Topic 12. Cells and Tissues of the Plant Body

I. Identifying Tissue and Cell Types in Cross Sections of Dicot Stems

Introduction: In this exercise you will make observations of various tissues and cells found in the stem of herbaceous eudicots: specifically a freehand section of Coleus stem, and the stained cross section of Medicago (Alfalfa) stem on a prepared slide. There are labelled images of these in our computer bank of images which your TAs can use to assist you. Further these same images are available on the course page for review.

Getting Started: If someone at you table has the knack for making good sections, have that person share with others.

Making a make freehand section of Coleus: Coleus plants are available on each student bench. Cut pieces of stem should be placed in the bowls of water at the front of each student bench for others to use. Before cutting into the stem, rub it between your fingers. Notice the square shape of the stem. This is a characteristic of all members of the Lamiaceae (the mint family). Do not use the razor blades in your drawer to make these sections. Use the razor blades provided in the petri dish at the front of each bench. These are particularly sharp. They should be used up one at a time, and everyone at a bench should use the same blade until it becomes dull at which point it should be thrown away and replaced by a fresh blade. Please be careful not to mix these blades with the ones in your drawers!

Preliminary Observations: After preparing a wet mount, observe the cross section with your 4x objective. The stem is made up of three different tissue systems. Please identify these in turn:

The dermal tissue system consists of the outer layer of cells of the stem. These cells make up the epidermis.

The vascular tissue system represented by distinct vascular bundles. These are arranged in a circular pattern inside the stem.

The ground tissue system consists of everything enclosed by the epidermis except the vascular bundles. Typically in eudicots the vascular bundles are arranged in a circular pattern embedded in the ground tissue. This arrangement of the vascular bundles divides the ground tissue into two regions: pith and cortex.

After identifying these tissue systems with your Coleus slide, repeat the process with the prepared slide of Medicago.
Ia. The Epidermis: The epidermis functions to control the loss of water from the plant. It accomplishes this primarily through the excretion of a waxy layer called the cuticle. Like a sheet of wax paper, the cuticle limits the passage of water. In doing this it also blocks the passage of gasses between the plant and its environment. To keep the tissues from suffocating, the epidermis must have openings. These openings are called stomata (singular = stoma).

Structure

Stomata: Stomata are not easily seen in a freehand section, hence, look for these using the *Medicago* cross section. First identify large intercellular spaces directly below an area of epidermis using low power. These are substomatal chambers and they are positioned directly below each stoma. Switch to 400x and observe the stoma and its two guard cells. The level of turgor of these two cells serves to open and
close the stoma controlling the movement of gasses and water into and out of the underlaying tissue.

**Trichomes**: Another way the epidermis limits the plant’s loss of water is through the growth of hairs called trichomes. Switch back to your section of *Coleus* and observe these hairs projecting from the epidermis. Can you think of any other possible adaptive function of trichomes?

![Trichomes](image)

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**Function of the Guard Cells**

Guard cells must react to environmental conditions. It is adaptive for stomata to open in the light during times that the plant is not water stressed. It is generally maladaptive for the stomata to be open at other times. The closing of a stoma by its guard cells is ultimately a phenomenon of osmosis though mechanisms exist to translate environmental conditions to the turgor pressure of the guard cells. In the following exercise we will simply observe the response of the stomata to a change in the turgor of the guard cells of *Setcreasea*.

**Procedure**: Work in pairs. Set aside your section of *Coleus* stem and prepared slide of *Medicago* for use next section.

1. Make an epidermal peel from the lower surface of a *Setcreasea* leaf. Mount the peel in distilled water and cover with a coverslip. Avoid trapping air bubbles under your wet mount!

