Discovering Epigenetics – It's a *bal*!
Student Laboratory

Background Information

When scientists mapped the Human Genome in 2001, it was viewed as the beginning of a new era. Now that we know every A, T, C and G it takes to build a human; there is renewed hope that we can find medical approaches to diseases such as Alzheimer's and cancer. Now we know that the bases of our DNA are just like words on a page. How they are read is just as important as what they are. Plants turn yellow when they're kept in the dark even though they have the genes to produce chlorophyll; some identical twins don't look exactly alike even though they have exactly the same genes; and you have genes for building bones in your eyeballs. WHY?

It turns out there is more going on than just our genes. The science of **epigenetics** studies what is going on "above" our genes. How are genes controlled? When are genes turned on? Why are genes turned off? What role does the environment play? Can parents pass down changes in the control of gene regulation without there being changes in the genes? Scientists studying epigenetics are trying to answer these questions.

Gene regulation occurs and is influenced by multiple complex factors. The method of gene regulation that this project investigates is gene repression associated with **methylation** of cytosine. Methyl groups are added to the DNA, effectively preventing the gene from being read for transcription and silencing the gene. Cytosine methylation can also suppress gene **mutations**, such as gene duplications that occur from DNA replication and recombination errors. In this way, cytosine methylation protects the genome from mutation.

Often when scientists want to study something, they will use a **model organism**, which is something that can be easily grown, makes lots of offspring, and has clear characteristics. ***Arabidopsis thaliana***, commonly called 'mouse-eared cress' is in the mustard family, is small in size, and grows rapidly in laboratory and classroom settings. It has a small genome and was the first plant to have a fully sequenced genome. It is a model organism used by plant scientists around the world to study a variety of biological processes, including growth, development, flowering time, circadian rhythms, environmental stress response, disease resistance, and epigenetics.

Scientists at the **Boyce Thompson Institute (BTI)** at Cornell University are using the model plant *Arabidopsis thaliana* to study epigenetics. You will be growing Arabidopsis plants, and looking out for strange looking plants that may be a sign of a defect resulting from a breakdown in epigenetic mechanisms. If you find any, the scientists at BTI want the seeds from those plants so they can study them further.

The "Let's Have a *bal*!" Project

Dr. Eric Richards, a research scientist at the Boyce Thompson Institute for Plant Research (BTI), studies the function of **DNA methylation** on the model plant *Arabidopsis thaliana*. ***DDM1*** is a gene found in *Arabidopsis* that when active, maintains methylation throughout the genome, ensuring that genes and other genetic elements are properly regulated.

Dr. Richards has identified a number of **mutations** that occur in plants with a faulty *DDM1* gene. One of these changes occurs at the *BAL* gene due to a duplication of the gene. This extra copy of the *BAL* gene results in an abnormally small plant with twisted leaves. This dwarf-like **phenotype** is referred to as "*bal*". The *bal* phenotype is caused by overexpression of a resistance like gene (R-gene) which activates a defense response**.** In this case, the extra copy of the BAL gene causes the plant to respond as if it is under attack by pathogens, even when pathogens are absent. The plant sacrifices normal growth, resulting in abnormally small plants.

The objective of the project is to isolate new, independent mutations that are generated in *ddm1* **lines** to determine whether they affect the *BAL* gene or a similar region in the genome. In the process, scientists will learn more about how cytosine methylation protects the genome against mutations. These insights may lead to a better understanding of human conditions, such as cancer, which have been linked to changes in cytosine methylation.

Arabidopsis Wild Type

Figure 1: A normal *Arabidopsis* plant prior to flowering. Note the diameter of the **rosette** of leaves at the base, and the flattened characteristics of the leaves.

Arabidopsis bal

Figure 2: A *bal* phenotype at the time of **bolting**. Note the shorter bolt height, the smaller rosette, and the curled nature of the leaves.

The fast growing nature of *Arabidopsis*, the high volume of plants that must be screened, and the very obvious appearance of the *bal* mutation make the investigation of the *bal* mutation a rare opportunity to involve students in the research process. Not only will students experience an authentic scientific experiment, they will have the opportunity to collaborate with a plant scientists and educators.

