Plant Curiosity Advanced

Summary: This kit is highly adaptable and can be used to demonstrate a variety of concepts including plant anatomy, phenotypic variation, inheritance and mutations. The six stocks contained in this kit include five mutant lines paired with their corresponding reference strain. By observing the growth of this set of seeds students will appreciate how different mutations can affect the phenotype of Arabidopsis plants.

Recommended Grade Level: Elementary and middle school

Duration: This module requires 19 weeks for completion of all planting procedures and assignments. For teachers looking for a shorter demonstration of these concepts, consider "Plant Curiosity Basic", which requires only seven weeks to complete.

Learning Objectives

Through this module students will:

- Define concepts and terms associated with the growing process, plant anatomy and reproduction.
- Plant and care for two generations of Arabidopsis.
- Make observations, compare phenotypes, and illustrate growth stages of mutant and reference strains of Arabidopsis
- Make predictions about the impact of different mutations on a plant's ability to survive
- Make predictions about the inheritance of specific traits

Alignment with Next Generation Science Standards

NGSS	
Standards	-From Molecules to Organisms: Structures and Processes (K-LS1-1, 4-LS1-1) -Heredity: Inheritance and Variation of Traits (1-LS3-1, 3-LS3-1, MS-LS3-1) -Biological Evolution: Unity and Diversity (2-LS4-1, 3-LS4-2)
Science & Engineering Practices	-Making observations -Developing and using models
Disciplinary Core Idea	-Structure and Function -Inheritance of Traits -Variation of Traits -Natural Selection -Biodiversity and Humans
Crosscutting Concepts	-Patterns -Systems and system models

Supporting Resources

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

- All about Arabidopsis Worksheet
- Growing Arabidopsis in the Classroom
- Greening the Classroom Terms & Concepts

Materials

Gloves
Vial of mixed Arabidopsis seeds (Catalog #CS4004, see below for individual strain details)
Potting soil
14-14-14 fertilizer (e.g. Osmocote)
Plastic pots (Recommended size: 1 quart round pots, 4.7"d x 4.75"h)
Solid trays (Suggested product – Hummert, Item #11-3050-1)
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
Scissors
Eppendorf tubes (Suggested product - Fisher Scientific, Item #05-408-138)

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 five mutants used in this module.

- ***bp-1*** (Catalog # CS30) – This strain carries a recessive, homozygous ethylmethane sulfonate (EMS)-induced mutation affecting the *BREVIPEDICELLUS* gene. The phenotype of this mutant includes reduced height, shortened pedicels and siliques that are bent downwards.

- ***cer1-1*** (Catalog # CS31) – This strain carries a recessive, homozygous EMS-induced mutation affecting the *ECERIFERUM 1* gene. The phenotype of this mutant includes reduced plant height, and a brighter green stem that lacks a waxy appearance when compared to the reference strain.

- ***ch1-1*** (Catalog # CS41) – This strain carries a recessive, homozygous X-ray-induced mutation affecting the *CHLORINA 1* gene. The resulting mutant is a small, yellow-green plant.

- ***clv1-1*** (Catalog # CS45) – This strain carries a recessive, homozygous EMS-induced mutation affecting the *CLAVATA 1* gene. The phenotype of this mutant includes bent, club-like siliques.

- ***gl1-1*** (Catalog # CS64) – This strain carries a recessive, homozygous mutation affecting the *GLABROUS 1* gene. The resulting mutant is glabrous (bald), with very few or no trichomes present on the stem and leaves.

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 crop 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.

With this module, students will grow five Arabidopsis mutants and one reference strain. At its most basic, this module will teach young students what plants need to survive and give students practice caring for live organisms in the classroom. Through the activities outlined in the module, students will practice important steps in the scientific process including observing, making predictions, recording and comparing results. By developing these skills, students are setting the stage for future, more complex investigations.

Adding complexity to these basic concepts, students will observe the growing plants to see first-hand how changes to an organism's DNA (genotype) can affect its appearance (phenotype). Through the process of growing the parent generation, collecting and planting seeds, and caring for the resulting offspring, students will understand that heritable traits are passed down through generations.

Schedule of Procedures and Assignments

Week	**Activity**
Week 1	Procedure 1 – Plant parent generation seeds
Weeks 2-6	Water plants
Week 7	Assignment 1 – Observe growth and record phenotypes; make predictions
Week 8-9	Do not water – Allow plants to dry for seed collection
Week 10	Procedure 2 – Collect offspring seeds
Weeks 11-12	Dry offspring seeds Assignment 2 – Make a prediction
Week 13	Procedure 3 – Plant offspring seeds
Weeks 14-18	Water plants
Week 19	Assignment 3 – Compare results

Laboratory Procedures & Assignments

PROCEDURE 1 – Plant parent seeds

Because of the small seed size, planting Arabidopsis requires patience and fine motor skills. This activity may not be suitable for younger students. In these cases, teachers can complete planting procedures outside of class time.

1. Divide class into small groups. The suggested group size is six students. However, if growth space or supplies are limited larger groups can be formed.

2. Each group should prepare 8 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.

3. 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.

