Leaf Physiology, a Simulation

Introduction

In this laboratory, you will perform simulations of experiments designed to study the reactions of photosynthesis as they occur in the leaves of different plants. Some of these plants perform C₃ or C₄ photosynthesis, some plants prefer shade over direct sunlight, while some plants in LeafLab have different numbers of chromosomes that will affect photosynthetic rates. By changing experimental parameters such as light intensity, light quality, temperature, gas flow, and carbon dioxide concentration, you will learn about the importance of each parameter by measuring the amount of carbon dioxide consumed by the plant cells in your experiment as they undergo the reactions of photosynthesis. Data collected from these experiments will be calculated to determine photosynthetic rates.

Objectives & Goals

The purpose of this laboratory is to:

- Demonstrate how photosynthetic rates in different plants can change in response to factors such as light intensity, light quality, CO₂ concentration, and temperature.
- Simulate measurements of CO₂ assimilation rates in leaves.
- Investigate dark respiration, photochemical efficiency, CO₂ conductance, carboxylation efficiency, light compensation points, and photosynthetic saturation.
- Compare photosynthesis in C₃ and C₄ plants.
- Study the effects of polyploidy on photosynthetic rates.

Before You Begin: Prerequisites

Before beginning LeafLab you should be familiar with the following concepts:

- The importance and functions of enzymes as biological catalysts, basic principles of metabolic pathways, and mechanisms involved in regulating the catalytic activity of an enzyme (see Campbell, N. A., Reece, J. B., and Mitchell, L. G. Biology 5/e, and Campbell, N. A., Reece, J. B., Biology 6/e, chapter 6).
- The structure and function of the chloroplast (chapters 7 and 10).
- The electromagnetic spectrum and the photoexcitation of chlorophyll by visible light (chapter 10).
- The reactions of photosynthesis including cyclic and noncyclic electron flow in the light reactions, and the Calvin cycle. Be able to describe the primary substrates required, reactions involved, and products generated by each of these reactions (chapter 10).
- Plotting, interpreting data, and fitting data points to curves in scatter plots; using y- and x-intercepts, slope of the line, and the asymptote to extrapolate data from a line plot.

Background Information

The Earth receives approximately $13 \times 10^{23}$ calories of light energy per year from the sun. Less than 1% of this energy is captured and used by living organisms, yet without this energy, life on Earth as we know it cannot exist. Plants capture the light energy from the sun and convert it to organic molecules such as carbohydrates, in the process called photosynthesis. Because plants
can produce organic molecules to feed themselves and support their metabolism without eating other organisms, they are referred to as autotrophs or producers. In addition to light energy, autotrophs also rely on carbon dioxide, water, and soil nutrients to produce organic molecules by photosynthesis. The summary equation showing the yield of products created by photosynthesis is shown below.

\[
\text{Light Energy} + 6 \text{CO}_2 + 12 \text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{O}_2 + 6 \text{H}_2\text{O}
\]

Other autotrophs include algae, certain protists, such as Euglena, and some photosynthetic bacteria. Plants are essential producers of energy for many animals, including humans. Members of the Animal Kingdom are known as heterotrophs because they must obtain their energy by eating other organisms. Plants are an excellent source of carbohydrates for heterotrophs. Of equal importance, plants also provide the oxygen necessary for heterotrophs to convert carbohydrates into ATP during the reactions of aerobic cellular respiration. It is important to remember that photosynthesis in plants is not a replacement reaction for cell respiration. Plants must still perform the reactions of cellular respiration to produce ATP; however, photosynthesis provides plants with their own source of carbohydrates. Approximately 50% of the carbohydrates produced by most plant cells are used to produce ATP via cell respiration.

The production of carbohydrates by photosynthesis can be grouped into two major metabolic stages: (1) the light reactions and (2) the Calvin cycle, also known as the dark reactions or light-independent reactions. In plant cells, both sets of reactions occur within chloroplasts. Similar to the way the reactions of aerobic cellular respiration rely on oxidation-reduction reactions, electrons produced during photosynthesis are transferred to an electron acceptor molecule called nicotinamide adenine dinucleotide phosphate (NADP⁺). Upon receiving electrons, NADP⁺ is reduced to NADPH. Reduced NADPH functions as an electron carrier in a manner similar to the way NADH functions to supply electrons to the electron transport chain in mitochondria to power ATP synthesis in oxidative phosphorylation. The reactions of photosynthesis are summarized below.

