ABRC: Greening the Classroom Module

Play Mendel Basic

Summary: This module guides students through the process of investigating reference and mutant strains of Arabidopsis to learn about Mendelian genetics. Through these activities students will learn about concepts such as growth and development, anatomy, variation, segregation, inheritance, phenotypes and genotypes.

Recommended Grade Level: Middle and high school

Duration: This module requires six weeks for completion of all planting and growth procedures. Student assignments can be completed within a single class period. An expanded version of this module is available in Play Mendel Advanced, as well as in the *American Biology Teacher* article, Following phenotypes: An exploration of Mendelian genetics using Arabidopsis plants (Price et al., 2018).

Learning Objectives

Through this module students will:

- Define concepts and terms associated with Mendelian genetics, the growing process, and plant anatomy
- Plant and care for two strains of Arabidopsis
- Make observations, compare phenotypes, and illustrate growth stages of mutant and reference strains of Arabidopsis
- Collect and analyze data
- Determine the inheritance of a unique trait
- Use evidence to support findings

Alignment with Next Generation Science Standards

NGSS	
Standards	-Heredity: Inheritance and Variation of Traits (MS-LS3-1, MS-LS3-2, HS-LS3-3) -Biological Evolution: Unity and Diversity (MS-LS4-4)
Science & Engineering Practices	-Developing and using models -Constructing explanations and designing solutions - Analyzing and interpreting data
Disciplinary Core Idea	-Inheritance of Traits -Variation of Traits -Natural Selection
Crosscutting Concepts	-Structure and function -Cause and effect -Scale, proportion, and quantity -Science is a human endeavor

Supporting Resources

The following supporting resources are available for download from the ABRC website:

- Play Mendel Protocol Video
  - Note: This video was developed for the Play Mendel Advanced module. Only a portion of the content of this video relates to the Play Mendel Basic module.
- Student handout: Laboratory procedures & assignments
- Grading rubric
- Greening the Classroom Terms & Concepts
- Growing Arabidopsis in the Classroom

Materials
2 strains of Arabidopsis seeds (See seed strain details below)
Potting soil
14-14-14 fertilizer (*e.g.,* Osmocote)
32 plastic pots (Recommended size: 1 quart round pots, 4.7"d x 4.75"h)
4 solid trays (Suggested product - Hummert, Item #11-3050-1)
4 trays with holes for sub-irrigation (Suggested product - Hummert, Item #11-3000-1)
Cheesecloth or paper towels
Weighing boats
Disposable Pasteur pipettes
Labeling tape and marker
Plastic wrap
Watering can
Lab notebook
Growth space with fluorescent lights

Seed Strain Details

- **Landsberg erecta** (Ler-0, Catalog # CS20) – This laboratory strain contains an X-ray induced mutation in the *ERECTA* gene, which causes the plants to have a more upright growth habit. Ler-0 is widely used to generate mutants, and serves as the reference strain for the *ag-1* mutant used in this module.

- ***ag-1*** (Catalog # CS25) – This strain is heterozygous for a mutation in the *AGAMOUS* gene, which encodes a protein involved in the production of floral organs (sepals, petals, stamens and carpels). The reference strain for this mutant has flowers with all four organs present. In the *ag-1* mutants, the stamens and carpels have been replaced by petals and sepals to produce a "double" flower. The term agamous means "asexual", which represents the phenotype of the mutant plant which is sterile.

Background Information
Arabidopsis thaliana (Arabidopsis) was the first plant to have its genome completely sequenced. Although technically a weed, this plant has been transformed into an important model system for plant research, and a useful tool in teaching a variety of science concepts in K-12 and college level instruction. Arabidopsis is member of the Brassicaceae family and is related to a number of common food plants including cabbage, radish and cauliflower. It is a small, relatively easy to grow plant with a fast life cycle, going from seed to mature plant in six to eight weeks.

This module explores how you can use different strains of Arabidopsis to teach Mendelian genetics. Gregor Mendel, an Austrian monk who is generally considered to be the "father of genetics", discovered basic principles of heredity through experimentation with pea plants. Mendel's laws include:

- The Law of Segregation
    - In most cells, genes occur in pairs. Each of the two copies of the gene is called an allele. During gamete formation, the two alleles separate resulting in gametes with only one allele for each gene.

- The Law of Independent Assortment
    - Alleles for one trait separate and are passed on to offspring independent of the inheritance of alleles for other traits.

- Mendel also demonstrated that a trait can be recessive or dominant. Recessive traits are displayed only when both alleles are recessive. Only one dominant allele must be present for a dominant trait to be displayed.

Through this module, students will grow a segregating population of Arabidopsis and analyze the inheritance of the *ag1* allele to learn about the concepts of variation, segregation and inheritance discovered by Mendel.

