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B.Sc. (Hons), M.Sc.

UNIT 04

Water potential Experiments

4.2.3: Investigates the concepts of acquisition of water and minerals

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PRACTICAL No: 21 (2019 AL (new))

Determination of water potential of *Colocasia* petioles / Potato strips

(A) Experiment 1: Determination of water potential of potato strips

Objectives
1. Measures the change in length of potato strips.
2. Plots a graph using concentrations of sucrose solutions (in X axis) against the percentage of change in length of potato strips (in Y axis)
3. Interprets experimental results.
4. Determines the water potential of potato tuber cells using the data obtained from the graph.

Materials and Equipment
- Fresh potato tuber
- Six Petri dishes of relevant solutions covered with lids (labeled 0.15M, 0.20M, 0.25M, 0.30 M, 0.35 M, 0.40 M)
- Six test tubes (labeled 0.15M, 0.20M, 0.25M, 0.30 M, 0.35 M, 0.40 M)
- Test tube rack
- Two (10 cm^3 or 25 cm^3) graduated pipettes
- Distilled water
- 1M sucrose solution
- Cork borer
- Two 100 cm 3 beakers
- Graph paper

Instructions
- Direct the students to follow the instructions given below.
- Cut 12 strips of tissue (5cm in length) using the cork borer.
- Keep a graph paper below each petri dish.
- Completely immerse at least 2 strips in each Petri dish.
- Immediately measure their lengths against the graph paper seen through the bottom of the Petri dishes.
- Leave in covered Petri dish for 30 minutes to 60 minutes (depending on the diameter of the tubers) to achieve osmotic equilibrium.
- Measure the lengths again and calculate the mean percentage change in length.
- Then plot a graph of the mean percentage change in length versus molarity of the sucrose solution.
- Determine the concentration of the solution which caused no change in length from the graph.
- Determine the water potential of potato tissue using the given table.

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7. Following diagram show a *Rhoeo* leaf epidermal cell immersed in 15% sugar solution. What is labeled as X in the diagram?
   (1) Water (2) Vacuole (3) Cell sap (4) Sugar solution (5) Protoplasm

8. Which of the following is not having a direct involvement in ascent of sap in tall plants?
   (1) Attraction between water molecules (2) Impermeability of the casparian strip
   (3) Attraction between xylem vessel wall and water molecules
   (4) Evaporation of water from the leaf mid cells
   (5) Diffusion of water from intercellular spaces to the atmosphere

9. Two pieces of a lower epidermal peel of a *Rhoeo* leaf were immersed separately in two sucrose solutions labelled A and B. Solute potential of solution A was – 1800 kpa and that of solution B was – 1300kPa. After the tissues had come to equilibrium with the solutions, it was found that 50% of the cells in the peel immersed in solution A were plasmolysed. Which of the following would be close to the pressure potential of the cells immersed in solution B?
   (1) 1450 kPa (2) 1120 kPa (3) 500 kPa (4) 0 kPa (5) -500 kPa

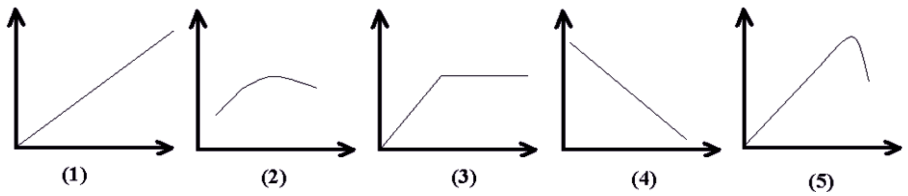
10. Which of the following statements is incorrect regarding water potentials?
    (1) Water potential of vacuolar solution in root hair cell is higher than of soil solution.
    (2) Pure water has the highest water potential.
    (3) Ascent of sap in the xylem takes place along a decreasing gradient of water potential.
    (4) Cells of halophytic plants normally has a lower water potential.
    (5) When transpiration takes place the water potential of outside air is lower than of air inside the plant leaf. (2012/12)
11. Passive movement of water and hydrophilic solutes across membranes with the help of transport proteins is called
    (1) diffusion. (2) osmosis. (3) imbibition. (4) facilitated diffusion. (5) bulk flow. (2022/15)

12. Transport of water molecules due to physical adsorption by hydrophilic materials called
    (1) imbibition. (2) osmosis. (3) facilitated diffusion. (4) bulk flow. (5) mass flow. (2023/13)

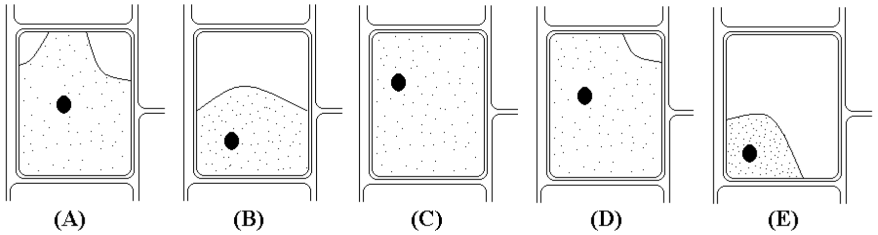
MCQ

1. Which of the following is least important for the ascent of sap of higher plants?
   (1) Adhesive forces (2) Cohesive forces (3) Water potential gradient (4) Root pressure
   (5) Suction of transpiration

2. Which of the following graph show the relationship between relative humidity and rate of transpiration? X axis - R^H, Y axis - Rate of transpiration.



