

Bromeliad lipidomics for adaptation to elevation

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Sample preparation

Over 30 individuals of *Pitcairnia flammaea* per population (according to population abundance) were collected from eight localities along an elevation gradient from 0 to ~2,200 m.a.s.l. for greenhouse cultivation under the same environmental conditions before measuring traits.

Leaf samples of the cultivated *P. flammaea* were harvested and immediately frozen in liquid nitrogen to stop enzymatic reactions and stored in -80°C in a 50 mL Falcon tube. The sample was ground to fine powder using a mortar and pestle in liquid nitrogen.

The processes used in this study were based on the method developed by Hummel *et al.* for plant lipid analysis using UHPLC-ESI-MS. In this method, the sample is separated into 3 phases: organic, aqueous and protein. It is noteworthy that the lower phase can also have other solid matter besides proteins, as fibers. 60 mL of solvent mixture was prepared with pre-cooled (-20°C) methanol (MeOH, grade HPLC, LiChrosolv® Reag. Ph. Eur.) and methyl-tert-butyl-ether (MTBE, grade HPLC, purity 99,9%, Sigma-Aldrich) in proportion (1:3 v/v). In a 2 mL tube, 50 mg of macerated sample were added and 1 mL of the solvent mixture. The samples were incubated for 5 min under agitation at 500 rpm at 4 °C (Microtube Shaking Incubator AccuTherm, Labnet International, Inc.), followed by an ultrasonication (Branson 5800 Ultrasonic Bath, Emerson, Danbury, USA) in ice-cold bath in 10 minutes. After adding 500 µL mixture of water type I:MeOH (3:1 v/v), the samples were vortexed and centrifuged for 5 min at 4 °C, 10000 rpm (Hettich Zentrifugen Mikro 220R, Tuttlingen, DE). The three phases were separated and dried in a vacuum concentrator (Concentrator Plus, Eppendorf AG, Hamburg, DE), at ambient temperature under vacuum - alcoholic mode, and stored at -80 °C until the chromatographic analysis.

Hummel, J.; Segu, S.; Li, Y.; Irgang, S.; Jueppner, J.; Giavalisco, P.; *Front. Plant Sci.* **2011**, 2, 1. [<https://doi.org/10.3389/fpls.2011.00054>]