Practical work n°4

Centrifugation

1- Introduction

Plant cells contain various organelles, including plastids, which store pigments and other important molecules. The study of these organelles often requires their isolation from other cellular components to analyze their contents.

2-Objective of the practical work

-Separation of cellular organelles (tomato lycopenes) by differential centrifugation.

3. Principle

Differential centrifugation is a technique used to separate cellular components (organelles) based on their size and density.

-Cells are first homogenized to break them open and release their contents into a uniform suspension (homogenate).

-The homogenate is then centrifuged.

3- Materials and chemicals

-Centrifuge

-Centrifuge tubes

-Precision balance

-Spatula

-Watch glass

-Beakers (100 mL each)

-Magnetic stirrer

-Stirring bar

-Mortar

-Pestle

-Funnels

-Micropipettes (1 mL)

-Vortex mixer

-Sucrose

-Distilled water

4- Experimental procedure

4-1- Preparation of 70%, 30%, 5% and 3% sucrose solutions

-Weigh 70 g of sucrose (table sugar) for the 70% solution.

- Add 100 mL of distilled water.

-Stir the solution using a magnetic stirrer until the sucrose is completely dissolved.

-Repeat the same procedure for the other concentrations:

- 30% solution: 30 g sucrose in 100 mL distilled water
- 5% solution: 5 g sucrose in 100 mL distilled water
- 3% solution: 3 g sucrose in 100 mL distilled water

4-2- Preparation of a discontinuous sucrose gradient

-In a centrifuge tube, add 1 mL of 70% sucrose solution vertically to form the bottom layer.

-Add 1 mL of 30% sucrose solution slowly along the side of the tube at an angle to avoid mixing.

-Finally, add 1 mL of 5% sucrose solution in the same angled/tube-side method to form the top layer.

4-3- Sample preparation

Tomato samples are cut and ground dry in a mortar, then a few milliliters of 3% sucrose solution are added to break the cells and release organelles, including plastids. The mixture is then double-filtered to remove cellular debris.

4-4- Centifugation

-Place 3 mL of the sample into centrifuge tubes.

-Centrifuge at 4000 rpm for 10 minutes.

-After centrifugation, discard the supernatant and recover the pellet, which contains cellular organelles, including plastids.

-Add 2 mL of 3% sucrose solution to the pellet and homogenize using a vortex.

-Take 0.5 mL of the sample and add it drop by drop onto the discontinuous sucrose gradient.

-Centrifuge at 2000 rpm for 10 minutes.

Questions

1- What is the purpose of double filtration?

2- Why is a discontinuous sucrose gradient used?

3- Why are there two centrifugation steps (4000 rpm and 2000 rpm)?

4- How can you tell that lycopene-containing chromoplasts were successfully isolated?

5-Illustrate the separation of tomato organelles in a discontinuous sucrose gradient in a figure.