

## *Arabidopsis thaliana*: Rise to Fame in Plant Genetics

### INTRODUCTION

At the beginning of the twentieth century, *Arabidopsis thaliana* (Figure 5.1) was known only as an inconspicuous weed. By the end of the 20th century, it became the major model system in plant genetics. This rise to scientific world fame suggests something about how geneticists choose a model organism. Mendel's peas were the first model system in genetics, and after the rediscovery of his work in 1900, other agricultural plants, such as maize (corn) and tobacco, became major model systems. These organisms have some qualities that make them well suited as genetic models. For example, they are relatively easy to grow and maintain, and in each case, a single plant can produce hundreds or even thousands of seeds in one growing season. However, one of the main reasons that plants such as maize and tobacco became major model systems was strictly economic — they are important crop species, and funding agencies could easily see the advantage of experiments using them. This distinction of economic importance has become less significant in recent decades.

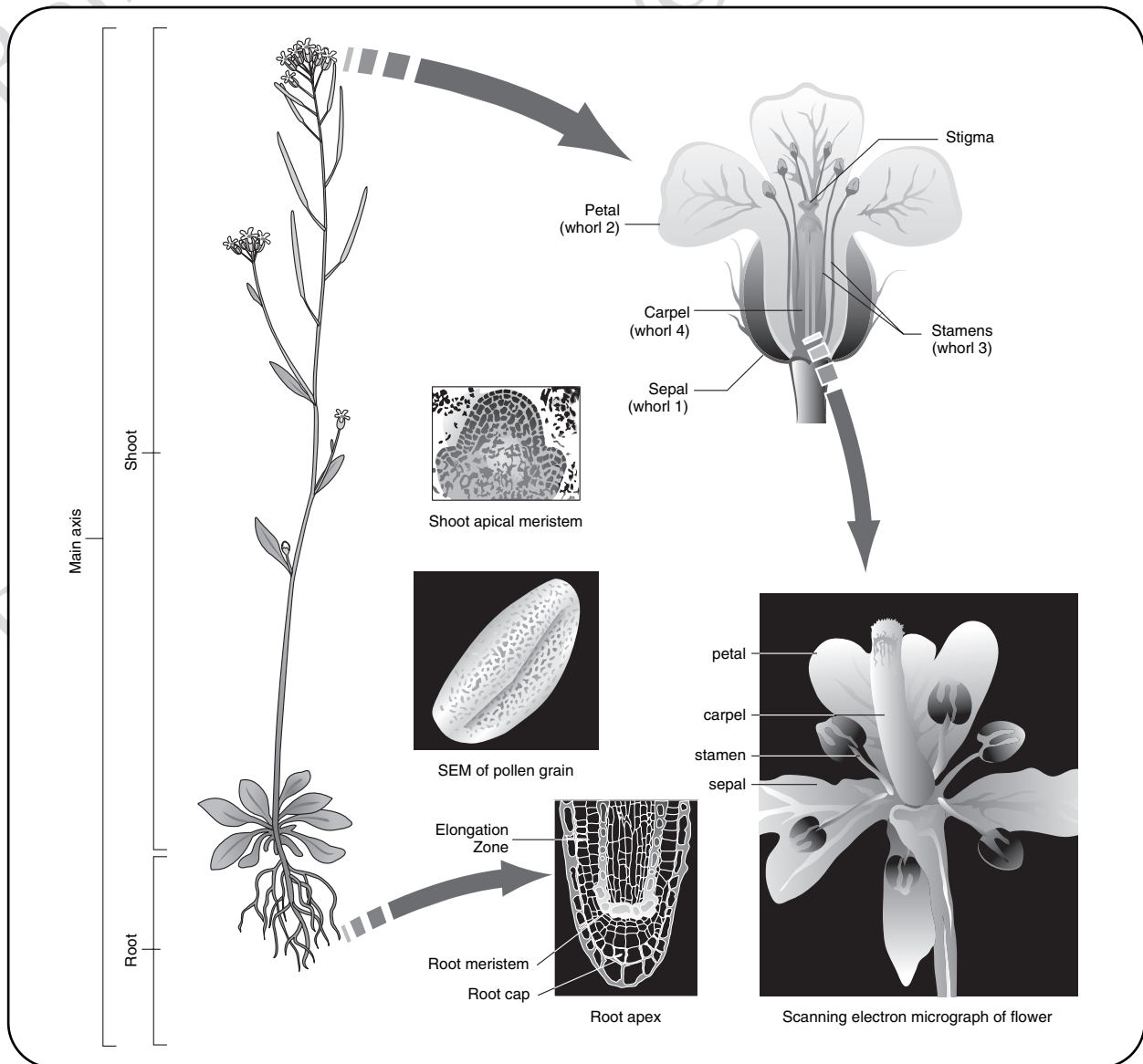
*Arabidopsis* is a tiny plant in the Brassica family, which also contains plants such as mustard, cabbage, and broccoli. However, unlike some of its close relatives and unlike the model systems of maize and tobacco, *Arabidopsis* has virtually no economic value whatsoever. But it does have several features that make it ideal as a model organism. *Arabidopsis* has been called the *Drosophila* of the plant world, and like *Drosophila*, it would just be considered another pest species if it weren't for some of its unique features. *Arabidopsis* is very small (compared to most plants, just a few centimeters high at maturity), it is easy to grow and breed, it matures quickly (in about 6 to 8 weeks), it can produce vast numbers of seeds (as many as 10,000 per plant), and it has a relatively small genome (with only five pairs of chromosomes).

