Topic 07: **Water Relations, Osmosis and Transpiration**

A. Water Relations

Water plays a critical role in plants. Water is the universal solvent that allows biochemical reactions to occur in all organisms, but that is not the only importance in plants. Water moves from the roots to the shoots and to every cell in between. Water movements across membranes (**osmosis**) results in the development of **turgor pressures** (positive pressures) in plant cells. These turgor pressures are what keep the primary plant body upright on land and, when they are lost, the plant wilts (cells become **flaccid**). In addition, the turgor pressures in young developing plant cells is directly responsible for the expansion of these cells. Therefore, growth of the plant depends on the movement of water to growing regions like the apical meristems.

In addition, water movements facilitate nutrient transfer (minerals) from the roots to the photosynthetic tissues. Water in the phloem keeps important photosynthate compounds in solution for transport from sources (photosynthetic leaves) to sinks (non-photosynthetic organs, rapidly growing regions). So obviously water is extremely important to the health of a plant. In today's lab you will have a chance to investigate the importance of water uptake and movement at the cellular and whole plant levels. Enjoy!

B. *To Transpire or Not to Transpire…*

Water moves through the plant continuously due to severe differences in water potential between the soil, the plant and the atmosphere. The xylem vascular tissue takes advantage of the physical properties of water (**surface tension, cohesion, adhesion**) in order to move water from the roots to the shoot. No energy is expended in this process but other sacrifices are made. Water availability is a major limiting factor for growth of a plant, and if the **rate of transpiration** exceeds the uptake of water from the soil, then wilting can occur. In the wilted state, the plant cells do not have any positive pressure in them and thus cannot grow. So the need for regulation of transpiration is present.

Guard cells regulate the flow of water out of primarily the leaves (where most stomata are), but due to the nature of photosynthesis, guard cells need to be open for CO_2 to be available. This interaction results in a complex regulation of **stomatal aperture** in order to maximize photosynthesis while minimizing water loss. Like it or not, water is going to be lost (and a large amount of it at that) but the rate will depend on other environmental factors in addition to the anatomy of the plant.

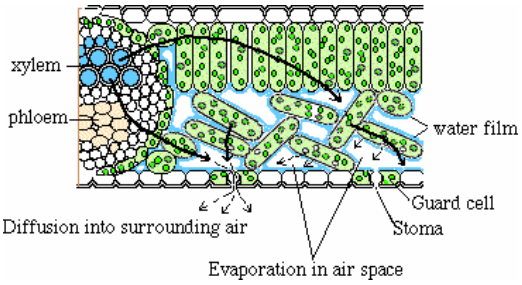

Fig. 1 Water movement from the xylem to the atmosphere.

C. Changes in Water Potential Reflect Changes in Plant Cell Turgor

Water transport in the xylem from the roots to the shoot (**transpiration**) and from cell to cell across a membrane (**osmosis**) responds to changes in **Water Potential (Ψ_W)**. Water Potential consists of several distinct components as seen in the equation below:

$$\Psi_W = \Psi_S + \Psi_P$$

Water moves in the direction of high to low water potential. Ψ_S refers to solute potential (also known as osmotic potential) and Ψ_P refers to **hydrostatic pressure** which is the pressure potential. This means that Water potential changes when either the concentration of solutes change or the pressure inside or outside the cell changes. When solutes like sodium ions (Na+) or chloride ions (Cl-) enter a cell the total solute concentration increases and this changes the water potential.

Keep in mind that there is a limit to how much solute can enter a cell. Changes in solute content also affect pressure such that increases in solutes result in increases of water intake which in turn gives rise to high cellular pressures. High cellular pressures mean that the cells are **turgid** (the environment is **hypotonic**). The cells of severely wilted plants have lost water in response to changes in the environmental water potential (such that water potential in the soil is lower that in the plant). These cells have **plasmolyzed** (the environment is **hypertonic**) under these extreme conditions. Plant cells that lose or do not gain water are referred to as **flaccid** (the environment is **isotonic** with respect to the plant) and also display wilting although not as severe as in plasmolysis.

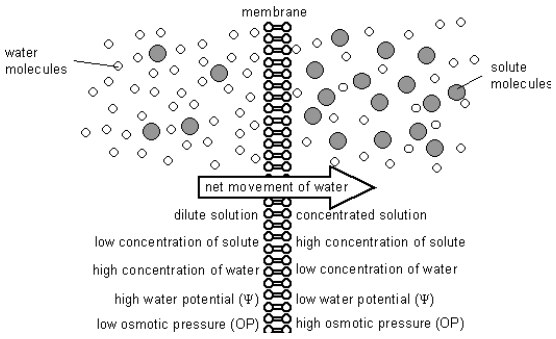

Fig. 2. Osmotic H_2O movements

D. The Transpiration Stream:

Work in pairs

1. Add approximately 1 inch of 0.1% toluene blue into a glass test tube.
2. Cut one corn plant (per pair of students) a ½ inch above the soil line.
3. Immediately insert the cut shoot into the toluene blue and place the tube in the rack near the high light environment.
4. Allow the treatment to continue for 30 - 40 minutes.
5. Observe the plant. Look for the presence of stain. Answer the following questions:

a) What is the furthest distance (into leaves) that the dye is visible (measure in cm)?

b) Can you discern a pattern regarding the presence of the dye? Describe it.

c) Cut a section of the shoot about an inch from the original cut and look at it with a dissecting scope.

