Metabolite Profiling of Arabidopsis by Shimadzu LCMS-IT-TOF with Phenomenome Profiler™ software

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Overview

Challenges

To understand the complex biological systems that apply to the functional genomics of mutant Arabidopsis and different growth conditions

Solutions

Integrate Shimadzu LCMS-IT-TOF analysis that delivers not only accurate MS measurement for MS^n analysis but also high mass resolution, high sensitivity, excellent MS stability and fast polarity switching (100msec) with Phenomenome Profiler™ software.

Phenomenome Profiler™ software is the world's first universal metabolomics software solution developed for metabolomics research projects

Key point

Discover important biomarkers

Summary

The development of a high performance mass spectrometer that delivers
high mass accuracy and high mass resolution independent of MS^n mode has resulted in new opportunities for metabolomics research. By bringing together high mass accuracy MS^n data and Phenomenome Profiler™ software tools has resulted in a bio-informatics platform that identifies specific biomarkers with disease states.

Introduction

Biological function is the sum of gene interactions and metabolic network interactions; both are affected by the environment and genetics. Metabolomics can detect cryptic changes and link unpredictable phenotypes to their biochemistry. The most common platforms for studying metabolites have traditionally included LC- and GC-MS, and NMR. With recent advances in MS instrument technology, such as the Shimadzu LCMS-IT-TOF, we can acquire high resolution and high mass accuracy in the MS data to help identify novel metabolite discovery. Although there has been a rapid advance in instrument performance for mass spectrometry, there is no corresponding developments in software which can help accelerate metabolomics research. To address this issue, we have developed Phenomenome Profiler™ software to work with MS spectrometry-based analysis of biological samples. Phenomenome Profiler™ comprises a suite of tools to manage metabolomic MS spectrometry experiments and raw data, view the data as an interactive array, and perform multivariable statistical analysis.

Methods

Details regarding nine Arabidopsis samples used in this study are listed below and the growth conditions and extraction methods are described at Ref. 1. HPLC was carried on a Shim-pack VP-ODS (2 x 150mm 5u) at a flow rate of 200uL/min and column temperature was 40˚C. Elution gradient with solvent A (0.1% Formic acid-H_2O) and solvent B (0.1% Formic acid-CH_3CN) and the following elution profile were used (0 min 5% B, 40 min 60% B, 40.1 min to 45 min 100% B, 45.1 min 5% B). Injection of samples were performed with a SPD-10A$_{VP}$ UV detector and a SIL-10AD$_{VP}$ auto-injector and LC-10AD$_{VP}$ pumps and a CTO-10A$_{VP}$ column oven. MS analysis was performed on an LCMS-IT-TOF using an ESI source. All above instruments and columns were
obtained from Shimadzu Corporation (Kyoto, Japan).

Name	Arabidopsis	Tissue	Condition	Name	Arabidopsis	Tissue	Condition
LLA	Mutant L	Leaf	Agar Medium	PLV1	Pap1-D mutant	Leaf	Soil
LRA	Mutant L	Root	Agar Medium	PLV2	Pap1-D mutant	Leaf	Soil
WLA	Wild type	Leaf	Agar Medium	WLV	Wild type	Leaf	Soil
WRA	Wild type	Root	Agar Medium	WFV	Wild type	Flower	Soil
				WSV	Wild type	Stem	Soil

Results

For the comprehensive metabolite analysis, we employed a Shimadzu LCMS-IT-TOF (Figure 1), a new hybrid MS spectrometer combining a quadrupole ion trap with time-of-flight. This instrument allows us to acquire MS and MS^n spectrum data having high resolution, high accuracy and high sensitivity due to the compressed ion introduction methods (continuous ionization), improvements to Dual Stage Reflection (high resolution and high sensitivity), ionic cooling using argon gas (high resolution) and a temperature controlled instrument interior (stability of MS accuracy).

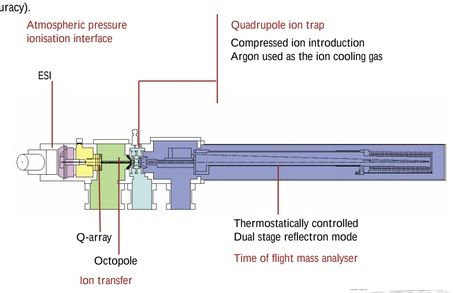

Figure 1. LCMS-IT-TOF schematic

The nine Arabidopsis samples were analyzed in triplicate in LCMS-IT-TOF systems using a positive-ion ESI with a scan range of m/z 250 to 1500. All unique detected MS/retention time pairs are representing sample-specific metabolites, and metabolites fragments are visualized as a metabolite array, as shown to the left (Figure 2). Darker shades of blue represent metabolites with greater intensity. Each column of the array represents one sample, and each row represents a single metabolite.

