



ABRC: Greening the Classroom Module

Think Green:

Student Handout – Lab Procedures & Assignments

Group Assignments & Seed Strain List

Group	Treatment	Seed Strain List
Group 1	Temperature stress	<i>chs1-2</i> (CS6256), Col-0 (CS70000), Lov-1(CS22574), Ler-0 (CS20)
Group 2	Temperature control	<i>chs1-2</i> (CS6256), Col-0 (CS70000), Lov-1(CS22574), Ler-0 (CS20)
Group 3	Drought stress	<i>aba1-1</i> (CS21), <i>abi1-1</i> (CS22), Ler-0 (CS20)
Group 4	Drought control	<i>aba1-1</i> (CS21), <i>abi1-1</i> (CS22), Ler-0 (CS20)
Group 5	Salt stress	<i>sos1-1</i> (CS3862), <i>abi3-1</i> (CS24), Col-0 (CS70000), Ler-0 (CS20)
Group 6	Salt control	<i>sos1-1</i> (CS3862), <i>abi3-1</i> (CS24), Col-0 (CS70000), Ler-0 (CS20)
Group 7	Heavy metal stress	Col-0 (CS70000), Lov-1 (CS22574), Ws-2 (CS28828), Santa Clara (CS28722)
Group 8	Heavy metal control	Col-0 (CS70000), Lov-1 (CS22574), Ws-2 (CS28828), Santa Clara (CS28722)

PROCEDURE 1 – Assign Groups, Prepare Pots & Plant

Materials

Arabidopsis seeds*	Weighing boats**	Potting soil
Fertilizer	Gloves	Plastic pots**
1 solid tray	1 tray with holes	Disposable Pasteur pipettes **
Plastic wrap (or dome)	Plastic teaspoon	Labeling tape
Fine-tip permanent marker		

* Your group will plant three or four strains of Arabidopsis, depending on your group assignment. Check the *Group Assignment & Seed Strain List* above for a list of the specific strains your group should plant. Your group will need nine seeds of each of your assigned strains.

** Your group will need either three or four of each of these items, depending on how many different strains you are planting.

1. Prepare a paired tray – Stack one tray with drainage holes inside a solid tray. From this point forward, this pair of stacked trays will be referred to simply as a tray.
2. Prepare a tray label - Using labeling tape and a fine-tip permanent marker, label your tray with your group number, treatment type and date (see example below).

Group 1 – Temp. stress
Date

3. Prepare pot labels - Label one pot for each of your group's seed strains (e.g. Group 1, temperature stress, will make four different pot labels). Each pot label should include your group number, treatment, and the strain type (see example below).

Group 1 – Temp. stress <i>chs1-2</i>

4. Put on gloves.
5. Prepare soil – 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.
6. Fill each pot loosely with soil. Do not compress the soil as you fill the pots as that will limit aeration.
7. Seeds should be planted individually on top of the soil. To start, fill a weighing dish with water. Working with one seed stock at a time, sprinkle nine seeds of one strain 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. Be sure to use a different weighing dish and pipette for each strain to prevent cross-contamination.
8. 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.
9. Once the planting is complete, place all of your group's pots your labeled tray. Cover the tray with either a plastic dome or plastic wrap. This will help to maintain soil moisture during germination.
10. Optional – If space is available, place your tray inside a cold room or refrigerator at 34-40° F 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.
11. Place your covered tray in the classroom growing area.
12. If the soil was prepared with adequate moisture, you should not need to water your pots while they are covered with the plastic dome or wrap. Remove the plastic dome or wrap once you see seedlings emerge from the soil (approximately seven days after planting).

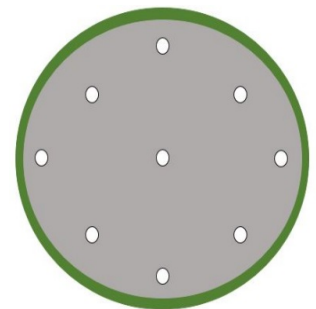


Figure 1. Placement of 9 seeds on soil surface (Price *et al.*, 2018).

