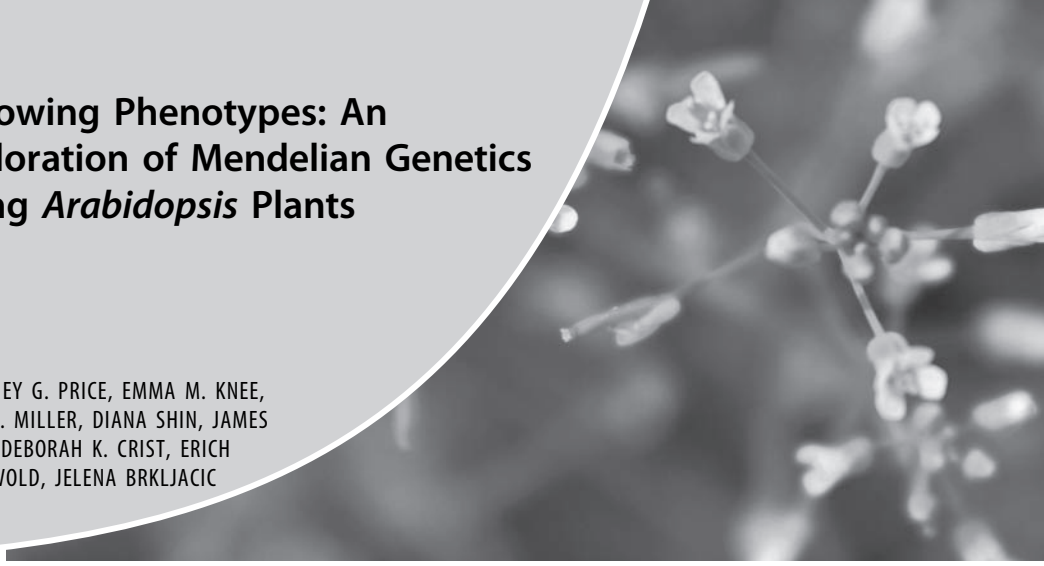
INQUIRY & INVESTIGATION

Following Phenotypes: An Exploration of Mendelian Genetics Using *Arabidopsis* Plants



COURTNEY G. PRICE, EMMA M. KNEE, JULIE A. MILLER, DIANA SHIN, JAMES MANN, DEBORAH K. CRIST, ERICH GROTEWOLD, JELENA BRKLJACIC

Abstract

Arabidopsis thaliana, *a model system for plant research, serves as the ideal organism for teaching a variety of basic genetic concepts including inheritance, genetic variation, segregation, and dominant and recessive traits. Rapid advances in the field of genetics make understanding foundational concepts, such as Mendel's laws, ever more important to today's biology student. Coupling these concepts with hands-on learning experiences better engages students and deepens their understanding of the topic. In our article, we present a teaching module from the Arabidopsis Biological Resource Center as a tool to engage students in lab inquiry exploring Mendelian genetics. This includes a series of protocols and assignments that guide students through growing two generations of Arabidopsis, making detailed observations of mutant phenotypes, and determining the inheritance of specific traits, thus providing a hands-on component to help teach genetics at the middle and high school level.*

Key Words: *Mendel's laws; genetics;* Arabidopsis; *inheritance; phenotype; segregation.*

○ Introduction

Topics related to genetics and genetic modification have been making regular appearances in the news for some time now. For today's students to develop the scientific literacy necessary to understand advances in this field, it is important to establish a solid understanding of basic genetic concepts. Presenting challenging content solely through lectures leaves many students with only a superficial understanding of the material (Kontra et al., 2015). By performing hands-on experiments designed to demonstrate foundational concepts such as Mendel's laws, student learning is elevated beyond what textbooks and lectures alone can accomplish (ACS, 2016; Wyatt & Ballard, 2007; Zheng, 2006).

Arabidopsis thaliana (*Arabidopsis*), the first plant to have its genome completely sequenced, has been transformed from being just a common weed to serving as a major model system for plant research worldwide (Somerville & Koornneef, 2002; Koornneef & Meinke, 2010). Its role expands beyond the laboratory to have considerable utility in science education. *Arabidopsis* is a member of the mustard family (Brassicaceae) and a relative of Wisconsin Fast Plants, which may be familiar to some science educators. *Arabidopsis*, whose common name is mouse-ear cress, provides a launching point from which students can investigate a wide variety of scientific concepts, including adaptation to environmental conditions, how plants sense light, and the role of environment and genetics in growth and development (ABRC, 2016; Provart et al., 2016). With a short life cycle (6–8 weeks from seed to seed), self-fertility, and relatively low-maintenance growing requirements, this plant can be easily incorporated into even the most modestly equipped science classrooms (Ausubel, 2000; Pang & Meyerowitz, 1987; Zheng, 2006).

The Arabidopsis Biological Resource Center (ABRC) at The Ohio State University (OSU) is one of two global stock centers providing seeds, DNA, and other resources to scientists and educators worldwide. ABRC, which is home to more than 1,000,000 *Arabidopsis* stocks, provides samples to approximately 30,000 researchers in more than 50 countries annually. The Center launched its education and outreach program in 2011 by releasing 20 teaching modules, consisting of *Arabidopsis* seeds and/or DNA resources combined with lab instructions. The instructional materials have been made available through ABRC's education and outreach website (ABRC Outreach, https://abrcoutreach.osu.edu/), and the seeds and DNA can be ordered through The Arabidopsis Information Resource (TAIR, https://www.arabidopsis.org/). The program provides centralized access to *Arabidopsis* resources and teaching tools for K-12 and undergraduate education. Seeds are provided free of charge to K-12 teachers, along with in-depth lesson plans and supplemental materials that guide educators through the process of incorporating *Arabidopsis* into their science curriculum. The activities presented in this article are based on ABRC's "Play Mendel" module, with additional support for the procedures presented in this article available on the ABRC Outreach website. Seeds for this lesson can be ordered from ABRC through TAIR using the module's catalog number, CS19985.

