Biology Lab
Stomata: The Gateway to the Leaf

Introduction: It is easy for us to think of roots as absorbing organs responsible for obtaining water and minerals for the plant. It is a little less obvious that leaves are also absorbing structures. They are structures well designed to absorb a gas (carbon dioxide) from the atmosphere. The stomata are, however, “necessary evils”. When the stomata are open to let in carbon dioxide, water vapor escapes into the atmosphere. The stomata are generally found on the underside of the leaf to help prevent water loss. A stomate is formed by two specialized epidermal cells called guard cells. Guard cells regulate the passage of water vapor, oxygen, and carbon dioxide into and out of the leaf. When the guard cells are very turgid, they pull apart, opening the stoma. When the guard cells lose water and become flaccid, they collapse, closing the stoma.

Purpose: To observe the opening and closing of the stomata, and to estimate the number of stomata in a leaf.

Materials: Fresh leaves Microscope Microscope slides
Cover slips Glucose solution

Procedure:
1. Hold the inner surface of the leaf toward you. Tearing action should peel off portions of the lower epidermis which will appear as a narrow, colorless border along the torn edge.
2. Remove a very small fragment of the colorless layer and immediately place it in a drop of water on a clean microscope slide. Cover with a cover slip. Do not allow the leaf fragment to dry out.
3. Look at the specimen under the low power of your microscope. You will see that it consists of epidermal cells which may look like pieces of a jigsaw puzzle. Look for the smaller, bean shaped cells which occur in pairs. These are the guard cells.

Observations:
1. Do you notice any differences between epidermal cells and guard cells?
2. Look at the specimen under high power. What differences in thickness, if any, do you see in the guard cell walls?
3. What is the name of the opening, or pore, between the guard cells?
4. Make a drawing of a pair of guard cells and the surrounding epidermal cells. Label the guard cells, epidermis, and chloroplasts.
5. By now you have observed that there are many, many of these pores that we call stomata in the epidermis of leaves. Using your 10x objective (with a field of view of 1500 microns) approximate the number of stomata in one field of view. Multiply this number by 170. This gives you a relative idea of the number of stomata in a circle one inch in diameter. How many stomata are present on the average leaf?
6. As long as these pores are open, there will be an opportunity for a free exchange of gases between the external atmosphere and the internal atmosphere of the leaf. What kinds of gases will pass through the stomata? In which direction will they be moving?
7. During photosynthesis, what gas will enter the stomata in greater amounts than it exits?
8. What gas will exit from the stomata in greater amounts than it enters during photosynthesis?
9. What is turgor pressure? What happens to the guard cells when they are very turgid?

Procedure:
Remove your slide from the microscope. Gently lift up the cover slip and blot off the water with a paper towel. Immediately add a drop of glucose solution to the specimen and replace the cover slip. Make a drawing of the stomata, guard cells, and epidermal cells as they now appear.

10. What change did you observe in the guard cells when the glucose solution was added?
11. What happened to the water in the guard cell when the glucose solution was added?
12. Explain the mechanism for the opening and closing of the stomata.
13. Why is it necessary for the stomates to close at night?
14. Where are the stomata usually found on a leaf? Where would the stomata be found on a floating water lily plant?
15. How would the number of stomata on a desert cactus be different than the leaf you used in this lab?

Name __________________________
1. Do you notice any differences between epidermal cells and guard cells?

2. Look at the specimen under high power. What differences in thickness, if any, do you see in the guard cell walls?

3. What is the name of the opening, or pore, between the guard cells?

4. Make a drawing of a pair of guard cells and the surrounding epidermal cells. Label the guard cells, epidermis, and chloroplasts if visible.

5. How many stomata are present on the average leaf?

6. As long as these pores are open, there will be an opportunity for a free exchange of gases between the external atmosphere and the internal atmosphere of the leaf. What kinds of gases will pass through the stomata? In which direction will they be moving?

7. During photosynthesis, what gas will enter the stomata in greater amounts than it exits?

8. What gas will exit from the stomata in greater amounts than it enters them during photosynthesis?

9. What is turgor pressure? What happens to the guard cells when they are very turgid?
10. Make a drawing of the stomata, guard cells, and epidermal cells as they now appear.