The light reactions of photosynthesis occur in the thylakoids of a plant's chloroplasts. These reactions are designed to provide the plant cell with the NADPH and ATP required for the carbohydrate-producing reactions of the Calvin cycle. As their name indicates, the light reactions cannot occur without light energy. Specifically, most plants have evolved to absorb blue (approximately 480 nm) and red (approximately 680 nm) wavelengths of light, which are part of visible light spectrum. Thylakoids can absorb these wavelengths of light because embedded in the thylakoid membrane are a number of photosynthetic pigments that are capable of absorbing visible light. Chlorophyll a, which absorbs blue and red light, is the predominant pigment in the thylakoid. In addition to chlorophyll a, other pigments -- including chlorophyll b and a group of pigments known as the carotenoids -- are capable of absorbing other colors of visible light. The overall color of most plant leaves is green because the chlorophyll a in the chloroplasts of these leaves reflects green light.

The light reactions begin when light energy strikes chlorophyll a and the other pigments in the thylakoid membrane, resulting in photoexcitation of these pigments. In photoexcitation, when light energy strikes the pigment molecules, some of the electrons in these molecules are elevated to higher electron shells. These excited electrons can be captured and used by the plant cell to drive the light reactions. Electron flow in the chloroplast can occur because the photosynthetic pigments are organized in the thylakoid membrane as units called photosystems. Each photosystem consists of a single molecule of chlorophyll a called the reaction center. The
reaction-center chlorophyll $a$ molecule channels excited electrons to a molecule called the primary electron acceptor. Other pigments surround the reaction-center chlorophyll molecule. The pigments act as "antennae" pigments that send their excited electrons to the reaction-center molecule of chlorophyll $a$. Two types of photosystems are important for the light reactions: photosystem I and photosystem II. Photosystem I utilizes a reaction-center molecule of chlorophyll $a$ called chlorophyll $a$ P700, while photosystem II relies on a reaction-center molecule of chlorophyll $a$ called P680. These molecules are designed to absorb visible light with a wavelength of 700 nm and 680 nm, respectively.

Excited electrons may follow one of two paths during the light reactions, noncyclic electron flow or cyclic electron flow. Noncyclic flow involves photosystem I and photosystem II. This path is the primary route for the majority of excited electrons released during the light reactions. Noncyclic electron flow begins when light energy strikes photosystem II and excited electrons from this photosystem are captured by the primary electron acceptor molecule. When this occurs, these electrons are subsequently transferred to a series of electron acceptor molecules in the thylakoid membrane. These molecules form an electron acceptor chain and many of them are very similar to those found in the electron transport chain used in cell respiration (e.g., the cytochromes). As was the case with cell respiration, electron transport along this chain results in the production of a H$^+$ gradient (proton-motive force) in the intermembrane space of the chloroplast.

As electrons are transferred along this chain, the electron carrier molecules pump hydrogen ions into the intermembrane space to create the H$^+$ gradient. This H$^+$ gradient provides the energy necessary for the enzyme ATP synthase, which performs the same function in photosynthesis and cell respiration. ATP synthase functions as an ion channel to allow H$^+$ flow down a gradient from the intermembrane space into the stroma. The H$^+$ flow through ATP synthase activates the enzyme to synthesize ATP from ADP and inorganic phosphate in a final stage called noncyclic photophosphorylation.

In the last part of noncyclic flow, excited electrons that have passed through the electron transport chain are now transferred to the reaction-center chlorophyll $a$ P700 molecule in photosystem I. These electrons are ultimately transferred to NADP$^+$ by the enzyme NADP$^+$ reductase. This reaction results in the production of the reduced electron carrier NADPH. This entire set of reactions is called noncyclic electron flow because excited electrons that leave photosystem II never return, or cycle back, to photosystem II. How, then, does this photosystem continue to function? Why don't the photosystem molecules exhaust their supply of electrons? The answer is that the electrons in the photosystem are replaced through a water cleavage reaction that involves the enzymatic splitting of water with the concomitant removal of two electrons from water and the release of one oxygen atom. Because water splitting is constantly occurring, oxygen atoms liberated in this reaction quickly combine to form the molecular oxygen (O$_2$) that animals rely on to support cell respiration. The electrons released from the splitting of water are then used to replace excited electrons that leave chlorophyll $a$. Consider this reaction the next time you wonder why all your plants are dying due to a lack of water!