3. Following diagrams show the stages of a *Rhoeo* epidermal peel in hypertonic sugar solution. Find the correct order.

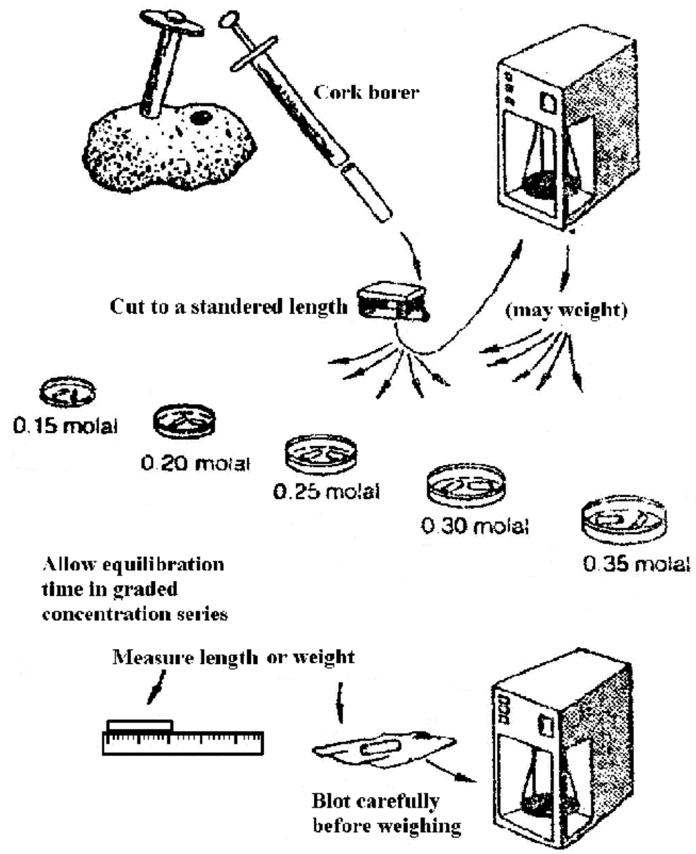


   (1) CADEB (2) EBADC (3) EBCAD (4) CDABE (5) ADEBC

4. Which of the following is not a part of the apoplast of the plant?
   (1) Cell walls of parenchyma cells (2) Lumen of xylem vessels
   (3) Lumen of sieve tube element (4) Cell wall of guard cell
   (5) Cell wall of conductive cells

5. Part of the leaf epidermal cells were immersed in distilled water for 30 minutes. All cells became turgid and reach to equilibrium. Which of the following statement is correct regarding these cells?
   (1) The water potential and the osmotic potential of the cell sap is having equal opposite values.
   (2) The water potential of the cell sap and the pressure potential are having equal values.
   (3) The osmotic potential and the pressure potential of the cell sap is having equal opposite values.
   (4) The water potential of the cell sap is less than the water potential of distilled water.
   (5) Osmotic potential of the cell sap is higher than the pressure potential.

6. Which of the following sentence is correct regarding water relation?
   (1) Entry and the loss of water from transpiration is through a water potential gradient.
   (2) Energy is required to transport water through symplast.
   (3) Only water is transported through apoplast and not solutes.
   (4) Movement of water through vacuolar path is against the concentration gradient.
   (5) Guttation is an active process which spends cellular energy.



Results

Solute potentials of sucrose solutions at 20 °C

Concentration of sucrose solution (molarity)	Solute potential/kPa	Solute potential/atm
0.05	-130	-1.3
0.10	-260	-2.6
0.15	-410	-4.0
0.20	-540	-5.3
0.25	-680	-6.7
0.30	-820	-8.1
0.35	-970	-9.6
0.40	-1 120	-11.6
0.45	-1 280	-12.6
0.50	-1 450	-14.3
0.55	-1 620	-16.0
0.60	-1 800	-17.8
0.65	-1 980	-19.5
0.70	-2 180	-21.5
0.75	-2 370	-23.3
0.80	-2 580	-25.5
0.85	-2 790	-27.5
0.90	-3 010	-29.7
0.95	-3 250	-32.1
1.00	-3 510	-34.6
1.50	-6 670	-65.8
2.00	-11 810	-116.6

7. Explain how cohesive forces are important in ascent of sap.

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8. What are cell types present in angiosperm xylem.

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9. What is the cell type present only present in angiosperms.

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10. What are lignified cell types present in Gymnosperm xylem.

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11. What are functions of xylem.

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12. What are cell types involve in ascent of sap.

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13. What are adaptation of those cells for ascent of sap.

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2019 AL (new)

(i) You are given 12 fresh potato strips immersed in distilled water, each of which is about 5 cm long and six petri dishes kept on graph papers, each containing sucrose solutions of 0.15 M, 0.20 M, 0.25 M, 0.30 M, 035 M and 0.40 M concentrations. State in correct sequence, the steps followed to determine the water potential of given fresh potato tissue.

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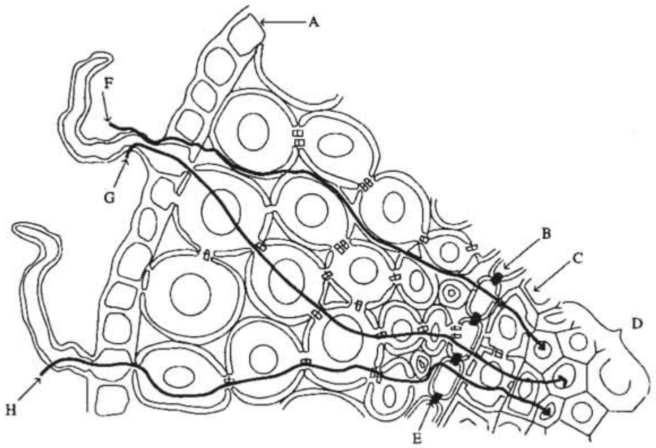
3. Answer questions based on the diagram.

