Protocol for abscisic acid (ABA) extraction from plant seeds

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- List of equipment:

Calibrated balance, Grinder (Mixer Mill MM 400, RETSCH®), Sonicator (Branson 5510R-DTH Ultrasonic machine), ultra-centrifuge (Centrifuge 5424 R, Eppendorf™), speedVac (Concentrator Plus, Eppendorf™), 0.2 um filter (Non-Sterile Syringe Filter 13 mm; Whatman™ Uniflo™), 0.1 mL Micro-insert clear glass tube (fisher scientific), 1.5 mL LCMS vial.

- To prepare before to start:

Prepare 2 mL of working solution per sample: 100% Methanol spiked with 1 ng/mL D_6-ABA stock solution.

- Sample preparation:

1. Grind 7-8 seeds in a Safe-Lock 2.0 mL Eppendorf tube with 3-4 beads for 1 min – frequency 25-26 Hz
2. Weight 100 mg fine powder of ground seeds
3. Add 1mL of Standard solution in each tube
4. Sonicate the samples for 15 min
5. Centrifuge for 5 min at 14000 rpm, then transfer the supernatant to a new 2 mL Eppendorf tube
6. Add another 1mL of Standard solution
7. Sonicate 15 min
8. Centrifuge for 5 min at 14000 rpm
9. Transfer the supernatant (2 mL) to a 2 mL Eppendorf tube
10. Dry the supernatant under vacuum by speedVac for 2 hours

11. After evaporating the solvent, either keep the extracted samples at -20ºC or proceed to the next step
12. The final extract was re-dissolved in 120 µL of acetonitrile : water [25:75 (v/v)] followed by 1 min sonication
13. Filter the re-suspended solution through a 0.22-µm filter into 0.1 mL micro-insert 29x5.7 mm clear glass tubes with inserted vials
14. Tap the bottle to remove any bubbles

- Sample quantification:

1. ABA quantification is performed by LC-MS/MS using a UHPLC-Triple-Stage Quadrupole Mass Spectrometer (Thermo Scientific Altis) machine
2. Chromatographic separation is achieved on the Hypersil GOLD C_{18} Selectivity HPLC Columns (150 × 4.6 mm; 3 µm; fisher scientific) with mobile phases consisting of water (A) and acetonitrile (B), both containing 0.1% formic acid, and the following linear gradient (flow rate, 0.5 mL/min): 0–10 min, 15%–100 % B, followed by washing with 100 % B for 5 min and equilibration with 15 % B for 2 min
3. Inject 10 µL of sample, maintain the column temperature at 35 °C for each run
4. Set the MS parameters of Thermo ScientificTM Altis™ as follows: negative mode, ion source of H-ESI, ion spray voltage of 3000 V, sheath gas of 40 arbitrary units, aux gas of 15 arbitrary units, sweep gas of 0 arbitrary units, ion transfer tube gas temperature of 350 °C, vaporizer temperature of 350 °C, collision energy of 20 eV, CID gas of 2 mTorr, and full width at half maximum (FWHM) 0.4 Da of Q1/Q3 mass. The characteristic Multiple Reaction Monitoring (MRM) transitions (precursor ion → product ion) were the characteristic MRM transitions (precursor ion → product ion) were 263.2 → 153.1 for ABA; 269.2 → 159.1 for D_6-ABA

- Author Contributions

S.A.-B. and J.Y.W. conceived the project. J.Y.W., and L.B. conducted experiments. L.B., J.Y.W., and S. A.-B. wrote, reviewed, and edited the protocol.

- Competing interests

The authors declare no competing interests.

- References

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