Although the reactions of noncyclic electron flow represent the predominant path of electrons during the light reactions, the reactions of cyclic electron flow are also important. The sole purpose of these reactions is to synthesize additional ATP, because noncyclic electron flow alone does not produce enough ATP to support the reactions of the Calvin cycle. This pathway involves photosystem I only. In cyclic electron flow, light strikes photosystem I and excited electrons from chlorophyll $a$ P680 are transferred to a primary electron acceptor molecule. These electrons then
travel down an electron transport chain. Similar to the events of cell respiration and the electron transport chain, the transfer of electrons to acceptor molecules in this chain results in the production of a H\(^+\) gradient that is used to power ATP synthesis by ATP synthase. However, unlike the reactions of noncyclic flow, during cyclic flow the electrons that leave the electron transport chain return (hence the name cyclic flow) to photosystem I; thus the splitting of water is not required to supply electrons to this pathway. The synthesis of ATP during cyclic electron flow is called cyclic photophosphorylation.

The aforementioned light reactions are designed to supply the reactions of the Calvin cycle with the ATP and NADPH necessary for the Calvin cycle. Because the reactions of the Calvin cycle do not directly require light energy, these reactions are known as the dark reactions of photosynthesis. The primary purpose of the Calvin cycle is carbon fixation--the conversion of carbon dioxide into organic molecules such as carbohydrates. This cycle is named after Melvin Calvin, an American biochemist who was awarded a Noble Prize in 1961.

The reactions of the Calvin cycle occur in the stroma of the chloroplast and involve three enzymatic steps to convert carbon dioxide into valuable carbohydrates for the plant cell. Although one molecule of carbon dioxide is converted into carbohydrates during each round of the cycle, it is convenient to follow the fixation of three molecules of CO\(_2\) (or three rounds of the cycle). In the first reaction, one molecule of CO\(_2\) is attached to a five-carbon sugar called ribulose bisphosphate (RuBp) to produce two molecules of a three-carbon sugar called 3-phosphoglycerate. For every three molecules of CO\(_2\) that enter the cycle, six molecules of 3-phosphoglycerate are produced. This reaction is catalyzed by an enzyme called rubisco (ribulose carboxylase). The six phosphoglycerate molecules are then phosphorylated using ATP from the light reactions to create six molecules of the three-carbon sugar 1,3-bisphosphoglycerate. The six molecules of 1,3-bisphosphoglycerate are then reduced, using NADPH from the light reactions, to generate six molecules of the three-carbon sugar glyceraldehyde 3-phosphate, abbreviated G3P. G3P is an important molecule because it can be converted by the plant cell into glucose intermediates that can be used to synthesize starch and other macromolecules for the cell.

The reactions of the Calvin cycle are not completed by the synthesis of G3P, however. Of the six molecules of G3P produced by three rounds of the cycle, five molecules are used to replenish the supply of RuBp in the plant cell. This is accomplished by phosphorylating G3P in another enzymatic reaction. Therefore, of the six molecules of G3P produced, only one molecule is available as a source of consumable energy for the cell. But because these reactions occur continually, the level of G3P (and the level of carbohydrates in a cell) can accumulate rapidly. It has been estimated that worldwide production of carbohydrates by photosynthesis produces approximately 160 million metric tons of carbohydrate per year--a very large cube of sugar indeed! Approximately 50% of the carbohydrates produced by most plant cells are consumed by the cell during cell respiration. The remaining carbohydrates may be stored as starch in various parts of the plant or used to make other necessary molecules. You may have heard that talking to plants helps them grow. You should now understand why: the CO\(_2\) that you are exhaling (and not the mellifluous sounds of your voice) promotes plant growth!

Because many plants convert carbon dioxide into the three-carbon sugar RuBp during the Calvin cycle, these plants are called C\(_3\) plants. Not all plants perform C\(_3\) photosynthesis. Some plants are called C\(_4\) photosynthetic plants because, prior to the Calvin cycle, carbon dioxide in these plants is enzymatically converted into a four-carbon molecule called malate. C\(_4\) plants include members of the grass family, such as crabgrass, and important agricultural plants such as sugarcane and corn. This reaction occurs in mesophyll cells and then the malate is shuttled into the chloroplasts.
of specialized cells, called bundle sheath cells, where the Calvin cycle occurs. Carbon dioxide is released from malate in the first step of the Calvin cycle, after which carbon fixation proceeds according to the reactions of a C3 plant.

