This science curricular material was developed by Tanya Manternach as part of the NSF-funded RET summer internship with the Iowa Turtle Army in Dr. Nicole Valenzuela’s Lab at Iowa State University, and is intended for use by other peer teachers with proper acknowledgement. Funding was provided in part by NSF grant IOS 0743284 and IOS-0924290 to Nicole Valenzuela.

Tanya Manternach
Hempstead High School
Dubuque, Iowa
							
tamanternach@dubuque.k12.ia.us
Studying Variation in Gene Expression of Tobacco Seedlings

I. Background Information:
An organism’s phenotype or physical appearance for a particular trait is the result of both its genetic makeup and the environment. In many instances, an organism may have genes for a particular phenotype, but they are turned-off by environmental factors such as light or temperature. The ability of certain genes to be turned-on or turned-off by environmental factors is theorized to have evolved in some species over many generations. The most likely reason this occurred is because organisms that can turn genes off, when the proteins they produce are not needed, are more energy efficient. This energy efficiency provided a survival adaptation (advantage) for individuals possessing this characteristic.

Tobacco seeds will germinate and grow in as little as three days if they are kept moist. The seeds contain an embryonic plant along with cotyledons that serve as a food source for the embryonic plant before its leaves develop, produce chlorophyll, and carry out photosynthesis. Tobacco seedlings that possess a dominant allele for chlorophyll production will produce chlorophyll if environmental conditions are favorable. However, tobacco seedlings that possess two recessive alleles are genetically programmed to be albino, and will not possess chlorophyll. Therefore photosynthesis cannot occur in albino plants.

II. The problem you will be investigating:
Will the presence of sunlight effect the expression of the genes for chlorophyll production in tobacco seedlings?

III. Your Hypothesis:
The presence of sunlight _______ (will or will not) effect the expression of the genes for chlorophyll production.

1. What is the independent variable you are manipulating/testing?
   A. presence of sunlight        B. expression of genes

2. What is the dependent variable that you will measure the effects of manipulating the independent variable (results)?
   A. presence of sunlight    B. expression of genes

IV. Your Experimental Design.
3. How will you design your experiment to test your hypothesis to see if the presence of light will impact the expression of the chlorophyll gene?

4. How will you control your experiment to make sure you are only testing for the presence of light and not something else like the amount of water or soil quality between the two test subjects? Complete the procedure:
   Step 1: Place a moistened filter paper (equal amounts of water) into two identical Petri dishes.
   Step 2: Add 20 tobacco seeds to each Petri dish. Use a toothpick to evenly spread them out.
   Step 3: Place a labeled cover over the dish and record the appearance of the seeds on day 1 in the chart.
   Step 4: 
   Step 5: Each day (for about 7-10 days), check the seeds and add equal amounts of water to maintain moisture.
   Step 6: When the leaves appear, record their color in the chart. You may choose to photograph the seeds.

V. Recording the results of the change in the independent variable on the dependent variable:

<table>
<thead>
<tr>
<th>Light Dish 1: Seedlings in light on Day 10</th>
<th>Data Table 1: Seedlings in dark on Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light Dish</td>
<td>Dark Dish</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>Chlorophyll</td>
</tr>
<tr>
<td>Albino</td>
<td>Albino</td>
</tr>
</tbody>
</table>

Chlorophyll production is controlled by one, dominant gene (C). Albinism is recessive (c). Based on your results can you guess the parent genotypes (use a punnett square)?

a. CC xCc = 100 % produce the chlorophyll production
b. CC x cc = 100 % produce the chlorophyll production
c. Cc x Cc = 75% with chlorophyll and 25% albino
d. Cc x cc = 50% with chlorophyll and 50% albino
e. cc x cc = 100% albino

Add on experiment: Now place the dark seedlings in the light for 48 hours and record your results in data table 3.

| Data Table 3: Dark Dish exposed to light for 48hours |
|-----------------|-----------------|
| Dark dish in light | Day 1 seedlings | Day 2 seedlings |
| Chlorophyll     | #    | %    |
| Albino          |      |      |

Add on experiment: Now place the dark seedlings in the light for 48 hours and record your results in data table 3.
Data Analysis Questions
1. What are the phenotypes of the tobacco seedlings growing in the light at the end of the experiment?
2. What are the phenotypes of the tobacco seedlings grown in the dark (before they were exposed to light)?
3. What are the phenotypes of the tobacco seedlings grown in the dark but then exposed to light for 24 hours?
4. From the data collected, what is the effect of light on the alleles for chlorophyll production?
5. Explain why did some of the seedlings in the light dish failed to produce chlorophyll and end up being albino?
6. Predict what will happen to the albino seedlings in the light dish by day 30 if growth was allowed to proceed. Explain:
7. Albino seedlings will not survive long enough to reproduce and pass their alleles on to the next generation. If this is so, explain how were albino seeds produced by the parent plants from which our seeds were harvested?
8. Were all of the tobacco seedlings that grew in the dark albino? Explain your answer below:
9. Is it energy efficient for plants to produce chlorophyll only when sunlight is present in the environment? Explain:

VI. Conclusion:
10. The experiment supports/does not support the hypothesis.
11. Explain how the data supports or refutes the hypothesis:
12. What possible sources of error could have interfered with your results?
13. Why is it important for scientists to use large sample sizes and repeat experiments?

VII. Reflection and Application Questions:
14. Review the lab introduction. What do they mean by gene expression?
15. Why might it be beneficial to the plant to not express the chlorophyll gene when there is no light?
16. How do you know that the plants grown in the dark had the gene, but were not expressing it at the time?
17. Could the non-expressed gene be turned on later in time? How do you know?
18. Some species of reptiles’ their gender is determined by the temperature of their nest or the amount of yolk in their egg. The reasons or benefits of this are not yet understood. Some scientists hypothesize, that having a turtle’s gender being determined by how much yolk is inside the egg or temperature of the environment may help the turtle produce a gender that might survive better at that temperature to ensure survival or more reproductive success. In humans, our genetic make up determines are gender and is not thought to be affected by temperature or yolk allocation.

In two different species of turtles (thought to be distantly related), both have the TSD gene (gene that allows the temperature of the nest to determine the gender/male and female of the babies), but in one species the gene is not turned on and the sex of the baby turtles is not affected by the temperature of their nest (similar to human sex determination). What could you hypothesize from this?
19. Scientists were able to measure whether or not this gene was being expressed in the embryonic turtles by sampling their yolk while they were still inside the egg developing. The more RNA being produced from a particular gene there is (more or less) expression of that gene at that time and it (is or is not) actively producing protein at that time.
20. How can you use the idea that organisms can turn certain genes in the DNA on and off to explain how your cells all have the same DNA, but can differentiate (specialize) to perform different functions or have a different structure (for example, bone, blood, and nerve cells all look and act differently, but have the same DNA)?