(i) Label A, B, C, D

A - ………………………………… B - ……………………

C - ………………………………… D - …………………...



(ii) From F, G, H pathways through which path more water enters and through which path less mount of water enters in to the root.

More Water - ………………………… Less Water - ………………...………

(iii) What are special characteristic features of tissue label as B

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(iv) State the chemical nature of structure E shown in the diagram.

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(v) What is the process of transport of water up along structure D.

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4. State 3 factors contribute for ascent of sap.

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5. What is called as suction of transpiration.

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6. What are cohesive forces.

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Concentration	Final Length (Cm)	Initial Length (Cm)	Mean % Change in length
0.15M	5.3 + 5.1	4.9 + 5.1	
0.20M	5.1 + 5.2	5.0 + 5.0	
0.25M	5.1 + 5.1	4.7 + 5.3	
0.30M	4.9 + 5.1	5.3 + 4.8	
0.35M	4.7 + 4.9	4.8 + 5.0	
0.40M	4.7 + 4.8	5.1 + 4.9	

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(iv) Radial movement of water occurs through 3 pathways across the plant root, namely, apoplast, symplast and transmembrane pathways. Describe briefly what are these pathways in (a) . Indicate what type of passive movement takes place through each of them in (b).

Apoplast pathway

(a)

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(b)

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Symplast pathway

(a)

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(b)

Transmembrane pathway

(a)

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(b)..

(iii) Briefly describe in column A, the types of movement of water, you mentioned in (ii) and in column b indicate where each type of passive movement occurs in plants.

A	B
Type of Passive movement	**Place of occurrence in plants**

(a)

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(b)

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(c)

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(d)

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(e)

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(B) Experiment 2: Determination of Water Potential by Measuring the Curvature of *Alocasia/Colocasia* petioles

Objectives

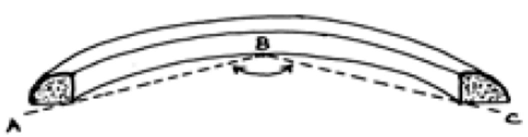
1. Develops methods for measuring curvature of *Alocasia /Colocasia* petiole strips.
2. Plots a graph using concentrations of sucrose solutions (in X axis) against the percentage of change in curvature (in Y axis)
3. Interprets experimental results.
4. Determines the water potential of *Alocasia /Colocasia* petioles using the data obtained from the graph.

Materials and Equipment

- Fresh petioles of *Alocasia /Colocasia*
- Six Petri dishes with lids (labeled 0.15M, 0.20M, 0.25M, 0.30 M, 0.35 M, 0.40 M)
- Six test tubes (labeled 0.15M, 0.20M, 0.25M, 0.30 M, 0.35 M, 0.40 M)
- Test tube rack
- Two 10.00 ml graduated pipettes
- Beaker with distilled water
- Beaker with 1M sucrose solution
- Fine forceps, razor blade
- Graph paper
- Protractor
- Blotting paper

Methodology

- Prepare 20 ml solutions of different concentrations as given above.
- Take six pieces of 6 cm long *Alocasia /Colocasia* petioles having uniform diameter and mark the centre of each piece.
- Split each of them radially in to 4 strips of equal size.
- Place each piece on blank paper and mark the three points as given in the diagram.



2. Describe how soil water enters in to root hairs.

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Past Paper Questions

AL 1997 Bot

1. (i) What do you understand by passive movement of water in plants?

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(ii) Name the **five** types of passive movement of water in plants.

(a) …………………………………………………………………………………

(b) …………………………………………………………………………………

(c) …………………………………………………………………………………

(d) …………………………………………………………………………………

(e) …………………………………………………………………………………

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B. (i) Name the pathways of movement of water from soil solution to inner cortical cells of a root and define each of them.

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(ii) Which one of these pathways is unavailable for movement of water through endodermis?

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(iii) Indicate the reason for unavailability of this pathway for movement of water through endodermis.

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(iv) What is the advantage to the plant of blocking this pathway at endodermis?

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(v) Name four factors that are important for movement of water up in the stem of a tall tree.

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- Measure the initial curvature as the angle $A\hat{B}C$.
- Immerse four strips in each of the sucrose solutions and set aside with the lid closed for at least one hour to achieve osmotic equilibrium.
- Remove the strips from the solutions. Blot the excess solution using blotting paper and place on a sheet of paper.
- Draw the outlines of each strip to record the curvature again and measure the angle $A\hat{B}C$.
- Determine the change in curvature of each strip.
- Plot a graph using concentration of sucrose solutions (in X axis) against the percentage change in curvature (in Y axis).
- Determine the water potential of *Alocasia /Colocasia* tissues using data obtained.

Question

Molarity of sucrose solution	Initial angle of *Alocasia / Colocasia* strips	Final angle of *Alocasia / Colocasia* strips	Mean % change in curvature
0.15M	170 + 172 + 168 + 170	90 + 85 + 95 + 90	
0.20M	170 + 172 + 168 + 170	140 + 145 + 135 + 140	
0.25M	173 + 172 + 167 + 170	190 + 192 + 188 + 190	
0.30M	170 + 172 + 168 + 170	240 + 241 + 239 + 240	
0.35M	169 + 172 + 169 + 170	290 + 290 + 292 + 288	

(a) Calculate the percentage change of the lengths of *Alocasia /Colocasia* strips and enter the results in column 4 of the above table.

(b) Draw a graph to show the relationship between morlarity and percentage change of length in the space given below.

(c) Form the graph determine the molarity of the sucrose solution having a water potential equal to that of leaf stalk strips.

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(d) Give another parameter which could be used in a similar experiment for determining the water potential of Leaf stalk strips.

