Bromeliad lipidomics for adaptation to elevation

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Liquid chromatography coupled to mass spectrometry analysis

The upper phase (lipid or organic phase) was re-suspended in a mixture of solvents corresponding to the initial of the chromatographic run: 60% mobile phase A and 40% mobile phase B. Mobile phase A was composed of acetonitrile:water (40:60, v/v) and mobile phase B was acetonitrile:2-propanol (10:90, v/v). In both chromatographic mobile phases, 10 mmol L^{-1} NH_4Ac was added. Quality control (QC) samples were prepared using 5 µL of each filtered extract to a vial with a 150 µL insert. One vial with acetonitrile was used as system suitability blank sample for further checks on impurities and equipment fluctuations. Reversed-phase liquid chromatography was performed in an UHPLC system (Thermo Scientific UltiMate™ 3000 RSLCnano system) using a Titan C18 column (100 mm x 2.1 mm x 1.9 µm particle size, Supelco Sigma-Aldrich, Bellefonte, PA, USA). The sample injection volume was set to 5 µL and the column and sampler temperature were kept at 40 ºC and 10 ºC, respectively. Separation was performed at a 250 µL min^{-1} flow rate under a gradient elution mode. Over the next 2 min, the column was re-equilibrated before the next injection. The total execution time was 14 min (Table 1).

Table 1. Gradient of solvents at chromatographic separation

Time of analysis (min)	% A	% B
0-2	60	40
2-3	50	50
3-6	50	50
6.1	30	70
6.1-8	30	70
8-9	0	100
9-11	0	100
11-12	60	40
12-14	60	40

Detection was performed using a Thermo Scientific Orbitrap Q-Exactive mass spectrometer equipped with a Heated-ESI source operating on the positive and negative ionization modes using the MS full scan followed by MS/MS analysis in the DDA mode

of the 5 most intense peaks. Full scan data were acquired between *m/z* 100 and 1500 in profile mode and at resolution 70000 (at *m/z* = 200). The automatic gain control was set as ACG target at 1 x 10^6, 1 scan s^{-1}, and injection time at 100 ms. Heated-ESI parameters were optimized for both ionization analysis as follows: sheath gas flow rate 35 arbitrary units; auxiliary gas flow rate 10 arbitrary unit; and capillary temperature 300 °C. For the spray voltage, the positive ion mode was + 3.5 kV, and the negative ion mode was - 3.2 kV. The ion optics setting was S-Lens RF level 50; S-Lens 25 V; skimmer 15 V; and C-Trap RF 1010 V. Stepped normalized collision energy was 20-30-40.

Data processing and metabolite identification

Manual extraction of UHPLC-ESI-MS chromatograms, signal intensity, and total ion detection were achieved using Thermo Xcalibur Roadmap 3.1 from the raw data. For pre-processing data on MS-DIAL 4.9 software (http://prime.psc.riken.jp/compms/msdial/main.html), UHPLC-ESI-MS raw data were converted to .mzML extension on MSConverter 3.0 from ProteoWizard (https://proteowizard.sourceforge.io/download.html). The parameter analysis were setting with MS^1 tolerance of 0.02 Da, MS^2 tolerance 0.06 Da, retention time 0-14 min, MS^1 and MS^2 *m/z* 100-1500 range, maximum charged number 1; peak detection with 10000 of minimum peak height and mass slice width of 0.1 Da; deconvolution with MS/MS abundance cut off of 30 amplitude and sigma window value of 0.5; alignment parameters with retention time tolerance 0.5 minutes, MS^1 tolerance of 0.02 Da and removed features based on blank information (Zandonadi *et al*, 2023). For identification, the default of accurate mass tolerance was used as 0.01 Da for MS^1 and 0.05 Da for MS^2, and identification score cut off 80%. Adduct forms selected were $[M-H]^-$, $[M-H_2O-H]^-$, $[M+Na-2H]^-$, and $[M+Cl]^-$ for negative ionization mode, and $[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$, and $[M+CH_3OH+H]^+$ for the positive one. The peak spot viewer was filtered to show just the peaks matched according to the reference libraries and with MS/MS information. MS-DIAL internal lipid annotation is based on LipidBlast (Kind *et al*, 2013).

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