Schedule of Procedures and Assignments

Week	**Activity**
Week 1	Procedure 1 - Plant seeds
Week 2-5	Assignment 1 – Observe growth and record phenotypes Water plants
Week 6	Assignment 1, continued - Observe growth and record phenotypes Assignment 2 - Analyze inheritance of the *ag-1* allele Assignment 3 - Formulate next-step research questions

Laboratory Procedures & Assignments

PROCEDURE 1 – Plant P generation seeds

1. Prepare 32 pots for planting. Cut pieces of cheesecloth or paper towel to fit the bottom of a pot. Place one piece in the bottom of each pot to prevent soil from escaping during watering.

2. Place potting soil in a container and add water to moisten. The moisture level of the soil should resemble a wet sponge. Add fertilizer according to package directions. Thoroughly mix soil for even distribution of water and fertilizer. Wear gloves when handling fertilizer and fertilized soil.

3. Fill each pot loosely with soil. Do not compress the soil as you fill the pots as that will limit aeration.

4. Stack one tray with drainage holes inside a solid tray. From this point forward, this pair of stacked trays will be referred to simply as a tray.

5. Divide the class into two groups. Each group will plant two trays containing eight pots each. Using labeling tape and a permanent marker, label each tray with your group number and the date (see example below).

Group 1 - Date

6. Label eight pots for the reference strain (Ler-0) and eight pots for the mutant strain (*ag-1)* (see examples below).

Group 1 Ler-0	Group 1 *ag-1*

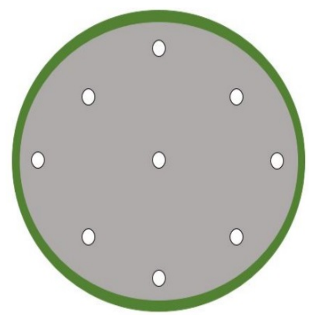

Figure 1. Placement of 9 seeds on soil surface (Price *et al.*, 2018).

7. Seeds are planted individually on top of the soil. To start, fill a weighing dish with water. Working with one seed stock at a time, sprinkle a portion of the seeds of one stock into the water. Mix the seeds in the water by pipetting up and down slowly using a disposable Pasteur pipette. This will help to separate the seeds and make it easier to capture them individually for planting. Be sure to use a different weighing dish and pipette for each stock to prevent cross-contamination.

8. Use the pipette to draw up individual seeds and place them on the surface of the soil. Plant nine seeds, evenly spaced, in each pot (Figure 1). Do not cover the seeds with soil.

9. Once planting is complete, place four reference strain pots (Ler-0) and four mutant strain pots (*ag-1*) in each tray (Figure 2). Wrap each tray tightly with plastic wrap to maintain moisture levels during germination.

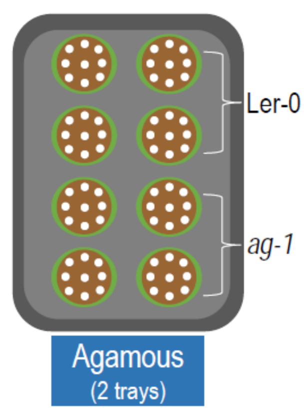

Figure 2. Illustration of how to organize pots in trays (Price *et al.*, 2018).

10. Optional – If space is available, place all of the trays inside a cold room or refrigerator at 4°C for 2-3 days. This process, known as stratification, mimics winter conditions and promotes uniform germination of the seeds. Skip this step if you do not have access to adequate refrigeration space.

11. Place the trays under fluorescent lights (see Growing Arabidopsis in the Classroom for lighting suggestions). If the soil was prepared with adequate moisture, you should not need to water your pots while they are covered with plastic wrap.

12. Remove the plastic wrap once you see seedlings emerge from the soil (approximately seven days after planting).

13. Once the plastic wrap has been removed, you will begin watering the plants regularly. Do not water directly into the pots. Add water to the tray to a depth of ½ inch once or twice a week. Be careful not to overwater the pots or allow the soil to dry out.

ASSIGNMENT 1 – Observe growth and record phenotypes

Since Arabidopsis has a fast life cycle, students will be able to observe morphological changes over a relatively short period of time. After germination, students should observe plants on a weekly basis and complete the following tasks:

1. Define key terms related to plant growth and anatomy such as rosette, inflorescence, stratification, germination, and bolting.

2. Make detailed drawings of the plants noting any visible differences between the two different strains.

3. Describe the Arabidopsis life cycle by identifying details about and onset of various growth stages such as the number of leaves present in the rosette stage, flowering, etc.

4. Identify the unique trait that differentiates the mutant from the reference strain (Figure 3). Describe the trait using drawings and notes. Make a note of when the trait was first noticeable (*e.g.,* in rosette stage or after flowering).

