

## CHLOROPHYLL ANALYSIS

1. Flash freeze one leaf punch per eppendorf in liquid N<sub>2</sub>. (NB. Take punches with a borer that gives 0.5 cm<sup>2</sup> leaf discs.)
2. Pre-freeze white re-useable plastic pestles in liquid N<sub>2</sub>.
3. Quickly grind leaf disc in eppendorf using a plastic pestle and add 1 ml 80% acetone, rinsing off last traces of chlorophyll from pestle.
4. Repeat for another 3 samples. Briefly pulse spin all samples and take to the spectrophotometer.
5. Make sure UV light is turned off and blank spec at 645 nm (chlorophyll B) and 663 nm (chlorophyll A) using 80% acetone in two plastic cuvettes.
6. Remove supernatant from one of the sample eppendorfs to plastic cuvette and record reading at 645 nm and 663 nm.
7. Repeat step 6 for remaining 3 samples.

NB. It is good to record chlorophyll readings for c. 4 samples at a time because the longer the sample is in acetone the more chlorophyll leaches out and this may bias readings. Also, acetone attacks the plastic cuvettes so it is good to re-blank with a new cuvette between sets of 4 readings.

Chlorophyll concentration can be calculated using this formula:

$$\text{Chl. Conc in } \mu\text{g/ml} = (\text{OD}_{645} \times 20.2) + (\text{OD}_{663} \times 8)$$

This value is then multiplied by 2 to obtain chlorophyll conc<sup>n</sup> per cm<sup>2</sup>.

Ref: Arnon, D. L. (1949). Copper enzymes in isolated chloroplasts. *Plant Physiology* 24: 1-15.

For analysis of silique chlorophyll concentration: individual siliques are harvested and weighed to the nearest 0.1 mg and flash frozen in liquid N<sub>2</sub>. Follow steps 1 - 7 above and then calculate chlorophyll concentration by dividing by the weight in mg to obtain total chlorophyll concentration per mg.