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Structured Essay

1. What is radial transport of water.

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2. What are 3 routes of radial transport.

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3. Define Apoplastic route.

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4. Define symplastic route.

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5. Define transmembrane route.

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6. Describe the process of upward movement of water in the plant body.

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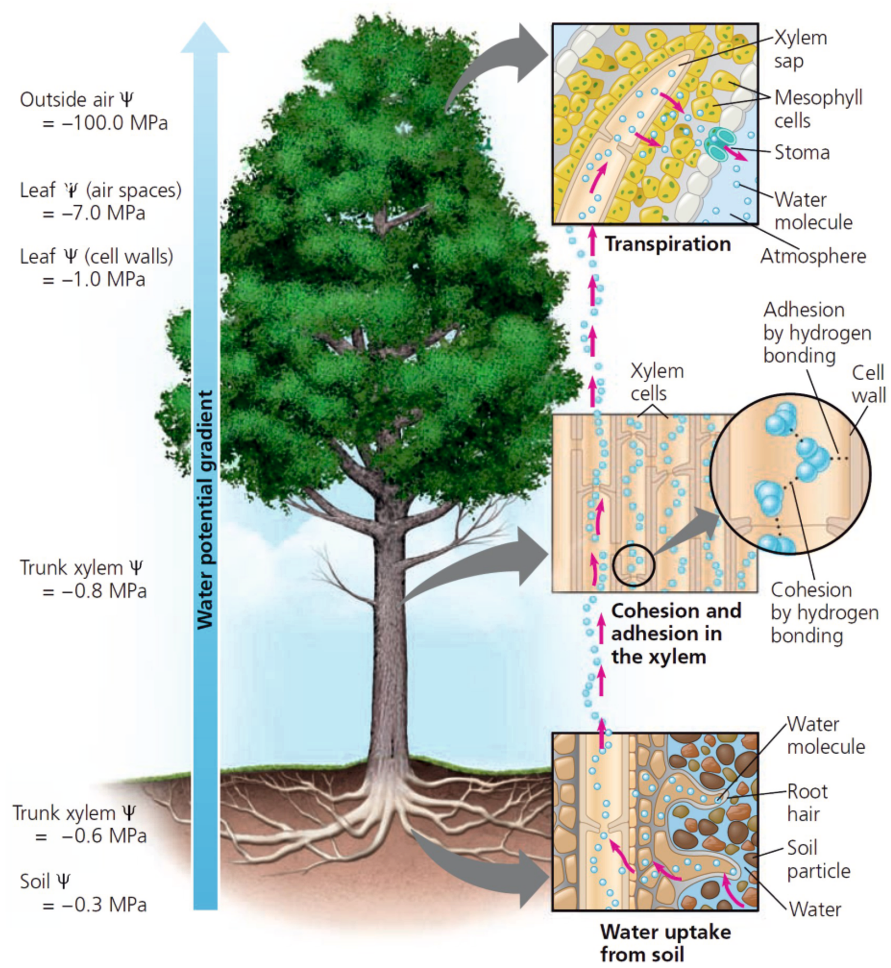
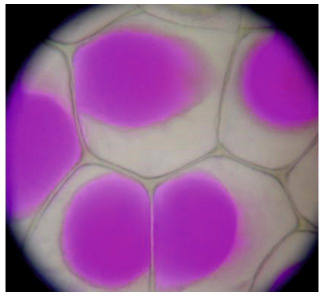
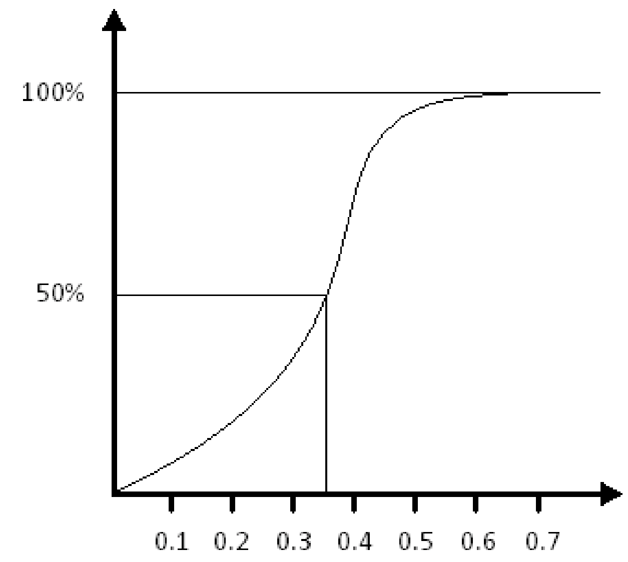


Mechanism of mineral absorption into root

Mineral ions are absorbed by the plant roots mainly from the soil solution. Epidermal cells are permeable to water and many epidermal cells are modified to form root hairs. Root hair cells are unicellular structures which absorb dissolved mineral ions from the soil solution. Soil solution has a lower concentration of ions than that of the cell sap of root hair cells. Therefore, absorption takes place against a concentration gradient.





PRACTICAL No : 20
Determination of solute potential of epidermal peels of *Tradescantia* (Rhoeo)/AL 2011

Objectives
1. Prepares solutions of known concentrations using stock solutions.
2. Differentiates between the status of flaccid, turgid and incipient plasmolysis of cells in Rhoeo epidermal peels through microscopic observations.
3. Prepares solutions of known concentrations using stock solutions.
4. Determines percentage plasmolysis of the tissue by making accurate observations under microscope.
5. Plots a graph to illustrate obtained data.
6. Determines solute potential of cells in *Rhoeo* epidermal peels using values obtained by the graph.