The American Biology Teacher, Vol. 80, No 4, pages. 291–300, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: https://doi.org/10.1525/abt.2018.80.4.291.

○ Lesson Details

This article presents three activities that can be combined or used as stand-alone units to engage students in the process of growing, breeding, and caring for two generations of *Arabidopsis*, as well as making observations, maintaining a detailed lab notebook, collecting data, and analyzing results. By performing these activities, students gain insight into concepts such as genotype, phenotype, inheritance, and segregation of traits, which lie at the core of understanding Mendel's laws. The activities are designed for use with middle and high school classes and are aligned with the Next Generation Science Standards (NGSS), which are listed within each activity section. This module provides a structured laboratory experience, and the skills developed through the procedures and assignments lay the foundation for future open-inquiry and student-driven experiments using *Arabidopsis*.

The full lesson (all three activities) spans four months. Together, these activities guide students through the process of growing *Arabidopsis* from the parent (P) generation (Activity 1), conducting phenotypic analysis of the segregation of a reproductive trait (Activity 2), and performing genetic crosses to obtain and analyze the phenotype of the first filial (F1) generation (Activity 3). Procedures and assignments are listed for each of the activities (Tables 1, 2, and 4). Students can be engaged in the entire process for a rich hands-on experience, or portions of the procedures can be performed by the teachers ahead of time to adapt to tighter schedules.

Table 1. Schedule of procedures and assignments for Activity 1.

Week	Activity	Estimated Time	Lab Learning Objective
1	Procedure 1: Plant P seeds Assignment 1: Observe growth	1 hour (prep) + 45 min (plant) 20 min	 1, 2, 3
2–4	Water plants Assignment 1 continued	20 min twice a week 20 min per week	 1, 2, 3
5	Water plants Assignment 1 continued Assignment 2: ID unique traits	20 min twice a week 20 min 1 hour	 1, 2, 3 3, 4

Table 2. Schedule of assignments for Activity 2.

Week	Activity	Estimated Time	Lab Learning Objective
6	Assignment 1: Analyze inheritance	45 min	1, 2

Table 3. Data table for analysis of the inheritance of select mutations.

	Reference phenotype	Mutant phenotype	Ratio
Group 1			
Group 2			
Groups 1 & 2			

Table 4. Schedule of procedures and assignments for Activity 3.

Week	Activity	Estimated Time	Lab Learning Objective
6	Procedure 1: Perform genetic crosses	2 x 45 min	1, 2
7	Water plants Assignment 1: Observe cross outcome	20 min 20 min	 1
8–9	Do not water plants Assignment 1 continued	20 min per week	1
10	Procedure 2: Collect F1 seeds	45 min	
11–12	No activity		
13	Procedure 3: Plant F1 seeds	45 min (prep & plant)	
14–15	Water plants	20 min twice a week	
16	Water plants Assignment 2: Compare phenotypes	20 min 45 min	 3

○ Seed Strain Background

- Columbia (Col-1, CS28169)—This is the reference strain of *Arabidopsis*. The genome of the closely related Col-0 strain has been completely sequenced and is used as a basis for comparison with other natural strains. Col-1 is a laboratory strain that has been used to generate many mutants, including the *gl1-1* mutant used in this lesson. Col-1 is used as a reference strain for the *gl1-1* mutant in this lesson.
- *gl1-1* (CS28175)—This strain has a mutation in the *GLABROUS1* gene, which encodes a protein involved in trichome (leaf hair) formation. The Col-1 reference strain has trichomes on its stems and leaves. The homozygous *gl1-1* mutant is glabrous (hairless), with very few trichomes present.
- Landsberg erecta (Ler-0, CS20)—This laboratory strain, which is widely used to generate mutants, carries an X-ray induced mutation in the *ERECTA* gene, causing the plant to have a more upright growth habit. This is the parent strain for the *ag-1* mutant used in this lesson. Ler-0 is used as a reference strain for the *ag-1* mutant in this lesson.
- *ag-1* (CS25)—This plant has a mutation in the *AGAMOUS* gene, which encodes for a protein that controls the production of floral organs (sepals, petals, stamens, and carpels) during flower development. The term "agamous" means asexual, referring to the phenotype of the mutant plant, which is sterile. The stamens and carpels in *ag-1* mutants have been replaced by petals and sepals, producing a double flower (see Figure 2).

○ Lesson Preparation

Seeds can be ordered from ABRC through TAIR (www.arabidopsis.org), which requires individuals to register before placing an order. To streamline the registration process, it is recommended that first-time users contact ABRC directly to set up an ordering account with TAIR. To contact ABRC about setting up an account, e-mail abrcedu@osu.edu with the following information: full name, email address, school/institution name, job title (elementary/middle/high school teacher/other), phone number (for shipping), and shipping address. Seeds should be ordered at least two weeks before the planned start date of the first activity.

