Protocol for plant growth, tissue collection, cytokinin extraction, and LC-MS

(as part of data submission to NMDR)

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04/20/2023

The *ap1 cal 35S::AP1-GR* and *drmy1 ap1 cal 35S::AP1-GR* plants were grown in soil under continuous light at 16°C to prevent premature floral induction.

After bolting, plants were induced daily with an aqueous solution containing 10 μM dexamethasone (Sigma-Aldrich), 0.01% (v/v) ethanol, and 0.015% (v/v) Silwet L-77 (Rosecare.com). When sepals initiated from the floral meristems (on the fourth day after three daily inductions), inflorescence samples (including inflorescence meristems and buds under stage 6) were collected and immediately put into liquid nitrogen. Five samples were collected for *ap1 cal 35S::AP1-GR* and six for *drmy1 ap1 cal 35S::AP1-GR*.

Cytokinin extraction was based on a previously published protocol[1] with modifications. Briefly, samples were ground in liquid nitrogen and twice extracted in methanol : water : formic acid (15:4:1). 200 pg of BAP per sample was added as an internal control. Extracts were centrifuged at 14,650 rpm in -4°C for 30 min, and supernatant was evaporated of methanol and reconstituted in 1% (v/v) acetic acid. Samples were passed through an Oasis MCX SPE column (Waters 186000252), washed with 1% acetic acid, washed with methanol, and eluted with 0.35 M ammonia in 70% methanol. Eluents were evaporated to complete dryness, reconstituted in 5% acetonitrile, and sent for LC-MS.

LC-MS was done as previously described[2], with modifications. Briefly, 1 μl of each sample was injected into a Thermo Fisher Scientific Vanquish Horizon UHPLC System coupled with a Thermo Q Exactive HF hybrid quadropole-orbitrap high-resolution mass spectrometer equipped with a HESI ion source. Samples were separated on a C18 ODS column (AQUITY UPLC BEH C18, 1.7 μm, 2.1 × 100 mm, Waters), at a flow rate of 0.3 ml/min, with linear gradients of solvent A (0.1% formic acid) and solvent B (0.1% formic acid in methanol) according to the following profile: 0 min, 99.0% A + 1.0% B; 4.0 min, 55.0% A + 45.0% B; 7 min, 30.0% A + 70.0% B; and then with isocratic conditions: 8 min, 1.0% A + 99.0% B; 12 min, 99.0% A + 1.0% B. Cytokinins were detected using the positive ion mode.

For tZ, tZR, iP, iPR, and the internal control BAP, peaks were identified from an external standard mix composed of 0.1 μg/ml each of BAP (Alfa Aesar A14678), tZ (Sigma Z0876), tZR (Sigma Z3541), iP (Cayman Chemical 17906), and iPR (Cayman chemical 20522) in 5% acetonitrile. For cZ and cZR, peaks were identified based on previously reported precursor m/z and retention time[3]. Using Xcalibur (Thermo Scientific), peak area was quantified for each cytokinin in each sample, normalized against the peak area of BAP (internal control) and sample fresh weight, and then normalized against the average abundance of tZ in WT samples.

Abbreviations:

BAP, 6-Benzylaminopurine

tZ, trans-Zeatin

tZR, trans-Zeatin riboside

cZ, cis-Zeatin

cZR, cis-Zeatin riboside

iP, N6-(Δ2-Isopentenyl)adenine

iPR, N6-Isopentenyladenosine

References:

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