Stomata Investigations

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Overview

In this lab you will learn a very simple technique to make a cast of the outer surface of plant tissues. Using your cast and a microscope, you will see different types of epidermal cells. After identifying the structures that define a pair of guard cells and their accompanying stoma, you will design an experiment to test the distribution and/or function of stomata in land plants.

Background

Stomata are gaps in the epidermis of plant tissues that are bordered by a pair of guard cells. Stomata are the avenue of gas exchange for the plant. They are also the avenues of water loss through the process of transpiration. The mechanism that controls the opening or closing of stomata is based on water potential of the guard cells compared to their surrounding cells or environment. Stomata open as a result of water moving into the guard cells. That process is regulated by the active transport of K+ ions into the guard cells. The active transport of K+ ions decreases the water potential of the guard cells, causing them to take up water. The increased water in the cells causes the guard cells to change shape and opens the stomata. Stomata usually are open during the day and closed at night, balancing the need for photosynthesis and water conservation.

Procedure

1. Obtain a leaf and dry it if necessary. Paint a small section (not more than 1 cm^2) of the underside of the leaf with clear fingernail polish. Let the polish dry completely (510 minutes).

2. Place a small piece of clear adhesive tape onto the painted portion of the leaf. Press gently. Lift the tape off the leaf. The patch of fingernail polish should adhere to the tape.

3. Place the tape and fingernail-polish cast sticky side down onto a clean microscope slide. There is no need for a cover slip because the tape keeps the sample aligned.

4. View the cast under a microscope. High power (400x) is the best magnification for viewing detail, but low power (100x) may be the best magnification for counting the number of stomata in a field of view.

5. Observe the stomata that appear as the space between pairs of guard cells. The stomata may be open or closed. See diagram.

http://www.colorado.edu/geography/class_homepages/geog_3251_sum08/01_stomata.jpg

Courtesy of Graham Kent

6. Count the number of stomata in one field of view under low power (100x). If there are too many to easily count under low power, switch to high power (400x). Record your data and move to two additional fields of view to count the stomata. Average your three counts.

   Microscope magnification: _____

   Number of stomata: Trial 1_____Trial 2_____Trial 3_____Average_____

7. Calculate the average density of stomata per mm2 using the following technique:

   Low power (**100x**) field of view diameter = 1.760 mm (verify this with a millimeter ruler and your microscope).

   Area of low power field of view = πr^2 or (3.14)(.880)(.880) = 2.43 mm^2

   Average stomata counted from your data table / 2.43 mm^2 = stomata/mm^2

   High power (**400x**) field of view diameter = .440 mm (verify this with a micrometer ruler).

   Area of high power field of view = πr^2 = (3.14)(.220)(.220) = .15 mm^2

   Average stomata counted from your data table / .15 mm^2 = stomata/mm^2

   Which magnification did you use?_____

Show calculations:

8. From your initial observations, ask a question about stomata. Think about the distribution and function of stomata on your leaf. Do you think that all leaves from this plant are the same? Do you think that leaves on different plants have the same distribution? Do all plants have stomata? What plan parts have stomata? Can you control the opening and closing of stomata? How are stomata formed? Do edible vegetables have stomata? How many stomata are on a typical leaf? What other questions do you have?

   State a question here:
9. Design a controlled experiment to answer your question:

   Design a data table to record your results. Think about how many observations you need to make to see a pattern. Is one sample enough? Think about ways to collaborate with your classmates. Can you find someone who is interested in a similar project?

10. Explain your results and share them with your class.

This activity is very useful in helping students understand the significance of a trend in observation. It is very unlikely that all stomata will respond the same way. How many open or closed stomata are necessary to see a pattern? Your students could record the numbers of stomata that are open or closed to find a percentage for comparison.

Some factors will increase success in student observations:

- Be sure to use clear or transparent adhesive tape. DO NOT USE magic, disappearing, or cloudy tape. Clear packing tape or very inexpensive clear adhesive tape works.
- If two or more conditions are being compared, students should put all samples on one slide. It is much easier to compare the samples by moving the slide around than by switching slides and refocusing.
- Be sure that the leaves or any plant parts that are experimented with are dry.
- Have students interested in the opening and closing of stomata work with leaves that are still or very recently connected to plants. Once a leaf is picked, it may change quickly.
- DO NOT take a stomata print of a very valuable leaf. The process of taking the print damages the leaf.

Possible Directions

While it is important for students to develop their own questions, be ready to point them in a direction. Projects to explore include:

- View stomata on one type of plant at different times of the day to determine when stomata open and close.
- Compare leaves from different parts of one plant to view density or action of stomata on leaves in the sun or shade.
- Compare stomata density of the upper and lower surfaces of leaves.
- Choose a plant such as dandelion that has stomata on both upper and lower plant surfaces to determine the difference in density. Explore other nonwaxy leaves to find other upper surface stomata.
- Look for stomata in ferns and possibly moss. Compare the density in these plants. (Most moss do have stomata, but they are hard to see.)
- Determine the effects of desiccation or changes in water potential on leaves with open stomata. (Dandelions are a good choice for this.) Try soaking leaves in 1 M sucrose solutions or just leave them in the air after picking them.
- Compare stomata density and location on different types of vegetables such as lettuce, cabbage, green onions, asparagus, broccoli, etc.

- Compare the arrangement or density of stomata on monocots and eudicots.
- Compare the arrangement or density of stomata on conifers and angiosperms.
- Compare the arrangement or density of stomata on plants that are sun tolerant and those that are not sun tolerant.
- Compare the size of stomata on different types of plants.
- Compare the action of stomata on plants kept under different wavelengths of light.
- Compare the density or arrangement of stomata on c3, c4, and CAM plants.

Expectations

Students should be able to articulate a finding with their experiment. Their data collection should include multiple trials, an obvious pattern, and a reasonable conclusion. While most students need to have an answer, it may not be possible to see differences with their experimental conditions. It is acceptable to state that no difference was observed. Students should record their results in a data table that makes sense for their observations. Help them understand that one data table would not work for all observations. With all students doing different experiments, it would be very meaningful to have a "stomata seminar" for them to share their results with their classmates.

References

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