Chlorophyll Determination:

Time required: 2day

Procedure

1. Weight 100 mg of leaf tissue in fractions into vial containing 7ml Dimethyl sulphoxide
2. Chlorophyll will extract into the fluid without grinding at 65 C by incubating for various times, depending on the degree of cutinization and thickness of the leaf
3. Transfer the extract liquid to a graduated tube and made up to a total volume of 10 ml with Dimethyl sulphoxide, assay immediately or transferred to vials and stored between 0-4 C until required for analysis
4. Take 3 ml of chlorophyll extract and transfer to cuvette,
5. Measure the optical density (OD) of the extract at the following wavelengths 645 and 663 nm using Dimethyl sulphoxide as a blank after 30 min, 1 hr, incubation.

Calculate total chlorophyll as mg/g of tissue, using the following equations:

Chlorophyll A (mg/g) =12.7 (OD663) – 2.69(OD645) x (V/(1000 x wt))

Chlorophyll B (mg/g) =22.9 (OD645) – 4.68(OD663) x (V/(1000 x wt))

Total Chlorophyll (mg/g) =20.2 (OD645) + 8.02(OD663) x (V/(1000 x wt))

Where;

OD: optical density at certain wave length (645 or 663 nm)

v: final volume (10 ml)

wt: weight of sample (100 mg)