4. Fill each pot loosely with soil. Do not compress the soil as you fill the pots as that will limit air flow to the roots.

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

6. Using labeling tape and a permanent marker, label each tray with your group number and the date (see examples below).

   Group 1 - Date

7. Label eight pots with your group number and the seed type (see example below).

   Group 1

   CS4004 Mix

8. Seeds are planted individually on top of the soil. To start, fill a weighing dish with water. Sprinkle a portion of the seeds 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.

9. 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.

10. Once planting is complete, place eight pots in each tray. Wrap each tray tightly with plastic wrap to maintain moisture levels during germination.

11. 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.

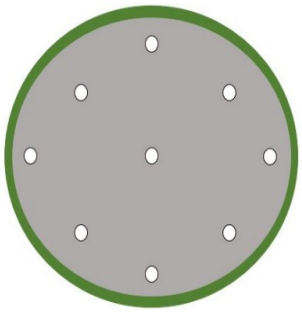

Figure 1. Placement of 9 seeds on soil surface (Price *et al*., 2018).

12. 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.

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

14. Once the plastic wrap has been removed, you will begin watering the plants regularly. Water when the pots feel light and the soil appears dry (1-3 times/week). Do not water directly into the pots. Add water to the tray to a depth of ½ inch and allow pots to soak. After 15 minutes, dump out any water that was not absorbed. 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 the morphological changes over a relatively short period of time. After germination, students should observe plants on a weekly basis and complete the following tasks in their lab notebooks:

1. Define key terms related to plant growth and anatomy such as rosette, inflorescence, silique, stratification, trichome, germination, bolting, and senescence.
   Adaptation for younger students (All about Arabidopsis Worksheet, Section 1) - Identify and label the following plant parts: Root, stem, leaf, flower, seed pod.

2. Make detailed drawings of each of the six strains of Arabidopsis, noting any visible differences between the strains.

3. Describe the Arabidopsis life cycle by noting the timing of various growth stages including flowering, the development of siliques and senescence, as well as developmental details such as the number of leaves present in the rosette and the height of the inflorescences.
   Adaptation for younger students (All about Arabidopsis Worksheet, Section 2) – Sort the stages of the Arabidopsis life cycle into the correct order.

4. Identify the unique traits for each of the six strains (Appendix A). Describe the traits using drawings and notes. Note when each trait was first visible (*e.g.*, in rosette stage or after flowering).
   Adaptation for younger students (All about Arabidopsis Worksheet, Section 3) – Use the photos provided to search for the six different types of Arabidopsis plants. Check off each different type of plant you find. As a class, decide on a name for each different type of Arabidopsis based on unique traits of each plant.

5. Make predictions about how each of the unique traits identified in #4 above may increase or decrease a plant's likelihood to survive and be healthy.
   Adaptation for younger students (All about Arabidopsis Worksheet, Section 4) –Identify what a plant needs to grow and be healthy.

PROCEDURE 2 – Collect offspring seeds

1. Allow the plants to dry out for two weeks until the color of the siliques changes from green to yellow-brown.

2. Prepare a 2 mL Eppendorf tube for each of the six strains of Arabidopsis identified. Label each tube with your group number, date, and strain name (see example below).

Group 1, date Ler-0

3. Use scissors to carefully remove 2 siliques from each type of plant and place them in the appropriately labeled Eppendorf tube. If students are working in groups of six, each student can be responsible for collecting siliques from one strain of Arabidopsis.

4. Gently tap the tube on a table several times to release the seeds.

5. At this point, the original plants can be discarded. Pots and trays can be disinfected for reuse.

6. Allow the seeds to dry in the Eppendorf tubes for two weeks. This process will reduce the internal moisture content of the seeds leading to higher germination rates.

NOTE: 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 2 – Make a prediction

1. Define key terms related to reproduction such as pollination, self-pollination, cross-pollination, and fertilization. *Adaptation for younger students: Discuss as a class how some types of plants are cross-pollinated and others are self-pollinated.*

2. Based on the knowledge that Arabidopsis is self-pollinating, make a prediction about how the plants grown from each strain of seed might look.

PROCEDURE 3 – Plant offspring seeds

1. Following the steps for planting outlined in Procedure 1, each group should plant one pot each of the six strains of Arabidopsis seeds collected in Procedure 2, as well as one pot of the parent seeds originally planted in Procedure 1.

2. Follow steps 10-14 in Procedure 1 for plant care.

ASSIGNMENT 3 – Compare results

1. Write down observations about the plants growing in each of the six pots representing the offspring generation.

2. Compare the phenotypes of the offspring plants with those of the parent generation plants.

3. Do all of the offspring resemble their parent plant? Why or why not?

4. Talk to your classmates, do their results match yours? How do you explain differing results?

References

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

The European Arabidopsis Stock Centre [NASC]. Teaching kits for Arabidopsis. Available online at http://arabidopsis.info/CollectionInfo?id=49

APPENDIX A

Photographs of the six strains of Arabidopsis included in the Plant Curiosity kit

Landsberg erecta (reference strain)

bp-1

cer1-1

ch1-1

clv1-1

gl1-1