Chapter 7

Regulation of Gas Exchange of Terrestrial Plants

Objectives

Leaves. Understand the relationship between structure and function of the cells and tissues of leaves.

Stomata. Know the function and regulation of stomata. Know the importance of water in agriculture and the rationale for studying guard cells. Understand how guard cells are expected to respond to some environmental conditions (*e.g.* CO_2 concentration, light, and drought).

Experimentation. Understand the principles and procedures of the experiment. Experience data collection and analysis.

Leaf structure[1]

As do roots and shoots[2], leaves consist of three major tissue systems: dermal tissue, ground tissue, and vascular tissue, which all arise from primary meristem activity. These systems are continuous throughout the plant body. Within leaves, sugars are produced by reduction of CO_2 during photosynthesis generally in parenchyma, which is part of the ground tissue system. Sugars produced from photosynthesis are transported (bulk flow mechanism) from leaves to sinks, which are heterotrophic (*e.g.* roots) by the phloem (vascular tissue). Phloem transport is driven by positive pressure created by osmotic influx of water. Water is transported throughout the plant body by the tracheary elements of the xylem (vascular tissue) via negative pressure driven by evaporation of water from the leaf. The water content in leaves is very high. The waxy cuticle excreted by the leaf epidermis prevents leaves from losing too much water; however, some water loss is necessary to bring nutrients to the leaves. In addition; however, the waxy cuticle prevents uptake of CO_2, into the leaf, which is necessary for photosynthesis. Thus, to allow gas (water vapor and CO_2) exchange in terrestrial plants, the leaf epidermis is perforated by adjustable pores, or stomata. Each stoma is flanked by a pair of guard cells, highly specialized epidermal cells, which are embedded in the tightly packed layer of epidermal cells. The pair of guard cells regulate the aperture of the stoma to balance water loss and CO_2 uptake. Guard cells balance water transport and photosynthesis; therefore, guard cells are regulated by many external (*e.g.* light) and internal (*e.g.* ABA synthesized by water-stressed roots and transport via xylem to guard cells) factors associated with both water status and photosynthesis. When the guard cells are stimulated (*e.g.* by light) to increase the aperture of the stoma between them, the first step to opening is activation of the H^+-ATPase in the plasma membrane of the guard cells. The energy from the hydrolysis of ATP drives the energetically uphill excretion of protons resulting in a more negative charge within the guard cells; thus creating a driving force for K^+ influx. The accumulation of K^+ causes osmotic water influx. Guard cells have specialized cell walls that are thick and rigid around the pore and radially wound cellulose microfibrils, so, as water osmotically enters guard cells, the cells bow out causing an increase in stomatal aperture. When stomatal closing is triggered (*e.g.* by ABA), anion channels are activated, allowing anions to passively move out of the guard cells, causing the charge in the guard cells to be less negative, resulting in

[1] pp. 559-566
[2] Review Chapter 6

efflux of K^+. K^+ and Cl^- efflux cause osmotic efflux of water that decreases the pressure in the guard cells thus decreasing the aperture of the stoma between them.

Specimen 1: Prepared slide of cross section of dicot leaf (*Ligustrum*)

1. Observe at 4x. Observe the upper and lower epidermis, the vascular bundles, and the remaining ground tissue. Notice that these are the same basic tissue systems that are found in roots and shoots.

2. Focus on the epidermis. Identify a stoma and observe at 10x. Each pore, stoma, is flanked by a pair of guard cells.

3. Observe at 40x the cells of the ground tissue, which in leaves is also called mesophyll (parenchyma cells of ground tissue). These cells are variable in shape and location. The closely packed columnar cells just below the upper epidermis are palisade parenchyma cells. Notice that each cell contains many chloroplasts. Below the rows of palisade parenchyma cells are the spongy parenchyma cells. Notice these cells also contain many chloroplasts. Also notice that there is much airspace between these cells. The organization of the leaf (air spaces near the stomata that allow CO_2 uptake and densely packed parenchyma with many chloroplasts that absorb light energy near the upper epidermis) is functionally efficient for photosynthesis.

