

Analytical Platform 9 (UPLC-Q-TOF) was conducted by the Sumner group at the Samuel Roberts Noble Foundation. Frozen plant material was lyophilized for 3 days and then powdered by grinding with a mortar and pestle before extraction. Metabolites were extracted from the dried material in 1 dram glass vial with 80% methanol (200 μ L extraction solvent per mg of dry weight) containing 18 μ g/mL of 7-hydroxycoumarin (common name = umbelliferone) as internal standard. The extract was sonicated for 30 seconds, followed by a 2-hour incubation with gentle agitation at room temperature. Samples were then centrifuged for 30 minutes at 2900 *g*, and the supernatant was transferred to LC/MS vial with a glass insert and analyzed by LC/MS.

UPLC separation was performed on a Waters Acquity UPLC equipped with a BEH C18 column (1.7 μ m, 2.1 x 150 mm), using a linear elution gradient starting at 95% solvent A (0.1% acetic acid) and 5% solvent B (acetonitrile) to 30% solvent A and 70% solvent B over a 30 min period, followed by a linear gradient to 5% solvent A and 95% solvent B in 3 min, and then held at 95% solvent B for 3 min, followed by re-equilibration to 95% solvent A, 5% solvent B for 3 minutes. The elution gradient was at a flow rate of 0.56 mL min⁻¹, and typically 5 μ L of metabolite extracts were injected.

The elution from the UPLC system was subjected to ESI-Qtof mass spectroscopy with a Qtof-Premier (Waters, Milford, MA) operated in the negative-ion mode. Electrospray ionization was achieved at 2.9 kV using a desolvation gas of nitrogen, and a source temperature of 120 °C. The Qtof-Premier was operated in a lock spray mode for internal *m/z* calibration using raffinose as reference compound. To ensure the quality of the analyses, prior to analysis and after every 21 samples, a standard Arabidopsis seed extract, a standard Soyasapogenol B mixture, an internal standard, and a solvent blank were analyzed for quality control and confirmation of mass accuracy.

Raw UPLC-QtofMS data were converted to cdf format. Multiple files from various Arabidopsis tissues were deconvoluted using AMDIS. The deconvoluted unique ions and retention times were compiled to generate a composite list of ion and retention times. The composite list was manually curated to remove redundancy. Putative identifications were then assigned to individual composite list components based upon accurate mass and/or relative retention times yielding a final composite. This list was imported into MET-IDEA (Broeckling et al., 2006) and used to extract quantitative data (i.e. peak areas) for all the ion/retention time pairs in the composite list from each data file. The data matrix was transposed, exported as a txt file, and imported into MS Excel. The data was normalized to the response area of the internal standard using a macro. More specifically, each metabolite peak area was divided by the internal standard (IS) peak area for each file. No background subtraction nor other post acquisition processing was performed.

Broeckling CD, Reddy IR, Duran AL, Zhao X, Sumner LW (2006) MET-IDEA: data extraction tool for mass spectrometry-based metabolomics. *Anal Chem* **78**: 4334-4341