The Huntington Library, Art Collections, and Botanical Gardens

Impressions of a Stoma

Overview

Students use two different methods to view stomata on the underside of leaves.

Introduction

Plants exchange the gasses involved in photosynthesis and respiration through **stomata**, which are pores in a leaf epidermis. Water also evaporates from the surface of the leaf through the stomata, a process called **transpiration**, which is one of the driving forces of water uptake and transport throughout a plant.

The plant can regulate gas exchange or water loss through its stomata by opening and closing them. This function is regulated by the **guard cells**, oval-shaped cells surrounding the pores. When the cells are turgid and full of water, they unevenly swell, and the stomata are opened up. To close the stomata, the plant sends water out of the guard cells and as they go flaccid, the pores are closed up.

Plants also can reduce transpiration by having stomata in recessed pits and/or surrounded by hairs. This will create local areas of humidity even in dry conditions.

Motivation

You probably heard at an early age that plants produce oxygen and use the carbon dioxide that animals breathe out. Taking that as it is, how are plants doing their half? We know that animals breathe in oxygen and breathe out carbon dioxide, but without lungs, how exactly do plants exchange gasses? Would you believe that they take air and water in and out of their mouths?

In this lab, we'll look closely at the pores on the leaf that are the primary sites of gas exchange and the transpiration of water. The pores are called stomata (singular stoma) which indeed comes from Greek word for "mouth".

Objectives

Upon completion of this lab, students should be able to

1. Correctly locate and identify stomata on a leaf.
2. Explain the role of stomata in the daily functioning of a plant.
3. Evaluate the effect of daily light cycles on stomata opening/closing.

Materials

- Razor blades
- Variety of plants, incl. Zebrina
- Glass slides
- Clear nail polish
- Clear Scotch tape
- Microscopes
- Permanent markers

Associated California State Biology Standards

1a. Students know cells are enclosed within semipermeable membranes that regulate their interaction with their surroundings.

1f. Students know usable energy is captured from sunlight by chloroplasts and is stored through the synthesis of sugar from carbon dioxide.

Procedure

Part I: Viewing Zebrina stomata

1. Give each student group a leaf from a Zebrina plant.
2. Have students carefully use a razor blade to slice a small square of the leaf, tiny enough to be placed on a microscope slide.
3. Students should place the leaf section on a slide or Petri dish and view under a dissection scope. It may help to use a dropper to apply some water to the section to prevent it from drying.
4. Have students look for and focus on the green guard cells surrounding the stomata. The surrounding epidermal cells are purple, so the stomatal areas stand out quite clearly.
5. Students should draw and label the cells and features in their viewing field.

Part II: Making an imprint slide of the stomata

6. Assign student groups to obtain leaves from a variety of available plants. It may help to leave the leaf on the plant for the experiment. They should record information about the source plant on the table on their student sheet.
7. Each group should carefully apply clear nail polish to a section on the underside of the leaf, let dry, then place clear tape to the area and peel off. Groups can repeat this procedure for a few different leaves.
8. Each student can apply the tape from each peel to a microscope slide then view the tape and impression under magnification.
9. Students should make approximations of the stomatal density and fill in the chart on their student sheet in order to make conclusions about the density in different plants under different conditions. In order to make the density approximation, they should count all stomata in a clear field of view on a given magnification. If you would like to convert this into stomata per millimeter, you will need a micrometer to measure the diameter of the field of view for the microscope your class is using. When you have that number, students should use it to find the radius and then the area of the field of view, which can be calculated with $pi*r^2$, the area of a circle. Finally, have students divide the number of stomata by the field of view area to get the stomata per square millimeter.

Evaluation

The following questions are listed under the Analysis section of the student handout and maybe used as part of a report, class discussion or assessment.

