Name: _____ Date/Lab time: _____

Lab 6: Comparison of Leaf Anatomy of C_3 and C_4 Species

Put details in your plant forms table C3 C4, monocot leaf and dicot leaf

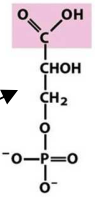
Supplies:

Fresh samples or prepared slides of the following: C_4 dicot ex. *Amaranthus* C_3 dicot ex. Bean C_3 monocot ex. wheat or lawn grass C_4 monocot ex. corn	Carrots for sectioning Lugol's iodine Razor blades (**new!**) Prepared slides of *Zea* and *Syringa* CAM plant (see *Aloe* in slide box)

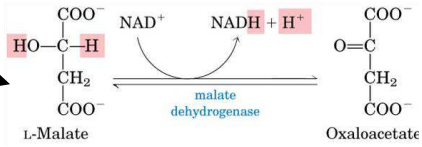
LAB SYNOPSIS:

We will examine the anatomical differences between leaves of plants that do C_3 photosynthesis with those that do C_4 photosynthesis.

Introduction



C_3 photosynthesis- the first product of photosynthesis is a 3 carbon molecule called phosphoglycerate. This is the first molecule formed as part of the Calvin cycle.



C_4 photosynthesis- the first product of photosynthesis is a 4 carbon molecule of oxaloacetate or malic acid. Eventually the carbon (COO^-) is released as CO_2 to enter the Calvin cycle.

We will look at how leaf anatomy differs between C_3 and C_4 plants. The main difference is the bundle sheath cells surrounding the leaf veins. Bundle sheath cells are cells that encircle the vascular bundles of leaves (leaf veins).

Recall, for C_4 plants, the goal is to concentrate CO_2 at the site of the enzyme rubisco in the bundle sheath cells. To do this, they initially, temporally, fix CO_2 into a C_4 molecule outside the bundle sheath cells. They then move the C_4 molecule to the bundle sheath cells and release the CO_2 onto rubisco. Thus avoiding photorespiration and increasing the efficiency of photosynthesis. Because of this, the bundle sheath cells of C_4 plants are BIG with BIG chloroplast. This structure is known as Kranz anatomy. The bundle sheath cells of C_3 plants on the other hand are less obvious.

PROCEDURE-Vein morphology in C_3 vs. C_4 plants. *Whole leaves*:

Set up 2 dissection microscopes. Remove leaves from *Amaranthus* (a C_4 dicot) and from bean (a C_3 dicot). Using bottom illumination examine each under the scopes. It is all about comparison. Look at the regions between major leaf veins (i.e. the minor veins). You should be able to identify C_4 leaves vs. C_3 leaves.

Which leaf, C_3 or C_4, shows dark green veins? _____

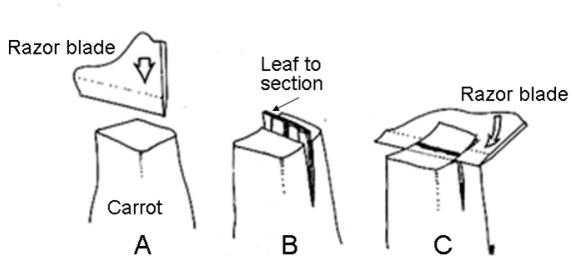
Which leaf, C_3 or C_4, shows transparent veins (lots of light showing through)? _____

Note how this relates to anatomical differences in C_3 vs. C_4 leaf cross sections that we will look at next. i.e. how does this relate to the chloroplasts in bundle sheath cells?

We will now examine the internal structure of C_3 vs. C_4 leaves.

PROCEDURE- Leaf sections (free-hand sectioning):

Getting good cross-sections of leaves takes some patience. The secret is to support the piece of leaf, and to cut many sections (10-12). You don't need perfect cross-sections. Do the *Amaranthus* leaf first, because it is thicker, it is easier to get sections; it's also more interesting to look at! Then try wheat, followed by corn and bean (use the ones your group started during out first lab).



To support the leaf, cut a carrot into a rod shape and make a small incision in one end (See A); into this slit the leaf, is carefully placed (B). Sections are now cut of carrot tissue with the inserted leaf at the same time (C). It is usually easier to get a thin section if you section at a slight angle. Good luck.

Float the sections onto a drop of water on a microscope slide. When ready to examine, put on a cover slip.

After making observations of the fresh sections, place a drop of iodine solution along one side of the cover slip and use a razor to lift the cover slip up and down a few times to pull the iodine solution around the sections. The starch grains will appear as black structures inside the green chloroplasts.

Hint: it might be easier to set up 3 microscopes, one with a leaf cross section of *Amaranthus* (a C_4 dicot) another with bean (a C_3 dicot) and of corn (a C_4 monocot). Then you can easily go back and forth, noting the differences in leaf anatomy.

PROCEDURE- Observations & Drawings

Draw what you see. Note the leaf veins. How do the bundle sheath cells vary between C_3 and one C_4 leaves? Label the tissues (upper and lower epidermis, mesophyll cells, bundle sheath, xylem and phloem). Make a note of the starch distribution, as well. Be sure to identify the bundle sheath in the C_3 leaves, so that meaningful comparisons can be made of that structure in the two leaf types.

Drawing of C_3 leaf (free-hand section)	Drawing of C_4 leaf (free-hand section)

PROCEDURE- Leaf sections (Prepared slides):

Hint: it might be easier to set up two microscopes, one with a leaf cross section of *Zea* (a C4 plant) and the other with *Syringa* (a C_3 plant). Then you can easily go back and forth, noting the differences in leaf anatomy.

C_4 leaves and Kranz anatomy

Examine a prepared slide of a leaf cross-section of the Monocot *Zea* (corn), identify the tissues present. Note the vascular bundles in the corn leaf. Surrounding each vascular bundle is a ring of cells called the bundle sheath. Note the large bundle sheath cells containing large chloroplasts. This type of leaf anatomy is called "Kranz anatomy" and is typical for C_4 leaves.

C_3 leaves

C_3 leaves do not have Kranz anatomy. C_3 leaves have small bundle sheath cells.

Examine a prepared slide of a cross-section of the Dicot *Syringa* (Lilac) leaf; identify the tissues present as above. *Syringa* (Lilac) is a C_3 plant.

How does C_3 leaf anatomy, compare to the Kranz anatomy of a corn leaf? Look at the bundle sheath cells.

In the plant kingdom, C_3 photosynthesis is the more common form, however, C_4 photosynthesis is found in some Dicots and in some Monocots.

PROCEDURE- Observation & Drawings:

Draw the appearance of the cellular patterns in cross sections of one C_3 and one C_4 leaf, showing a vein in cross-section. Label the tissues (upper and lower epidermis, mesophyll cells, bundle sheath, xylem and phloem). Be sure to identify the bundle sheath in the leaves, so that meaningful comparisons can be made of that structure in the two leaf types.

Drawing of C_3 leaf (prepared slide)	Drawing of C_4 leaf (prepared slide)

If time permits, take a look at leaf anatomy of a CAM plant "*Aloe* leaf". Note how it differs from both C_4 and C_3 plants.

Questions from lecture notes relative to your drawings above:

l. In which cells (bundle sheath cells or mesophyll cells) is atmospheric CO_2 initially fixed in a C_3 leaf? In a C_4 leaf?

2. What is the name of the carboxylase for initial CO_2 fixation in each (C_3 vs. C_4)?

In which cells is the carboxylase located?

3.a. In which cells is the Calvin cycle and rubisco in a C_3 leaf?
b. In a C_4 leaf?