

Electronic Supplementary Information

Quantification of free auxins in semi-hardwood plant cuttings and microshoots by dispersive liquid-liquid microextraction / microwave derivatization and GC/MS analysis

Sara Porfírio^{1,3}, Roberto Sonon³, Marco D. R. Gomes da Silva^{2}, Augusto Peixe¹, Maria J. Cabrita¹, Parastoo Azadi³*

Sara Porfírio^{1,3}, Roberto Sonon³, Marco D. R. Gomes da Silva^{2*}, Augusto Peixe⁴, Maria J. Cabrita⁴, Parastoo Azadi³

¹ Instituto de Ciências Agrárias e Ambientais Mediterrânicas / Instituto de Investigação e Formação Avançada – ICAAM/IIFA, Universidade de Évora, 7002-554 Évora, Portugal

² LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

³ Complex Carbohydrate Research Center, The University of Georgia, 315 Riverbend Road, Athens, Georgia 30602

⁴ Escola de Ciências e Tecnologia, Instituto de Ciências Agrárias e Ambientais Mediterrânicas Universidade de Évora, 7002-554 Évora, Portugal

Corresponding author

*E-mail: mdr@fct.unl.pt

Phone: +351-212948351

Fax: +351-212948550

Table S1. Ions used in auxin quantification. The suffix –tms1 and –tms2 corresponds to the mono-silylated and di-silylated derivatives, respectively.

Phytohormone	m/z (molecular ion)	m/z ₁ (fragment ion)
IAA-tms1	247	130
IAA-tms2	319	202
IBA-tms1	275	130
IBA-tms2	347	202
[¹³ C ₆]IAA-tms1	253	136
[¹³ C ₆]IAA-tms2	325	208
IPA-tms1	261	130
IPA-tms2	333	202

