# Designing Arabidopsis experiments

**Additional Resource:** PREP Designing a PREP experiment
http://www.prep.biotech.vt.edu/expinfo/expinfo_light.html

The above website suggests parameters for stressing *Arabidopsis*. Your students can use this as a guide to a brief summary of the *Arabidopsis* literature. Please do not feel compelled to have your students design an experiment based on any of these ideas or parameters listed on this page. I am looking for creativity, but relativity. In other words, encourage the students to think of environmental stresses to test; encourage students to explore the literature and determine the parameters, but you don't want the experiments to all result in plant death (teaching moments are one thing - pure frustration and a dead lesson plan is totally different).

Most importantly, the students need to accurately record their experimental conditions in order for the experimental data to contribute to my research and the *Arabidopsis* research community as a whole. Whatever conditions the students decide to subject the plants to, it needs to be a consistent or regular regime that can be reproduced by other scientists.

**Replicates.** The best way to conduct experiments and generate meaningful data is to have replicates. This is easily accomplished with *Arabidopsis* as you will hopefully have 4 replicates per pot. Ideally, your students will set up at least 2 pots per sample (meaning 8 replicates per plant line, per condition). You can definitely have more replicates than this (more is better in this case), but space is usually a constraint for classroom replicates.

**Mutant vs.Wild-type.** The experimental design is to observe differences in stress response between a mutant (gene knock-out) plant line and a wild-type (Col) plant line. These are the experiments your students will design. There should be only one variable for the experiment, all other conditions should be held constant.

**Experimental vs. Control conditions.** The other set of plants that you need to include in your classroom (even if its just one set of plants per classroom) is the "control". You need to grow a set of *Arabidopsis*, both mutant and wild-type, under ideal conditions. This way, the students have a controlled environment set (we would call a control group) to compare their experiments to. This could be YOUR project and a back-up set of plants for students who kill their plants early on in the semester.

**Analysis of these experiments can be challenging because they are really analyzing 2 variables, the genetics and the environmental condition.**

**As your students design and analyze their experiments, try to have them put their research into the context of the "Big Picture". It may help to ask them:**

- Why would their chosen environmental conditions affect the plant?
  - Are you affecting photosynthesis? nutrient transport? water uptake?
- How do your experimental conditions relate to the true environment?
- What would be the significance of finding a phenotype under these conditions?

# Designing Arabidopsis experiments (continued)

**Phenotypes to look for.** While there are MANY phenotypes your students can measure, below is a suggestion of what I think are significant (and easily measurable) phenotypes.

- **Timing**- days until "X" happens (all these measurements start at planting = day 0)
  - days to stage 1.04 (4 rosette leaves)
  - days to stage 1.10 (10 rosette leaves)
  - days to stage 5.10 (reproductive bud)
  - days to stage 6.0 (first open flower)

- **Number of rosette leaves** or size (diameter) of entire rosette at time of first bolt.

- **Bolt height** (at whichever stage you want to measure - either at certain days post planting or at a specific rosette leaf stage). This is easy to measure but very variable! And not very meaningful unless there are dramatic differences (for example stunting vs. normal growth like we see with virus infections). **If you want to teach statistical analysis this kind of data would be great.**

- **Leaf color** (our RDR6 and DCL4 mutants, when stressed (sometimes), will produce flavonoids (antioxidants, anthocyanin, purple pigment) http://en.wikipedia.org/wiki/Flavonoid
  - HINT: look under the leaves and at the petiole (the leaf stem)
  - If you see darkening of leaves or a purple color and the students want to measure this quantitatively (and you have a spectrophotometer) I can provide a protocol for extracting the anthocyanins and measuring their levels

**Photodocumentation.** If you have a digital camera that students can use for photodocumentation of phenotypes, it is a great tool for allowing the students to look back through their experiment and see if they missed any phenotypes from earlier time points.

## A student worksheet:

# Planning Your Experiment with Arabidopsis

*by John Kowalski*
*Roanoke Valley Governor's School for Science and Technology*

Complete the questions below after participating in the introductory discussion about the *Arabidopsis* experiment.

What is the overall goal of this investigation?

Why is *Arabidopsis* being used for this investigation?

Describe the relationship between genotype and phenotype? Provide two examples of how changing the genotype affects an organism's phenotype.

Provide two examples of how changing the environment affects an organism's phenotype even though the genotype does not change.

What do the terns "wild-type" and "mutant" mean?

What is the difference at the gene level between the wild-type and mutant *Arabidopsis* plants used in this investigation?

How was the mutant plants produced in the laboratory?

List the gene names for the mutant plants being used in this investigation.

What, if anything, is known about this mutant plant?

# Planning Your Experiment with Arabidopsis (cont.)

List three possible environmental variables that you might wish to investigate.
Discuss the rationale for selecting each of these variables.
You choices must meet the following criteria:

1. Your variable must be relevant to plant growth and development
2. Your must not intentionally try to kill the plants.
3. Your experiment can be conducted in your classroom setting.

After discussion with your teacher pick one variable to pursue in more detail. List it below.

For this environmental variable indicate:

Preliminary title for the investigation.

Purpose of the investigation.

Hypothesis (a testable question).

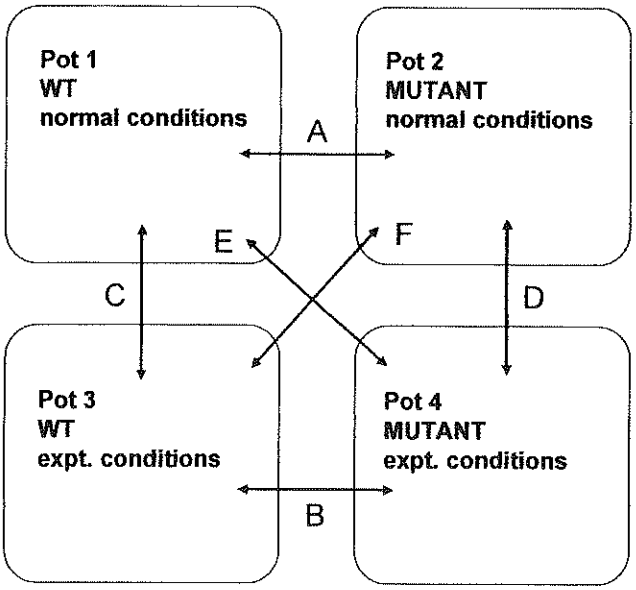
Details about your independent variables (type of seed and environment).

Important constants.

List five possible dependent variables that you could collect data for. Indicate for each why you selected it and what can be learned from studying that variable.

The diagrams below represent the four posts you will use for this experiment. Indicate in each pot what environmental variable the plant will be exposed to.

What can be learned by comparing the data collected from each pot? Describe the relationships between the four pots. Which comparisons are the most useful to make?



# Data collection format (example)

**Names in group:** _____

**Date of Observations:** _____

**Stress (independent variable):** _____

**Observations: Measure the dependent variable for EACH plant in EVERY pot (raw data). Calculate the average for each measured variable (each square).**

| Dependent Variable | wild-type pot no stress | mutant pot no stress | wild-type pot experimental | mutant pot experimental |
|---|---|---|---|---|
| number of plants per pot | | | | |
| # of rosette leaves | | | | |
| **AVERAGE** | | | | |
| # of flowers | | | | |
| **AVERAGE** | | | | |
| bolt appearance | | | | |
| leaf appearance | | | | |
| stem (bolt) straight, angled, flopped, curled | | | | |
| general observations | | | | |