Extraction of Ions from Leaf Sections

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[Abstract] The concentration of ions in plant cells and tissues is an important factor to determine their functions and conditions. Here, we describe the method to extract ions from leaf sections for measurements with an ion chromatogram. This method is available for not only barley but also other plant species.

Materials and Reagents

1. Barley seedlings
2. Milli-Q water
3. Hydroponic culture solution (see Recipes)

Equipment

1. 1.5 ml and 2 ml plastic tubes
2. Scissors and forceps
3. Sample crusher (Kurabo Industries, model: SH-48)
4. 7 mm zirconia beads (Kurabo Industries, model: Z-07)
5. Vortex mixer
6. Dry thermo unit (Taitec, catalog number: DTU-18)
7. 0.45 µm filters (EMD Millipore, catalog number: SLLHH04NL)
8. 1.0 ml syringe
9. Centrifuge
10. Ion chromatogram (Thermo Fisher Scientific, Dionex, model: ICS-1500) equipped with an ion exchange column (AS-12 for anions and CS-16 for cations)

Procedure

1. Barley plants are germinated on moist filter paper for 2-3 days and then seedlings are grown in hydroponic culture for 7-8 days.
2. Leaves are sampled using scissors and forceps and weighed.

3. Samples are cut into small pieces, then put into 2 ml plastic tubes with 7 mm zirconia beads and frozen in liquid nitrogen.
4. Frozen leaves are crushed to a powder using a sample crusher.
5. 1 ml distilled water is added to each sample which is then homogenized on a vortex mixer at the maximum speed (*e.g.* 3,000 rpm) until the mixture becomes uniform.
6. Samples are centrifuged at 20,400 *x g* for 10 min at 4 °C. The supernatant is put into a 1.5 ml plastic tube and boiled at 100 °C for 7 min with a dry thermo unit to denature enzymes like phosphatases in Reference 1. If samples are heated for longer than 7 min, some metabolites may begin to be degraded.
7. Samples are cooled on ice and again centrifuged at 20,400 *x g* for 10 min at 4 °C.
8. The supernatant is sucked into a 1 ml syringe, and then filtered through a 0.45 µm filter to remove debris.
9. The filtrates are stored at -20 °C until measurement.
10. Ion levels in each sample are determined with an ion chromatogram.

Notes

1. All steps should be performed on ice to avoid degradation of organic ions.
2. Depending on the purpose of the experiment, any part of the leaf can be used. The extract from at least 5 mg of sample is needed for the ion chromatogram. Sample amounts may vary according to the nutritional status of the plants, but must be enough for the detection by the apparatus.
3. The size and number of zirconia beads in each plastic tube should be adjusted according to the hardness of the sample. In case of a barley primary leaf (about 100 mg), we used two beads with our sample crasher.
4. To crush a larger sample, a mortar and pestle is available. In that case, samples are put into a mortar with liquid nitrogen, and ground by a pestle.
5. For details about the ion chromatography, see Weiss and Weiss (2014).

Recipes

1. Hydroponic culture solution

9 mol/m^3	KNO_3
6 mol/m^3	$Ca(NO_3)_2$
3 mol/m^3	$MgSO_4$
1.5 mol/m^3	KH_2PO_4
0.125 mol/m^3	Fe-EDTA

Micronutrients: 10 mmol/m^3 $MnSO_4$, 1 mmol/m^3 $CuSO_4$, 1 mmol/m^3 $ZnSO_4$, 30 mmol/m^3 H_3BO_3, 30 µmol/m^3 $(NH_4)_6Mo_7O_{24}$, 0.1 mmol/m^3 $CoCl_2$

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References

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