Arabidopsis grows best at 120–150 μmol/m^2s continuous light and a temperature of 22–23° C (Rivero et al., 2014). Detailed protocols for growing and maintaining *Arabidopsis* plants can be found on the ABRC website (https://abrc.osu.edu/seed-handling). Please note that growth rates will vary when seeds are grown in different conditions. Plants may grow faster or slower than what is indicated in these protocols if the light intensity and duration, as well as the temperature, vary from what is recommended.

Supplemental materials for teachers are provided to facilitate easier implementation of this unit. Appendix 1 provides definitions of terms related to plant anatomy and Mendelian genetics. Appendix 2, designed to serve as a student hand-out, contains simplified laboratory protocols for all of the procedures.

○ Activity 1

Observation of Growth and Development of *Arabidopsis* Plants

The first activity in this lesson allows students to observe both vegetative and reproductive growth and development of *Arabidopsis* plants over a five-week period. Students will learn about plant anatomy while making detailed observations throughout this dynamic period of growth. Instructors can use this activity to discuss how the development of plant organs, tissues, and cells contribute to overall anatomy and to identify the different functions each perform in a plant organism. The schedule of procedures and assignments for this activity is listed in Table 1. This activity is aligned with the following Next Generation Science Standards (NGSS):

- MS-LS1-4, From molecules to organisms: Structures and processes
  - Disciplinary Core Idea (DCI) LS1.B, Growth and development of organisms
- MS-LS4-4, Biological evolution: Unity & diversity
  - DCI LS4.B, Natural selection

Lab Learning Objectives

1. Make detailed observations of the various growth stages in mutant and reference plants.
2. Define terms associated with the growing process as well as with the phenotypes of different *Arabidopsis* mutants.
3. Compare the phenotypes of mutant and reference strains of *Arabidopsis*.
4. Label the anatomy of a plant and identify the role of specific features in reproduction.

Materials

- 4 strains of *Arabidopsis* seeds (Catalog #: CS28169, CS28175, CS20, CS25)
- Potting soil
- 14-14-14 fertilizer (e.g., Osmocote)
- Teaspoon
- 64 plastic pots (Recommended: 1-quart round pots, 4.7″d × 4.75″h)
- 8 solid trays (Hummert, Item #11-3050-1)
- 8 trays with holes for sub-irrigation (Hummert, Item #11-3000-1)
- Cheesecloth (Fisher Scientific, Item #06-665-2513) or paper towels
- Weighing boats
- Disposable Pasteur pipettes
- Labeling tape and marker
- Plastic wrap
- Watering can
- Lab notebook
- Growth space with fluorescent lights (http://abrcoutreach.osu.edu/growing-arabidopsis-classroom)

Procedure 1: Plant the P Generation Seeds (Week 1)

In this procedure, students will plant two reference strains of *Arabidopsis*, a homozygous *gl1-1* mutant, and a segregating population (heterozygous for gamete formation) of the *ag-1* mutant. Divide the class into two groups, with each group completing the following planting procedure:

1. Place 1.0 cubic feet of potting soil in a bucket or other large container and wet thoroughly with water. The moisture content of the soil should resemble that of a wet sponge. Add 14-14-14 fertilizer to the soil according to the ratio provided on the product label and blend thoroughly.
2. Line 32 pots with a piece of cheesecloth or paper towel cut to fit the bottom of the pot such that soil will not escape through the drainage holes.
3. Place a tray with irrigation holes inside a solid tray. This set of two trays (one with holes, one without) will be referred to as a tray throughout the remaining procedures. Label four trays per group with the group number or name, and the name of the experiment (glabrous or agamous). Next, label eight pots with each of the seed stock names (Col-1, *gl1-1*, Ler-0, or *ag-1*).
4. Fill the pots loosely with the prepared soil, taking care not to limit aeration by compressing the soil.
5. Put a small amount of water in a weighing dish. Select the first seed stock to be planted and sprinkle a portion of the seeds into the water. Use a disposable pipette to mix the seeds by pipetting the water up and down slowly until the seeds are dispersed throughout the water. Use the pipette to draw in individual seeds and water. Dispense the seeds onto the surface of the soil, placing nine seeds evenly spaced in each pot. Continue until you have completed eight pots per seed stock. Do not cover the seeds with soil.
6. Repeat Step 5 until all four stocks have been planted (Figure 1). Use a new weighing dish and pipette for each seed stock to avoid cross-contamination.
7. Place four mutant pots and four of the corresponding reference strain in each tray. When complete, each group will have two trays with four pots each of Col-1, and *gl1-1*, and two trays with four pots each of Ler-0 and *ag-1* (Figure 1).
8. Cover pots tightly with plastic wrap. This will help maintain humidity until the seeds germinate. If possible, immediately after covering, place the trays in a dark refrigerator for 2–3 days to promote uniform germination. This process is known as stratification.
9. After planting (or after stratification), place the trays under the light source. Once the seeds have germinated and growth is seen (3–7 days after planting), remove the plastic wrap. Fill the bottom of the tray with ½ inch of water once or twice a week. It is important not to overwater the plants or let the soil dry out completely.

Observation of Genetic Traits

The short life cycle of *Arabidopsis* provides students with an opportunity to make detailed observations of the morphological changes that occur in the plants throughout all stages of growth. To prepare for the following assignments, have students research the life cycle of *Arabidopsis* and become familiar with terms for basic plant structures and processes (see Appendix 1). There are a number of online resources where this information can be gathered including the ABRC Outreach website (http://abrcoutreach.osu.edu/) and the American Society of Plant Biologists K-12 education page (https://aspb.org/education-outreach/k12-roots-and-shoots/).

