Plant Physiology (BOT 104) Answer Key Exam 2

March 4, 2009

Name ______________________________

1. **Legumes** have a unique ability to form a symbiosis with Rhizobium bacteria. During establishment of the symbiosis, the infecting Rhizobium bacteria must also change from a free-living form suited to life in the soil to a symbiotic form designed to live inside the plant host.

   a. Rhizobia first enter the plant root through what **cell type**? (1 point)

   Root hair cells

   b. Once the infecting Rhizobium in the infection thread reach the center of the nodule, they enter the plant cells.

      i. Do the Rhizobium bacteria actually **enter** the cytoplasm or are they **separated** from it by a membrane? (1 point)

   No, they stay separated from the cytoplasm by a membrane.

      ii. Please describe the process of Rhizobium **release** from the infection thread into the nodule cells. (2 points)

   - the cell wall at the tip of the infection thread disappears
   - the plasma membrane of the cell pinches off vesicles containing a single Rhizobium
   - the Rhizobium is now enclosed in a vesicle called a symbiosome

   c. What is the symbiotic form of Rhizobium called? (1 point)

   bacteroid

   d. What is the name of the **enzyme complex** that fixes nitrogen? (2 points)

   nitrogenase

   e. Is this nitrogen-fixing enzyme made by the **plant cell** or by the **Rhizobium**? (1 point) by Rhizobium

2. Some mutants of the model legume, *Medicago truncatula* (the species that you are working with in lab!), have been found that are unable to nodulate with Rhizobium. Surprisingly, these mutants are ALSO completely unable to form mycorrhizae.

   a. Name two similarities between the rhizobium-legume symbiosis and the mycorrhizal symbiosis. (2 points) Name some of the following points (or others!)

   - They both involve nutritional transfer between the microbe and the plant root
   - In each case, the microbe pushes into the plant cell, but remains surrounded by plant cell plasma membrane
   - The bacterial signal molecule is based on a piece of chitin. Fungal cell walls are made of chitin.
   - Both the mycorrhizal fungus and the rhizobium get past the plant defenses (i.e. enter without activating plant defenses)

   b. Bonus question: Speculate on why these mutants might be unable to form both mycorrhizae and nitrogen-fixing nodules. (+ 1 point)

Perhaps because establishment of both mycorrhizae and nodulation share some common steps.

Perhaps because nodulation evolved from the more ancient mycorrhizal symbiosis.

3. Leaves capture a broader spectrum of visible light than is absorbed by chlorophyll.

    a. How does **leaf anatomy** help maximize light capture? (2 points)

    Mention at least two of the following:

    Epidermal cells shaped like lenses can focus light into palisade cells

    Palisade cells can send light into the interior of the leaf

    Air spaces around mesophyll cells can lead to light scattering

    b. How does an **antenna complex** help increase the amount of light energy captured for photosynthesis? Please be specific! (4 points)

    In an antenna complex, there are lots of pigment molecules. Each can absorb a different wavelength of light, thus extending the range of visible light that can be used for photosynthesis. It also provides a larger target for the photons to hit.

    The energy absorbed by the pigment molecules is transferred directly to the reaction center.