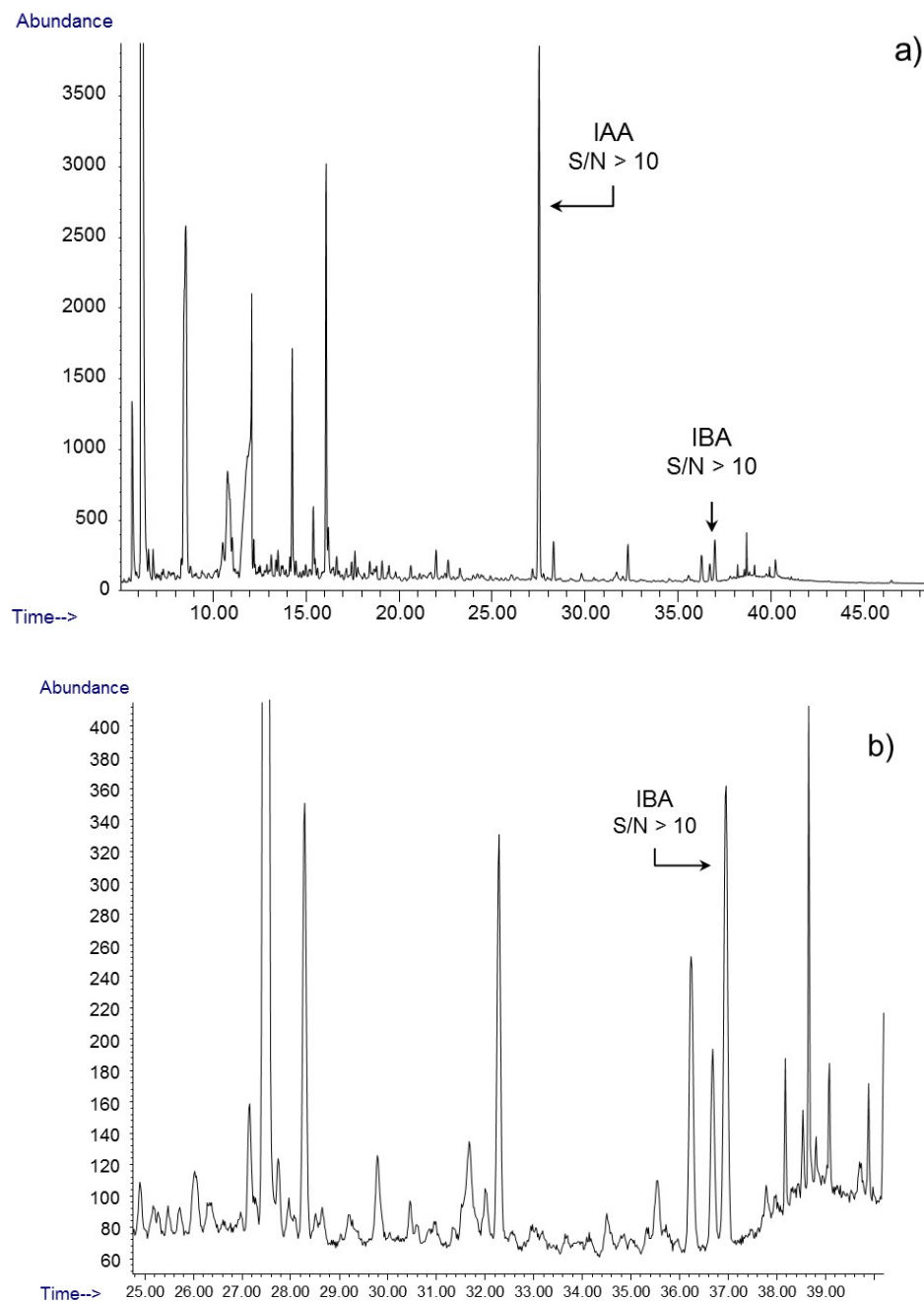


Fig. S1. SIM chromatogram (m/z 202) of a [0.25 ng/mL] standards mixture following DLLME-MAD. a) Full view of the chromatogram; b) Detail of the chromatogram shown in a) where IBA peak is visible. IAA and IBA peaks are identified as well as the respective S/N. This concentration corresponds to the LOQ, based on the S/N ratios for IAA (S/N 15) and IBA (S/N 10), determined by serial dilution of standard solutions analyzed following DLLME-MAD.

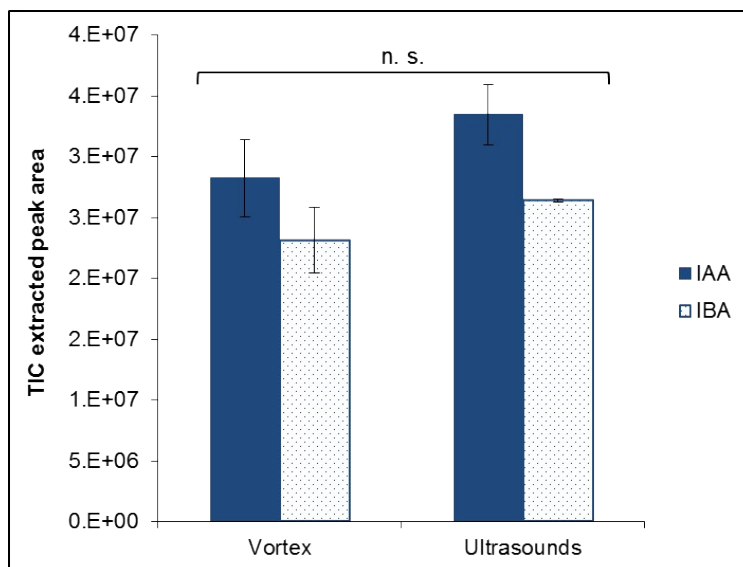


Fig. S2. Effect of vortex- and ultrasounds-assisted extraction on chromatographic response (n=3). Blanks were spiked with [10 µg/mL] of standard mixture. Other DLLME conditions: 3 mL sample, 200 µL CHCl₃, 1000 µL acetone, pH 4, NaCl 15% (w/v), one-step extraction. Mean peak areas correspond to extracted TIC signal. Data analyzed using Student's t-test and shown as mean values and standard deviation bars.