*Arabidopsis* was first recommended to the scientific community as a model organism by Friedrich Laibach in 1943. However, for many years, only a small group, including Laibach and his students, utilized this organism. However, during the last several decades, *Arabidopsis* has become the major model system among plants. It is currently studied by hundreds of major laboratories, and in 1990, it became the focus of the first plant genome project (which was completed at the end of 2000).

As with other model organisms in genetics, there are hundreds of known mutants of *Arabidopsis*. In this lab activity, you will observe several mutant strains of this organism and compare them with the wild type. You will then have the opportunity to identify and isolate plants that may contain new mutations. To find these potential new mutant plants, you will be visually screening large numbers of seedlings derived from a mutagenesis experiment (which used ethyl methane sulfonate as a mutagen). The seeds that produced these plants are called M2 *seeds* (mutagenized generation 2) because

This lab activity was inspired in part by a poster presented by Jonathan Monroe to the Council on Undergraduate Research in 1994. The poster, which describes his use of *Arabidopsis* for a plant physiology course, is located at [http://csm.jmu.edu/biology/courses/bio455\\_555/atlab/poster1.html](http://csm.jmu.edu/biology/courses/bio455_555/atlab/poster1.html)

they represent a large batch of seeds derived from plants grown from mutagenized seeds (M1 seeds). Because most mutations are recessive and because *Arabidopsis* is a diploid organism, most mutations are generated by mutagenesis would never be observed in the M1 generation. Therefore, it is a common practice to allow M1 plants to self-fertilize and screen M2 seeds for possible new mutations. If your class is able to isolate several putative mutants, your instructor may have you grow these plants and self-fertilize them to produce seeds so that the mutation can be confirmed.



**Figure 5.1** – Diagram of *Arabidopsis thaliana* root tips and flowers. See the text for a description.

## PROCEDURE

### Observing Wild-Type *Arabidopsis* Plants

Obtain several wild-type plants at different stages of development, observe the features described here, and answer the questions related to these features. After you become familiar with some traits of the wild-type strain, you will be given plants from several mutant strains and asked to identify the mutant traits. In most cases, these mutant traits will be associated with one of the features that you will be directed to observe in the wild-type strain during this part of the activity.

### General Shape, Color, and Appearance

Begin your observations of the wild-type strain by noticing the general features of its appearance. First, notice the basic arrangement of the parts of the plant itself. Observe the general shape and arrangement of parts in both young seedlings and in more mature plants. Notice that the youngest seedlings are very simple in appearance, having little more than a primary root (with root hairs), a hypocotyl (the portion of the stem below the cotyledons), and two obvious cotyledons (seed leaves). There are also less-obvious leaf primordia (precursors to future leaves) nestled between the two cotyledons. In slightly older seedlings, both cotyledons and young vegetative leaves will be obvious. The mature plant has a much more complex organization. The oldest leaves on mature plants form a “basal rosette” just above the soil from which a comparatively long, branched stalk emerges. Notice the placement of branches, leaves, and flowers on the mature plant.