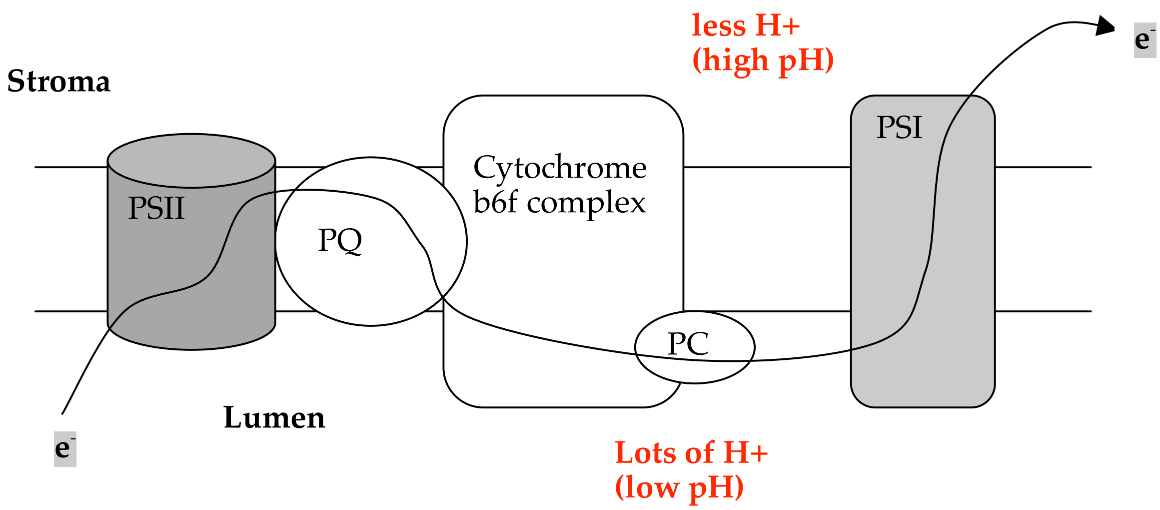
4. You are working in a nursery and move a flat of **oxalis** (a shade-loving plant) into the shade of a shed before moving them to the sale rack. You get busy and forget them until after lunch. By now the plants have been in full sun for some time.

When you move the wire screen that has fallen over some of the plants, you notice that the leaves underneath the screen now have **a light green/dark green pattern** that matches the wire mesh.

    a. What happened? Why are some areas of the leaf light green and others dark? (4 points)

Chloroplast movement! In the areas where there was high light, the chloroplasts moved to the side walls and stacked up against the walls. These cells look lighter green because some of the chloroplasts are hidden. In areas where there was low light (covered by the wire mesh) the cells are still dark green, because the chloroplasts are spread out across the surface of the leaf cells.

5. **Electron transport** powers the first steps of Photosynthesis. The following diagram shows the passage of electrons through the photosynthetic electron transport chain.



a. What is the **source** of the electrons (e^-) that enter Photosystem II (PSII)? (2 points)

H_2O

b. What **membrane** are these complexes embedded in? (2 points)

Thylakoid membrane

c. What is the **final e^- acceptor** for e^- leaving Photosystem I (PSI)? (Please indicate both the unreduced and the reduced form of this acceptor) (4 points)

NADP+ (oxidized)

NADPH (reduced)

6. The light energy captured by the photosynthetic reaction centers is used to establish a pH gradient across this chloroplast membrane. (This means a gradient of H^+ ions.)

a. Please indicate on the diagram above, where the concentration of H^+ is low and where it is high. (2 points)

See diagram

b. How is this pH gradient established? There are 3 different ways, please specify all of them. (6 points)

1. Splitting H_2O into O_2 and H+ in the lumen

2. Pumping 4 H+ from stroma to lumen by Plastoquinone (PQ)

3. Reducing NADP+ to NADPH it picks up an H+ to neutralize charge (in stroma)

c. The difference in concentration between the two ends of the gradient is approximately 10,000-fold. Such a steep gradient contains a lot of potential energy that can be harnessed to do work. **How is potential energy in the chloroplast pH gradient used?** [Be specific: explain what the **product** is and what the role of the pH gradient is in making it.] (4 points)

As the H+ ions flow down the gradient (from lumen to stroma), they can do work. They flow through the ATP synthase molecule, and as they do, they turn it, providing the energy to synthesize ATP from ADP and phosphate.

7. RUBISCO is the key enzyme in the Calvin cycle, catalyzine the 1st step in this pathway. Rubisco stands for "RuBP carboxylase oxygenase". This means that Rubisco can catalyze two reactions.

   a. When RUBISCO functions as a **carboxylase**, what does it do? (2 points)

   Adds CO_2 to RuBP

   b. When RUBISCO functions as an **oxygenase**, what does it do? (2 points)

   Adds O_2 to RuBP

   c. The fact that Rubisco catalyzes two competing reactions tells us something about the early atmosphere in which Rubisco evolved. Please explain. (4 points)

   The ancient atmosphere had a lot of CO_2 but very little O_2. Thus the fact that Rubisco could mistakenly use O_2 was not a problem and did not really interfere with its carbon fixation function.

   d. In **C3** plants, oxygenation by Rubisco can result in as much as a 50% reduction in productivity. Please explain how this happens. (3 points).