n.s. = Non significant differences at 95% confidence interval

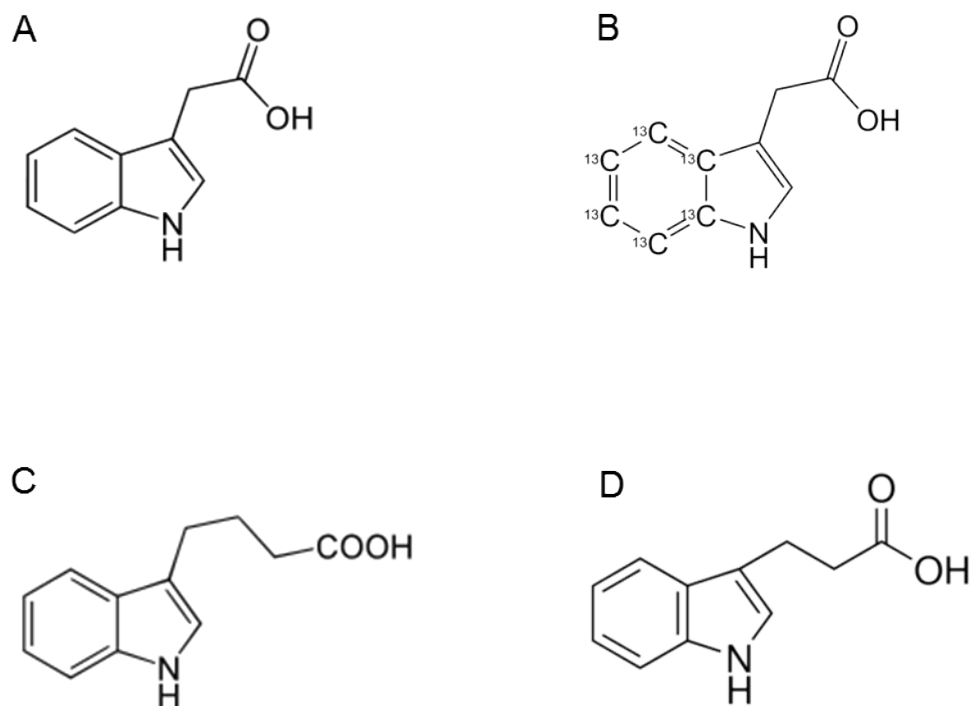


Fig. S3. Chemical structures of the analytes and their respective internal standards. (A) Indole-3-acetic acid (IAA); (B) [$^{13}\text{C}_6$]IAA; (C) Indole-3-butyric acid (IBA); (D) Indole-3-propionic acid (IPA)

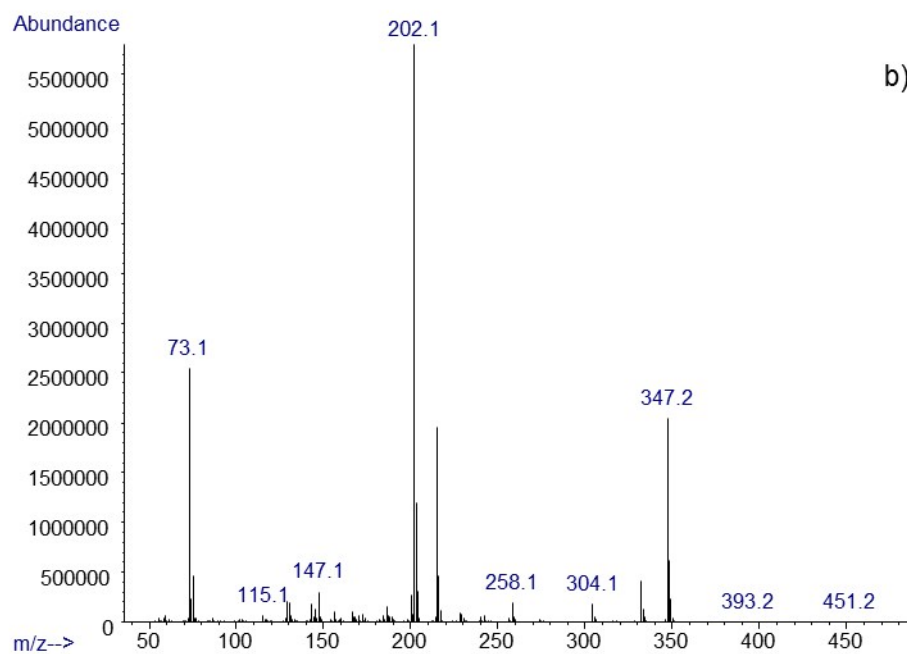
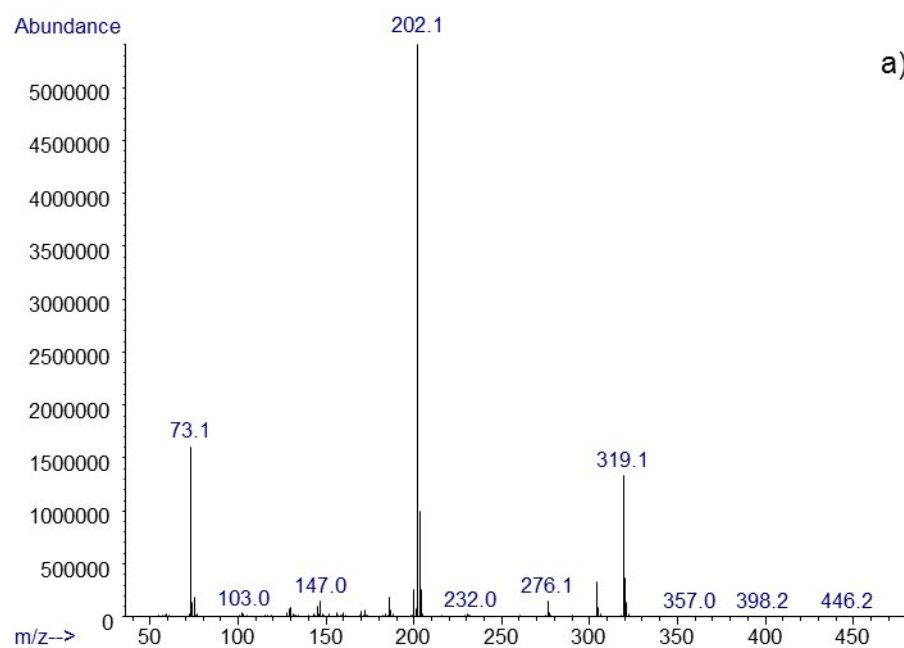


Fig. S4. Mass spectra (MS) of IAA (a) and IBA (b) peaks found in TIC chromatograms of samples.