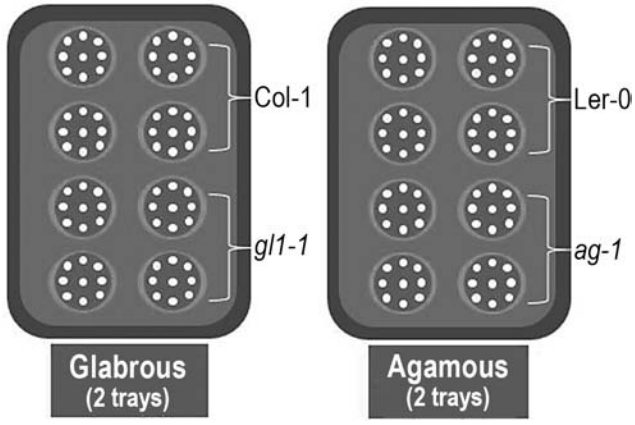


Figure 1. Illustration of seed spacing and how groups should organize pots in trays for the experiment. In the Glabrous experiment, the glabrous (hairless) *gl1-1* mutant (containing a loss-of-function mutation in the *GLABROUS* or *GL1* gene) will be compared to the Col-1 reference strain (containing a functional *GL1* gene). In the Agamous experiment, the agamous (asexual) *ag-1* mutant (containing a loss-of-function mutation in the *AGAMOUS* or *AG* gene) will be compared to the Ler-0 reference strain (containing a functional *AG* gene).

Assignment 1: Observe Growth (Weeks 1–5)

After planting is complete, have students record observations of the plants in their lab notebooks several times a week for the first five weeks.

Through this assignment students should:

- Make detailed drawings and notes about each of the four strains of *Arabidopsis*.
- Define growth stages of the plants based on the number of leaves, onset of flowering, silique maturation, and senescence.
- Define terms related to the growing process including stratification, germination, bolting, flowering, senescence (see Appendix 1).

Assignment 2: Identify Unique Traits (Week 5)

Have students compare all four strains of plants to identify the traits that differentiate each mutant from its corresponding reference strain. Images in Figure 2 show the distinguishing traits for each of the four seed strains.

Through this assignment students should:

- Understand the anatomy and function of plant organs.
- Describe the traits using illustrations and notes.
- Record the growth stage when the differences were first noticed.

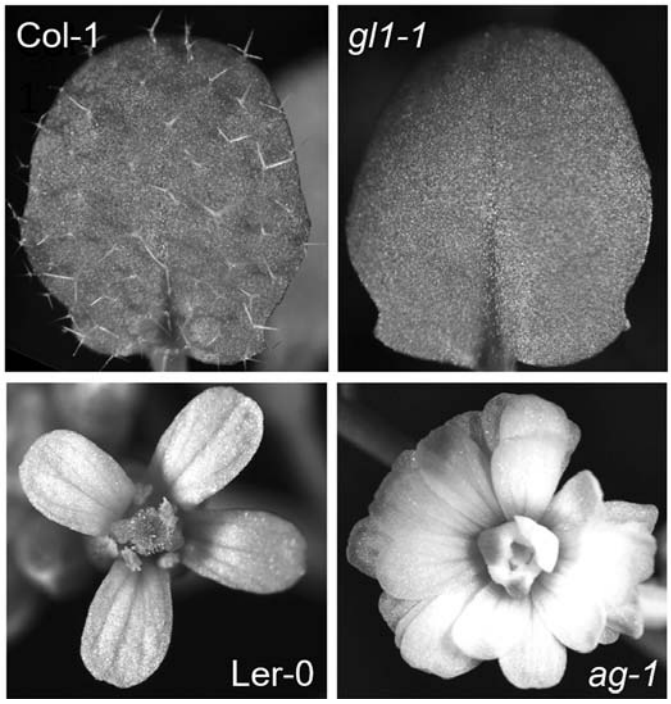


Figure 2. Different phenotypes associated with the reference (Col-1 and Ler-0) and mutant (*gl1-1* and *ag-1*) plants used in this unit.

- Compare the two different mutants with respect to growth stage when each plant's unique trait was first visible.

○ Activity 2

Phenotypic Analysis of the Segregation of the Agamous Trait

An investigation of the inheritance of a mutation in the *AGAMOUS* gene can be completed as a stand-alone activity within a single class session. This activity, which requires only six weeks of grow time, serves as a demonstration of Mendelian genetics. The schedule of assignments for Activity 2 is listed in Table 2. This activity is aligned with the following NGSS:

- MS-LS3-1 & MS-LS3-2, Heredity: Inheritance & variation of traits
  - DCI LS3.A, Inheritance of traits
  - DCI LS3.B, Variation of traits
- HS-LS3-3, Heredity: Inheritance & variation of traits
  - DCI LS3.B, Variation of traits

Lab Learning Objectives

1. Collect and analyze data to determine the inheritance of a specific trait in *Arabidopsis* mutants.
2. Define concepts central to Mendelian genetics (see Appendix 1).