2. Scan your slide to find an area with few air bubbles and with open stomata.

3. Switch to high power. In the space below draw an open stoma including guard cells and subsidiary cells.

4. While the field of view of your microscope is centered on an open stoma, place a large drop of 10% solution of NaCl next to one side of your coverslip. On the other side place a piece of tissue to wick up the distilled water to draw the salt solution under the coverslip. Observe any changes. Make a drawings of the stomata below on the next page.
Ib. Ground Tissue

Collenchyma in the Cortex

Retrieve your section of Coleus stem, place it on the stage of your microscope and observe with your 4x objective. Switch to a higher power and observe the cells types at the corners of the stem in the cortex. This region of the ground tissue includes collenchyma tissue. Collenchyma has thickened primary walls. Primary walls are elastic and collenchyma provides static resistance to turgor pressure. This balance of forces in herbaceous stems and leaves provides support. Switch to your Medicago slide. This stem also has collenchyma positioned at the ribs. Note, however, that in this preparation the cells are dehydrated and their walls are uniformly thick. Collenchyma is both a cell type and a type of tissue consisting of collenchyma cells.
Collenchyma in the Petioles of Celery

The strands that can be peeled from celery are made of collenchyma tissue. Get a piece of celery at the front. You may eat this tissue, but while you do so break a piece of it and peel out these stands. Note that they are elastic.

Make a cross section of your celery petiole to observe the collenchyma.

**Draw collenchyma and parenchyma cells at a boundary between the two tissue types.**

Parenchyma of the Pith

Using your *Coleus* section observe the pith (the region of the ground tissue inside the ring of vascular bundles). In *Coleus*, this region is composed entirely of parenchyma tissue consisting of **parenchyma cells**. Parenchyma is both a cell type and a tissue type made up of parenchyma cells. Parenchyma cells have thin primary walls. Stain your section with I₂KI. Based on your observation of the staining, what is one obvious function accomplished by this tissue?

Ic. Vascular Tissue: Switch to the slide of *Medicago* stem. Vascular tissue includes **xylem** and **phloem** both of which are complex tissues: that is they each consist of more than one cell type. In these two dicot stems the **xylem** occurs in the innermost area of each vascular bundle facing the pith. The most prominent cells here in the xylem are **vessel elements**. These are large dead cells with secondary walls that stain red. Vessel elements function as pipes through which water moves by mass flow. These are surrounded by **parenchyma cells** which are also part of the xylem tissue. The **phloem** is positioned to the outside of each vascular bundle. The phloem of *Medicago* includes **sieve-tube elements**, **companion cells**, **parenchyma cells**, and **fibers**. Try to identify as many cell types as possible. The cell types in vascular tissue will be more fully explored using *Cucurbita* slides later.

**Label the Figure on the next page** - include xylem and phloem; use your own arrows to label epidermis, pith, cortex, and collenchyma.
II. Demonstration of Sclerenchyma Cells: Sclerenchyma cells have thick secondary walls.

IIa. Fibers: Observe the two views of fiber cells at the front bench. One microscope has a prepared slide of a woody Tilia stem. Fibers on this slide can be seen in cross section in the bark. Note their thick secondary walls. Compare this view with that of the second microscope. On this microscope is a slide of macerated Tilia bark. Here the fibers have been physically disassociated from the bark and can be seen on their side. Note their length. Fibers are like cables, and lend strength to plant tissues.

IIb. Stone Cells: These cells are not elongated but have massive secondary walls. Observe the preparation on the third microscope of the macerated pear flesh. These stone cells have simple pits which are also forked. Stone cells give pear fruit its gritty texture.
III. Studying Vascular Tissues in Cross and Longitudinal Sections of the Stem of Cucurbita

Take a prepared slide of Cucurbita and examine it by holding it up to the light. Note that there is both a cross section and longitudinal section on the slide. Now observe the slide under your microscope.

IIIa. The Xylem

Cross Section: Survey the cross section of the stem at 40x. Note the two rings of vascular bundles arranged around a hollow pith. Observe a vascular bundle at 100x. Note the huge empty cells with the secondary walls. These are vessel members. The xylem tissue is made up of these vessel cells together with the parenchyma around them.

Longitudinal Section: Move your stage to view the stem in longitudinal section. Note, because of the hollow pith, it forms two sections seemingly unconnected. In this view the vessels can be viewed for what they are, continuous tubes running longitudinally through the stem. The most prominent vessels are those that differentiated after the stem stopped elongating. Xylem tissue that differentiates after elongation ends in a plant organ is termed metaxylem. The metaxylem vessels have a complete cylinder of pitted secondary wall. If you look carefully you should also see vessels with incomplete secondary walls. The secondary walls here consist of either hoops or spirals. These are protoxylem vessels. Protoxylem differentiates while elongation is occurring. The incomplete secondary walls inside the primary walls prevents the vessel from collapsing and yet allows it to be stretched without ripping. Protoxylem vessels with hoops are said to have annular thickenings. Those with spirals are said to have helical thickenings. It is unlikely that you will see both on your slide so observe the demonstration scopes of these vessels on the side bench.