Materials and Equipment
- Fresh leaves of *Rhoeo*
- Six Petri dishes with lids (labeled 0.15M, 0.20M, 0.25M, 0.30 M, 0.35 M, 0.40 M)
- Six test tubes (labeled 0.15M, 0.20M, 0.25M, 0.30 M, 0.35 M, 0.40 M)
- Test tube rack
- Two 10.00 ml graduated pipettes
- Beaker with distilled water
- Beaker with 1M sucrose solution
- Fine forceps, razor blade/Microscope/Slides and cover slips/Graph paper

Instructions
- Instruct the students to prepare 20 ml of sucrose solutions of different concentrations as given (0.15M, 0.20M, 0.25M, 0.30 M, 0.35 M, 0.40 M) in each of the labeled test tubes by using the graduated pipettes, 1M sucrose solution and distilled water.
- Direct them to pour the prepared solutions from test tubes into Petri dishes.
- Ask students to take small fragments from the lower epidermis (purple coloured) of *Rhoeo* and place a few (2-3) fragments in each of the sucrose solutions in Petri dishes.
- Instruct them to set the Petri dishes aside with their lids closed at least for 20 minutes for the cells to achieve osmotic equilibrium.
- Direct the students to mount fragments of each of the epidermal peels on slides in a drop of the sucrose solution from which the peel is immersed.
- Let students examine under low power of microscope and select a clear field of cells and turn to mid power .
- Instruct the students to count the number of plasmolysed cells and total no. of cells within that particular field.
- Ask them to calculate percentage plasmolysis.
- Instruct the students to plot a graph of concentration of sucrose solution on X axis against percentage plasmolysis on Y axis.
- Direct students to determine the molarity of the sucrose solution that would give 50 % plasmolysis, from the graph. Calculate the solute potential of the sucrose solution from the table.
- Discuss the results obtained.
- Solute potentials of given sucrose solutions at 20^0C

(i) Following table shows experimental results.

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- Adhesion and cohesion facilitate transport water by bulk flow. Due to high adhesion water molecules are attracted to cellulose molecules in the xylem walls. Cohesion of water molecules is unusually high due to hydrogen bonds among water molecules. Therefore, a continuous water column is formed within xylem vessels and tracheids.
- Transpiration pull can extend down to the root only through an unbroken chain of water.
- As water evaporates from the mesophyll cells, water potential of mesophyll reduces, and water moves from cells of petioles to the mesophyll cells.
- It reduces the water potential of cells of petioles. Then water pulls upward due to this transpiration pull.
- The xylem sap is driven by difference in pressure potential. Therefore, the water potential gradient within xylem is essentially a pressure gradient.
- The tensile force on xylem sap is transmitted all the way from the leaves to the root tips and even into the soil.
- Therefore, water potential gradient between the soil solution and atmosphere through the plant body also help ascent of xylem sap, against the gravity.
- The plants do not need energy to lift the xylem sap.

- Some substances can use more than one route. The least resistance for the transport is found in apoplastic route. Therefore, more water use apoplastic route.
- Finally, water and minerals enter into the tracheids and vessel elements of xylem. These waters conducting cells lack protoplasts when mature and therefore they are parts of the apoplast.
- Endodermal cells and living cells of the vascular tissues discharge minerals from their protoplast to their own cell walls.
- Both diffusion and active transport involve in transport of solutes from symplast to apoplast. Then water and minerals can enter the tracheids and vessel elements to the transport to shoot system by bulk flow only through the apoplast.

Upward movement of water and minerals in a plant

	Number of turgid cells	Plasmolyzed cells	
0.15M	46	4	
0.20M	34	16	
0.25M	18	32	
0.30M	5	45	
0.35M	2	48	
0.40M	0	50	

(ii) Plot a graph to show above results. Find concentration of isotonic solution.

(iii) Calculate solute potential of epidermal peels.
(Solute potential of 0.05M sucrose solution at 20^0C= -130kPa)

(iv) What is the pressure potential of cells immersed in 0.1M, 0.2M, 0.3M 0.4 and 0.5M solutions

(v) Why it is important to mount epidermal peels using the same solutions which the peels were immersed.
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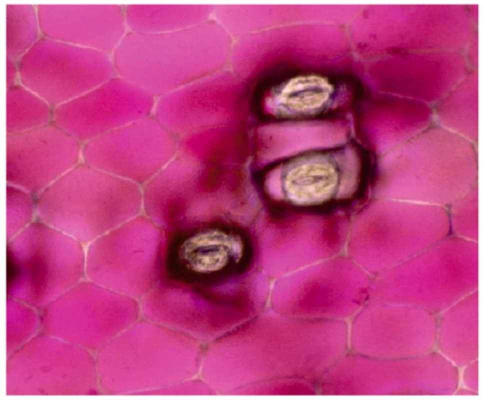
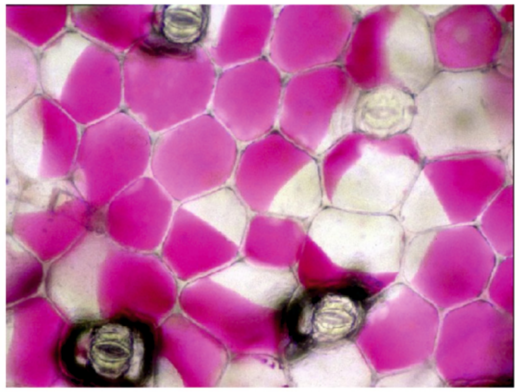
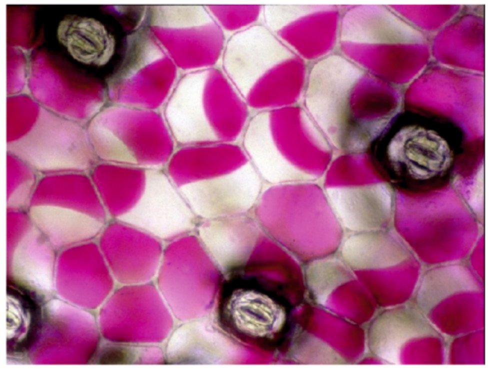
(vi) Determine the solute potential of the solutions in which the following X, Y, Z tissues were immersed.