ASSIGNMENT 1 – Define Key Terms

Complete the following tasks in your lab notebook:

1. Define key terms related to genetics, plant development and anatomy:
 - a. Genetics terms - Natural accession, mutant (strain), reference strain, genome, genome sequence, genotype, phenotype, adaptation, susceptible, resistant
 - b. Plant development terms - Stratification, germination, bolting, senescence
 - c. Anatomy terms - Cotyledon, rosette, inflorescence, silique

ASSIGNMENT 2 – Experimental Design for Temperature Stress & Temperature Control (Groups 1 & 2)

Review the information below about temperature stress and your groups' assigned seed strains.

Stress Treatment Details

- **Cold stress** – *Arabidopsis thaliana* is a temperate plant that grows best at room temperature (approximately 71°F-77°F). *Arabidopsis* can tolerate higher or lower temperatures, particularly if it has been acclimated. The ability of plants to respond to cold stress varies between species. Plants originating from temperate regions, such as *Arabidopsis*, demonstrate an ability to acclimate to periods of cold stress. Many different aspects of plant growth and development can be affected by cold stress, with a variety of hormones involved in the plant's response. In order to survive changing temperatures a plant must make adjustments at the cellular level, for example by the production of cryo-protectant molecules, or by changing the composition of lipids that make up the plasma membrane of the cell. The objective of this experiment is to subject a group of natural accessions and mutant strains to cold shock treatments and evaluate their response.

Seed Strain Details

- **Columbia** (Col-0, Catalog # CS70000) - The genome of this laboratory strain has been completely sequenced and is used as a reference for comparison with the genome sequences of other strains of *Arabidopsis*. This strain has been maintained in the laboratory for many generations, grows well in laboratory conditions, and has relatively low levels of seed dormancy. Col-0 serves as the reference strain for the *chs1-2* and *sos1-1* mutants used in this experiment.
- ***chs1-2*** (Catalog # CS6252) – This mutant strain was generated by treating Columbia with a chemical mutagen called ethylmethane sulfonate (EMS) that causes single base changes in the plant's DNA. As a result of this mutation, *chs1-2* is defective in chloroplast protein accumulation at low temperatures and is more sensitive to cold than Col-0.
- **Lovvik** (Lov-1, Catalog # CS22574) – This strain is a natural accession of *Arabidopsis* from Lövvik, Sweden (60° N, 16° E). It grows in an area where there is significant snow cover for up to seven months out of the year. This area has an average of 81 days of measurable snowfall annually. Average high temperatures in Lövvik are 88°F in the warmest month (July) and 29°F during the coldest month (February). Average low temperatures in the area are 40°F in July and 17° in February.
- **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 *aba1-1*, *abi1-1* and *abi3-1* mutants used in this experiment.

Review the experimental protocols outlined in *Procedure 3*. Consider what you know about what plants need to grow and survive. Based on this information, respond to the prompts below in your lab notebook. Be sure to explain your reasoning.

1. Identify the variable and the control for your stress treatment.
2. Formulate a hypothesis - How will your group's stress treatment affect the plants in general?
3. Formulate a hypothesis - Which genotype will be the most susceptible to your group's environmental stressor?
4. Formulate a hypothesis - Which genotype will be the most resistant to your group's environmental stressor?

ASSIGNMENT 2 – Experimental Design for Drought Stress & Drought Control (Groups 3 & 4)

Review the information below about temperature stress and your groups' assigned seed strains.

Stress Treatment Details

- **Drought Stress** – Though water is essential for plant growth and survival, plants often experience drought conditions. As the effects of climate change become more intense and widespread, more regions around the world are experiencing altered precipitation patterns including periods of drought and/or flooding. Drought affects plant growth and productivity. Drought conditions can cause molecular, physiological and architectural changes that increase a plant's likelihood of surviving a drought event. For example, sensing the onset of drought conditions a plant may flower earlier than usual in order to set seed before conditions become too inhospitable. In other cases, plants may respond to drought with structural changes such as elongated roots and reduced stomata. Environmental stressors such as drought can have significant negative effects on our food supply. The ability to produce crops that are adapted to drought conditions could increase the amount of land available for food production. The objective of this experiment is to subject natural accessions and mutant strains of *Arabidopsis* to drought and evaluate their responses.