What change did you observe in the guard cells when the glucose solution was added?

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12. Explain the mechanism for the opening and closing of the stomata.

13. Why is it necessary for the stomates to close at night?

14. Where are the stomata usually found on a leaf? Where would the stomata be found on a floating water lily plant?

15. How would the number of stomata on a desert cactus be different than the leaf you used in this lab?
1. Do you notice any differences between epidermal cells and guard cells?

The guard cells will be bean shaped and will be seen in groups of two. They are smaller in size than the larger, boxier epidermal cells.

2. Look at the specimen under high power. What differences in thickness, if any, do you see in the guard cell walls?

The cell wall of the guard cell is thicker. Turgor pressure inside a guard cell is high. The cell wall is thicker and stronger to withstand the pressure inside the guard cell.

3. What is the name of the opening, or pore, between the guard cells?

The opening is the stoma.

4. Make a drawing of a pair of guard cells and the surrounding epidermal cells. Label the guard cells, epidermis, and chloroplasts.

![Diagram of leaf structure]

5. How many stomata are present on the average leaf?

Student answers will vary.

6. As long as these pores are open, there will be an opportunity for a free exchange of gases between the external atmosphere and the internal atmosphere of the leaf. What kinds of gases will pass through the stomata?

Carbon dioxide will enter. Water vapor and oxygen will exit.

7. During photosynthesis, what gas will enter the stomata in greater amounts than it leaves them?

Carbon dioxide

8. What gas will exit from the stomata in greater amounts than it enters them during photosynthesis?

Oxygen and water vapor

9. What is turgor pressure? What happens to the guard cells when they are very turgid?

Turgor pressure is the pressure of water inside a plant cell. When guard cells are very turgid, they stretch farther apart causing the stoma to open.

10. What change did you observe in the guard cells when the glucose solution was added?

The guard cells collapse and the stoma closes.

11. What happened to the water in the guard cell when the glucose solution was added?

The guard cells will lose turgor pressure and will collapse. The glucose solution is hypertonic to the guard cells causing the guard cells to lose water.
12. Explain the mechanism for the opening and closing of the stomata.

There are several explanations for the opening and closing of the stomates.

1. The movement of water into and out of the guard cell is controlled by the movement of potassium ions. When potassium ions accumulate inside the guard cells, it lowers the water potential and water moves into the guard cell making them more turgid. An exodus of potassium causes water to leave the guard cell and the guard cell collapses. The movement of potassium into and out of the guard cell is probably a passive movement. It is controlled by the proton pumps. Proton pumps actively pump hydrogen ions out of the guard cells. The resulting membrane potential drives potassium into the cells through ion channels.

2. Plants have a biological clock and the opening and closing of the stomata is a circadian rhythm. Stomata open during the day and close during the night. Stomata continue to open and close on an approximately 24-hour clock even when switched to continuous light.

3. Low concentrations of CO₂ inside the leaf cause stomata to open.

4. Light causes the stomates to open.

13. Why is it necessary for the stomates to close at night?

Photosynthesis cannot take place at night so there is no need for the stomata to remain open. If the stomates stay open, the plant will lose too much water and wilt. To conserve water it is necessary for the stomates to close when there is no need for carbon dioxide.

14. Where are the stomata usually found on a leaf? Where would the stomata be found on a floating water lily plant?

Stomata are usually found on the lower surface of the leaf. This helps to conserve water loss by keeping the stomates out of direct sunlight. In a water lily the stomates would be found on the upper surface.

15. How would the number of stomata on a desert cactus be different than the leaf you used in this lab?

A desert plant would have fewer stomata due to the need to conserve water.

Teacher Preparation Notes

1. Any type of fresh leaf will work for this lab. Ask students to find and identify various plants on campus for a comparison.

2. A 10% glucose solution works well. Mix 10 mL of Karo syrup with 90 mL of water.

3. An alternative for visualizing stomata:
   a. Coat the underside of a leaf with clear nail polish and let dry.
   b. Place a small piece of clear tape on the polish and press down to ensure even contact.
   c. Slowly peel up the tape and place it sticky side down on a clear microscope slide. On the tape should be a thin (clear) layer of epidermis along with the polish.