1) Do you see the vascular tissues? How do you know?

2) Can you see any patterns regarding the presence of dye?

3) What is going on in the center of the shoot? Why?

d) Cut other sections of the shoot to continue following the transpiration stream.

1) Do you notice any changes in the number of bundles stained? Explain why.

e) Cut thin sections of various leaves – both stained and apparently unstained and compare them.

1) What staining pattern can you detect?

2) Is there any variation between leaves? Why?

E. "Transpirometer" Measurements under Environmental Conditions

*** Work in groups of 3-4 ***

1. Remove the plunger and needle (if present) from a 3ml syringe.
2. Seal the plastic "needle end" with parafilm.
3. Add 2ml of water to the sealed syringe using a second syringe with needle.
4. Place the syringe in a glass test tube → this is your transpirometer!
5. Cut a sunflower plant just above the cotyledons and IMMEDIATELY place the cut stem into the prepared syringe. Do this very quickly.

6. Bring the water volume up to 3ml with the plant in the transpirometer. Label the tube so you can recognize it (don't cover the number scale on the syringe).
7. Prepare a total of three transpirometers as described above.
8. Place the one plant/transpirometer in each of the conditions below for 60-75 minutes:

    a. High Light (back bench)
    b. Darkness (under benches)
    c. Medium Light + Wind (fans)

9. Record the amount of water lost (beginning volume minus final volume).

10. Weigh the leaf blades for each experiment (do this last) and calculate area as cm^2.

11. Calculate the rate of transpiration as $ml/min/cm^2$.

Hint: Determine total leaf area by first calculating the weight/cm^2 of the bean leaf by cutting a square leaf section 3 cm X 3 cm and weighing this leaf section. Divide the leaf section weight by 9 cm^2 to find the weight of 1cm^2 section of leaf. By dividing the total mass of the leaves by the mass of 1cm^2, you will determine the surface area in cm^2 of the leaves on your bean plant.

Transpiration Rate Data:

	Bean Leaf Treatments		
	High Light	Wind	Dark
Starting H_2O Volume			
Final H_2O Volume			
H_2O Lost			
Time			
Total Leaf Weight			
Weight/cm^2			
Total Leaf Area			
Transpiration Rate ($ml/min/cm^2$)			

16. NOW, plot the transpiration rates for each treatment on the graph below. Determine what graph format would be the best for comparison. Label the graph appropriately.

Which environment caused the most water loss? Why?

How does light environment affect transpiration?

How does a windy environment affect transpiration?

Why does total surface area need to be considered for all treatments?

When would you expect the most transpiration to occur naturally?

What plant adaptations can you think of that might decrease leaf water loss?

F. Turgor Pressure at the Cellular Level

Work in groups of 3-4

1. Prepare 11 beakers with 100ml of the following sucrose solutions:

   0 M; 0.1M; 0.2M; 0.3M; 0.4M; 0.5M; 0.6M; 0.7M; 0.8M; 0.9M; 1.0M

2. Using the vegetable or fruit available on your table, prepare five $1cm^3$ squares for each solution (for a total of 55 squares). Use the French fry cutter to make sticks and cut these sticks in 1cm lengths to obtain $1cm^3$ squares. Do not use squares that have skin or dead spots on them.

3. Rinse the squares in sets of five, blot them dry and weigh them prior to adding to a solution beaker (this is the Wt_I). Be sure to correlate weight to the specific solution.

4. Imbibe the tissues in the solutions for a minimum of 60 minutes.

5. After 60 minutes, remove the squares, blot dry and weigh them again (this is the Wt_F). Record this weight in the provided table.

	Wt_I	Wt_F	% Change	Wt_I	Wt_F	% Change	Wt_I	Wt_F	% Change
0 M									
0.1 M									
0.2 M									
0.3 M									
0.4 M									
0.5 M									
0.6 M									
0.7 M									
0.8 M									
0.9 M									
1.0 M									

	Wt_I	Wt_F	% Change	Wt_I	Wt_F	% Change	Wt_I	Wt_F	% Change
0 M									
0.1 M									
0.2 M									
0.3 M									
0.4 M									
0.5 M									
0.6 M									
0.7 M									
0.8 M									
0.9 M									
1.0 M									

6. Calculate the % weight change for each solution: $(Wt_F - Wt_I)/Wt_I$

7. Graph your results below. Report your findings to the rest of the class.

8. Record and graph the results from the other groups.

What are the hypotonic and hypertonic solutions for your specific tissue?

What are the isotonic points for each different tissue?

Which tissue had the highest amount of solutes? Explain your rationale.

Which tissue had the lowest amount of solutes? Explain your rationale.