The array can then be analyzed and interesting metabolites identified using numerous statistical (unsupervised analysis) and Boolean search tools (supervised analysis) present in the Phenomenome Profiler™ software. In the figure, the array has been clustered hierarchically by sample and metabolite, as indicated by the dendrogram at the top and left of the array.

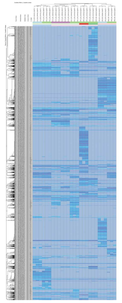

Figure 2. Arabidopsis metabolites array

Results

To identify the key determinant factors of the metabolites, principle component analysis (PCA) was performed with approximately 3,000 unique MS/retention time pairs. By this analysis (Figure 3a), nine experimental groups, each of three independent plant lines, were classified into five major clusters: leaves grown on agar (WLA and PLA) and leaves grown on soil (WLV, PLV1 and PLV2) and roots grown on agar (WRA and LRA) and stems (WSV) and flowers (WFV) grown on soil. The heat map displays all of the metabolites that were used in the PCA analysis. The heat map screenshot (Figure 3b) shows a group of metabolites all having the strong positive loadings in the first component of the PCA results (28% variance; indicated by green histograms) and another group having the strong negative loadings (indicated by red histograms). As you can see, metabolites that are high in the root are the most important metabolites associated with the separation observed along PC1.

Advanced Boolean filters allow you to perform an infinite number of pair-wise comparisons between individual samples or between groups of samples with different variable assignments. The data filters rapidly identify metabolites that are differentially expressed between distinct experimental variables. For example, a pair-wise comparison of samples within the variable assignment set of pap1-D mutant (PLV) vs. the wild type in leaf tissue (WLV) was performed, and the software identified the significant metabolites. Real-time interaction exists between the array tables and the underlying raw
chromatogram and MS spectrum data. Figure 4a shows the extracted ACCURATE MS ion chromatogram (EIC) of pap1-D mutant (PLV) and the wild type in leaf tissue (WLV) at m/z of 1343.366. In this figure, the variable assignments are grouped together and displayed as an EIC overlay. In Figure 4b, average spectra of all samples within a variable assignment of PLV were subtracted from WLV, displayed as subtracted ACCURATE MS spectrum, and listed as ACCURATE MS, intensity and peak%. In this example, m/z of 1343.366 was present in PLV at retention time of 12.4 min. According to the experimentally derived accurate mass information, we can calculate the molecular formula of likely candidates using Profiler™ software, as shown in Figure 4c.

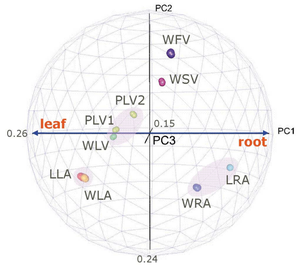

Figure 3a. PCA of Arabidopsis metabolites

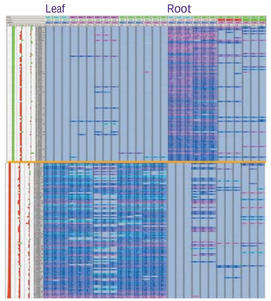

Figure 3b. Heat map of Arabidopsis metabolites

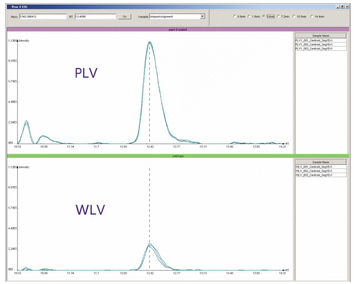

Figure 4. Extracted ACCURATE MS chromatogram of PLV and WLV at m/z of 1343.366.

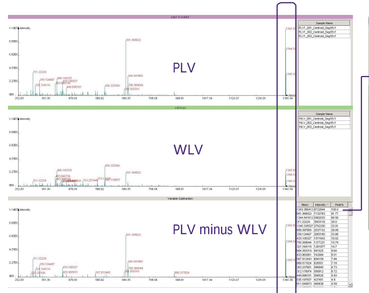

Figure 4b. Variable subtraction of extracted averaged ACCURATE MS spectrum of PLV minus WLV at retention time of 12.4 min.

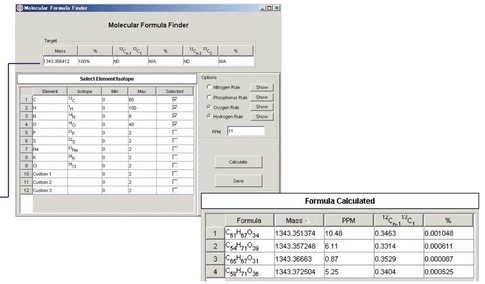

Figure 4c. Molecular Formula Finder assign the molecular formula based on ACCURATE MS.

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