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Endogenous ABA Extraction and Measurement from *Arabidopsis* Leaves

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Abstract

The endogenous messenger, the phytohormone abscisic acid (ABA) plays a major role in plant's adaption to drought, salinity, cold and other abiotic stresses. In addition to abiotic stress signaling, ABA is involved also in developmental regulation and in responses to diverse biotic stresses. Dehydration stress results in a strong increase in endogenous ABA levels, which can be perceived by RCAR/PYR1/PYL receptors, initiating the ABA signaling pathway to coordinate the genome-wide gene expression, and plants adaptive physiological responses. ABA biosynthesis triggered by environmental cues as well as developmental signals occurs predominantly in vascular parenchyma cells. The measurement of ABA content in different organ/tissues is required to understandings how ABA is produced and delivered within the plants upon various stress conditions and to elucidate its regulatory role in both physiological and transcriptional responses.

Quantitation of ABA can be achieved by two approaches: 1) the use of gas chromatography–tandem mass spectrometry (GC-MS) and 2) the use of immunoassays. Both methods are sensitive to trace amount of ABA down to the low pico-gram (10-12 g/ml FW) range. The GC-MS method needs special facilities, however the antibody based method is relatively simple and can be carried out in laboratory. Here we describe an easy method for ABA extraction from Arabidopsis seedlings, and further determination of ABA levels by competitive ELISA kit.

Materials and Reagents

1. 3-week old *Arabidopsis thaliana* (*A. thaliana*) plants.
2. Methanol (Thermo Fisher Scientific, Acros Organics, catalog number: AC124790010)
3. Sterile deionized H_2O
4. Sodium diethyldithiocarbamate trihydrate (Sigma-Aldrich, catalog number: D3506)
5. Trizma® base (Sigma-Aldrich, catalog number: T1503)

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6. Magnesium chloride (Sigma-Aldrich, catalog number: M8266)
7. Sodium chloride (Sigma-Aldrich, catalog number: S3104)
8. Phytodtek ELISA kit (Agdia, catalog number: PDK 09347)
9. Extraction buffer (see Recipes)
10. Methanolic Tris buffer (see Recipes)

Equipment

1. Microcentrifuge (Eppendorf, model: 5415R)
2. Tweezers or forceps
3. Mortar and pestle
4. Siliconized borosilicate tube (VWR International, catalog number: EPCTS-13100)
5. Liquid nitrogen
6. pH meter (Corning, catalog number: 443i)
7. Refrigerator 4 °C
8. Refrigerated CentriVap Benchtop Vacuum Concentrator (Labconco, catalog: 7310020)
9. Vertical light path photometer for microtiter plate (Dynex Semiconductor, MRX revelation microplate reader)

Procedure

1. *Arabidopsis* seedlings were grown in potting soil under regular growth conditions (at 22 °C with a 12-h light photoperiod and light intensity of 180 μmol/m^2/s) until rosettes were fully expanded (~4 cm diameter).
2. Collect ~15 rosette leaves (~200 mg) from ~3 week old *Arabidopsis* seedlings with sharp tweezers, and measure the fresh weight (FW).
3. Freeze leaf tissues with liquid nitrogen immediately.
4. Carefully grind them in a mortar and pestle to get the fine powder.
5. Add 500 μl extraction buffer to mix them thoroughly by gently pipetting.
6. Transfer extracts to a covered, siliconized borosilicate tube.
7. Incubate the extracts overnight in darkness at 4 °C.
8. Centrifuge at 8, 000 × *g* for 10 min at 4 °C.
9. Transfer supernatant to pre-cold new 1.5 ml Eppendorf tube.
10. Vacuum centrifuge at 4 °C to evaporate the supernatant.
11. Dissolve the residue with 500 μl methanolic Tris buffer (if necessary, gently pipette up and down a few times).

12. Measure the ABA content with Phytodtek ELISA kit according the manufacturer's instructions.

Notes

1. In our procedure, the extractions tube should always be kept on ice during steps 5-11, and a lightproof cover is recommended to prevent extracts from degradation.
2. In addition to *Arabidopsis* leaf, other tissues like root, flower, seed, *etc.*, can be used for extraction. Depending on the type of tissues, sufficient material should be collected until ~200 mg of fresh weight.

Recipes

1. Extraction buffer

   90% (v/v) methanol

   200 mg/L sodium diethyldithiocarbamate trihydrate
2. Methanolic tris buffer

   10% methanol

   50 mM Tris (pH 8.0)

   1 mM $MgCl_2$

   150 mM NaCl

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