

Plant hormone extraction for LC/MS

COMPOUNDS -- NEGATIVE MODE

	Chemical	Abbreviation	internal standard
1	Abscisic acid	ABA	d6-ABA
2	Salicylic acid	SA	d4-SA
3	Jasmonic acid	JA	d5-JA
4	Jasmonic Acid-Isoleucine	JA-IIU	X
5	Methyl jasmonate	MeJA	X

PROCEDURE

To be done by laboratory submitting samples:

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.
2. Grind the tissues into powder with a mortar and pestle, weigh and transfer each sample (100-150 mg fresh weight) to 2-50 ml tube. Keep the samples in liquid nitrogen (for technical). (Fresh weight)
CRITICAL STEP It is important to accurately weigh frozen plant tissues. Plant tissues need to be weighed as powder.
3. Lyophilize the sample overnight by using a lyophilizer (freeze-dryer).
4. Weighing the dry sample. (Dry weight)
(You may choose to use fresh weight or dry weight when submitting your samples.)

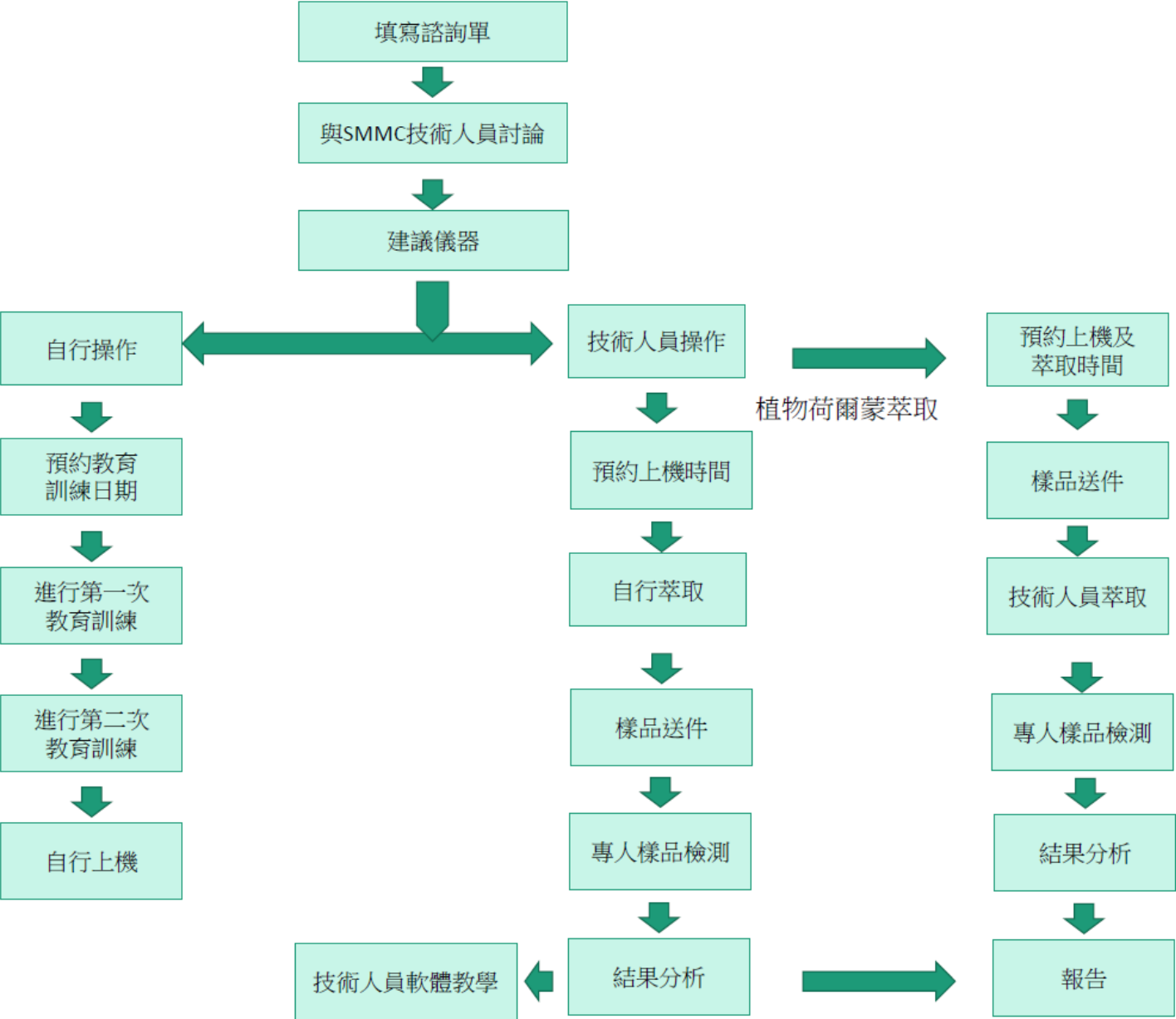
Extraction: Performed by the core (see below for extraction charges) or by user.

1. Add 1 ppm of stable isotope plant hormone 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.
2. Add 3 ml extraction solvent, 2-propanol/H₂O/concentrated HCl (2:1:0.002, vol/vol/vol), to each tube. keeping the ratio of sample: solvent at 1:10 (mg/ul).
3. 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.
4. Put the samples into a refrigerated centrifuge at 4 °C and centrifuge at 13,000g for 10 min, then save supernatant.
5. Add 6 ml dichloromethane to each sample and shake for 30 min in a cold room at 4 °C.
6. Put the samples into a refrigerated centrifuge at 4 °C and centrifuge at 13,000g for 5 min. After centrifugation, two phases are formed; plant debris is between the two layers.

7. 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 ~1-2 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.
8. 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.

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Workflow to Access Metabolites Analysis

