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Photosynthesis, a Look inside the Leaf

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This cheap and easy photosynthesis lab is an alternative or complement to gas exchange and light absorbance labs. Students will understand the relations between structure and function in photosynthesis. After learning the functional differences between C3 and C4 plants, students will try to locate in easily available fresh leaves where they think most cells are doing photosynthesis based on chloroplast abundance. Students prepare fresh leaf cuts, and epidermal peels. Learn basic leaf anatomy and look for evidence of photosynthetic locations. Students draw the structure and explain the path from sugar producing cells to the phloem and from outside CO_2 to the fixation sites. If time allows, students are provided leaves from different habitats and relate adaptations such as leaf cuticle, sunken or exposed stomata, leaf thickness and other traits to the environment the leaves are from and the challenges they face. Prepared slides of standard C3 and C4 leaves, mono and dicots are desirable to complement fresh samples that turn out to be a little thicker. This lab requires only microscopes, razor blades, glass slides and leaves picked around campus or at the grocery store.

Keywords: photosynthesis, leaf anatomy, leaf epidermis, stomata

Introduction

This lab attempts to connect the physiology of gas exchange in photosynthesis to the anatomy and histology of where it happens. It intends to have students relate structure to function in a practical hands-on way. In its simplest version, the observation of life leaf epidermis, students will see that epidermal cells do not have chloroplasts and are completely clear, a protective sheet from water loss and parasite invasion. They will see the stomata, hopefully some open, and see that they are gates that open for CO_2 at the risk of water loss. If using leaves from different ecosystems, observing the adaptation to drought or lack of them in the leaf cuts, as thickness of cuticle and possible sunken stomata (De Micco *et al.* 2012; Rotondi *et al.* 2003). Comparing the number of stomata on the upper and lower epidermis, (better use the nail polish approach for this one) will give students the opportunity to thing about where the danger of water loss is highest, the exposed top or the bottom, and relate and draw the path of carbon dioxide into the parenchyma palisade cells where it will be most needed, students will see on the fresh leaf slices that this area has the highest density of cells with chloroplasts.

If comparing C3 to C4 leaves students will be able to look at the different distribution of vessels and pattern of cells containing chloroplasts. At this point is it desirable to have some stained prepared slides to relate how a different physiology goes with a different anatomy, and to see how convenient it is for sugar producing cells to be so close to the phloem. It can be related to how factories were built on the river banks to facilitate shipping the goods by boat.

The easiest part of this exercise is the epidermal peel, with a succulent broad leaved plant students can do it fairly easily if they have the slide ready with a drop of water to immediately apply the peeled epidermis.

Epidermal imprints with nail polish are also easy and fast.

Leaf cuts can be trickier, it is easier with high quality new blades and on a surface other than glass, the top of a plastic Petri dish or styrofoam work well. The choice of leaves should fall on softer leaves, such as geraniums or similar, brittle leaves will be very hard to work with, those are better looked at in prepared rather than fresh slides. Not all cuts will be good, using about four in each slide, and again having the water drop waiting for the leaf slices does help. Everybody can easily have their own peeled epidermis, good leaf cuts can be shared. Students can draw and labels their leaf epidermis and leaf slices, it is helpful to have some textbooks or plant anatomy atlases in lab to look at and label drawings, but a projected slide or drawing on the board will also do the job well.

Student Outline

Objectives

Relate structure and function in photosynthesis
Understand the value of leaf adaptations to avoid water loss
Have a better understanding of plants and how they function

Introduction

Why would you care about photosynthesis? Think about your last meal, it all came from photosynthesis, usually by a plant.

Land plants originated from aquatic plants about 400 million years ago (Berner 1997). To adapt to living out of water several protective structures evolved to protect the plant from water loss, since plants cannot move to find water at a more suitable location, and to provide support for a self-standing structure. The epidermis with its flat tightly bound cells on the outer layer of tissues is important to prevent water loss, much like skin cells in animals.

Plant cells are eukaryotic, they have a nucleus and typical eukaryotic organelles: mitochondria, ribosomes, endoplasmic reticulum and Golgi apparatus. They also have extra structures found exclusively in plants: cell walls made out of cellulose, cytoplasmic connections between cells through the walls called plasmodesmata, a large central vacuole, and plastids, such as chloroplasts. In plants, as in animals, cells specialize to serve specific functions and are grouped into tissues.

Just as a reminder of the ins and outs in photosynthesis look at the equation:

$$6\ CO_2 + 6\ H_2O \longrightarrow C_6H_{12}O_6 + 6\ O_2$$

We will focus on the tissues that do photosynthesis and on the path of gases in and out of them, you will have a chance to have a look right into a fresh plant leaf that you will cut, and see for yourself where all the action on making your food happens.