   Photorespiration (the process of adding O_2 instead of CO_2 to RuBP) is a very wasteful process. One of the products of the Rubisco oxygenation activity is a 2-carbon molecule. In order to regenerate RuBP so that the Calvin cycle can continue, the 2-carbon molecule **must go through three organelles** (chloroplast, peroxisome and mitochondrion) and many enzymatic reactions in order to produce RuBP. In the process, these reactions **consume ATP** and **release 1 molecule of CO_2.**

e. In **C4** plants, oxygenation does **NOT** compete with carboxylation, and thus there is no loss of productivity on warm days. Why? What changes occurred in C4 plants make this possible? Please make sure to mention both **biochemical** and **anatomical** modifications. (4 points)

1. The C4 plant uses **PEP carboxylase** to fix carbon. The first product is a C4 acid (oxaloacetate). PEP carboxylase does NOT ever use O_2 as a substrate. So it works well even at low concentrations of CO_2. This happens in the mesophyll cytoplasm.
2. The oxaloacetate is transported to the bundle sheath cells and then into the chloroplasts there.
3. There it is broken down to pyruvate and CO_2.
4. The CO_2 is used as a substrate by Rubisco, and enters the Calvin Cycle. Because there is no O_2 around (these chloroplasts don't have PSII and so don't evolve O_2) and lots of CO_2 (because the CO_2 is being released right next to Rubisco), there is no problem with photorespiration.

Summary of differences:
C4 plants use a different enzyme to fix carbon (PEP carboxylase)
Carbon fixation is physically separated from the Calvin cycle

- PEP carboxylase fixes carbon in the mesophyll cytoplasm
- The calvin cycle happens in the bundle sheath chloroplasts
- This is called "Kranz anatomy" because it looks like a green wreath

8. Some paleontologists discover bones of a previously undescribed mammal. Analysis of its teeth indicate that the mammal is clearly a plant-eater. Pollen from that area indicates that the region must have been a hot, grassy plain with some trees and bushes. **They want you, as a plant physiologist, to help them determine what the mammal ate: grass or leaves**. How can you figure this out? How can an understanding of the physiology of these kinds of plants (especially photosynthesis!) help you? Be specific! (4 points).

[Hint: I will be looking for 1) What kind of test you would do, 2) data that supports grass, 3) data that supports leaves and 4) the plant physiology behind the data.]

- **isotope discrimination**: measure ratio of C^{13} to C^{12}. Both are commonly occurring non-radioactive isotopes. Most Carbon (C) in the atmosphere is C^{12}, a tiny bit is C^{13}.

- Leafy plants are C3, tropical grasses are C4.

- C4 plants use PEP carboxylase, which uses both C^{12} and C^{13} indiscriminately.

- C3 plants use Rubisco for Carbon fixation. Rubisco prefers C^{12} and discriminates against C^{13}.

- So, you would expect that a grass eater would have a Carbon isotope ratio similar to the plants it ate, i.e. a C^{12}:C^{13} ratio similar to that found in the atmosphere.

- A leaf eater would have mostly C^{12} in its bones, just like the leafy plants that were its food.

9. The final step in **nitrogen assimilation** is the addition of ammonium (NH_4) to a carbon skeleton to make **glutamine**.

   a. What molecule serves as the **carbon skeleton** in this reaction? (2 points) glutamate

   b. What kind of a **molecule** is glutamine? (2 points)

   Amino acid

10. The activity of the enzyme **nitrate reductase** is regulated by **phosphorylation**. When the enzyme is phosporylated (has phosphate groups added), it is inactive. When the phosphate groups are removed, the enzyme is active again. **What is the advantage of this kind of regulation?** (2 points)

It's very rapid and easily reversible.

