaluation of immunotoxicity induced by diazinon in C57bl/6 mice. *Toxicology* 196 (2004) 173-179.
- [5] Y. Zhang, Y. Hou, F. Chen, Z. Xiao, J. Zhang, and X. Hu, The degradation of chlorpyrifos and diazinon in aqueous solution by ultrasonic irradiation: Effect of parameters and degradation pathway. *Chemosphere* 82 (2011) 1109-1115.
- [6] A. Çakir, and R. Sarikaya, Genotoxicity testing of some organophosphate insecticides in the *Drosophila* wing spot test. *Food and chemical toxicology* 43 (2005) 443-450.
- [7] T. Obata, and A. R. Fernie, The use of metabolomics to dissect plant responses to abiotic stresses. *Cellular and Molecular Life Sciences* 69 (2012) 3225-3243.

[8] J. Zhou, L. Zhang, Y. Chang, X. Lu, Z. Zhu, and G. Xu, Alteration of leaf metabolism in Bt-transgenic rice (*Oryza sativa* L.) and its wild type under insecticide stress. *Journal of Proteome Research* 11 (2012) 4351-4360.

[9] U. Roessner, C. Wagner, J. Kopka, R. N. Trethewey, and L. Willmitzer, Simultaneous analysis of metabolites in potato tuber by gas chromatography-mass spectrometry. *The Plant Journal* 23 (2000) 131-142.