**Q1** Sketch the appearance of a young wild-type seedling, labeling the structures noted in the prior section.

**Q2** Describe the general morphology of the more-mature plant you observed. In your answer, compare the appearance and arrangement of the basal leaves with those features of the upper leaves. Also describe the branching pattern and the flowers' position and arrangement.

- Q3** Look closely at the surface of the leaves and stems of the seedlings and more-mature plants. How would you describe the color and the sheen (i.e., the brightness or shininess) of these tissues? Is the coloration and sheen of the plant uniform over the entire surface of a given plant, or does it vary?

### Trichome Morphology

Now, using a dissecting or compound microscope, look carefully at the surfaces of the leaves and stems of the wild-type plants. Notice that tiny hairlike structures (called *trichomes*) are present on some of these surfaces. Many of the mutant strains of *Arabidopsis* have unusual, or even absent, trichomes. After looking carefully for trichomes on the various surfaces of seedlings and more-mature plants, answer the following questions:

- Q4** Were trichomes present on both the seedlings and the more-mature plants that you observed? Which parts of each of these plants had trichomes, and which did not?
- Q5** There are several different types of trichomes on wild-type *Arabidopsis* plants. What kinds of variations did you observe in trichome shape, and where were the different kinds of trichomes located?

### Flower Organization

Flowers are complex reproductive structures that are usually very characteristic in their organization from species to species. In general, flowers are organized with concentric whorls of flower parts. From the outside in, the whorls of parts include the leaflike sepals; the more showy petals the male reproductive structures, called *stamens* (each with a filament and an anther); and the female structures, called *pistils* (each composed of a basal ovary, a long style, and a stigma where pollen is received). Using a dissecting microscope, carefully examine a typical flower from a mature *Arabidopsis* plant, and answer the following question:

**Q6** How many sepals, petals, stamens, and pistils does a wild-type *Arabidopsis* flower contain?

### Observing Various Mutant Strains of *Arabidopsis*

Your instructor will provide plants representing several different types of mutations. Some will be very young seedlings, and others will be more mature plants (since some mutations will be more obvious at certain stages). These plants will be in containers labeled with “code” designations, but not the names of the mutant phenotypes. You should carefully observe these plants, using dissecting and/or compound microscopes as needed, and determine which phenotypes are represented by each labeled culture.

The following are descriptions of some of the possible phenotypes that may be present in the strains available in your lab. Your instructor may provide more information regarding which of the following mutants may or may not be present among the plants provided in your lab. You may also be provided with names and descriptions of other mutant phenotypes besides those listed here.

**albina** — These plants lack normal chlorophyll and are therefore cream colored. They only live a short while (as seedlings) and must be maintained in heterozygous stocks. If your instructor has provided some of these plants for you to observe, they may be present as a few seedlings in a mixture of F<sub>2</sub> seedlings (including lots of green ones).

**apetala** — Several types of *apetala* mutants exist. They are homeotic mutations in which some part of the flower (often the sepal or petal) is absent or is transformed into another part of the flower, or into a leaflike structure.

**chlorina** — Due to a chlorophyll deficiency, these plants have yellowish-green shoots, instead of the normal darker green.

**chloroplast mutator** — These plants have sectors of green, yellow, and white variegation.

**constitutively photomorphogenic** — Plants with this category of mutation respond abnormally to light signals that control growth and development. When grown in the light, they accumulate purplish anthocyanin pigments in the cotyledons. When grown in darkness, they do not exhibit the normal, dark-grown, etiolated (i.e., “spindly”) appearance. These plants are maintained in heterozygous stocks and may be mixed with normal plants as F<sub>2</sub> progeny.