Materials

- 2 trays containing 16 pots of 6-weeks old plants (*ag-1* and Ler-0, Figure 1) per group (plants obtained as described in Activity 1)
- Lab notebook

Assignment 1: Analyze Inheritance (Week 6)

In this assignment, students will analyze the inheritance of the *ag-1* mutation. Have students determine the number of plants displaying the reference strain flower phenotype and the mutant flower phenotype in the *ag-1* pots. Students should arrange their results in a table (Table 3) and conclude whether this mutation is dominant or recessive. A dominant mutation requires that only one copy of the mutant gene be present in order for the mutant phenotype to be apparent. However, a recessive mutation requires that both copies of the gene contain the mutation in order for the mutant phenotype to be displayed. This activity will demonstrate the importance of having a statistically significant sample size when analyzing data. Students will appreciate that the Mendelian ratio of 3:1 may not be observed with a random sample of small size, but that the ratio approaches 3:1 with a larger sample. The number of plants of the segregating *ag-1* mutant used in this experiment may not be sufficient for the collected data to accurately reflect the Mendelian ratio. In this case, teachers are advised either to use the data generated by previous classes or to reach out online for additional data from other teachers to aggregate with the data collected by their class. Teachers can use this experiment as an example to convey the importance of large sample sizes. Demonstrating the effect of a large sample size on the outcome of the experiment will also help reinforce student understanding and appreciation of Gregor Mendel's original discovery. Mendel counted and scored phenotypes for thousands of specimens to conclude the approximate 3:1 ratios.

Through this assignment students should:

- Calculate the ratio of reference to mutant flower phenotypes in each group.
- Predict what will happen with the ratio if the results from the two groups are combined.
- Calculate the ratio with the results from both groups combined, and explain how and why the ratio may have changed.
- Conclude whether the *ag-1* mutation is dominant or recessive (keeping in mind that a segregating population of the *ag-1* mutant was planted).
- Use a Punnett square to support the conclusion with evidence (Figure 3).

Figure 3. Punnett square demonstrating the possible outcomes of a genetic cross in which each parent possesses one each of the dominant (A) and recessive (a) gene variants (alleles).

At this point in the module, the group is done working with the Ler-0 and *ag-1* plants, and they can be discarded. Once emptied, pots and trays can be disinfected and reused for future procedures in Activity 3. To disinfect pots and trays, dilute ¼ cup of Lysol per one gallon of warm water. Allow the material to soak for 10 minutes. Use a sponge or scrub brush to remove any plant or soil material, then rinse and air dry.

○ Activity 3

Perform Genetic Crosses to Obtain F1 Heterozygotes for the Glabrous Trait

In this activity students will perform genetic crosses between a *gl1-1* mutant and Col-1 reference strain, collect seeds and grow the F1 generation, and analyze the phenotype of the resulting F1 plants. The schedule of procedures and assignments for this activity is listed in Table 4. This activity is aligned with the following NGSS:

- MS-LS3-1 & MS-LS3-2, Heredity: Inheritance & variation of traits
  - DCI LS3.A, Inheritance of traits
  - DCI LS3.B, Variation of traits
- HS-LS3-3, Heredity: Inheritance & variation of traits
  - DCI LS3.B, Variation of traits.

Lab Learning Objectives

1. Generate a segregating population of plants by performing a genetic cross between mutant and reference plants.
2. Label the anatomy of a plant and identify the role of specific features in reproduction.
3. Compare the phenotypes of F1 plants to the mutant and reference strains of *Arabidopsis* to hypothesize whether the glabrous trait is dominant or recessive.

Materials

- 2 trays containing 16 pots of 6-week old plants (*gl1-1* and Col-1, Figure 1) per group (plants obtained as described in Activity 1)
- Potting soil
- 14-14-14 fertilizer (*e.g.,* Osmocote)
- Teaspoon
- 18 plastic pots (Recommended: 1-quart round pots, 4.7″d × 4.75″h)
- 3 solid trays (Hummert, Item #11-3050-1)
- 3 trays with holes for sub-irrigation (Hummert, Item #11-3000-1)
- Cheesecloth (Fisher Scientific, Item #06-665-2513) or paper towels
- Weighing boats
- Disposable Pasteur pipettes
- Labeling tape and marker
- Plastic wrap
- Scissors
- Headband magnifier (Lehle Seeds, Item #DA-10)
- Fine-tip tweezers (Lehle Seeds, Item #DV-30)
- Eppendorf tubes (Fisher Scientific, Item #05-408-138)
- Watering can
- Lab notebook
- Growth space with fluorescent lights (http://abrcoutreach.osu.edu/growing-arabidopsis-classroom)

Procedure 1: Perform Genetic Crosses (Week 6)

Arabidopsis is a self-pollinating plant. Once the flower has opened, pollination has already occurred. To avoid self-pollination and perform a successful cross, students will need to select buds with barely visible petals to become the female parent plant. Figure 4 demonstrates the various steps outlined in this procedure. This is a challenging procedure that requires patience. To help prepare students, have them view the Play Mendel Protocol Video available on the ABRC Outreach website (http://abrcoutreach.osu.edu/educational-kits). At this stage of growth, each *Arabidopsis* plant should contain multiple flower buds. This will allow students the freedom to practice preparing a flower for a cross with the understanding that mistakes will be made, while still providing enough flower buds for the completion of a successful cross. However, if students are demonstrating difficulty with this procedure or are unsure of flower anatomy, teachers may purchase cut flowers with obvious anatomy (such as lilies) from a grocer or flower shop to use as a practice specimen.

