Teaching Activity: Nitrogen Fixation in Root Nodules

Introduction: Plants and animals use nitrogen to build proteins and enzymes. Nitrogen makes up about 78% of the Earth's atmosphere. However, most living things cannot use atmospheric nitrogen (N2). Instead, they must rely on nitrogen compounds, such as nitrates and nitrites, that are found in soils. The process that converts nitrogen gas into compounds and animals can use is called the nitrogen cycle. This cycle includes 4 major processes: *nitrogen fixation, ammonification, nitrification and denitrification.*

- During nitrogen fixation, bacteria such as *Rhizobium japonicum,* convert nitrogen gas (N_2) into ammonium compounds (NH_4OH). These bacteria live in the roots of legumes, including alfalfa, clover, peas, and beans. Some blue-green bacteria can also fix bacteria.

- In the second process, ammonification, bacteria breakdown nitrogen -containing amino acids from animal wastes and dead organisms, forming ammonia compounds.

- During nitrification, chemosynthetic bacteria oxidize ammonia compounds to produce nitrates and nitrites. Nitrates can also enter the soil from other sources. The enormous energy in a bolt of lightning can cause nitrate ions to form atmospheric nitrates.. Plants also use nitrates and nitrites to form amino acids.

- In the final process, denitrification, anaerobic bacteria break down nitrates, releasing nitrogen gas into the atmosphere. This process requires much energy and so occurs very slowly. As a result, nitrogen availability often limits the productivity of an ecosystem.

Plants remove nitrogen from the soil. Traditional farming practice has been to increase the nitrogen content of soils by growing legumes. However, rapid growth of the world's population has lead to intensive agricultural practices in which traditional methods of nitrogen replenishment are too slow. Large quantities of artificially produced nitrogen compounds are now manufactured for use in agriculture. A key process in the production is the "Haber process", which provides a relatively cheap and convenient way to artificially "fix" atmospheric nitrogen. All of the presently used nitrogen fertilizers are derived from ammonia. Through the production and widespread use of nitrogen containing fertilizers, as well as large scale cultivation practices, humans have more than doubled the amount of nitrogen available to agriculture, at the same time over - loading the natural cycle.

Objectives:

- To examine the structure and function of root nodules in legumes;
- To evaluate the effect of over-use of fertilizers on the natural cycling of nitrogen;

Important Terms: Nitrogen fixation, nitrification, denitrification, ammonification, symbiosis, root nodules , nitrogen cycle;

Materials: Living alfalfa, bean or other leguminous plant infected with symbiotic Rhizobium; the same type of plant without Rhizobium infection; scalpel;, microscope, prepared slide of the cross section of legume nodules, pencil/ **Student Lab Sheet**, colored pencils;

Procedure:

1. Students should carefully dig a plant from the pot labeled "NONINFECTED".
   - Shake free any soil that clings to the roots.
   - Examine the root carefully and then set the plant aside.

2. Students should then Dig a plant from the pot labeled "INFECTED" and repeat step #1.
   - They should then locate a root nodule in the root system using the 20X setting on the microscope.
   - Inside the nodules are cells filled with Rhizobium bacteria. These bacteria fix atmospheric nitrogen (N_2) by converting it into ammonia compounds (NH_4OH). Excess ammonia is expelled into the soil and plant tissues surrounding the nodule.

3. Using the scalpel, students should carefully cut open one of the root nodules.

4. Students should then examine the inside of the nodule carefully.
   - They should record their observations on the lab sheet.
   - If the infecting strain of Rhizobium is able to fix nitrogen in this species of legume, the inside of the nodule wall will be pink. The pink color indicates the presence of plant protein necessary to fix nitrogen.
   - If the bacteria cannot fix nitrogen, the inside will be green.

5. Student should use the scalpel to cut through several other nodules.
   - Students should record their observations.

6. Student should use the microscope and the prepared slide to view sections of root nodules and attempt to determine if all the nodules contain Rhizobium.
   - They should use the low power to locate an area they wish to view, and then switch to high power.

7. Students should then complete all sections of the LAB: **Observations, Analysis and Conclusions**

Student Activity Sheet: Nitrogen Fixation in Root Nodules

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- In the final process, denitrification, anaerobic bacteria break down nitrates, releasing nitrogen gas into the atmosphere. This process requires much energy and so occurs very slowly. As a result, nitrogen availability often limits the productivity of an ecosystem.

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Objectives:

- To examine the structure and function of root nodules in legumes;
- To evaluate the effect of over-use of fertilizers on the natural cycling of nitrogen;

Student Activity Sheet

Procedure:

1. Carefully dig a plant from the pot labeled "NONINFECTED".
    - Shake free any soil that clings to the roots.
    - Examine the root carefully and then set the plant aside.

2. Dig a plant from the pot labeled "INFECTED" and repeat step #1.
    - Locate a root nodule in the root system using the 20X setting on the microscope.
    - Inside the nodules are cells filled with Rhizobium bacteria. These bacteria fix atmospheric nitrogen (N_2) by converting it into ammonia compounds (NH_4OH). Excess ammonia is expelled into the soil and plant tissues surrounding the nodule.

3. Using the scalpel, carefully cut open one of the root nodules.

4. Examine the inside of the nodule carefully.
    - Record you observations on the lab sheet.
    - If the infecting strain of Rhizobium is able to fix nitrogen in this species of legume, the inside of the nodule wall will be pink. The pink color indicates the presence of plant protein necessary to fix nitrogen.
    - If the bacteria cannot fix nitrogen, the inside will be green.

5. Use the scalpel to cut through several other nodules.
    - Record your observations.

6. Use the microscope and the prepared slide to view sections of root nodules and attempt to determine if all the nodules contain Rhizobium.
    - Use the low power to locate and area you wish to view, and then switch to high power.

7. Complete all sections of the LAB: **Observations, Analysis and Conclusions**

Student Activity Sheet #1

Part I: Observations:

1. Draw and label the root nodule(s) that your located in the root system.

2. Describe the root nodule that you observed.

_____
_____
_____
_____
_____

3. Draw the root nodule that you dissected. Be sure to include the correct color.

4. What color was the inside of the nodule?

_____
_____

Part II: Analysis

1. How many of the nodules that you observed were nitrogen fixers? _____
   How could you tell? _____
   _____
   _____

2. Does the fact that some of the nodules are not effective in fixing nitrogen mean that they do not have a role in the relationship between the legume and the bacteria? _____

3. What possible role might the uninfected modules have? _____

_____

_____

Part III: Conclusions

1. Given the need of all green plants for nitrogen, how would you describe the relationship between the bacteria and the legume? _____

_____

_____

_____

2. If you were to grow legumes without root nodules to use as experimental controls, why should you plant the seeds in sterile soil? _____

_____

_____

_____

3. Why would a farmer want to plant legumes in a field one year and a non-legume crop the next year? _____

_____

_____

4. How would the removal of trees from an ecosystem result in increased nutrient (nitrate/nitrate) runoff? _____

_____

_____

_____

5. If nitrogen compounds are important for plant growth, how would the excess nutrients in the runoff affect aquatic plants? _____

_____

_____

6. The "Haber process" needs hydrogen to produce artificial nitrogen fertilizers. The hydrogen is generally extracted from natural gas, which is a fossil fuel. What in industrial nitrogen fixation putting into the nutrient cycle?

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