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 *aba1-1*, *abi1-1* and *abi3-1* mutants used in this experiment.
- ***aba1-1*** (Catalog # CS21) – This mutant strain was generated by treating Ler-0 with the chemical mutagen EMS. The *aba1-1* mutation causes loss of function of a gene involved in the biosynthesis of abscisic acid (ABA), a hormone involved in plant stress responses. The resulting mutant plant is ABA deficient and demonstrates increased water loss and wilt in low humidity and water stress conditions. This strain shows decreased sensitivity to sodium stress during germination. In general, *aba1-1* plants show reduced growth, size and vigor when compared to Ler-0.
- ***abi1-1*** (Catalog # CS22) - This mutant strain was generated by treating Ler-0 with EMS. This resulting mutant plant is less sensitive to ABA. Unlike *aba1-1*, this mutant is able to produce ABA in response to stress, however the plant is not able to respond to it. The *abi1-1* mutant plants demonstrate increased water loss in low humidity and water stress conditions, and a reduced sensitivity to sodium stress during germination.

Review the experimental protocols outlined in *Procedure 4*. Consider what you know about what plants need to grow and survive. Based on this information, respond to the prompts below in your lab notebook. Be sure to explain your reasoning.

1. Identify the variable and the control for your stress treatment.
2. Formulate a hypothesis - How will your group's stress treatment affect the plants in general?
3. Formulate a hypothesis - Which genotype will be the most susceptible to your group's environmental stressor?
4. Formulate a hypothesis - Which genotype will be the most resistant to your group's environmental stressor?

ASSIGNMENT 2 – Experimental Design for Salt Stress & Salt Control (Groups 5 & 6)

Review the information below about temperature stress and your groups' assigned seed strains.

Stress Treatment Details

- **Salt Stress** – Salt stress negatively affects plant growth and development, and can have significant negative impacts on our food supply. This is especially true in arid and semi-arid regions around the world. Saline soil can result from natural processes such as mineral weathering, as well as human activities such as the use of road salt and irrigation practices. Salt damages soil structure, reduces its ability to hold water, affects soil pH and alters nutrient availability. Some of the effects of salt stress on plants are similar to those caused by drought stress, such as a limited ability to take up water and access nutrients in the soil. The objective of this experiment is to subject mutant and reference strains of *Arabidopsis* to salt stress and evaluate their responses.

Seed Strain Details

- **Columbia** (Col-0, Catalog # CS70000) - The genome of this laboratory strain has been completely sequenced and is used as a reference for comparison with the genome sequences of other strains of *Arabidopsis*. This strain has been maintained in the laboratory for many generations, grows well in laboratory conditions, and has relatively low levels of seed dormancy. Col-0 serves as the reference strain for the *chs1-2* and *sos1-1* mutants used in this experiment.
- **sos1-1** (Catalog # CS3862) – This mutant strain was generated by exposing Columbia plants to fast neutrons. The resulting mutant strain demonstrates sodium and lithium sensitivity. The mutation causes loss of function of a sodium ion exporter located in the plasma membrane. The mutant plants accumulate more sodium and retain less potassium when exposed to sodium stress. The plants are unable to grow in low potassium conditions. The export of sodium is necessary for the plant to tolerate salt.
- **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 *aba1-1*, *abi1-1* and *abi3-1* mutants used in this experiment.
- **abi3-1** (Catalog # CS24) – This mutant strain was generated by treating Ler-0 plants with EMS. The resulting mutation causes ABA resistance, affecting the plant's response to stress. This strain demonstrates reduced sensitivity to water stress, as well as decreased sensitivity to sodium stress during germination.

Review the experimental protocols outlined in *Procedure 5*. Consider what you know about what plants need to grow and survive. Based on this information, respond to the prompts below in your lab notebook. Be sure to explain your reasoning.

1. Identify the variable and the control for your stress treatment.
2. Formulate a hypothesis - How will your group's stress treatment affect the plants in general?
3. Formulate a hypothesis - Which genotype will be the most susceptible to your group's environmental stressor?
4. Formulate a hypothesis - Which genotype will be the most resistant to your group's environmental stressor?

ASSIGNMENT 2 – Experimental Design for Heavy Metal Stress & Heavy Metal Control (Groups 7 & 8)

Review the information below about temperature stress and your groups' assigned seed strains.