Parenchyma or general purpose cells are the most common type of living differentiated cell. They have thin primary cell walls, different shapes, some of them are specialized for photosynthesis others in storage of food or water in different organs. They are alive and can usually divide. We will look those that are doing photosynthesis, and will notice differences in most leaves between the upper part of the leaf, palisade parenchyma and lower spongy parenchyma. On top facing sunlight you have cells that have the most chloroplasts, those on the lower leaf are more spaced allowing the CO_2 and O_2 to diffuse between them on the way to the top layer of palisade parenchyma cells.

If you compare C3 and C4 leaves you will be able to see that in C4 leaves there are cells surrounding the phloem vessels, the bundle sheet cells, remember that their function is to produce the sugars that they conveniently load on the phloem right next to them, much like how factories were built on the river banks to facilitate shipping the goods by boat. It is helpful if you pull out your textbook or find a plant atlas in lab to help you identify/label your samples.

Materials

- Fresh leaves of flat leaved succulent and other like geranium (3 per student)
- Single edge new razor blades (1 per student)
- Plastic Petri dish or Styrofoam for cuts, 1 per student
- Forceps 1 per 3 students
- Needle 1 per student
- Microscope with 40X objective (1 per two students)
- Glass slides and coverslips (2 per student)
- Textbook or plant histology atlas (1 per 4 students)
- Prepared leaf transversal cut slides of C3 and C4 plant if doing C4 (1 per 4 students)
- 15% NaCl

Methods and Data Collection

Part A: Leaf Epidemis

This tissue forms the outer surface layer of cells of plant structures, leaves, stems, roots or fruits. The cells of this tissue are flattened and tightly packed. Epidermal cells have a protective function; they protect the plant from water loss while allowing gas exchange. Epidermal cells may secrete waxes to further waterproof the plant, the cuticle, abundance of waxes, is usually related to drought protection. In leaves stomata will be among epidermal cells, they open and close to allow gas exchange and minimize water loss (Weyers and Travis, 1981).

Leaf Peel

Take a few leaves provided. Hold the leaf with the lower side up, fold the leaf and pull sideways to peel out the lower epidermis (Fig. 1). Mount it on a drop of water on a glass slide, add cover slip. Observe on the microscope the tightly bound epidermal cells, they look transparent. Observe also the stomata scattered among the epidermal cells, they are the ones made of two bean shaped cells (guard cells) and have chloroplasts. Some may be open. If you wish to see them close add a drop of 15% NaCl to the side of the cover slip. Draw and label.

Figure 1. Fold and pull to obtain lower leaf epidermis.

Leaf Epidermis Print

Use this method when the leaf peel does not work.

Paint about 1.5 cm of the leaf epidermis with a thin layer of clear nail polish. Let dry at least two minutes, cover with glossy sticky tape, peel off slowly and stick the tape with the epidermal peel on a glass slide and observe. This method is suitable to count stomata. Draw and label.

Part B: Leaf Transversal Cuts

In a leaf transversal cut you will have a view of the whole inside of a leaf. Get a glass slide, put a drop of water on it. Obtain a leaf, place it on an inverted plastic Petri dish. Trim the area you want to cut to a width of about 0.5 cm. Get a new single edged razor blade and hold it perpendicular to the leaf. Make 4 to 6 very thin cuts. So thin that they are barely visible. You may have to try a few times. Place each cut immediately on the water drop, add a cover slip. Observe on the microscope. If cuts are too thick, repeat. Share the best cuts with your colleagues. You may find stomata visible in the middle of the epidermal cells. Try to follow the path of the gases that have to get in and out through the stomata to the cells that do photosynthesis. Draw and label.

Notes to the Instructor

Leaf choice depends on what you want to do. For the basic relation of structure/function in C3 plants any plant you can get epidermis from will do, try what you have around, you will not need much material, usually giving a student about three leaves is good because they may fail a few times. For the leaf cuts choose a leaf that is not hard, any C3 will do, but some are harder to cut than others, geranium works well and works also for the epidermis peel, although it is harder than succulents. The produce section of the grocery store can also be a source of leaves. The better razor blade you get the easier it will be. I divide students in groups of 4. They will share slides and help each other. This lab is thought as an addition to your regular photosynthesis lab, but can be an independent lab if you add an ecological component to it, as comparing adaptations of leaves from different ecosystems, or comparing C3 and C4 plants. Asking students to draw and label may be old fashioned but it does work.

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About the Author

Catarina Mata teaches Biology for both majors and non-major and Plant Biology at the Borough of Manhattan Community College since 2007. She did her PhD At the University of Utrech, The Netherlands, in Plant Ecophysiology. She is still interested in plant ecology and physiology and does research with students on environmental topics and sustainability.

Mission, Review Process & Disclaimer

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