Measurement of chlorophylls by spectrophotometer

Sample Preparation

How you prepare your samples depends on whether you want to work on a leaf area basis or a leaf mass basis. The easiest approach is to cut discs of known area from the leaf and use these for analysis. But if you don't want to use discs, you'll have to weigh the material as described below

- Use scalpel to cut frozen or fresh leaves into 0.6 cm 2 sections. Use care when attaching blade and cutting sections as scalpel is razor sharp
- Bend lid of Eppendorf and rest Eppendorf on top of scales
- Weigh approximately 20 mg (0.6 cm 2) frozen or fresh leaf material into Eppendorf
- Record weight and label Eppendorf with corresponding label of plant sample
- Insert one 5 mm ball bearings into Eppendorf with leaf, and seal lid.
- In matrix mill room, clasp an Eppendorf carefully with long-handled tweezers, and lower into 1 L Dewar of liquid nitrogen. Hold for 20 seconds, so as to freeze the Eppendorf and its contents. Remove Eppendorf from liquid N and place in ball mill Eppendorf holder. Prepare a total of 8 Eppendorfs in that manner before setting off the ball mill. Ensure that sample is well ground

Extraction

The next step involves delivering DMSO to a vial. DMSO is a hazardous substance. Before handling, ensure nitrile gloves, lab coat and safety glasses are worn. All work with DMSO must take place under a fume hood, as DMSO is an irritant if inhaled. DMSO is a C1 combustible liquid. It should not be used or stored near any source of ignition and should be stored well away from oxidising agents. DMSO is not a dangerous good.

- Add 1.0 mL DMSO to each Eppendorf containing macerated leaf
- Place eppendorf tube into matrix mill and mix.extract at 30 hz for 2 minutes.
- Centrifuge and remove supernatant
- Add 1.0 mL of DMSO to pellet and re-extract
- Centrifuge, remove supernatant and add to other 1.0 mL

Spectrophotometer

- Calibrate at zero absorptance using a blank of pure DMSO
- Measure absorbance of blank and samples at 645 and 663 nm no longer than 20 minutes after extraction procedure completed.
- A blank of pure DMSO will be included in each run. The absorbance of this blank will be subtracted from the absorbance readings of each sample before any calculations have been made.

Calculations

There are a bunch of different equations for calculating amounts of chlorophyll. Feel free to choose your favourite. Arnon's (1949) equations for calculation of chlorophyll extracted in 90% acetone were proven by Hiscox & Israelstam (1979) to be virtually identical to chlorophyll extracted in DMSO. Arnon's (1949) equations are as follows.

Chla (g l-1) = 0.0127 A663 – 0.00269 A645
Chlb (g l-1) = 0.0029 A663 – 0.00468 A645
Total Chl (g l-1) = 0.0202 A663 + 0.00802 A645

Arnon DI. 1949. copper enzymes in isolated chloroplasts, polyphenoxidase in beta vulgaris. plant physiology 24: 1-15.

Hiscox JD, Israelstam, GF. (1979). a method for the extraction of chlorophyll from leaf tissue without maceration. canadian journal of botany 57: 1332-1334

Richardson AD, Duigan SP, Berlyn GP. (2002). an evaluation of noninvasive methods to estimate foliar chlorophyll content. new phytologist 153: 185-194.