Apoplastic route

- The apoplastic route consists of everything external to the plasma membrane of living cells and includes cell walls, extracellular spaces and the interior of dead cells such as vessel elements and tracheids.
- Water and solutes move along continuum of the cell walls and extracellular spaces and it is known as apoplastic route.
- Uptake of soil solution by the hydrophilic walls of root hairs provides access to the apoplast.
- Water and minerals then can diffuse into cortex along this matrix of walls and extracellular spaces.
- Endodermis blocks apoplastic route by a barrier located in the transverse and radial walls of endodermal cells, called the casparian strips.
- It is a belt made of suberin which is impervious to water and mineral salts. Thus water and minerals cannot cross the endodermis and enter the vascular cylinder via apoplast.
- Therefore, water and minerals cross the selectively permeable plasma membrane before entering the vascular tissue and keep unneeded and toxic materials out.
- The endodermis also prevents solutes that have accumulated in the xylem from leaking back into the soil solution.

Symplastic route

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- In the symplastic route, water and solutes move along the continuum of cytosol.
- This route requires substance to cross a plasma membrane once, when they first enter the plant. After entering one cell, substances can move from cell to cell via plasmodesmata.

Transmembrane route

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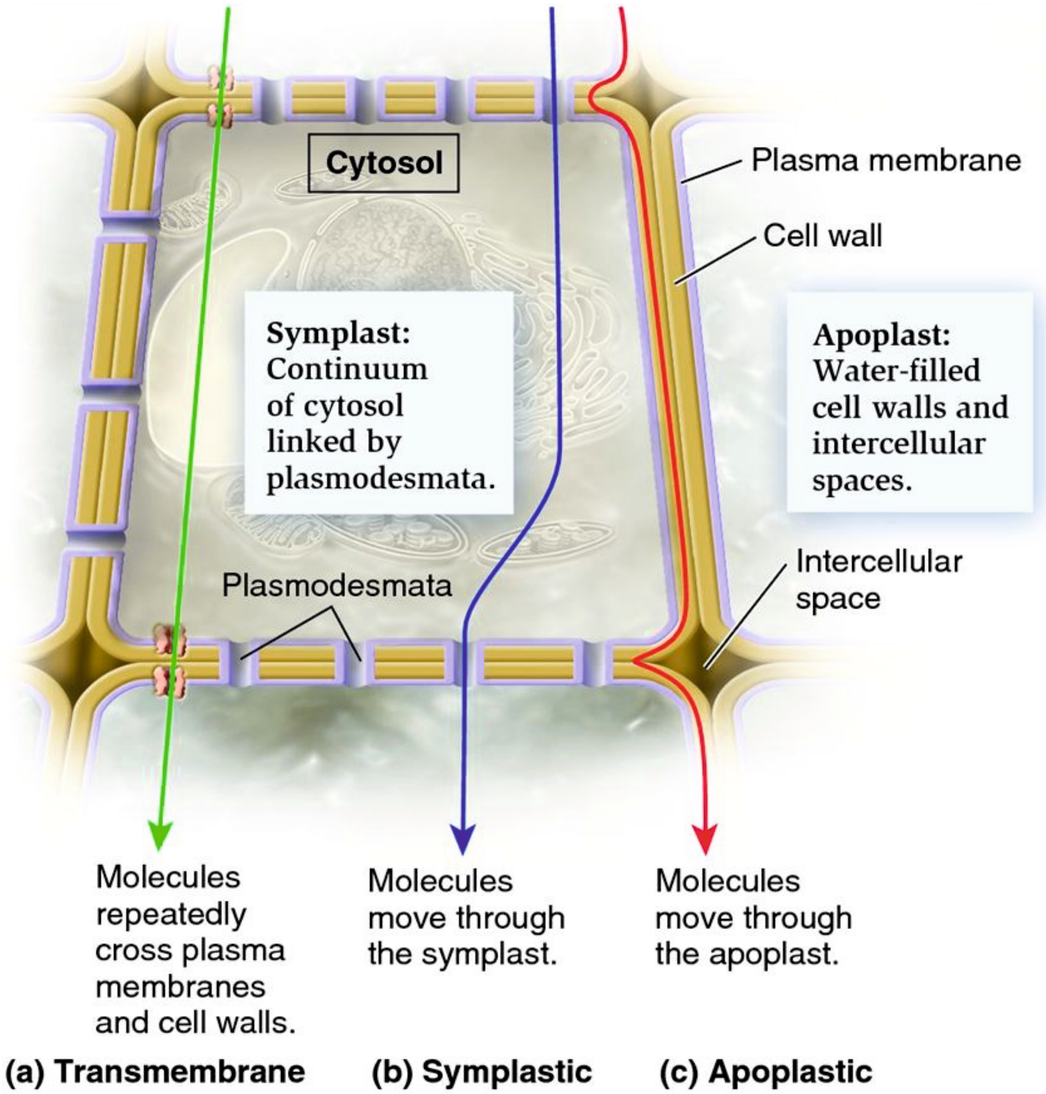
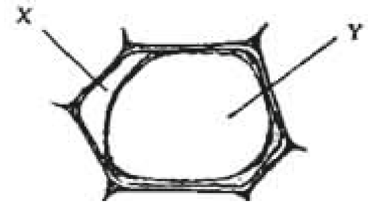
Radial transport

- Transport of water and minerals entered from soil to root cortex into the xylem of the root is known as radial transport.
- The endodermis, the innermost layer of cells in the cortex, functions as the last check point for selective passage of the minerals from the cortex into the vascular cylinder.
- All materials which enters root through cell walls and extracellular spaces should cross the membranes of endodermis.
- Therefore, unwanted materials can be selectively excluded. Three routes are used in the radial transport. They are:
  1. Apoplastic route
  2. Symplastic route
  3. Transmembrane route



AL 1995 Bot

(a) The diagram given below shows a cell of the lower epidermis *Rhoeo* leaf plasmolyzed by immersion in a sucrose solution. Indicate what you would find in the space labeled X and Y.