4. Observe the major vascular bundle in the center that corresponds to the mid-vein of the leaf and the smaller vascular bundles that correspond to the minor veins of the leaf. Notice the orientation of the xylem and the phloem.

5. Draw the leaf cross section at 10x or 40x and label the upper epidermis, lower epidermis, guard cells, stomata, palisade parenchyma, spongy parenchyma, vascular bundle, xylem, and phloem.

Water is a limiting resource for plant growth

Fresh water is the limiting resource for terrestrial life. For perspective, about 85% of consumed water is by agriculture and, in the US, about 1,700 gallons of water are required to grow food for one adult per day[3]. Farmers strive to optimize growth with minimal water usage. Therefore, it is important that we understand how plants optimize water-use efficiency and, because guard cells regulate water loss, it is important that we understand guard-cell physiology. Experiments, as we are conducting in this laboratory, are vital for understanding biological systems.

Specimen 6: Epidermal peels of *Vicia faba* to observe guard cells.

1. Obtain a leaflet from a *Vicia faba* plant. Peel the lower epidermis back (as you did with the onion epidermis early in the semester).

2. Make a wet mount of the epidermal peel and observe at 4x and 10x.

3. Stain with a drop of Neutral Red for ~30 sec. Blot away most of the neutral red. Rinse the peel with a drop of tap water. Observe at 4x.

[3] National Geographic, 1993 Special Issue

4. Draw at 40x and label guard cells, stoma, and epidermal cells.

5. While observing at 40x, count and record in your notebook the number of open versus closed stomata.

Experimentation of guard-cell response to CO_2

Guard cells provide the major pathway for gas exchange in higher plants; they regulate CO_2 entry into, and water loss from leaves of higher plants. Thus, guard cells regulate the balance between photosynthesis and water conservation. We will use the model plant *Vicia faba* L. to determine how guard cells respond to atmospheric CO_2 concentration.

In your lab notebook, write the following.

1. The question that the experiment is designed to answer

2. Your hypothesis regarding how guard cells respond to atmospheric CO_2 concentration and rational for your hypothesis

3. A numbered procedure based on the procedural outline below

Outline of procedure (Your lab notebook should have more details of what you plan to do and what you do.)

Notice that at each node of the *Vicia faba* stem, there is a pair of leaflets on one petiole. Cut, under water to prevent an embolism in the xylem, one pair of leaflets including the petiole and one inch of the stem that is below the node and half of an inch of stem above the node. Quickly transfer into an Erlenmeyer flask containing water. Repeat this process for another pair of leaflets. One pair will be the control (atmospheric CO_2) and the other will be treated with low CO_2. Place the flask in a large beaker containing ~ 50ml of water or 0.5N NaOH[4]. Cover the beaker with Saran Wrap and leave under the light for 25 minutes. Peel the lower epidermis from leaves incubated under the two conditions. Stain the epidermal peel with neutral red for 1 minute then rinse it with tap water. Observe and measure at least 25 stomatal apertures from each sample with a microscope that has a micrometer in the ocular. Record the data in a table in your lab notebook. Mathematically analyze the results of the experiment and, independently, write an interpretation. Based on your data, what would you expect if you exposed droughted plants to low CO_2 conditions?

Write a short report as described in Chapter 1 of the lab manual. Note that all data analysis and writing must be done independently. Failure to compose lab reports independently will result in complete loss of credit for the report.

Review Questions

1. Why would non-functional guard cells result from symplastic connections via plasmodesmata between guard cells and other cells?

2. Assuming that the humidity inside leaves is 100%, under which atmospheric humidity, 90% or 40%, do you expect to see more open stomata? Why?

[4] NaOH depletes the concentration of CO_2 in the atmosphere.

3. If you wanted to use a drug to inhibit stomatal closing, but not stomatal opening, what protein would you target the drug towards and why?