Stress Treatment Details

- **Heavy metal stress** – Heavy metals can occur in soil as a result of the slow and natural weathering of soil parent material. The amount of heavy metals present due to natural processes varies by geographic location, but these trace amounts of heavy metals are usually not considered to be toxic. However, heavy metals can be present in much higher quantities due to human activities such as mining, improper waste disposal, petrochemicals and application of fertilizers, manures and bio-solids. Some plants are adapted to grow in the presence of heavy metals, while others cannot survive in these conditions. There are two strategies plants employ to survive heavy metal stress. Some plants are able to avoid taking up heavy metals when they take up water and nutrients from the soil. These plants can be useful in stabilizing soils in contaminated areas to prevent erosion. Other plants are able to accumulate heavy metals and store them in a form that is not lethal to the plant. These plants are useful in the decontamination process, as they can be grown and harvested as a means of removing the heavy metals from the soil. The objective of this experiment is to subject natural accessions of *Arabidopsis* to a heavy metal (nickel or copper) and evaluate their response.

Seed Strain Details

- **Columbia** (Col-0, Catalog # CS70000) - The genome of this laboratory strain has been completely sequenced and is used as a reference for comparison with the genome sequences of other strains of *Arabidopsis*. This strain has been maintained in the laboratory for many generations, grows well in laboratory conditions, and has relatively low levels of seed dormancy. Col-0 serves as the reference strain for the *chs1-2* and *sos1-1* mutants used in this experiment.
- **Lovvik** (Lov-1, Catalog # CS22574) – This strain is a natural accession of *Arabidopsis* from Lövvik, Sweden (60° N, 16° E). It grows in an area where there is significant snow cover for up to seven months out of the year.
- **Wassilewskija** (Ws-2, Catalog # CS28828) – This strain is a natural accession of *Arabidopsis* that was originally collected in Wassilewskija, Russia (53° N, 30° E). This strain has shown a resistance to heavy metal contamination when compared to Columbia.
- **Santa Clara** (Catalog # CS28722) – This strain is a natural accession of *Arabidopsis* collected from a rocky outcrop in Santa Clara, California (37° N, -121° E). This strain accumulates significant quantities of nickel inside its cells.

Review the experimental protocols outlined in *Procedure 6*. Consider what you know about what plants need to grow and survive. Based on this information, respond to the prompts below in your lab notebook. Be sure to explain your reasoning.

1. Identify the variable and the control for your stress treatment.
2. Formulate a hypothesis - How will your group's stress treatment affect the plants in general?
3. Formulate a hypothesis - Which genotype will be the most susceptible to your group's environmental stressor?
4. Formulate a hypothesis - Which genotype will be the most resistant to your group's environmental stressor?

PROCEDURE 2 – Water

Materials

Watering can

1. Start watering the pots after removing the plastic dome or wrap from the top of the tray. Water the pots regularly (Mondays, Wednesdays and Fridays) unless otherwise instructed in subsequent procedures.
2. Do not water directly into the pots. Add water to the bottom of the tray to a depth of $\frac{1}{2}$ inch and allow pots to soak for 15-30 minutes. Dump out any water that was not absorbed. Be careful not to overwater the pots or allow the soil to dry out.
3. Groups 1, 2, 4, 6 and 8 should continue with this watering schedule for the duration of the experiment.

ASSIGNMENT 3 – Weekly Observations & Measurements

Materials

Lab notebook

Ruler

Begin making weekly observations of your group's pots once the seeds have germinated and the plastic dome or wrap has been removed. Complete the following tasks and record the data in your lab notebook.

1. Note the number of living plants in each pot.
2. Note the age of each plant. If the pots were stratified, this date should reflect the number of days since the pots were removed from the refrigerator. If the pots were not stratified, this date should reflect the number of days since the seeds were planted.
3. Note the stage of each plant (seedling, rosette, bolting, flowering, or setting seed).
4. Once the plants move to the rosette stage, make weekly illustrations of the plants noting any visible differences between the different strains. Pay specific attention to differences between control and treatment plants, as well as between natural accessions, reference and mutant strains.
5. Once the plants develop true leaves, use a ruler to measure the diameter of the rosette weekly.
6. Record the time to bolting. If the pots were stratified, this data should reflect the number of days from the time the pots were removed from the refrigerator to bolting. If the pots were not stratified, this data should reflect the number of days from the time the seeds were planted to bolting.
7. Once the first flower opens, use a ruler to measure the height of the inflorescence weekly.