IIIb. The Phloem

Cross Section: Look again at a vascular bundle in cross section. Note that in Cucurbita, phloem lies both to the inside and outside of the xylem. Carefully search the area of the phloem for views of sieve-tube members with a sieve plate in clear view. Locating a sieve plate is the surest way of identifying this cell type. Once you locate a sieve tube member note its size relative to the other cells. Also note that, in Cucurbita, these cells have primary walls that seem thicker than that of their neighbors. If you have difficulty locating a sieve tube after three minutes ask your TA for help. The smaller cells with the dense cytoplasm associated with the sieve tube members are companion cells. Also note that in Cucurbita phloem includes not only sieve tube members and companion cells, but also parenchyma cells.

Longitudinal Section: Switch back to the longitudinal section of this stem. Observe an area inside or outside of the xylem to locate a region of phloem. Note the arrangement of the sieve tube members. These are stacked directly one on the other to form a continuous structure, the sieve tube. Look for sieve plates at the junctures of these cells to confirm their identity. Also look for companion cells in this section.

Observe and label the figures on the next two pages.
Cucurbita: Longitudinal Sections of Vascular Tissue

Vessels in the Xylem

Annular Secondary Wall Thickening
Helical Secondary Wall Thickening
Pitted Secondary Wall Thickening

Phloem Tissue

cc = companion cells
stm = sieve tube member
*Cucurbita*: Cross Sections of Vascular Tissue

Phloem:

A = ________________

B = ________________
IV. Function of Vascular Tissues

IVa. Movement of Water Through the Xylem

In the xylem, water moves through tracheary elements which are dead at maturity. Living protoplasts are unnecessary for this movement to occur. In this activity you will determine the validity of the idea. At the start of lab, your TA placed a shoot of Coleus into a dye solution containing a poisonous salt (CuSO₄). The copper ions will kill any cells contacted and the dye will stain the areas to which the water is drawn.

**Procedure:** Work with the people who sit at your table (three groups per section). Take the shoot at your table and determine if the dye was drawn up the stem and how far. Make a cross section of the stem stained with the dye and determine in what tissue the dye is located. Record the distance the dye moves up the stem. Also record the results from the other two tables below. One bench will have the Coleus shoot placed in moving air (from a fan). Another will have the shoot exposed to ambient room conditions, and the third will have the shoot covered with a plastic bag. Record your observations and those of your classmates in the table below.

<table>
<thead>
<tr>
<th>Moving Air</th>
<th>Ambient Air</th>
<th>Covered</th>
</tr>
</thead>
</table>

Did the dye move up the stem? ________________________________

If so how far? __________________________

In what tissue did you view the dye in your cross section? __________________________

Explain the differences in the rate of movement, if any, between the three conditions.

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IVb. Movement through the phloem

The most widely accepted theory to explain the movement of photosynthate from source to sink (often from leaf to root) is the pressure flow hypothesis. By this idea, substances move through the sieve-tube members along a gradient of turgor pressure. This is created by the active transport of sucrose into the phloem at the source and by its active export out at the sink. The loading and unloading of sucrose results in the osmotic movement of water into the phloem at the source and out of the phloem at the sink. For this mechanism to work, an area bounded by living membranes is necessary, hence, sieve elements must be alive to function.

If the pressure flow hypothesis is true would you expect the pressure in the phloem at the hypocotyl of a squash seeding to be (check one):

- At ambient (air) pressure
- Less than ambient pressure
- Greater than ambient pressure

In this activity we will see which is correct.

Procedure:

- Work in pairs.
- On the side bench is a flat of squash seedlings with a cylinder of alcohol in front of the flat. While both you and your partner are observing, quickly cut off the shoot at the ground level, and place the cut stump into the alcohol and observe the emersed stump. What do you observe?

Based on your observations, is the phloem under pressure relative to air in the room?
Discuss your observations after cutting the shoot a second time with your TA. This relates to the p-protein found in the sieve-tube elements.

Notes:
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