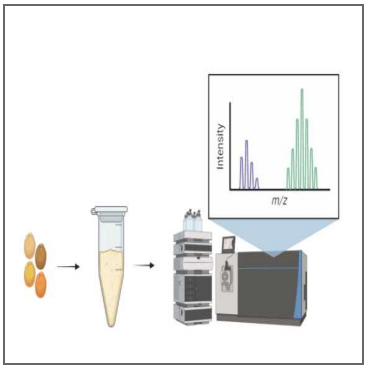

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Protocol status: Working
We use this protocol and it's working

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Protocol for abscisic acid (ABA) extraction from plant seeds

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DISCLAIMER

The authors declare no competing interests.

ABSTRACT

The plant hormone abscisic acid (ABA) regulates seed dormancy and germination. Here, we present a protocol for ABA extraction from plant seeds. We describe necessary steps required for preparation and extraction, followed by liquid chromatography-mass spectrometry (LC-MS/MS) analysis for ABA quantification.

ATTACHMENTS

851-294.pdf

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PROTOCOL integer ID: 88357

Keywords: abscisic acid, plant seeds, LC-MS/MS, plant hormone

GUIDELINES

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.

MATERIALS

List of equipment

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

BEFORE START INSTRUCTIONS

To prepare before to start:

Prepare 2 mL of working solution per sample: 100% Methanol spiked with 1 undetermined D6-ABA stock solution.

Sample preparation

2h 42m

1 Grind 7-8 seeds in a Safe-Lock 2.0 mL Eppendorf tube with 3-4 beads for 00:01:00 – frequency 25-26 Hz. 1m

2 Weight 100 mg fine powder of ground seeds.

3 Add 1 mL of Standard solution in each tube.

4 Sonicate the samples for 00:15:00. 15m

5 Centrifuge for 14000 rpm, 00:05:00, then transfer the supernatant to a new 2 mL Eppendorf tube. 5m

6 Add another 1 mL of Standard solution.

7 Sonicate 00:15:00. 15m

8 Centrifuge for 14000 rpm, 00:05:00. 5m

9 Transfer the supernatant (2 mL) to a 2 mL Eppendorf tube.

10 Dry the supernatant under vacuum by speedVac for 02:00:00. 2h

11 After evaporating the solvent, either keep the extracted samples at -20 °C or proceed to the next step.

12 Re-dissolve the final extract in 120 µL of acetonitrile : water [25:75 (v/v)] followed by 00:01:00 sonication. 1m

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 7m

15 ABA quantification is performed by LC-MS/MS using a UHPLC-Triple-Stage Quadrupole Mass Spectrometer (Thermo Scientific Altis) machine.

16 Chromatographic separation is achieved on the Hypersil GOLD C18 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, follow it by washing with 100 % B for 00:05:00 and equilibration with 15 % B for 00:02:00. 7m

17 Inject 10 µL of sample, maintain the column temperature at 35 °C for each run.

18 Set the MS parameters of Thermo ScientificTM AltisTM 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.

Note

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 D6-ABA.