Study no. – 03

Name of the study: Study on determination of cell sap concentration of *Rhoeo discolor* leaf by plasmolytic method.

Plasmolysis: The shrinkage of protoplasm by the influence of external hypertonic solution is known as plasmolysis. Plasmolysis is a specialized exo-osmosis which occurs in nature in extreme condition. However, it generally does not occur in nature.

Incepient plasmolysis: It is the starting or initial stage of plasmolysis when protoplasm just starts to shrink. In this stage, protoplasm moves away from the cell wall towards inside.

Evident plasmolysis: In case of evident plasmolysis the protoplasm is shrinked and moves to the one side of the cell wall or become concentrated in the centre of the cell. In this stage the highest shrinkage of protoplasm is observed.

Deplasmolysis: During deplasmolysis, water enters to the cell by the influence of hypotonic solution and protoplasm gets back to its original condition after fulfilling the whole cell, but certain damage occurs in case of protoplasm.

Normal solution: If the equivalent weight of a substance in grams is dissolved in a solvent and the solution is made to 1 litre (1000 cc) and the solution is called normal solution.

Molar solution: When the molecular weight of a substance in gram is dissolved in a solvent and the solution is made to 1 litre (1000 cc) and the solution is called molar solution.

Materials required:

1. *Rhoeo discolor* leaf
2. Salt/Sugar solution
3. Petridish
4. Pipette
5. Slide
6. Cover slip
7. Microscope
8. Blade/Scalpel
9. Watch glass
10. Distilled water
11. Brush
12. Conical flask

Procedure:

One molar sugar solution was prepared for the experiment. From that molar solution different strengths were made as mention in the table no. 1. A healthy *Rhoeo discolor* leaf was taken and thin scrape was taken from the lower purple portion and kept in fresh water. Different scrape of the leaf were placed in different watch glasses containing different strengths of solution in table no. 1.

Sl. No.	Strength of sugar solution (M)	Amount of stock sugar solution (ml)	Amount of water added (ml)	Total volume of desired solution (ml)
01	0.00	0.00	10.0	10
02	0.05	0.50	9.50	10
03	0.10	1.00	9.00	10
04	0.15	1.50	8.50	10
05	0.20	2.00	8.00	10
06	0.25	2.50	7.50	10

After sometime the different portion of the leaf was observed under microscope.

Observation:

The specimens were observed carefully. The specimens of the solution from 0.0 to -0.15 M not be changed. Plasmolysis of the cells was observed in the solution 0.20 M and also in 0.25 M solution.

Sl. No.	Strength of sugar solution (M)	State of plasmolysis	State of external solution compared to cell sap
01	0.00	No plasmolysis	Hypotonic
02	0.05	No plasmolysis	Hypotonic
03	0.10	No plasmolysis	Hypotonic
04	0.15	No plasmolysis	Hypotonic or isotonic
05	0.20	Plasmolysis occurs	Hypertonic
06	0.25	Plasmolysis occurs	Hypertonic

Inference:

It was evident that the solution from 0.05-0.15 M was hypotonic solution and 0.20 M was hypertonic solution. So, it is assumed that the cell sap conc. is in between 0.15 M-0.20 M.

Precautions:

1. *Rhoeo discolor* leaf should be healthy.
2. Purple portion of the leaf should be taken for experiment.
3. The solution should be made carefully.
4. Brushes should be washed carefully and dried properly to avoid inter-mixer of solution.

Fig: Fresh water

Fig: 0.05M

Fig: 0.10M

Fig: 0.15M

Fig: 0.20M

Fig: 0.25M

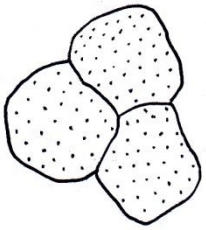

Fig: No plasmolysis (0.0M)

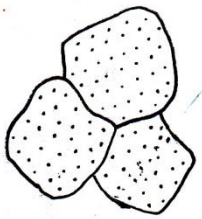

Fig: No plasmolysis (0.05M)

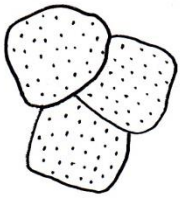

Fig: No plasmolysis (0.10M)

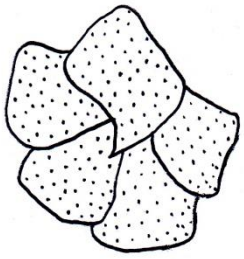

Fig: No plasmolysis (0.15M)

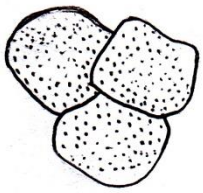

Fig: Incepient Plasmolysis (0.20M)

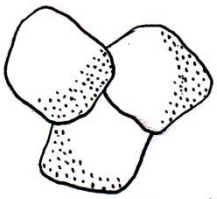

Fig: Evident Plasmolysis (0.25 M)