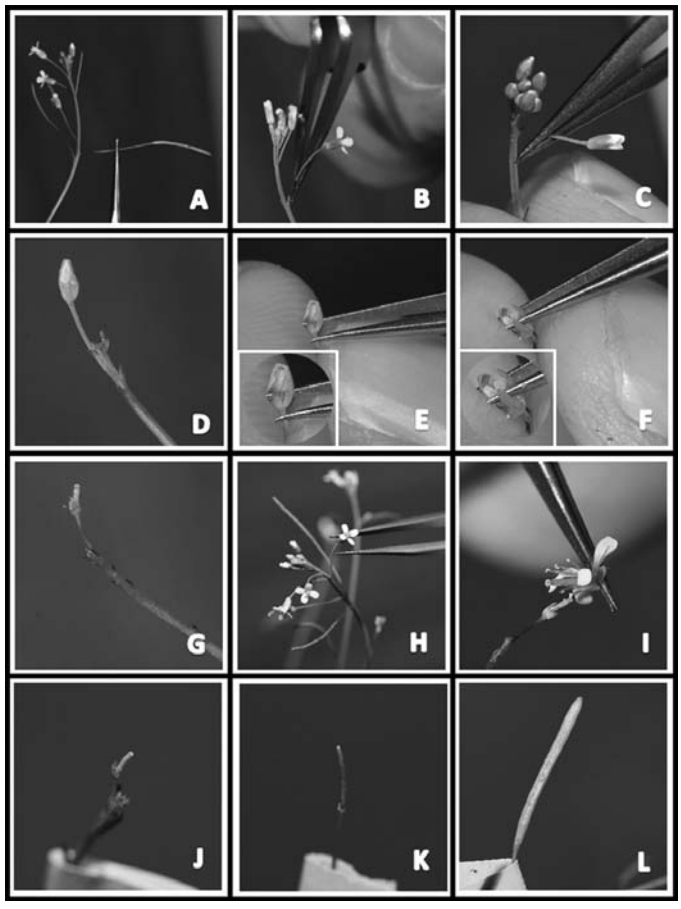


Figure 4. Steps involved in performing a cross between two Arabidopsis flowers. Steps A–L are explained in Procedure 1.

In this procedure, students will be performing two types of crosses. First, students will use pollen of a Col-1 reference strain plant to pollinate a *gl1-1* mutant (*gl1-1* x Col-1). Then students will use pollen from a *gl1-1* mutant to pollinate a Col-1 reference strain plant (Col-1 x *gl1-1*).

1. Have students select a *gl1-1* plant and locate an inflorescence branch containing at least two buds with barely visible petals. Students should use scissors or tweezers to remove any siliques and open flowers from the branch (Figure 4 A–D).
2. Using a headband magnifier and two pairs of fine-point tweezers, students should remove the sepals, petals, and stamens from two buds, being careful not to damage the carpel (Figure 4 E–G). If the carpel is damaged, remove the flower and proceed to the next bud.
3. Students should use tweezers to grasp a fully open flower from the Col-1 reference plant (male parent) at the peduncle level, squeezing the base to expose the anthers. Students may then pollinate the *gl1-1* female parent by brushing the anthers of the male parent over the emasculated carpel of the female flower (Figure 4 H–I). To increase the likelihood of success, each student should perform multiple crosses.
4. Students should use tape to label the cross and observe the branch for the formation of siliques (Figure 4 J–K). Let the siliques fully mature (Figure 4 L) before proceeding to Procedure 2. This process normally takes 2–3 weeks after pollination, during which time the students will have an opportunity to observe the development of siliques as part of the next assignment. A similar procedure should be performed for a reciprocal Col-1 x *gl1-1* cross, using Col-1 as a female and *gl1-1* as a male parent.

Assignment 1: Observe Cross Outcome (Weeks 7–9)

Have students observe the female parent plants and note the outcome of the crosses they performed. Approximately 1–2 days after the cross, the students should be able to tell the difference between a successful and unsuccessful cross. A successful cross will result in the formation of a silique (Figure 4 K), an unsuccessful cross will not. Have students sketch the female parent plant, noting the presence or absence of a silique in their illustration. Continue to water the plants for approximately one week after performing the crosses. Stop watering the plants and let the siliques dry out for at least two weeks, until they change from green to yellow-brown (Figure 4 L).

Through this assignment students should:

- Illustrate the results of a successful and unsuccessful cross.
- Calculate the percentage of successful crosses for the class.
- Discuss what occurs during the two weeks after pollination.
- Draw the *Arabidopsis* flower and label the structures listed below (see Appendix 1). For those structures that have a direct role in reproduction, have students define that role.
  - Stigma, stamen, carpel, inflorescence, petal, silique, sepal, peduncle, anther, pollen

Procedure 2: Collect F1 Generation Seeds (Week 10)

In this procedure, students will collect the seeds from the successful crosses and allow them to dry out to reduce the internal moisture content. This drying out process leads to improved seed germination.

1. Use scissors to carefully remove the dry siliques of successful *gl1-1* x Col-1 crosses (F1 generation) and place them in an Eppendorf tube, one silique per tube. Close tubes and label with the group number or name, plant number, generation (F1), and the type of cross.
2. Tap the tube several times to release the seeds from the siliques.
3. Repeat Steps 1–2 with the siliques of successful Col-1 x *gl1-1* crosses.
4. At this point in the module, the group is done working with the P generation plants and they can be discarded.
5. Allow the F1 seeds to dry for 2 weeks before planting.

Procedure 3: Plant the F1 Generation Seeds (Week 13)

In this procedure students will plant the F1 generation of the *gl1-1* x Col-1 and Col-1 x *gl1-1* crosses.