X— ....

Y— ....

(b) What is the most important advantage of using the lower epidermis of *Rhoeo* leaf in experiment of plasmolysis.

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(c) In an experiment to determine the osmotic potential of epidermal cells of Rhoeo, epidermal peels were immersed in a series of sucrose solutions of different concentrations. After 30 min the peels were observed under the microscope and cells were counted to determine the percentage of plasmolysed cells. The table given below shows the result of this experiment.

Concentration of sucrose solution	Percentage of plasmolysed cells
0·5 M	3
1·0 M	15
1·5 M	60
2·0 M	80
2·5 M	85
3·0 M	100

Using the above result draw a graph in the cage given below and determine the osmotic potential of the epidermal cells. (osmotic potential of 1M solution of sucrose = - 22.4 atm)

BioLanka.net
96×96 dots/inch

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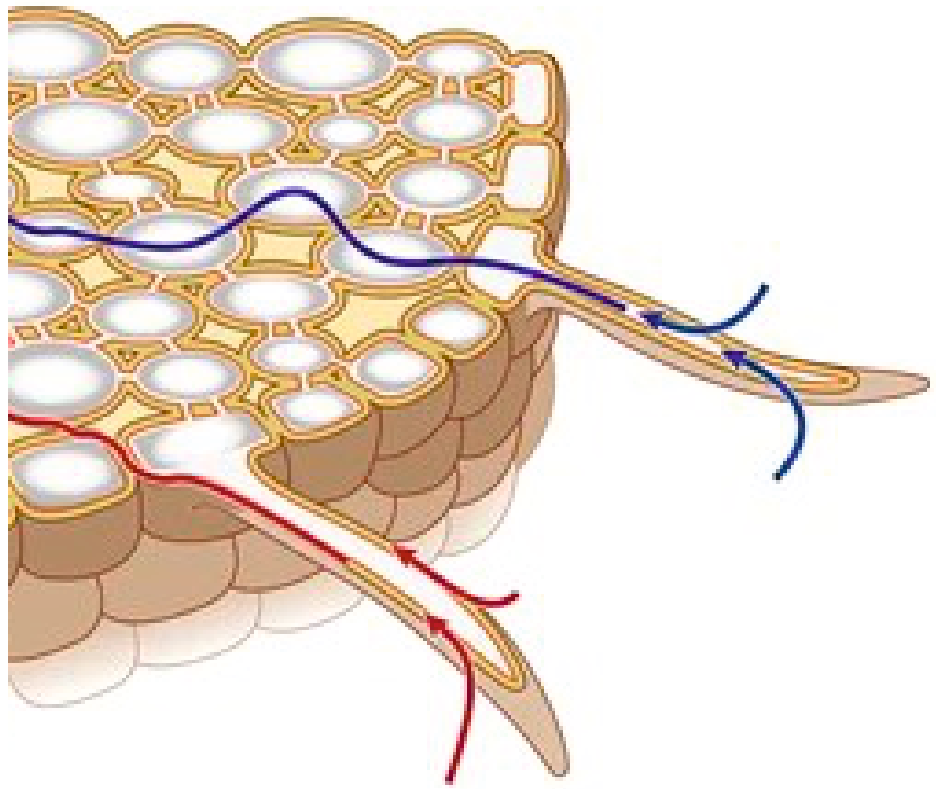
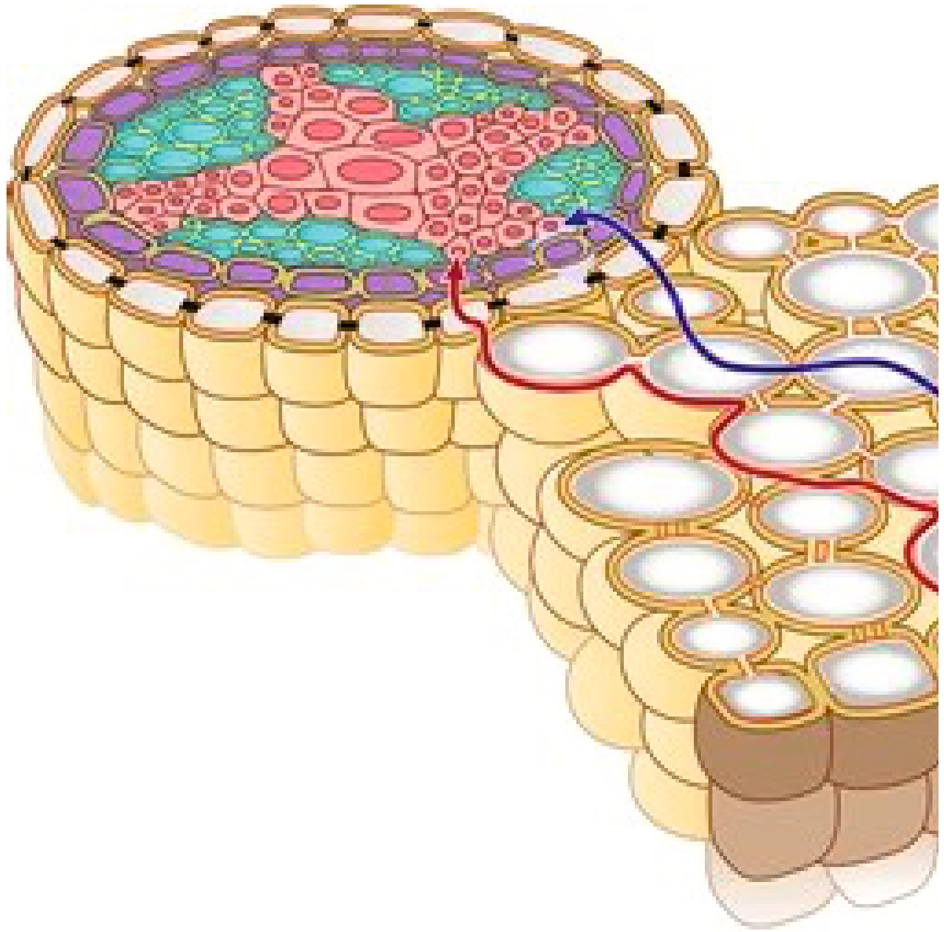
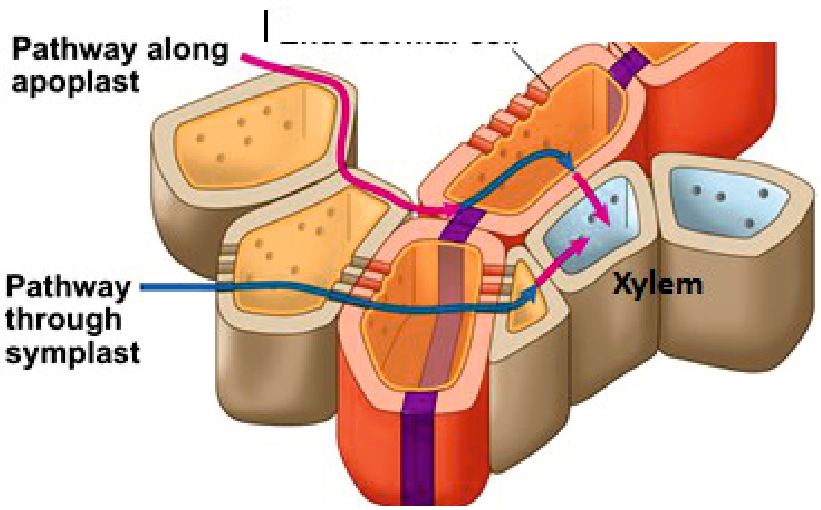