The Boyce Thompson Institute will supply high school classrooms with seeds derived from a line of *Arabidopsis* plants that have a mutation in the *DDM1* gene. Students will grow the plants, and report back to BTI the number of mutant plants, including those with the *bal* phenotype, that they observe. This information and the seeds from mutant plants will then be returned to Dr. Richards for further studies.

The purpose of this lab is to participate in an ongoing scientific experiment by growing and characterizing lines of *Arabidopsis thaliana* that may contain mutations resulting from epigenetic defects.

Key Scientific Vocabulary

Bolting: The production of a flowering stem

Chord: A straight line joining two points on a circle

Dehiscence: The spontaneous opening at maturity of a plant structure, releasing its contents (usually seeds)

DNA Methylation: The addition of a methyl group to a DNA molecule, which alters the expression of the gene

Epigenetic: The study of changes in gene expression or phenotype caused by mechanisms other than changes in the DNA sequence

Epigenetic Trait: A stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence

Genotype: The two specific alleles a diploid individual has for a trait

Genetic Marker: A variation in easily identify piece of genetic material, usually DNA, at a known location in the genome

Model Organism: An organism chosen by scientists for study, usually based on characteristics such as short generation time, high reproductive rates, easily observed characteristics, and close relative relationship to other organisms.

Mutation: A change in the DNA sequence.

Phenotype: The physical appearance of an individual

Plant or Seed Lines: A variety of species that is relatively uniform genetically because of continued inbreeding and artificial selection. Certain characters appear in successive generations as a result of inbreeding or self-fertilization, creating varieties within a single species that share similar, but unique genetic backgrounds.

Plant Architecture: Structures of the plant, leaves, stems roots, and the patterns they take during growth and development

Rosette: A circular arrangement of leaves

Silique: Any dry fruit that separates at maturity into two or four segments called valves, leaving a persistent partition that bears the seeds. A typical silique is an elongated capsule and is characteristic of plants in the mustard family

Stratification: A brief cold-treatment (few hours - days) of water soaked seeds to break seed dormancy, results in an even germination

Variation: Diversity within a species or population

Wild Type: Considered the typical phenotype for the species, considered "normal" when compared to new, possibly mutant traits

Zygote: a eukaryotic cell formed by a fertilization event between two gametes containing all genetic information necessary to form a new individual

Lab Materials

BTI Provided Materials

Light Rack:

- This will be picked up from BTI or purchased from Carolina Biological and sent to your school address if requested.

Environmental Monitoring Meters:

- Light meter for measuring light intensity in lux
- Temperature and humidity meter- measures min and max of both as well as current readings

Arabidopsis Seeds (seed packets)

- Columbia wild type (a control)
- known *bal* mutant (a control)
- 1-2 different experimental *ddm1* lines

Planting Materials:

- 2 x 2 potting cells (32 cells per large flat)
- 4 large watering flats or 8 small flats
- 4 humidity domes (Applied until seeds germinate, then remove)
- Plant labels
- 3 ml Pipettes

You will need to provide:

- A sunny classroom location, where temperatures are fairly constant throughout the experiment
- Three full Spectrum Florescent Light Bulbs for light rack (standard shop light size)
    - Example: Florescent light bulbs: Ex: Sylvania Octron XPS 32 W 4100K and F032/841/XPS/ECO3
- Soil, Seedling Starter Mix (fine particles size to promote germination)
- Refrigeration (seed **stratification** overnight)
- Markers
- Scissors
- 100 ml beakers
- Rulers (10 cm)
- Camera (cell phone cameras fine too) (at weeks 2 and 4 following germination)
- Watering Can (.5, 1, and 2 liter markings recommended but not required)
- Toilet paper Roll – 1 ply or 2 ply that has been separated