1. Compare the impressions from the under and upper surface of the leaves. Explain your findings.
2. Compare the stomatal density among different species of plants. Do they differ? Why or why not?
3. Compare the stomatal density between two plants of the same species grown in different conditions. Do they differ? Why or why not?
4. On which plant(s) and where on each plant are stomatal densities highest?
5. Why do stomata from the Zebrina leaf sections tend to be closed when viewed under the scope?
6. When might stomata be found more on the upper surface of a leaf?

Extension Activities

1. Based on the observations from this lab, students could form hypothesis about the stomatal densities on a given plant or in a given environment, then test these hypotheses.
2. The class or groups of students could devise tests to determine whether the density of stomata on an individual plant can be determined by environmental factors. For instance, two plants (or two groups of plants) could be grown from seed, with one grown under normal conditions and the other under high light, CO2, water stress, etc. The density of stomata can be tested after a sufficient growth period.

Test Preparation:

1. Which of the following, circle all that apply, occur during the light-dependent reactions?
   - **(A) Oxygen is released**
   - (B) Carbon gets reduced
   - **(C) Oxidative phosphorylation**
   - **(D) ATP is produced**
   - **(E) Electrons flow through an electron transport chain**
   - (F) Oxidation of NADPH
   - **(G) Reduction of NADP**

2. Which of the following, circle all that apply, occur during the light-independent reactions?
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   - (C) Oxidative phosphorylation
   - (D) ATP is produced
   - (E) Electrons flow through an electron transport chain
   - **(F) Oxidation of NADPH**
   - (G) Reduction of NADP

Student Sheet: Impressions of a Stoma Name:_____

Procedure

Part I: Viewing Zebrina stomata

1. Obtain a leaf from a Zebrina plant.
2. Carefully use a razor blade to slice a small square of the leaf, tiny enough to be placed on a microscope slide.
3. Place the leaf section on a slide or Petri dish and view under a dissection scope. It may help to use a dropper to apply some water to the section to prevent it from drying.
4. Focus on the green guard cells surrounding the stomata. The surrounding epidermal cells are purple, so the stomatal areas stand out quite clearly.
5. On a separate sheet of paper, draw and label the cells and features in their viewing field.

Part II: Making an imprint slide of the stomata

6. Obtain leaves from a variety of available plants. It may help to leave the leaf on the plant for the experiment. On the table below, record the appropriate information about your source plants.
7. Carefully apply clear nail polish to a section on the underside of the leaf, let dry, then place clear tape to the area and peel off. Repeat this procedure for a few different leaves, from different species and/or from the same species in different conditions. Pick one leaf do a peel on the upper surface of the leaf instead.
8. Apply the tape from each peel to a microscope slide then view the tape and impression under magnification.
9. Make approximations of the stomatal density (in stomata per millimeter) for each peel. To do this
    a. Count all stomata in a clear field of view on a given magnification. Record your count on a separate sheet of paper.
    b. Your teacher will use a micrometer to measure the *diameter* of the field of view for the microscope your class is using.
    c. When you have that number, use it to find the *radius* of your viewing field and then the *area* of the field of view, which can be calculated with $pi*r^2$ (the area of a circle).
    d. Divide the number of stomata you counted by the field of view area you just calculated to get the stomata per square millimeter. Record your findings in the table provided below.

	Species	Part of leaf from which peel was taken	Leaf description (color, succulence, texture, etc)	Description of plant's environment (wind, temp, shade, etc.)	Stomata density (stomata/square mm)
Sample A					
Sample B					
Sample C					
Sample D					
Sample E					
Sample F					

Analysis

On a separate sheet of paper please complete the following:

1. Compare the impressions from the under and upper surface of the leaves. Explain your findings.
2. Compare the stomatal density among different species of plants. Do they differ? Why or why not?
3. Compare the stomatal density between two plants of the same species grown in different conditions. Do they differ? Why or why not?
4. On which plant(s) and where on each plant are stomatal densities highest?
5. Why do stomata from the Zebrina leaf sections tend to be closed when viewed under the scope?
6. When might stomata be found more on the upper surface of a leaf?