Plant hormone extraction for LC/MS

REAGENTS

Extraction solvent mixture Add 100 µl of concentrated hydrochloric acid to 100 ml of 2-propanol and 50 ml of distilled H2O to make the extraction solvent, i.e., 2-propanol: H2O: concentrated HCl (2:1:0.002, vol/vol/vol). The stock solutions are stable for ~3 months.

PROCEDURE

1| Freeze plant tissues with liquid nitrogen in mortar.

CRITICAL STEP Wounding can induce changes in plant hormones, hence it is important to freeze the tissues in liquid nitrogen immediately after detaching the tissues from the plants.

2| Grind the tissues into powder with a mortar and pestle, weigh and transfer each sample (300 mg) to 50 ml screw-cap tubes. Keep the samples in liquid nitrogen (for technical triplicate).

CRITICAL STEP It is important to accurately weigh frozen plant tissues. Plant tissues need to be weighed as dried powder before they are defrosted.

3| Add 0.04 ppm of Leucine Enkaphalin as internal standards to each tube containing the frozen plant material; **For absolute quantitation, the isotope-labeled standards are required (1mg tissue/1ng isotope-labeled standards)**.

CRITICAL STEP It is important to accurately transfer the specified amount of internal standard to each sample because all quantitative measurements are based on the accurate addition of the internal standard mixture.

4| Add 3 ml extraction solvent, 2-propanol/H_2O/concentrated HCl (2:1:0.002, vol/vol/vol), to each tube. keeping the ratio of sample : solvent at 1:10 (mg ul^{-1}).

5| Put the tubes on a shaker at a speed of 100 r.p.m. for 30 min at 4 °C.

CRITICAL STEP Plant hormones are unstable, and it is important to keep them at 4 °C during solvent extraction.

6| Put the samples into a refrigerated microcentrifuge at 4 °C and centrifuge at 13,000*g* for 10 min, then save supernatant.

7| Add 6 ml dichloromethane to each sample and shake for 30 min in a cold room at 4 °C.

8| Put the samples into a refrigerated microcentrifuge at 4 °C and centrifuge at 13,000*g* for 5 min. After centrifugation, two phases are formed; plant debris is between the two layers.

9| Transfer the solvent from the lower phase using a Pasteur pipette into new tube and concentrate the solvent mixture (not completely dry) using a nitrogen evaporator with nitrogen flow or SpeedVac dry for ~1 hr. The samples are redissolved in 60ul methanol.

CRITICAL STEP Samples should be transferred to storage at − 20 °C until analysis. During analysis, samples are kept in a temperature-controlled autosampler tray at 8 °C until analyzed.

10| Centrifuge at 10,000g, 4 °C for 10 min, then transfer the supernatant to the sample vial for LC-MS/MS analysis.

Ref: X. Pan, R. Welti, and X. Wang.(2010)Quantitative analysis of major plant hormones in crude plant extracts by high-performance liquid chromatography–mass spectrometry.Nature Protocols.

The method is modified by **Ying-Lan, Chen.**