Development of Tandem Mass Spectral Libraries for Plant Metabolomics and Metabolite Identifications

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Overview

• Purpose: Large-scale identification of plant metabolites in M. truncatula using both systematic and biologically directed approaches

• Methods:
  - Aerial and root tissues of M. truncatula were sampled and extracted.
  - UHPLC-ESI/MS and UHPLC-ESI/MS/MS profiling was performed.
  - MS and MS/MS libraries were created for authentic, putatively annotated and unknown compounds.
  - Mass spectral deconvolution and dereplication were evaluated using M. truncatula extracts spiked with a mixture of authentic compounds.
  - MS and MS/MS libraries were created from empirical MS/MS spectra and structures predicted by in silico computational analysis.
  - MS and MS/MS libraries were used to annotate compounds found in lignin pathway mutants and control plants of M. truncatula.

• Results:
  - MS and MS/MS libraries were created containing 126 authentic compounds to aid in the systematic identification of plant metabolites.
  - As the relative intensity of spiked compounds decreased, the deconvolution algorithm became less sensitive in identifying peaks.
  - Metabolic and aglycone aglycone were identified in the mutant and control plants of M. truncatula.
  - Novelties: Extensive mass spectral libraries are being generated and used in the large-scale annotation of plant metabolites found in M. truncatula. These efforts represent the first plant metabolome ‘sequencing’ project.

Introduction

The fundamental understanding of plant biochemistry, including discovery of novel metabolites and gene function along with the elucidation of plant response mechanisms to unknown whether they be biotic, abiotic or environmental, is being advanced by large-scale profiling of plant metabolites. The metabolome, the complete inventory of all the metabolites in a given sample, is a complex mixture containing information on the plant response mechanisms to stimuli whether they be biotic, abiotic or environmental, is being advanced by large-scale profiling of plant metabolites. The metabolome, the complete inventory of all the metabolites in a given sample, is a complex mixture containing information on the plant response mechanisms to stimuli whether they be biotic, abiotic or environmental.

Materials and Methods

An extensive effort has been assembled for the systematic identification of plant metabolites. Compounds in the libraries are assigned by empirical mass values, elemental compositions and MS/MS fragmentation patterns. Empirical MS/MS libraries were created for authentic compounds and in silico computational methods were employed to predict structures. Empirical MS/MS libraries are created for authentic compounds and in silico computational methods were employed to predict structures.

Results

High resolution MS and MS/MS spectral libraries, consisting of the mass spectra of plant metabolites, including commercially available and gift reference standards, were created for the annotation of plant metabolites. The libraries were created using empirical MS/MS spectra and structures predicted by in silico computational analysis.

Conclusions

A sophisticated UHPLC-ESI/MS-SPE-NMR system has been assembled and is being used for metabolite identification and annotation with the ultimate goal of providing the metabolome context for plant metabolome experiments. MS-MS/MS libraries of authentic compounds and in silico computational methods were employed to predict structures. Empirical MS/MS libraries of authentic compounds were incorporated into a plant metabolome database. This database is being used to annotate compounds found in lignin pathway mutants and control plants of M. truncatula (MTR-900 NMR).

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References