Figure 3. Phenotypes associated with Ler-0 and *ag-1* (Price *et al.*, 2018).

ASSIGNMENT 2 – Analyze the inheritance of the *ag-1* allele

For this experiment, students planted a segregating (heterozygous) population of the *ag-1* mutant. In this experiment, the reference plant has a flower with four petals and all reproductive organs. In the mutant plant the male reproductive organs are absent, having been replaced with a second whorl of petals creating a "double" flower. In this assignment students will analyze the inheritance of the *ag-1* allele by completing the following tasks:

1. Collect phenotypic data (Student Handout, Agamous Worksheet, Table 1) to analyze the inheritance of the *ag-1* allele. Remember, there are multiple plants in each pot and care should be taken to accurately identify each individual plant.
    a. Count the number of plants with flowers displaying the reference phenotype.
    b. Count the number of plants with flowers displaying the mutant phenotype.
    c. Calculate the ratio of plants with reference to mutant flowers for your group.

2. Make a prediction about how combining the data from both groups in the classroom may affect the ratio determined by each individual group.

3. Add the data from both groups and calculate the ratio again. Use the student worksheet to answer the following questions:
    a. Did aggregating the data from both groups cause the ratio to change?
    b. If so, explain how and why the ratio changed.
    c. Why is this important to the process of science?

4. Based on the ratio your class obtained, conclude whether the *ag-1* allele is dominant or recessive.

5. Support your finding with evidence by completing a Punnett square (Student Handout, Agamous Worksheet, Table 2) and answering the corresponding questions.

6. Define key genetic terms such as genotype, phenotype, dominant and recessive.

NOTE: At the conclusion of this experiment the pots and trays can be disinfected for reuse. To disinfect materials, add ¼ cup Lysol to one gallon of water and soak for 10 minutes. Use a scrub brush to remove any soil residue or plant material then rinse and allow to air dry.

ASSIGNMENT 3 – Formulate next-step research questions

In this assignment, students will review what they have learned about the *ag-1* mutation, the Ler-0 reference strain, and the life cycle of Arabidopsis to consider future experiments. Through this assignment, students will complete the following tasks:

1. Consider and summarize what aspects of Arabidopsis they would like to investigate should the class continue working with this model system.

2. Draft a research question that could serve as the launching point for a new experiment using Arabidopsis.

3. Formulate a hypothesis to test based on the new research question.

References

Arabidopsis Biological Resource Center [ABRC]. (2016). Education and Outreach. Available online at https://abrcoutreach.osu.edu/

Price, C., Knee, E., Miller, J., Shin, D., Mann, J., Crist, D., Grotewold, E., & Brkljacic, J. (2018). Following phenotypes: An exploration of Mendelian genetics using Arabidopsis plants. *The American Biology Teacher*.

Additional Reading

Ausubel, F.M. (2000). Arabidopsis genome: A milestone in plant biology. *Plant Physiology, 124,* 1451-1454.

Knee, E.M., Rivero, L., Crist, D., Grotewold, E., and Scholl, R. (2009). Germplasm and molecular resources. In: Genetics and Genomics of the Brassicaceae. Jinnie Kim, Senior Editor in revision.

Koornneef, M. & Meinke, D. (2010). The development of Arabidopsis as a model plant. *The Plant Journal*, *61(6),* 909-921.

Pang, P.P. & Meyerowitz, E.M. (1987). Arabidopsis thaliana: A model system for plant molecular biology. *Nature Biotechnology, 5(11),* 1177-1181.

Provart, N.J., Alonso, J., Assmann, S.M., Bergmann, D., Brady, S.M., Brkljacic, J. *et al.* (2016). 50 years of Arabidopsis research: Highlights and future directions. *New Phytologist*, *209(3),* 921-944.

Rivero, L., Scholl, R., Holomuzki N., Crist, D., Grotewold, E., & Brkljacic, J. (2014). Handling Arabidopsis plants: Growth, preservation of seeds, transformation, and genetic crosses. *Methods in Molecular Biology, 1062,* 3-25.

Somerville, C. & Koornneef, M. (2002). A fortunate choice: The history of Arabidopsis as a model plant. *Nature Reviews Genetics, 3(11)*, 883-889.

Wyatt, S. & Ballard, H.E. (2007). Arabidopsis ecotypes: A model for course projects in organismal plant biology and evolution. *American Biology Teacher, 69(8),* 477-481.

Zhang, Z.-L. (2006). Use of the *gl1* Mutant & the *CA-rop2* Transgenic Plants of *Arabidopsis thaliana* in the Biology Laboratory Course. The American Biology Teacher online publication November/December: 148-153.