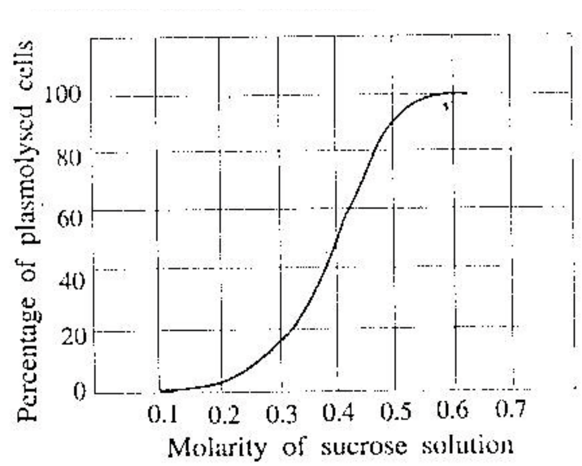
Pattern 5

18. In *Rhoeo* epidermal questions 50% plasmolysis occurs only at Isotonic solutions.
At Isotonic stage $\psi_w^{sol} = \psi_w^{cell}$
At isotonic stage cells are at incipient plasmolysis.
$\psi_w^{cell} = \psi_s^{cell} + \psi_p^{cell}$ $(\psi_p^{cell} = 0)$
$\psi_w^{sol} = \psi_w^{cell} = \psi_s^{cell}$

When two solutions given and when same epidermal peels are immersed in them, based on isotonic solution find ψ_s^{cell}
Two pieces of a lower epidermal peel of a Rhoeo leaf were immersed separately in two sucrose solutions labeled A and B. Solute potential of solution A was – 1450 KPa and that of solution B was – 1120kPa. After the tissues had come to equilibrium with the solutions, it was found that 50% of the cells in the peel immersed in solution A were plasmolyzed. Calculate pressure potential of the cells immersed in solution B?

A/L 2008

1. **I**n an experiment to determine the water potential, pieces of *Rhoeo* epidermal tissue were immersed in a series of sucrose solutions of different molarities. After one hour they were examined under the microscope and the percentage of plasmolysis in each was counted. The results are plotted in the graph given below. A table values of solute potential of sucrose solutions of different molarities is also provided.



Molarity of Sucrose solution	Solute potential in KPa
0.1	– 260
0.2	– 540
0.3	– 820
0.4	– 1120
0.5	– 1450
0.6	– 1800

A. (i) According to the results of the experiment what is the molarity of the sucrose solution causing incipient plasmolysis?

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(ii) What is the solute potential of the sucrose solution causing incipient plasmolysis?

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(iii)What would be the water potential of the epidermal cells at incipient plasmolysis?

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(iv) What would be the water potential of the epidermal cells immersed in sucrose solution of 0.1 molarity?

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(v) Is the value of solute potential of the above cells immersed solution of 0.1 molarity lower, higher or equal to their water potential?

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Movement of water and minerals from soil solution to plant root (2005/2009/2011)

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