Safety

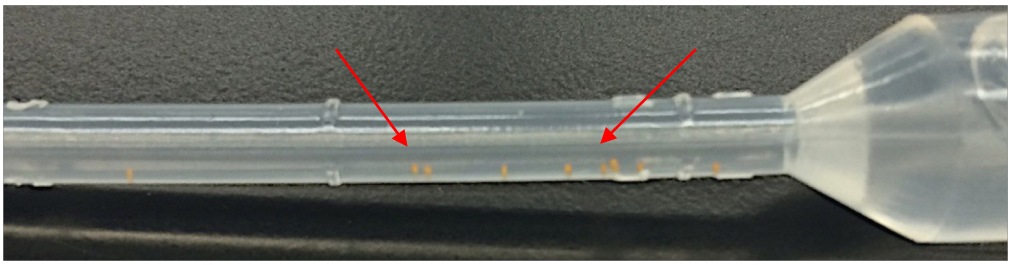
- Any students with potential allergies to specific plants should notify the teacher and should avoid contact with the plants
- Any injuries while working with the laboratory materials should be reported to the teacher immediately
- Students should not manipulate the light set-up while experiment is in progress

Lab Procedure

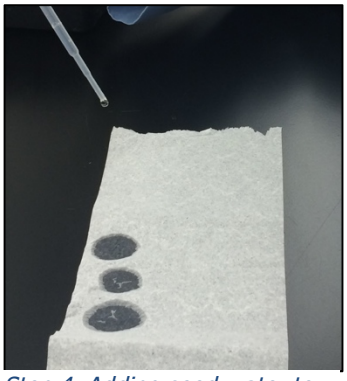
Lab Day 1: Making Seed Paper

Arabidopsis thaliana seeds are very, very small, almost dust-like, and require patience, as well as a plan for careful handling throughout the project to be successful.

1. Fill your beaker about halfway with water. Open seed packet and **very gently** tap on the sides until you see some seeds go into the beaker.
2. Take a square of toilet paper and lay it down flat.
3. Place the transfer pipet in the beaker and squeeze until it fills with water. You should also get some seeds. Look in the pipet to make sure there are seeds inside (shown below).



4. One drop at a time, squeeze the seed-water onto the toilet paper, making rows of drops. Not every drop will have a seed in it, and some drops will have more than one seed. Allow the paper to dry on the lab bench (20-40 min. depending on classroom conditions).

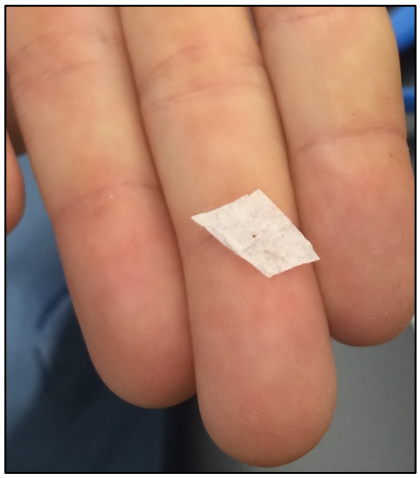


Step 4. Adding seed-water to the paper one drop at a time

Lab Day 2: Planting the Seeds

1. Fill all planting cells to the top with soil
2. Place the cells in the planting trays
3. Add about 2 liters of water to the bottom of the trays and allow the water to soak up through the soil from below (at least 30 minutes)
4. While soil is soaking, Label the planting tags with the Plant ID numbers of the seeds to be planted. Students' names and planting dates should be written on the back of the planting labels

5. A piece of masking tape with the plant ID number should be put on the outside of the 2x2 cell pack, just in case planting labels are lost or moved
6. Take your dry seed paper created in the previous lab. Use scissors to cut out small squares containing a single seed (shown on right).



7. Take one seed square from your seed paper and press it gently into the soil. Do not cover the seeds with soil; doing so will prevent germination.
8. Cover trays with humidity domes and place in the refrigerator overnight. This will help to synchronize seed germination.
    a. If you do not have enough space to refrigerate all of the trays, you may alternatively put the **dry** seed paper in the refrigerator overnight. If this is done, it is essential to plant the seeds immediately upon removing the paper from the refrigerator. (Check with your school cafeteria about using walk-in cooler space)
9. Remove the tray from the refrigerator and place under a 24-hour light source. Leave the humidity dome in place until seeds have germinated. It is recommended to elevate the trays, or lower the light bulb rack just above the surface of the plants in order to provide adequate light for growth. Remember to raise the lights up as the plants grow.
10. Record the date, temperature in °C, % humidity, and light level in Lux.

