

Week One Goals:

1. Plant seeds
2. Build and/or test a greenhouse if time permits (there's plenty of time next week, if necessary).

1. Plant Seeds

Before starting, split the class into 6 groups - each group will be doing a different treatment. Each group is doing a different environmental condition - light/dark or length of time in the cold (stratification).

GOAL

Test how plant variations influence germination in response to different environmental stimuli - light, dark, and cold.

OBJECTIVES

- Plant seeds of 3 variants of Arabidopsis (a plant used in labs): 2 natural variants (ecotypes) and a laboratory strain used as control.
- Germinate seeds in water under light and darkness, and with or without cold.
- Observe germination and collect data.
- Analyze the differences in responses between different genotypes and discuss the collected data.

BACKGROUND (for students)

Germination is a process by which a seed begins to sprout and grow into a seedling. We will be looking at the germination of a plant called *Arabidopsis thaliana*. There are many natural variants of *Arabidopsis thaliana* (*Arabidopsis*). These variants are adapted to particular environments and require different light and temperatures to grow!

The seeds start out in a dormancy phase and will not grow until this phase is broken. We can get the seeds out of the dormancy phase with light and temperature. The seeds sense the light and temperature and know that it's the right time to grow! Plants that evolved in different environments need to sense different things in order to germinate.

PROTOCOL

We will be taking the plates back to UW after class - the cold/light/dark treatments will be done there, since they have to be done on Sunday and Thursday.

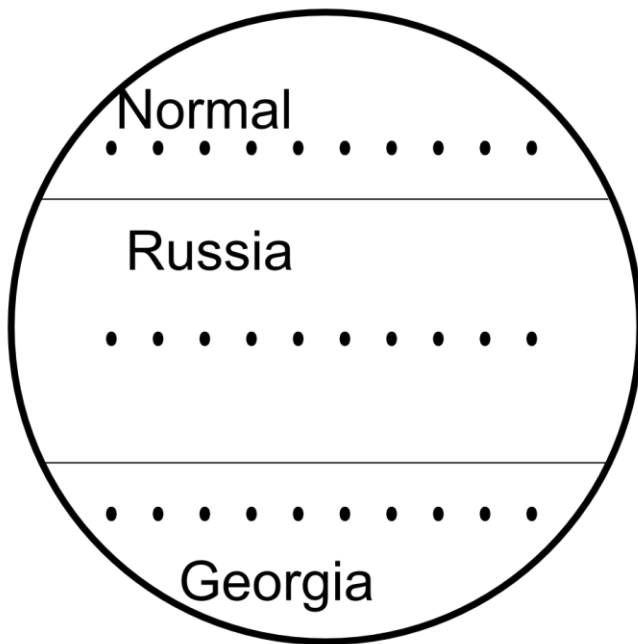
Treatments:

- A: Room temperature, light
- B: Room temperature, dark
- C: Cold 3 days, light

- D: Cold 3 days, dark
- E: Cold 7 days, light
- F: Cold 7 days, dark

Protocol:

1. Grab 4 petri dishes per group (ideally this works out to be ~1 per student)
2. Write "Normal" and the date on **bottom** of one of the petri dishes
3. Write the treatment and date on the bottom of the other plates.
4. Get filter paper or paper towels and cut out 16 circles (4 per plate) that will fit in the petri dishes.
5. Place 3 of the circles in each plate
6. Label one circle as "Normal" and put it on the top of the "Normal"-labeled plate.
7. For the remaining 3 circles, label as replicate 1, 2, or 3, then divide into 3 sections (shown below). Place them in the petri dishes.



8. Label 3 paper towels, one for each type of Arabidopsis - Normal, Russian, or Georgian (the country). Grab 10 seeds for each type (make sure to keep them separate). The seeds are **very** tiny, so be careful!
9. Plant the seeds into the appropriate sections of the paper circles using a toothpick.
10. Plant a bunch (~40) of "Normal" seeds on the "Normal" plate! We will use this to simulate the effects of acid rain in a later week, if time permits.
11. Pour 5-10 mL of water into each petri dish very carefully (at the edge). Don't wash away the seeds!
12. Put the petri dishes in a box for transporting back to UW. **KEEP THEM LEVEL.**

BACKGROUND (for teachers)

Large genetic variation is present among the many natural variants (ecotypes) of *Arabidopsis thaliana* (*Arabidopsis*). These variants are adapted to particular environments and exhibit great genetic variation in light and temperature requirements for seed germination. This variation leads to large differences in seed dormancy behavior. Genetic variation for germination-related traits can be detected when genotypes are compared in identical conditions.

Germination is a process by which a seed begins to sprout and grow into a seedling under favorable growth conditions. Dormancy is a temporary inability of an intact, viable seed to germinate despite the presence of favorable growth conditions.

Many *Arabidopsis* natural variants undergo a dormancy phase, and generally will not germinate until this dormancy is broken. Seed dormancy in *Arabidopsis* can be broken by germination-promoting factors such as dry storage (after-ripening), light, low temperature (stratification), as well as by applying certain chemicals such as gibberellins or nitrogen-containing compounds. The requirement for external germination-promoting factors can differ greatly depending on the genotype.

Stratification is a process of subjecting seeds to both a short-term cold (2°C - 5°C) and moist conditions to simulate natural winter that a seed commonly endures before germination in non-tropical climates. The required time to stratify *Arabidopsis* seeds depends on the genotype and temperature, but 3 to 7 days is usually enough. *Arabidopsis* seed germination and dormancy are controlled by both environmental and genetic factors.

Light and temperature are the key external factors in control of these processes. Plants must correctly perceive and respond to these stimuli to ensure that seedlings emerge and grow in the most favorable time for mature plant establishment. Photoreceptors called phytochromes are mainly responsible for sensing and responding to light during seed germination. A number of phytohormones act as internal regulators to determine whether a seed will germinate or remain dormant. Germination in *Arabidopsis* is stimulated by stratification, resulting in increased hormone biosynthesis and phytochrome action.

Seed dormancy plays a significant role in the adaptation of numerous plant species to their habitats. Plants have developed effective dormancy mechanisms to survive in disadvantageous environmental conditions, delaying the germination of mature seeds until favorable conditions return. Seed dormancy is crucial for grain production of some species grown in humid areas (corn, wheat, rice and canola) because it prevents seed germination before harvesting (vivipary), thus averting considerable grain damage and financial loss. However, long seed dormancy can be a problem in forestry and horticulture where germination of mature seeds may need to be induced with chemical treatments. Hence, the optimal level of seed dormancy for the specific growth environment is an important characteristic for any type of crop production.