1. Following Steps 1–6 outlined in Procedure 1 of Activity 1, plant the seeds from the *gl1-1* x Col-1 and Col-1 x *gl1-1* crosses to produce one tray (eight pots) of each type of cross. Label the pots with your group number or name, plant number, generation (F1), and the type of cross.
2. In addition, plant one pot each of Col-1 and *gl1-1* seeds, placing 10–20 seeds in each pot. These plants will serve as controls for phenotypic observations.
3. Follow Steps 8 and 9 in Procedure 1 of Activity 1 for *Arabidopsis* growth and care.

Assignment 2: Compare Phenotypes (Week 16)

Have students observe and compare the phenotypes of the F1 and control plants. Through this assignment students should:

- Illustrate and describe the phenotypes of the F1 generation.
- Investigate if any phenotypic differences occur between the F1 plants coming from *gl1-1* x Col-1 crosses versus the Col-1 x *gl1-1* crosses. If differences are noted, have students document those in their illustrations and notes.
- Based on the comparison of the F1 plant phenotype to reference and mutant plants, have students conclude whether *gl1-1* mutation is dominant or recessive.

○ Conclusions

This unit demonstrates one of many ways that *Arabidopsis* can be utilized in a science curriculum to reinforce key concepts and engage students in hands-on learning. Although originally designed for instruction at the high school level, this module now aligns with many NGSS for other grade levels, which introduce scientific concepts such as genetic inheritance and variation of traits as early as middle school. The unit also offers teachers the ability to adjust the depth at which they cover the material, resulting in a flexible format. Therefore, this unit can easily be adapted to suit the needs of middle school classes, as well as advanced placement or other specialized high school biology classes, and college-level courses.

Additional experiments that utilize *Arabidopsis* to demonstrate a variety of other science concepts can be downloaded from the

ABRC's Outreach website (https://abrcoutreach.osu.edu/). As students become more comfortable with the scientific process, *Arabidopsis* represents a simple system with which they can design and conduct their own investigations (Wyatt & Ballard, 2007). Learning opportunities with *Arabidopsis* are plentiful, and educators are encouraged to fully integrate students in the inquiry process using this model system for plant research.

○ Acknowledgments

We greatly appreciate the National Science Foundation (NSF) for their continued support of ABRC, as well as the support received from the American Society of Plant Biologists (ASPB) to generate educational resources at ABRC. We recognize and appreciate the hard work that Marcelo Pomeranz and Nick Holomuzki put into the initial development of the experiments and original protocols on which this lesson is based. We are also very grateful to Benson Lindsey and Nikolas Grotewold for their assistance with the images contained in this article, and to Chris Bartos for the design of the ABRC outreach website.

References

American Chemistry Society (ACS). (2016). Importance of Hands-on Laboratory Science: ACS Position Statement. Available at https://www.acs.org/content/acs/en/policy/publicpolicies/invest/computersimulations.html

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

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

Kontra, C., Lyons, D. J., Fischer, S. M., & Beilock, S. L. (2015). Physical experience enhances science learning. *Psychological Science*, *26*(6), 737–749.

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., . . . McCourt, P. (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.

Zheng, Z. L. (2006). Use of the gl1 mutant and the CA-rop2 transgenic plants of *Arabidopsis thaliana* in the biology lecture course. *American Biology Teacher*, *68*(9), 148–153.

COURTNEY PRICE (corresponding author), Education Specialist, price.1217@osu.edu. EMMA KNEE, Consultant, knee.2@osu.edu. JULIE MILLER, Program Coordinator, miller.2909@osu.edu. DIANA SHIN, Associate Curator, shin.260@osu.edu. JAMES MANN, Curator, mann.129@osu.edu. DEBORAH CRIST, Database and Financial Manager, crist.30@osu.edu. ERICH GROTEWOLD, Co-Director, grotewold.1@osu.edu. JELENA BRKLJACIC (corresponding author), Associate Director, brkljacic.1@osu.edu. All are with The Ohio State University, Arabidopsis Biological Resource Center.

Appendix 1. Key terms and definitions.

General Terms	
Reference strain	Strain used as the phenotypic benchmark against which mutant phenotypes are compared.
Mutant	Strain containing a mutation (a change in the DNA sequence) that is not present in the reference strain.
Genotype	Genetic makeup of an organism that can refer to the specific gene. Examples of different genetic makeups: TT, Tt, tt represent different variants of that gene (alleles).
Phenotype	Physical appearance of an organism for a given trait. Results from the interaction of the genotype with the environment.
Terms related to Mendelian genetics	
Inheritance	The process in which genotypes are passed down from one generation to the next
Segregation	Separation of pairs of gene variants into reproductive cells
Genetic variation	Genetic differences found in nature among different individuals of the same species

Terms related to plant anatomy	
Inflorescence	A cluster of flowers including the branches of the stem
Peduncle	A branch supporting an inflorescence
Stamen	Male reproductive organ that includes the anther
Anther	The pollen-producing, oval-shaped portion of the stamen
Pollen	A carrier of the male reproductive cells. Has an appearance of powder or dust.
Carpel	Female reproductive organ that includes the stigma
Stigma	The bulb-shaped portion of the carpel, where pollen lands and pollinates the plant
Petal	Modified leaves that often function to attract pollinators
Silique	Seed pod of the plant
Terms related to processes	
Senescence	Final developmental stage of the plant; the aging process that leads to death
Stratification	Cold treatment of seeds that mimics "winter." Seeds taken out of cold treatment have higher rates of uniform germination, a response to "spring."

