



Acidic Plant Hormone Analysis - Customer Instructions

Scope:

Compounds quantified: Jasmonic acid (JA), Jasmonyl Isoleucine (JA-Ile), 12-oxophytodienoic acid (OPDA), Salicylic acid (SA), Abscisic acid (ABA), Indole-3-acetic acid (IAA), Indole-3-acetyl-aspartic acid (IAA-Asp), cis/trans-Zeatin, Dihydrozeatin, and Zeatin-riboside.

Biological materials: The method is suitable for most vegetative plant tissues, including leaves, roots, fruits and seeds. It is recommended that you provide a few extra samples together with your batch samples for testing the grinding procedure, extraction, and matrix effects.

Experimental design

- Experimental design is the responsibility of the researcher. However, please feel free to contact the facility if you would like advice or help in designing your experiment. Below are some guidelines to help minimize biological and experimental variation:
 - Replication reduces variability in experimental results. We suggest analyzing at least 3 biological replicates per sample or treatment to reduce the variability, and increase the confidence level of each treatment.
 - A replicate sample should be a pool of several samples (ex: one biological replicate within a treatment is derived from harvesting and pooling several leaves from several plants and not just one). Experiments were performed internally to test the biological variation between samples coming from within the same plant and between individual plants. Results showed that there is a high variation if replicates are not a pool of tissue from several plants.

Procedure

Please follow the instructions below to ensure prompt analysis of your samples

Preparation of samples

- Weigh out the appropriate amount of tissue for each sample, and send the information electronically (Excel file). Additionally, send a hard copy of the list of samples submitted with the samples. 100-150 mg of plant tissue (FW) is sufficient for analysis in most cases. Additional sample preparation time will be charged to the customer if facility staff needs to weigh out submitted samples.
- Please provide your samples in 2mL microfuge tubes (preferably with snap-lock top; ex Eppendorf Tube cat # C-3247-1). Make sure your sample tubes are clearly labeled.
- Freeze samples as quickly as possible after harvesting, and store at -80°C prior to submission. Send frozen samples with sufficient dry ice, to ensure they remain frozen in transit to the facility.
- Lyophilized plant material is also suitable for analysis. In this case, only 50-70mg tissue is required.

Publication Acknowledgement:

If any results from this method are being used for publication or grants, it is greatly appreciated to acknowledge the Proteomics & Mass Spectrometry Facility at the Danforth Plant Science Center for its contribution. Our NSF grant that funds the instrument used should be acknowledged using this citation:

“This material is based upon work supported by the National Science Foundation under Grant No. DBI1427621 for acquisition of the QTRAP LC-MS/MS.” A write up of the hormone methods is available upon request.