11. **Sulfur** is an important nutrient.

    a. **Where** in the cell do most of the enzymatic reactions of sulfur assimilation take place? (2 points)

    chloroplast

    b. **What amino acid** is the first assimilated form of sulfur? (2 points)

   cysteine

12. In plants undergoing **CAM photosynthesis**, the vacuole becomes increasingly acidic as the night wears on.

   a. What molecule builds up inside the vacuole over the course of the night? (2 points) malate

   b. Why does this happen? Be specific! (4 points)

   At night, the stomata are open in a CAM plant. At this time, CO_2 is first fixed into a C_4 acid by PEP carboxylase, in the cytoplasm. This C_4 acid is transported into the vacuole where it builds up all night. By the end of the night there is a lot of malate in the vacuole!

   [During the day, the malate is transported into the chloroplast, where it is broken down to pyruvate and CO_2 and the CO_2 is fixed by the Calvin cycle, using ATP and NADPH from the light reactions.]

   c. Why does CAM photosynthesis give an advantage to plants growing in arid regions? (2 points)

   They can keep their stomata closed during the day and only open them at night. This reduces water loss.

   d. If CAM and C_4 photosynthesis are so successful in dry conditions, why haven't they taken over the world? Is there any **disadvantage** to doing CAM or C_4 photosynthesis? Be specific! (4 points)

   <u>Yes. There is an energy cost:</u>

   -It costs 2 ATP to regenerate PEP

   - C_4: there is the cost of transporting the C_4 acid into the bundle sheath cell chloroplasts where the Calvin Cycle enzymes are located

   - CAM: there is the cost of transporting malate into and out of the vacuole

13. The nursery you work for is trying to figure out the best soil for starting tree seedlings. Based on what you learned in Plant Physiology, you decide to start some seedlings in pots containing forest soil. Six weeks later, you notice that the seedlings started in pots containing forest soil are noticeably more vigorous and contain significantly larger shoots than those planted in a commercial non-soil mix. You are curious, and you tell your boss that you suspect those growing in forest soil have formed mycorrhizae.

    a. Your boss asks you to check whether the apple seedlings have **ectomycorrhizae or endomycorrhizae**. You study the roots to tell which they are (imagine you have all the microscopes you desire). How do these two kinds mycorrhizae differ? What characteristics determine whether you are looking at **endo** or **ectomycorrhizae**? (6 points)

Ectomycorrhizae have:

- a sheath or mantle of hyphae surrounding the roots
- a Hartig net between the cortical cells
- very obvious hyphae around the root

Ectomycorrhizae have:

- very fine hyphae like spiderwebs leaving the root
- **arbuscules** within plant cells!

    b. How do mycorrhizae improve the nutrition of the apple seedlings? Be specific! (3 points)

- They extend the root system by spreading farther into the soil
- They increase transfer of phosphate to the plant (also other nutrients, but those are less well studied).

14. Micronutrients are essential nutrients required by plants in small amounts. Please pick **one micronutrient** from the following list and name a **molecule** that uses or incorporates this micronutrient: (2 points)

Some examples:

- Molybdenum – a prosthetic group on **nitrate reductase** OR on one of the subuits of **nitrogenase**
- Copper - **plastocyanin**
- Manganese – the **water splitting complex** associated with PSII

15. Iron (**Fe**) is an essential nutrient, but is often limiting in soils.

a. **What are the 4 steps plant roots use to mine iron from the soil?** Don't just list the steps → explain what is going on in each step. In particular, note **what oxidation state of Fe** is involved in each step. (8 points)

Step 1: **secrete H^+** (Acidify the soil). This pulls Fe^{3+} off negatively charged soil particles.

Step 2: **chelation**. Secrete organic acids like **citrate** (which has 3 negative charges from the three carboxylic acid groups). Citrate then chelates the Fe^{3+}, keeping it from sticking back onto the soil particles.

Step 3: **Reduction**: ferric reductase, an enzyme in the plasma membrane of root epidermal cells, reduces Fe^{3+} in the soil (still chelated to citrate) to Fe^{2+}. The electrons come from NAD(P)H.

Step 4: **Transport**! IRT1 and other transporters take Fe^{2+} across the membrane into the cytoplasm of root cells.