What is the advantage of C4 photosynthesis compared with C3 photosynthesis? To understand this, consider what happens to a C3 plant when the plant is exposed to excess heat. At high temperatures, the stomata on most plants will partially close to prevent dehydration. But while this response prevents excessive evaporation of water through the stomata, it also restricts the amount of O2 that can leave the leaf and it limits the amount of CO2 that can enter the leaf. Under these conditions, the elevated levels of O2 can compete with CO2 for binding to the active site of rubisco. When O2 binds to the active site of rubisco instead of CO2, rubisco attaches O2 to RuBp to create a wasteful molecule called glycolate, which is useless to plant cells. The production of glycolate is called photorespiration.

The C4 plants have adapted to avoid photorespiration when the climate is warm. The light reactions and the conversion of CO2 into malate occur in a different set of cells (mesophyll cells) from the ones where the Calvin cycle occurs (bundle sheath cells). In addition, the attachment of CO2 to malate requires an enzyme called PEP carboxylase. This enzyme has a higher affinity for CO2 compared with O2; therefore, even under conditions where the level of CO2 in a cell is low relative to O2, PEP carboxylase will produce malate. Because the light reactions (and the release of O2) are occurring in mesophyll cells, when malate is pumped into the bundle sheath cells and CO2 is released, the concentration of CO2 in the bundle sheath cells is high enough to prevent photorespiration by rubisco. The rate of photosynthesis in both C3 and C4 plants can be determined experimentally by measuring the amount of CO2 consumed by a plant's leaf.

Measuring photosynthetic rate in a variety of different leaves is a primary purpose of LeafLab.

A number of different photosynthetic adaptations also occur in plants that prefer shade compared with plants that prefer direct sun. Shade-tolerant plants (e.g., ferns) often grow in the dim sunlight of a forest floor while sun plants (e.g., marigolds) prefer direct exposure to sun. Shade and sun plants have developed a number of special adaptations in response to light exposure. These include differences in photosynthetic enzymes and differences in leaf structure. Because of these adaptations, photosynthetic rate and other parameters of photosynthesis can differ in sun and shade plants when they are exposed to the same light intensity. For certain plants, both sun and shade leaves can be found on the same plant. You can use LeafLab to learn about photosynthetic rates in shade and sun plants.

Another aspect of plant biology that you will investigate using LeafLab involves the influence of plant genetics, specifically chromosome number, on photosynthetic rates. Approximately half of all flowering plant species are polyploid with respect to their chromosome number. Polyploidy is the presence of more than two complete sets of chromosomes in an organism's somatic cells. Unlike in animals, where polyploidy typically leads to spontaneous loss of an embryo, polyploidy in plants is partly responsible for the diversity of phenotypes in flowering plants.

Polyploidy in plants occurs when gametes contain the same number of chromosomes as somatic cells. These gametes are sometimes called unreduced gametes and they can arise due to nondisjunction of chromosomes during meiosis. Recall that during gamete formation by meiosis, the number of chromosomes is typically reduced to half so that gametes have a haploid (n) number of chromosomes compared with diploid (2n) somatic cells. Unreduced gametes can lead to a number of different polyploidy conditions in plants, including triploid (3n; typically sterile plants) and tetraploid (4n) plants. Hexaploids (6n) and octaploids (8n) are also fairly common,
and several plant species have been studied with significantly higher numbers of chromosomes. Plant breeders sometimes induce conditions of polyploidy to create new plant species. Examples include certain ryes and fruits, such as seedless watermelons and bananas.

When studying photosynthesis, botanists are routinely interested in learning about many different aspects of photosynthesis in addition to photosynthetic rate. Processes such as dark respiration, photosynthetic saturation, photochemical efficiency, and light compensation points are other important measures of photosynthesis. LeafLab can be used to study all of these processes. You will use LeafLab to learn about many of the factors presented in this background section by simulating experiments that modern-day botanists use to study the environmental and genetic factors that influence the rate of photosynthesis in plant leaves.

References