4 PHOTOREDUCTION OF DYES BY ISOLATED CHLOROPLASTS

4.1 INTRODUCTION

In the process of photosynthesis, photolysis (splitting in the presence of light) of water releases oxygen and electrons. The electrons travel through a series of carriers—cytochromes, plastoquinone and ferridoxin and finally reduce $NADP^+$ to NADPH. In 1937 Robin Hill and his colleagues first showed evolution of O_2 by illuminated suspension of isolated chloroplasts when provided with artificial electron acceptors such as ferricyanide. Ferricyanide is reduced to ferrocyanide by photolysis of water. This experiment demonstrated that artificial electron acceptor can substitute naturally occurring acceptors. The photoreduction of dyes by chloroplasts is commonly referred to as Hill reaction. In this exercise you will observe photoreduction of 2, 6 -Dichlorophenol indophenol an artificial electron acceptor by isolated chloroplasts. It is blue in oxidised state (quinone form) but becomes a colourless compound when reduced (phenol form).

$$\text{DCPIP (blue)} + H_2 \xrightarrow[\text{chlorophyll}]{\text{light}} \text{DCPIP–}H_2 \text{ (colourless)} + \frac{1}{2} O_2$$

Objectives

After performing this experiment you should be able to:

- isolate chloroplasts from leaves and
- show photoreduction of dye by illuminated suspension of chloroplasts.

4.2 MATERIALS REQUIRED

30 g leaves of any of the following plants: amaranthus, mursa, green cabbage, cauliflower leave or spinach

0.5 M sucrose

0.4 M phosphate bufler (pH 6.5)

0.1%, 2, 6 -dichlorophenol indophenol (DCPIP)

cheese cloth, nylon or fine muslin

mortar and pestle

ice bucket

centrifuge

4.3 PROCEDURE

A. Isolation of Chloroplasts

(work in a team of four students)

1. Keep leaves in the dark overnight before use. Keep solutions, leaves, motar and pestle and glassware handy under refrigeration at 0°C for maintaining the activity of chloroplasts.
2. Take 30 g of washed prechilled leaves and grind them with 40 ml of ice cold 0.5 M sucrose in a precooled mortar and pestle kept in the ice bucket.
3. Filter the homogenate through four layers of cheese cloth, or muslin. Centrifuge the

filtrate at 500 × g in a laboratory centrifuge for 5 minutes. (maximum speed of laboratory centrifuge is about 3000 rpm).

4. Discard the supernatant and suspend the pellet in 3 ml of sucrose solution and centrifuge again at 2000 × g. Resuspend the pellet. Keep it in the dark in refrigerator or in an ice bucket.

B. Photoreduction of the Dye

1. Take 3 test tubes and label them 1 to 3. Add 8 ml of 0.4 M phosphate bufler (pH 6.5) to each.
2. Now add 1 ml of chloroplast suspension and 1 ml of 0.1% dye solution in test tube 1 and 2. Leave tube 1 in light and 2 in the dark.
3. Inactivate 1 ml of chloroplast suspension by keeping it in boiling water for 5 minutes. Mix it with the buffer in test tube 3 and add 1 ml of dye solution. After 10 minutes observe change in the colour in three test tubes.

   If centrifuge is not available the filtrate can directly be used for the reduction of DCPIP.

4.4 RESULTS

Record your results as follows:

Test Tube No.	Chloroplast Suspension	Treatment	Change in the colour of dye
1.	Untreated	Light	Yes/No
2.	Untreated	Dark	Yes/No
3.	Boiled	Light	Yes/No

4.5 PRECAUTIONS

1. Leaves must be left in the dark overnight before use.
2. All the solutions, materials and equipment used for the isolation of chloroplast must be chilled before and during the isolation.
3. The various operations should be performed as quickly as possible.
4. It is necessary to grind leaves gently.

SAQ

1. Why is it necessary to chill apparatus and solution for the isolation of chloroplasts?

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2. Why is the dye not reduced by chloroplasts in the dark?

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3. Why is sucrose added to the isolation mixture?

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4. Why is it necessary to minimise grinding?

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