Chlorophyll and starch assays

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Introduction

Chlorophyll, starch, and sugar contents are good indicators of growth vigor in plants. To measure the chlorophyll content, we used a modified protocol[1,2]. The starch content was determined using iodine staining[3] and enzymatic reactions[4-6].

Procedure

Chlorophyll assay

1. Collect at least 300 mg of fresh leaves in 4-week old seedlings. If the leaves have high water content, partially dehydrate them by soaking them in 95% ethanol for 2-5 minutes.
2. Slice the leaves into small pieces, place them in a mortar, add liquid nitrogen, and grind the leaves with a pestle.
3. Add 5 ml of 80% acetone to a 15 ml Falcon tube, transfer the powder in to the tube, and mix them in dark for 15-30 min (note: chlorophylls degrade under light).
4. Centrifuge the tube at 4°C for 15 min (3,000 rpm), transfer the supernatant to a new centrifuge tube, and keep in dark.
5. Repeat steps 3 and 4, and transfer the supernatant to the centrifuge tube in step 4.
6. If necessary, repeat a couple of more times and combine the supernatant.
7. Mix the tube thoroughly and measure the absorbance (A) of chlorophyll content using spectrophotometry.
8. The chlorophyll concentrations are calculated as follows (use 80% acetone as a blank control). Ca (mg/g) = [12.7xA663 - 2.69xA645] ×V/1000×W (Chlorophyll a)

Cb (mg/g) = [22.9xA645 - 4.86xA663] ×V/1000×W (Chlorophyll b)

Ca+b (mg/g) = [8.02×A663 + 20.20xA645] ×V/1000×W (Chlorophyll a+b)

Where V = volume of the extract (ml); W = Weight of fresh leaves (g).

Starch and Sugar Assays

1. Collect at least 500 mg of leaves and freeze them using liquid nitrogen.

2. Grind the frozen leaves with mortar and pestle to fine powder.
3. Add 5 ml of 80% ethanol to 15 ml centrifuge tube, and vigorously mix and rotate for 30 min at 80°C (note: the lid should be tightly closed to prevent ethanol evaporation and leaking).
4. Centrifuge the tube at 4,000 rpm for 30 min at 4°C and transfer the supernatant into a new 50 ml tube.
5. Add 5 ml of 80% ethanol to the tube with the pellet, repeat steps 3 and 4, and transfer the supernatant into the tube in step 4 (note: the pellet is the insoluble carbohydrate fraction and the supernatant is the soluble carbohydrate fraction).
6. Repeat steps 4 and 5 twice and combine all supernatants.
7. Dry the tube with the pellet for starch content measurement.
8. Dissolve the pellet in water (V/W) to yield a solution of 0.01-0.1 mg/ml (based on the amount of starting materials) (note: the color is yellowish).
9. Transfer 500 µl of solution into a 1.5 ml tube and incubate at 90-95°C for 1-2 hours (note: the cap should be tightly closed).
10. Starch (insoluble carbohydrate) content measurement: Cool down the tube to room temperature and determine the starch content using 30 µl of resuspended pellet from step 9 in a reaction with amyloglucosidase following the manufacturer's instructions (Cat. No. 10 207 748 035, R-Biopharm, Darmstadt, Germany) (note: if the concentration is too high, dilute the solution).
11. Sugar (soluble carbohydrate) content measurement: The tube with the combined supernatants (from steps 5 and 6) should have 20-25 ml of solution.
12. Add ~10 ml of water and 5 ml of chloroform to the tube (note: chloroform removes pigments including chlorophylls in the sample, if water is not enough, the chloroform will not separate from the ethanol).

13. Centrifuge the tube at 3,000 rpm for 30 minutes at 4°C and transfer the aqueous phase into a new centrifuge tube.

14. Use a speed vacuum to reduce the volume of the sample until it is less than half of the original volume and add 10 ml of distilled water the tube. The sugar concentration was determined enzymatically using Maltose/Sucrose/D-Glucose (Cat. No. 11 113 950 035) and D-Glucose/D-Fructose (Cat. No. 10 139 106 035) kits (Boehringer Mannheim, R-Biopharm), respectively, following the manufacturer's instructions.

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