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)
- chlorophylll 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 $-CH_3$ side-chain
- **chlorophyll b** has a −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 → 0.4 mol/l sucrose - 0.06 mol/l Potassium phosphate buffer ph 6.5

Reaction medium (0.03 mol/l Potassium phosphate buffer ph 6.5 containing 0.01 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 ^{0}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:

Chlorophyll concentration (mg/ml) = Absorbance X **5.8**

Results Sheet