**distorted trichomes** — The trichomes on these plants are short, bent, and clublike.

**dwarf** — As the name implies, these plants are short (even for *Arabidopsis*!).

**eceriferum** — The stems of these plants are bright green and glossy, due to defects in the epicuticular wax layer.

**erecta** — These plants have short petioles and siliques (seed pods) and compact inflorescences.

**floral mutant** — The flowers of these plants look normal on the outside (sepals, petals, and stamens) but may have additional numbers of stamens and/or organs that appear to be fusions of stamens and carpels, and/or they may be completely or partially lacking in female reproductive structures.

**glabra** — There are several glabra mutations. All either have defective trichomes (i.e., unbranched) or are missing the trichomes, or the trichomes have been reduced in number on certain parts of the plant.

**gnom** — Seedlings with this mutation may lack a root and cotyledons, and they are cone or ball shaped. Obviously, this is a lethal mutation, and this gene must be maintained in a heterozygous stock.

**lepida** — This is another dwarf mutant. This strain is distinguished by having round, dark leaves.

**root hair defective** — The root hairs on these mutants may be short and wavy, branched, and/or short and bulging.

**unusual floral organs** — This floral mutation affects the second and/or third whorl of floral organs (petals and stamens), causing them to be absent in some cases or transformed into sepal or carpel-like organs.

**yellow inflorescence** — Unlike wild-type strains that have white flowers, this strain has yellow flowers.

The preceding list is just a small sampling of the hundreds of *Arabidopsis* mutants that have been discovered. By characterizing many of these odd phenotypes, investigators have learned some of the secrets of what controls normal growth and development in this tiny plant and in other plant systems as well.

**Q7** List the code designations of each type of plant you observed and indicate the name of the mutant phenotype exhibited in each case.

### Searching for New Mutations in *Arabidopsis*

Now that you have become familiar with the appearance of normal, wild-type *Arabidopsis* plants and have seen several mutant strains, you will have the chance to search for new mutations. Your instructor will provide you with one to several Petri plates with agar-solidified medium containing hundreds of small *Arabidopsis* seedlings. As was noted earlier, these are M2 seedlings that were derived from plants grown from mutagenized seeds (M1 plants). You should observe these with a dissecting microscope and look for mutant phenotypes like those described earlier (or anything else that looks unusual).

Although mutations occur very infrequently (even when a mutagen like EMS has been used), if you search carefully, there is still a good chance that you (or at least several people in your lab) will find a plant with a new mutation. Mutation rates vary with the amount and type of mutagen used, and they also vary for different genes. However, based on typical spontaneous mutation rates for eukaryotes (ranging from ca.  $10^{-7}$  to ca.  $10^{-4}$ ), one might expect an “average” gene under optimally effective concentrations of EMS to become mutated in something like 1 out of 100,000 seeds. Finding one mutant plant out of 100,000 seedlings would be like searching for a needle in a haystack. However, when you visually screen the M2 *Arabidopsis* seedlings in this experiment, you will be looking for any visible mutant phenotype (not just one phenotype associated with one gene).

Presumably, there are thousands of genes (maybe even tens of thousands) that could produce a visible mutant phenotype that you could detect. This means that if you make careful observations and look for any apparently non-wild-type phenotype, you (and your classmates) should be able to find several possible mutants for every few thousand seedlings you screen. That’s still a lot of seedlings to look at, but your chances of success are pretty high!

To ensure that you carefully view all the M2 seedlings on the Petri plate(s) you are given, you should draw a grid on the bottom of the plate(s) and view each square of the grid separately. Use a ruler and a permanent marker or a wax pencil to draw a grid with boxes that are about 1 cm square. As you carefully scan the seedlings in your plate(s), remove any with unusual phenotypes as possible mutants. Remove these by gently pulling them out of the agar-solidified medium with a pair of sterilized forceps. Using sterile technique, transfer possible mutants to small Petri plates containing fresh, agar-solidified growth medium, and label the Petri plates accordingly.

During the weeks to come, you should continue to observe any possible mutants that you isolated to determine whether the phenotype is stable during later development. If possible, you should transfer some or all of the putative mutants isolated by your class into soil, and keep them growing until they produce seeds (within about 6 to 8 weeks). At this point, the seeds can be harvested and grown to verify whether a heritable mutation is actually present. If one or more true mutations have been isolated, these could be further characterized by student researchers outside this class.

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