Appendix 2. Play Mendel—Handout for students

In this exercise you will plant and observe growth of *Arabidopsis thaliana* (*Arabidopsis*), a small plant used as a research model. The whole genome sequence of this plant is known, enabling scientists to understand the relationship of a gene sequence (genotype) and the resulting appearance of this plant (phenotype). You will compare the phenotypes of two different mutants (having a change in the sequence of a mutated gene) to a reference plant with a normal (common, wild type) phenotype. The first mutant, named *gl1-1*, has a mutation in the *GL1* (*GLABROUS1*) gene, resulting in the hairless leaf trait. The respective Col-1 reference plant has leaf hairs. The second mutant, named *ag-1*, has a mutation in the *AG* (*AGAMOUS*) gene, resulting in a double flower (multiple whorls of sepals and petals) trait. The respective Ler-0 reference plant has a flower with just one whorl each of sepals and petals. You will have an opportunity to follow these specific traits as they segregate, and make conclusions about their inheritance based on the analysis of the phenotypes in the progeny resulting from crossing a mutant with a reference plant. This will help you understand the principles of Mendelian genetics, which are the same for all organisms with sexual reproduction, including humans.

Activity 1. Observation of Growth and Development of *Arabidopsis* Plants

Procedure 1: Plant the Parent (P) Generation Seeds

In this procedure, you will work as part of a group. Each group should complete the same procedure.

1. Place potting soil in a bucket and add water and fertilizer. Use gloves when handling fertilizer and fertilized soil.
2. Line the bottom of 32 pots with a piece of cheesecloth or paper towel.
3. Prepare 4 sets of two trays by placing a tray with holes into the one without. Label the four outside trays with your group number or name. Label two trays "glabrous" and two trays "agamous". Label eight pots with each of the seed stock names (Col-1, *gl1-1*, Ler-0, and *ag-1*).
4. Fill the pots with soil.
5. Four students should each select one of the seed stocks and fill a weighing dish with water. For each stock, sprinkle approximately 100 seeds into the water. Use a disposable pipette to mix the seeds by pipetting up and down. Dispense nine seeds on top of the soil of each pot (see Figure 1). Continue until you have completed eight pots per seed stock. Do not cover the seeds with soil.
6. Place four *gl1-1* mutant pots and four Col-1 pots in each of the two "glabrous" trays. Place four *ag-1* mutant pots and four Ler-0 pots in each of the two "agamous" trays (see Figure 1).
7. Cover trays with plastic wrap and place them in a dark refrigerator for 2–3 days.

8. After 2–3 days, take the trays out of refrigerator and place them under the light source. Remove the plastic wrap after seeds have germinated and the seedlings look green. Fill the bottom of the tray with ½ inch of water once or twice a week. Continue watering the plants while proceeding with the assignments, as instructed by your teacher.

Activity 3: Perform Genetic Crosses to Obtain F1 Heterozygotes for the Glabrous Trait

Procedure 1: Perform Genetic Crosses

After approximately six weeks of growth, the plants should be ready for crosses. Performing crosses on small flowers of a plant such as Arabidopsis requires patience and practice. Your teacher will show you a video and give you other detailed instructions to help you master this technique. Don't be discouraged if you are unsuccessful at the beginning – there are plenty of flowers to practice on and you will get better!

1. You will start by preparing the *gl1-1* flower for crossing. Select an inflorescence branch containing at least two buds with barely visible petals. Remove open flowers and any siliques from the branch.
2. To expose the carpel (female reproductive organ) of the *gl1-1* plant, remove the sepals, petals and stamens from two buds using a headband magnifier and two pairs of fine point tweezers. If you damage the carpel, remove the flower and proceed to the next bud.
3. To prepare the pollen of the Col-1 plant, select a fully open flower and use tweezers to squeeze the flower at the base to expose the anthers (male reproductive organs that carry pollen). Pollinate the prepared carpel of the *gl1-1* plant by brushing the anthers of the Col-1 plant over it.
4. Use tape to label the cross and observe the branch for the formation of siliques (seed pods). This is part of the assignment that you should complete as instructed by your teacher.
5. Perform the same procedure (Procedure 1, steps 1–4) for a reciprocal Col-1 x *gl1-1* cross, using Col-1 as a female and *gl1-1* as a male parent. Continue watering the plants for 2–3 weeks until siliques mature.

Procedure 2: Collect F1 Generation Seeds

After 2–3 weeks of silique growth and maturation, the seeds are ready to be collected.

1. Use scissors to remove and carefully place the dry siliques of successful *gl1-1* x Col-1 crosses (F1 generation) in an Eppendorf tube, one silique per tube. Close tubes and label with the group number or name, plant number, generation (F1) and the type of cross.
2. Tap the tube several times to release the seeds from the siliques.
3. Repeat steps 1–2 with the siliques of successful Col-1 x *gl1-1* crosses.
4. Keep the closed tubes in a dry place for two weeks to let the seeds dry out.

Procedure 3: Plant the F1 Generation Seeds

After two weeks of drying, the F1 seeds of both types of crosses are ready for planting.

1. Plant the seeds from the *gl1-1* x Col-1 and Col-1 x *gl1-1* crosses to produce one tray (eight pots) of each type of cross. Label the pots with your group number or name, generation (F1) and the type of cross.
2. In addition, plant one pot each of Col-1 and *gl1-1* seeds, placing 10-20 seeds in each pot. These plants will serve as controls for phenotypic observations.
3. Continue watering plants as described in Procedure 1 of Activity 1. After three weeks of growth, your teacher will provide the instructions related to the assignment at the end of this activity.