

## Measuring the Water Potential of a Plant Tissue

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week #1 (06 September)

week #3 (20 September)

week #4 (27 September)

On 6 September you will be given three large potato tubers. Over the course of a two-week period you should subject your 3 tubers to different storage or treatment conditions that you think may have some effect on their water potential. At the end of the two weeks you will then measure the water potential of each tuber to determine the effects of each of your treatments.

In many cases the water potential ( $\Psi$ ) of a plant tissue can be considered to be equal to the solute potential ( $\Psi_s$ ) plus the pressure potential ( $\Psi_p$ ) and other terms (e.g. gravitational potential) can be ignored. This is indeed the case for potato tubers and we will assume that:

$$\Psi = \Psi_s + \Psi_p$$

You will measure the water potential ( $\Psi$ ) and the solute potential ( $\Psi_s$ ) which will then allow you to calculate the pressure potential ( $\Psi_p = \Psi - \Psi_s$ )

### PART I - measuring $\Psi$

One common method of measuring water potential ( $\Psi$ ) in plant tissues involves placing uniform sample pieces of tissue into a series of solutions of known water potential ( $\Psi$ ). It is best to use a solute such as mannitol, which is not readily taken up by plant cells. If the  $\Psi$  of the external solution is greater than that of the plant tissue, water will move into the tissue, causing an increase in weight. If  $\Psi$  of the solution is lower than that of the tissue, water will move out of the tissue, causing a decrease in weight. The object is to find that solution in which the weight of the tissue remains unchanged, indicating neither a loss nor gain of water. Such a situation would mean that the water potential of the solution was equal to the water potential of the tissue. Thus if one can calculate the  $\Psi$  of the external solution in which no weight change of the tissue occurred, one will know the  $\Psi$  of the tissue.

Prepare 12 beakers each containing 100 ml of one of the following solutions: distilled water, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50 and 0.60 molal mannitol solutions (the molecular weight of mannitol is  $182 \text{ g mol}^{-1}$ ).

Do the next operation quickly to prevent drying of the tissue from evaporation. Using a 6 mm diameter cork borer, obtain from a single potato tuber 12 cylinders, each about 4 cm long. Cut all 12 sections to a measured uniform length with a razor blade, leaving a clean transverse cut at the ends of each cylinder. Be sure to remove any peel from the ends of the cylinders. Place the cylinders between folds of a moist paper towel, where you have denoted the positions of the cylinders by the series of concentrations of mannitol that you will use. Weigh each cylinder (after quickly blotting it dry) to the nearest milligram. Immediately after weighing each cylinder, place it into its test solution. Do this for each cylinders, being sure that you accurately *record the initial weight of the cylinders placed in each test solution*. [Take the remains of the tuber, dice it

into small pieces, place it into a small flask covered with parafilm, and store it in the freezer so that you can use this tissue next week to determine  $\Psi_s$ ]. Repeat this process for each of your three tubers. Since each beaker will have three cylinders in it, one from tuber, be sure to identify the cylinders in some way so that you can tell them apart.

After 1 hr of incubation at room temperature, remove the cylinders comprising each sample, blot gently on paper towels, and *record the final weights*. Repeat this procedure until you have weighed all samples, in the same order in which you initially placed them in the test solutions.

*Organize the data in a table showing original weight, final weight, change in weight, and percentage change in weight, where:*

$$\% \text{ change in weight} = \frac{\text{final weight} - \text{original weight}}{\text{original weight}} \times 100$$

*Construct a graph plotting % change in weight (y axis) versus molality of the mannitol solution (x axis). Calibrate the x axis by calculating the water potential ( $\Psi$ ) for each mannitol solution.*

$$\Psi_s = -RTC$$

where:

R = gas constant ( $8.3 \times 10^{-3} \text{ kg MPa mol}^{-1} \text{ K}^{-1}$ )

T = absolute temperature (K)

C = solute concentration (molality =  $\text{mol kg}^{-1}$ )

*Using the graph, find by interpolation the mannitol concentration in which no change in weight occurred. Calculate the  $\Psi$  for this solution. This value then equals the water potential ( $\Psi$ ) of the tissue.*

## **PART II - measuring $\Psi_s$**

A common method of determining the solute potential of a solution is to measure the freezing point depression. You will carefully determine the freezing point of the sap from each of your three potato tubers. Since each degree of freezing point depression corresponds to -1.3 MPa of solute potential it will then be easy to calculate the  $\Psi_s$ .

Grind one of your tubers completely to a pulp with a mortar and pestle and filter it through cheesecloth into a clean flask. Store it on ice until you need it later.

Before making your actual measurements, it is important to make sure that your thermometer is reading accurately. In a small beaker, prepare a well-stirred mixture of ice and distilled water. This should have a temperature of exactly  $0.0^\circ$ . Place your thermometer into the mixture and if it reads  $0.0^\circ$  then you are all set to continue.

Place 15 ml of the secret solution X into a test tube containing a magnetic stir bar. Place the tube into a beaker containing a mixture of sodium chloride, ice, and water. Put

the whole apparatus on top of a magnetic stirrer. Stir the sap vigorously until it begins to freeze. Keep cooling the sap (with constant swirling) until you have a slurry containing a mixture of both frozen and unfrozen sap. It should have the consistency of a Slushie. Insert your thermometer into the slurry and carefully record its temperature. This will be the freezing point of the secret solution X.

Repeat this same process to measure the freezing point of each of your three tuber sap samples

*Once you have determined the freezing point depressions, calculate the  $\Psi_s$  for each of the tubers. Calculate the  $\Psi_p$  for each of the tubers.*

*How did  $\Psi$ ,  $\Psi_s$ , and  $\Psi_p$  vary between the three tubers that you measured? How might you explain this difference?*