

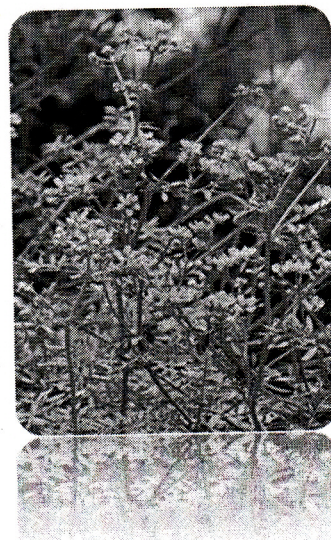
## Experiment-2

### Isolation of Chloroplast and assay of Chlorophyll

#### Introduction:

#### Chlorophyll:

- the most abundant pigment in plants
- the principal light-absorbing pigment in photosynthesis
- from Greek **chloros** "yellowish green"
- porphyrine ring similar to heme (of hemoglobin), but magnesium (not iron) central atom
- not water soluble (grass stain)
- forms tight molecular complexes with some carcinogens: aflatoxin-B1, polyaromatic hydrocarbons (tobacco smoke) & heterocyclic amines (cooked meat)
- chlorophyll absorbs red & violet light strongly
- chlorophyll reflects green light (making leaves green)
- chlorophyll in leaves decays in autumn, leaving carotenoid colors
- **chlorophyll a** has a  $-\text{CH}_3$  side-chain
- **chlorophyll b** has a  $-\text{CHO}$  side-chain
- plants contain both chlorophyll a and chlorophyll b
- chlorophyll b is missing from cyanobacteria
- (**cyanobacteria** are the toxin-producing pond scum bacteria known as "blue-green algae")
- chlorophyll a absorbs red light more strongly
- chlorophyll b absorbs violet light more strongly



## A-Isolation of Chloroplast:

### Material:

Spinach leaves

Buffered sucrose

Isolation medium  $\rightarrow 0.4 \text{ mol/l}$  sucrose -  $0.06 \text{ mol/l}$  Potassium phosphate buffer ph 6.5

Reaction medium  $0.03 \text{ mol/l}$  Potassium phosphate buffer ph 6.5  
containing  $0.01 \text{ mol/l}$  Potassium chloride

### Method:

- 1- Wash the spinach leaves, remove the midribs
- 2- Weigh 100gm leaves, add 100ml buffered sucrose isolation medium in blender for 2min.
- 3- Filter the mixture by muslin
- 4- Centrifuge the filtrate at 1000 r.p.m for 2min in cold centrifuge
- 5- Take the supernatant and centrifuge for 5min at 6000 r.p.m.
- 6- Remove the supernatant, wash the sediment with the isolation medium 3ml and centrifuge for 5min at 6000 r.p.m.
- 7- Discard the supernatant and repeat again under the same conditions
- 8- Suspend the chloroplast in 20ml ice-cold reaction medium

**(Store at -20 °C if the assay step will carry on the next lab)**

B- Assay of chlorophyll content:

- (a) Add 1ml of the suspension to 10ml acetone (80% v/v acetone in water)
- (b) Shake.
- (c) Filter with filter paper into 25ml volumetric flask.
- (d) Wash with the acetone to complete the volume to 25ml.
- (e) Read the extinction of green solution at 652nm against a solvent blank acetone.

Calculations:

$$\text{Chlorophyll concentration (mg/ml)} = \text{Absorbance} \times 5.8$$

## Results Sheet