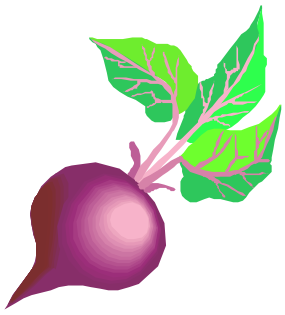
9. Demonstration of the effects of high temperature and chemicals on the permeability of cell membranes



Living beetroot cells are suitable materials to demonstrate the effects of high temperature and chemicals on the permeability of cell membranes. Beetroot contains a red pigment called anthocyanin, which is located in the large central vacuoles of the beetroot cells. As long as the cells and their membranes are intact, the anthocyanin will remain inside the vacuoles. However, if the membranes are damaged, anthocyanin will leak out and produce a red colour in the water surrounding the beetroot. The intensity of red colour in the water can be used to assess the degree of damage to living membranes by different factors.

High temperature and organic solvents e.g. alcohols, denature membrane proteins and increase the fluidity of membrane lipids. Organic solvents at high concentrations can also dissolve lipids. Acetone, alcohol and chloroform are organic solvents that severely destroy membranes.

A. By high temperature

Procedure

1. Use a cork borer to cut cylinders of tissue from a beetroot.
2. Cut the cylinders of beetroot into thin discs of about 3 mm thick.
3. Rinse the beetroot discs in running water to wash off pigment that leaked out as a result of cutting.
4. Pipette 5 cm^3 of water into six test tubes and labelled as 30, 40, 50, 60, 70 and 80.

5. Use a water bath to heat a boiling tube containing water up to 80° C.
6. Gently lower 5 pieces of beetroot discs into the hot water and leave them immersed for exactly 1 minute.
7. Carefully remove the discs and place them in the prepared test tube labelled as 80.
8. Leave the tubes for 20 minutes. Shake the tubes occasionally for the pigment to leak out of the cells.
9. Repeat steps (5) to (8) for temperatures at 70° C, 60° C, 50° C, 40° C and 30° C respectively.
10. Remove the discs from each test tube after 20 minutes.
11. Compare the intensity of red colour in each tube. Use a one to ten ' +' sign to indicate the relative colour intensity.

B. By chemicals

Procedure

1. Use a cork borer to cut cylinders of tissue from a beetroot.
2. Cut the cylinders of beetroot into thin discs of about 3 mm thick.
3. Rinse the beetroot discs in running water to wash off pigment that leaked out as a result of cutting.
4. Blot dry the discs with paper towels.
5. Prepare the following test tubes with 5 cm^3 of solutions as below:

Tube	A	B	C	D	E	F
Solution	water	10% alcohol	30% alcohol	50% alcohol	chloroform	paraffin oil

6. Put equal number of discs into tubes A to F. Stoppered the tubes with cork.
7. Shake the tubes occasionally.
8. After an hour, take out all discs from tubes A to D.
9. Take out the discs from tube E (chloroform) carefully without damaging them and blot to remove the solution on the surface of the discs.
10. Put the discs from step (9) into another test tube labelled as E1, which contains 5 cm^3 of water. Shake occasionally.
11. Repeat step (9) and (10) for tube F (paraffin oil).
12. After an hour, take out the discs from tubes E1 and F1.
13. Record and compare the colour intensity of the solutions in tubes A, B, C, D, E1 and F1.

CAUTION Alcohol and chloroform are volatile and harmful. Teachers should advise students not to inhale the vapour from these chemicals when handling them.

CAUTION Alcohol is inflammable. Avoid using naked flames near this solvent.

Note

1. Chloroform and alcohol can destroy the structure of cell membrane of beetroot, and the red pigment will diffuse out. The pigment is soluble in alcohol and water, but not soluble in chloroform.
2. Paraffin oil and water do not destroy the cell membranes.