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Measuring Endogenous GA and IAA

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Abstract

Plant hormones regulate many physiological processes that largely influence growth, differentiation, and development. Contents of phytohormones were analyzed using a liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) system. This protocol describes a detailed procedure to extract and quantify indole-3-acetic acid (IAA) and gibberellin acid (GA) in rice (*Oryza sativa*) tissues using high-performance liquid chromatography (HPLC)-based method.

Keywords: Phytohormones, IAA, GA, *Oryza sativa*, HPLC

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Background

Plant hormones are the trace organic substances synthesized in plants that are transported from the synthetic site to the active site to significantly regulate plant growth and development (Luo *et al*., 2016; Chen *et al*., 2020a and 2020b). Generally, plant hormones are classified according to their molecular structure and physiological function: auxins, cytokinins (CKs), abscisic acid (ABA), jasmonates (JA), salicy acid (SA), gibberellines (GAs), brassinasteroids (BRs), ethylene (ET), *etc*. The regulation of plant hormones to plant physiological activities involves the change of their own concentration. Uncovering the composition and content changes in plant hormones enables the discovery of possible mechanisms of action and more interactions between them. There are many methods to detect plant hormones, based on as enzyme-linked immunosorbent assays (ELISA), gas chromatography (GC), and high-performance liquid chromatography (HPLC) (Owen and Abrams, 2009; Prado *et al*., 2014). Nowadays, LC-MS and GC-MS have overcame the limitations of HPLC and GC in the qualitative and quantitative determination of plant hormones and become widely accepted and recognized methods.

Materials and Reagents

1. Centrifuge tubes (Eppendorf) (1.5 mL) (CAS 0030125150)
2. Ethanol (CAS 64-17-5), methyl alcohol (CAS 78-83-1), acetonitrile (CAS 75-05-8) (http://www.merck-chemicals.com)
3. Ultrapure water (Millipore, Bedford, MA)
4. Standards: IAA, GA (Olchemim Ltd) IAA (CAS 87-51-4); ME-IAA (CAS 1912-33-0); GA1 (CAS 545-97-1); GA3 (CAS 77-06-5); GA4 (CAS 468-44-0); GA7 (CAS 510-75-8); GA8 (CAS 7044-72-6)
5. Acetic acid (Sinopharm Chemical Reagent, Shanghai, China)
6. Liquid nitrogen
7. Mobile phase (see Recipes)
8. Organic phase (see Recipes)

Equipment

1. Scissors
2. UPLC (Ultra Performance Liquid Chromatography), Shim-pack UFLC SHIMADZU CBM20A, http://www.shimadzu.com.cn
3. MS/MS (Tandem mass spectrometry), Applied Biosystems 4500 QTRAP, http://www.appliedbiosystems.com.cn
4. WatersACQUITY UPLC HSS T3 C18, 1.8 μm, 2.1 mm * 100 mm

Software

1. Analyst 1.6 software (AB Sciex)

Procedure

1. Plant 15 rice seeds of the transgenic plants in a container filled with soil with 2 × 2-cm spacing in a phytotron. The phytotron is set at 30°C and 70% humidity with an 8 h light/16 h dark photoperiod.

2. Harvest fresh 15-day-old seedlings, weight them, and then immediately ground them into powder in liquid nitrogen. Since tissue close to the soil may be contaminated, remove a length of approximately 0.5 cm of tissue near the soil with scissors.
3. Use 100 mg of the powder for subsequent phytohormone extraction, with 1 mL of 80 % (v/v) methanol at 4°C for 12 h.
4. Rotate the extract three times and centrifuge at 12,000 × *g* and 4°C for 15 min.
5. Collect the supernatant and evaporate it to dryness under a nitrogen gas stream at 35°C. Reconstitute the dry supernatant in 100 mL of 95% (v/v) acetonitrile.
6. Centrifuge the solution at 12,000 × *g* and 4°C for 15 min and collect the supernatant for liquid chromatography-mass spectrometry analysis.
7. Prepare quality control (QC) samples from a mixture of sample extracts for analysis of samples under the same treatment method. To assess the repeatability of the analysis procedure, over the course of instrumental analysis, insert one QC sample for every ten test samples.
8. Analyze the sample extracts using an LC-ESI-MS/MS system, using the following analytical conditions: HPLC: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm*100 mm); solvent system: water (0.1% acetic acid): acetonitrile (0.1% acetic acid); gradient program: 100:0 V/V at 0 min, 5:95 V/V at 11.0 min, 5:95 V/V at 12.0 min, 95:5 V/V at 12.1 min, and 95:5 V/V at 15.0 min; flow rate: 0.40 mL/min; temperature: 40°C; injection volume: 5 μL. The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap (Q TRAP)-MS.
9. Acquire LIT and triple quadrupole (QQQ) scans on a triple quadrupole-linear ion trap mass spectrometer (Q TRAP), API 4500 Q TRAP LC/MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in a positive ion mode, and controlled by Analyst 1.6 software (AB Sciex). The ESI source operation parameters were as follows: ion source: turbo spray; source temperature: 550°C; ion spray voltage (IS): 5,500 V; ion source gas I (GSI), gas II (GSII), and curtain gas (CUR) were set at 55, 60, and 25.0 psi, respectively; the collision gas (CAD) was high. Instrument tuning and mass calibration were performed with 10 and 100 μmol/L polypropylene glycol solutions, in QQQ and LIT modes, respectively. Acquire QQQ scans as MRM experiments with collision gas (nitrogen) set to 5 psi. DP and CE for individual MRM transitions were done with further DP and CE optimization. Monitor a specific set of MRM transitions for each period, according to the plant hormones eluted within this period.

Data analysis

1. Three biological replicates were used for the quantification of IAAs and GAs.
2. Perform qualitative analysis on the first and second spectrum data of mass spectrometric detection, based on the self-built database and the public database of metabolite information.

Recipes

1. Mobile phase
Ultrapure water with 0.1% methanoic acid (99.9 mL of ultrapure water and 0.1 mL of methanoic acid).

2. Organic phase
Acetonitrile with 0.1% methanoic acid (99.9 mL of acetonitrile and 0.1 mL of methanoic acid).

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Competing interests

The authors declare no financial or non-financial competing interests.

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