

## Extraction and Quantification of Photosynthetic Pigments

*All extraction steps were carried out in dim light*

A known weight of fresh shoot tissues stored in acetone was ground with a little acid-washed sand using least amount of pre-chilled absolute acetone using a mortar and pestle on ice-bath.

A pinch on  $\text{MgCO}_3$  and anhydrous  $\text{Na}_2\text{SO}_4$  was added during grinding to neutralize acidic pH of plant tissue and to dehydrate the plant tissue, respectively.

The slurry was transferred to centrifuge tube and the mortar washed with absolute acetone then added to homogenate.

After centrifugation, at 4000 g for 15 minutes, at 4 °C. The clear supernatant was collected and pellet washed with ice cold acetone several times till complete extraction of pigments.

The clear extracts of all washes were combined in a volumetric flask and brought up to a known volume with acetone.

Absorbance of pigment extract was carried out at wavelength 440.5, 644 and 662 nm against 100% acetone as blank. Dilute if necessary.

The content of each pigment was determined using the following equations then calculated as  $\text{mg pigment g}^{-1}$  fresh weight:

$$\text{Chlorophyll } a \text{ (}\mu\text{g ml}^{-1}\text{)} = 9.78 \text{ E662} - 0.99 \text{ E644}$$

$$\text{Chlorophyll } b \text{ (}\mu\text{g ml}^{-1}\text{)} = 21.4 \text{ E644} - 4.65 \text{ E662}$$

$$\text{Chlorophyll } (a + b) \text{ (}\mu\text{g ml}^{-1}\text{)} = 5.13 \text{ E662} + 20.41 \text{ E644}$$

$$\text{Carotenoids (}\mu\text{g ml}^{-1}\text{)} = 4.69 \text{ E440.5} - 0.268 \text{ Chl } (a + b)$$

To express measurements in “**mg/g FW**” unit, multiply results in:

$$\frac{\text{Extraction volume (ml) X Dilution (if present)}}{\text{Mass of tissue (g) X 1000}}$$

$$\text{Mass of tissue (g) X 1000}$$