Lab Day 3: Germination

1. Depending on temperature of your room, germination will occur around Day 3-5. Check the trays daily for any sign of germination. If more than one seedling emerges in a cell, carefully remove them so that just the most vigorous plant is allowed to grow per cell. Choose the best looking seedling to remain. This is called "thinning" your plants.

2. Once seeds germinate, the humidity dome should be slowly removed over the next 2-3 days so as not to stress the seedling and maintain adequate moisture in the pots. On day one after germination, open the domes to the side and keep them loosely propped up (not locked in place) over plants, so water can evaporate and air circulates gently. On day 2 and 3 after germination, remove domes completely during the day to allow air and light

to circulate around plants. In the evenings partially cover with the domes. Remove domes completely three days after germination begins.

Lab Day 4: Characterize Plant Phenotypes (2 weeks after germination)

Two weeks after germination, seedlings will begin to show small differences in appearance. Compare experimental lines to the **Wild Type** control and the *bal* mutant control plants to determine if there are any differences in the plants. Record the number of plants growing, temperature, % humidity, lux level, and date. Take pictures of individual plants and entire groups, and label the pictures by line, as shown:

Teacher Name. Plant ID Number. Date

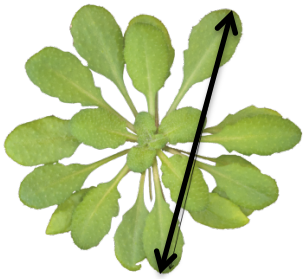
Example: Smith. 1903. 4/3/14

Lab Day 5: Characterize Plant Phenotypes (4 weeks)

Four weeks after germination, plants are mature and **bolting**. Pay close attention to the plants that appeared to be different during the first seed check, as well as new **traits** that have developed since then. Make as detailed observations as possible.

1. Record number of plants growing, temperature, humidity, lux level, and date.

2. Measure and record the longest **chord** on each plant's **rosette**. Find the longest leaf on the plant and place one end of a string on the tip of that leaf. Pull the string straight across to the tip of whichever other leaf gives you the biggest line of string. Lay the string on top of a ruler, and record this chord's length in cm.



Measuring the chord length

3. For each plant, record some detailed observations regarding the appearance of the plant in the area on your worksheet labeled "**Qualitative Observations**". For example, compared to the Wild Type, is this plant smaller?

Does it have very curly leaves? Is it missing bristles on its leaves? Is it a different color? Does it have a taller flower structure? Does it have fewer leaves? How about compared to the *bal* control plants?

4. If you think any of your plants is very different from the others, it may be a **mutant.** Make sure you make a note of this in your observations. If it is determined to be a mutant, it will be set aside and allowed to grow for several more weeks. Once it has flowered and produced seeds, the seeds will be sent to the scientists at BTI for further study.

Lab Day 6: Wrap up, about 8 weeks after planting

Seeds from mutant plants should be collected when seedpod or **silique** is brown but not open or showing signs of **dehiscence.** This often occurs between 6-8 weeks after germination depending on growth conditions. When dry, but not open carefully cut off the bolt containing the mature pods and place the entire structure into individual coin envelopes. Seed envelopes should be labeled with the teacher's name, the date collected, and the ddm1 experimental line number from the corresponding planting label. If you assigned an additional unique plant number to each individual plant in your experiment, please indicate that number too. Photos of the mutant plants for which seeds were collected through the experiment are also helpful to share.

Questions

1. List the challenges you had planting the seeds, growing the plants, or making observations.

2. How might these challenges affect your results?

3. What was the purpose of growing **Wild Type** and ***bal*** mutants?

4. How many of the plants appeared to be Wild Type? _____

   How many appeared to be mutants? _____

5. Did some Arabidopsis line numbers make more mutants than others? Which one(s)?

   Why do you think this is?

Student Data Sheet

Name_____
Plant ID #_____

Partner (s)_____
Teacher_____

Environmental Data

	Date	**Temp ^{0}C**	**% Humidity**	**Lux**	**Number Viable**
Planted					
2 Weeks Post Plant					
4 Weeks Post Plant					

Observational Data (at Bolting)
Date_____

Plant ID#	**Chord Length (cm)**	**Qualitative Observations**	**Mutant? (Y/N)**