EXPERIMENT No:

ISOLATION OF CHLOROPLAST

INTRODUCTION

Isolated chloroplasts are required to study the electron transport system of photosynthetic apparatus. There are as many techniques as there are research groups in the field of chloroplast research. Any one of these methods can be followed, provided the isolated chloroplasts are biochemically active as the chloroplasts *in vivo*. A general procedure is given below.

PRINCIPLE

The cell organelles depending upon their size and weight sediment at different centrifugal fields.

REQUIREMENTS

Spinach leaves, Mortar and Pestle, Beaker, Funnel, Refrigerated Centrifuge, Muslin Cloth, Varied capacity (25 ml, 50 ml, and 100 ml) centrifuge tubes made up of Polypropylene.

REAGENTS

- *Isolation Medium*: Weigh 2.42g Tris (20mM); 72.8g Sorbitol (0.4M); 1.168g NaCl (20mM); 0.610g $MgCl_2.6H_2O$ (3mM) and dissolve in one liter of distilled water. Adjust pH to 7.8.
- *Non-linear Sucrose Gradient*: Sucrose solution of 1.8M (52.1% *w/v*), 1.5M (43.1%), 1.2M (35.6%) and 0.6% (19.1%) separately prepared in 10mM MOPS or Phosphate buffer(*p*H 7.2), 0.1%(*w/v*) BSA

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PROCEDURE

1. Cut spinach leaf into small bits. Add 2ml of prechilled isolation medium.
2. Homogenize it with the help of mortar and pestle.
3. Filter the brei through eight-layered cheese/muslin cloth.
4. Take the filtrate in 25ml polypropylene centrifuge tube and centrifuge it at 4000 rpm for 15 minutes.
5. Discard the mark and take the supernatant.
6. Centrifuge the supernatant at 4000rpm for 15- 20 minutes.
7. Now discard the supernatant and resuspend the mark in small amount (0.5 to 1.0 ml) of isolation medium.