

Analytical Platform 2 (Fatty Acid Profiling) was conducted by the Nikolau group at the W.M. Keck Metabolomics Research Laboratory, Iowa State University. Frozen plant material (approximately 50 mg) was homogenized with 0.5 mL 10% (w/v) barium hydroxide and 0.55 mL 1,4 dioxane, containing 20 µg/mL nonadecanoic acid (Sigma Chemical Co., St. Louis, MO, USA) as an internal standard. The mixture was incubated at 110 °C for 24 hr. After cooling to room temperature, the mixture was acidified using 6M aqueous HCl, and fatty acid analytes were recovered by extracting the aqueous phase with 4 mL n-hexane and vigorously shaking for 3 minutes. Following centrifugation (3,400 g for 5 min) to achieve phase separation, the hexane phase was transferred to a new tube and evaporated to dryness using a gentle stream of nitrogen. The recovered fatty acids were derivatized by methylation with 2 mL HCl:MeOH (1:5.25 v/v) at 80°C for 60 min. After the mixture cooled to room temperature, 2 mL 0.9% (w/v) NaCl and 2 mL n-hexanes were added, the mixture was shaken vigorously for 3 min, and tubes were centrifuged as above. The hexane phase was transferred to a 2 mL glass vial and evaporated to dryness using a gentle stream of nitrogen. 500 µL Acetonitrile and 35 µL N,O-Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + TMCS) were added to the residue, the sample was shaken vigorously for 30 sec, and heated for 20 min at 60°C. Mixture was evaporated to dryness as above, 200 µL n-hexanes were used to dissolve residue and mixture was transferred to a 2 mL auto-sampler glass vial containing a 200 µL conical glass insert and evaporated to appropriate volume for GC-MS analysis.

GC-MS analyses were performed on an Agilent 6890N GC coupled to an Agilent 5973 inert MSD detector. Samples were loaded (injection volume 1 µL) with an Agilent 7683 G2613A auto-sampler onto a HP-5MS fused silica column (30 m x 250 µm; 0.25 µm film thickness). The initial temperature of the injector and MSD interface were set at 275°C and 280°C, respectively. Metabolites were separated at a flow rate of 1.2 mL/min using He as carrier gas, and using a thermal gradient that started at 80°C (2 min), ramped first to 260°C at 4°C/min, held at this temperature for 5 min, and then ramped to 320°C at 5°C/min. Data were acquired using MSD Chemstation (D.00.01) from Agilent Technologies. Metabolites were identified based on their mass fragmentation patterns by comparison with those of authentic standards using the NIST (National Institute of Standards and Technology) Mass Spectral Search Program (Version D.05.00). Peaks that were not identified were given a unique identifier (Bino et al., 2004). Peak areas were obtained from the Total Ion Chromatogram (TIC), raw data were exported to Microsoft Excel and peak areas were normalized to frozen tissue mass and the internal standard.

Bino RJ, Hall RD, Fiehn O, Kopka J, Saito K, Draper J, Nikolau BJ, Mendes P, Roessner-Tunali U, Beale MH, Trethewey RN, Lange BM, Wurtele ES, Sumner LW (2004) Potential of metabolomics as a functional genomics tool. Trends Plant Sci 9: 418-425