PROCEDURE 3 – Temperature Stress (Group 1)

Materials

Thermometer

Timer

1. Use a thermometer to record the temperature of the freezer.
2. Place each pot in the freezer (temperature should be approximately 0° F) for five minutes.
3. Return the pots to the growth area and continue to water according to *Procedure 2*.
4. NOTE: Group 2, temperature control, should not perform the cold shock on their plants. Control plants should remain in the growth area for the duration of the experiment.

PROCEDURE 4 – Drought Stress (Group 3)

1. Begin withholding water from all pots at the start of the fourth week. Check the pots daily. Do not water until the surface of the soil is completely dry, the pots feel light and the plants are just starting to show signs of wilt.
2. When the plants meet the criteria listed in the previous step, water them according to step two of *Procedure 2*.
3. After watering, resume withholding water. Continue with this cycle through the duration of the experiment.
4. NOTE: Group 4, drought control, should not withhold water from their plants. Control plants should be watered according to *Procedure 2* for the duration of the experiment.

PROCEDURE 5 – Salt Stress (Group 5)

Materials

Table salt

Digital scale

1-liter jar

Tap water

1. Prepare salt-water solution – Mix 8 grams of table salt (sodium chloride) for every 1 liter of water.
2. Begin watering all pots with salt-water solution at the start of week four. Follow the same watering schedule as outlined in *Procedure 2*, using the salt-water solution in place of tap water.
3. If you prepare the salt-water solution in advance, be sure to mix the solution thoroughly before watering to ensure that all salt crystals dissolve.
4. NOTE: Group 6, salt control, should not use the salt-water solution with their plants. Control plants should be watered with tap water according to *Procedure 2* for the duration of the experiment.

PROCEDURE 6 – Heavy Metal Stress (Group 7)

Materials

Nickel or copper sulfate

Tap water

1. Prepare an 8 mM solution of nickel sulfate or copper sulfate.
2. Begin watering Group 7 pots with the heavy metal solution at the start of week four. Follow the Monday, Wednesday & Friday watering schedule, using the heavy metal solution in place of tap water for a total of five consecutive waterings.
3. Resume normal watering with tap water on the Friday of week five.
4. NOTE: Group 8, heavy metal control, should not use the heavy metal solution to water plants. Control plants should be watered with tap water according to *Procedure 2* for the duration of the experiment.

ASSIGNMENT 4 – Measure Aboveground Fresh Weight

Materials

Lab notebook

Plastic bags or large envelopes*

Fine-tip permanent marker

Digital scale

Scissors

* You will need one bag or envelope for each seed strain your group planted (three or four).

Complete the following tasks and record the data in your lab notebook:

1. Label one plastic bag or large envelope for each pot in your group's treatment. The information on the bag or envelope should match the information on the pot label.
2. Weigh the bag or envelope. Be sure to record this weight in your lab notebook.
3. Use scissors to cut off the aboveground parts of the plants in each pot. Be sure to cut the plant material as close to the soil surface as possible. Place all of the plant material in the appropriately labeled bag or envelope.
4. Weigh the bag or envelope with the plant material. Record weight.
5. Subtract the weight of the bag or envelope from the total weight to find the weight of the plant material.

ASSIGNMENT 5 –Display & Interpret Data

Complete the following tasks and record your answers in your lab notebook. Be sure to explain your reasoning.

1. For each type of measurement you made, decide as a group how to visually display the data. Create the display.
2. As a group, use the data to determine how the environmental stressor affected each strain of Arabidopsis in the experiment. Compare these results with the hypotheses made in Assignment 2.
3. Did any of the results surprise you? Identify conditions when the hypothesis did not match the results of the experiment. How do you explain the discrepancy?
4. Present your group's results to the class.
5. Consider what you know about each strain of Arabidopsis and the results of each stress treatment. Predict which strains would be most likely to survive in the following growing conditions or locations:
 - a. An extended period of drought for rosette stage plants
 - b. A late frost following germination
 - c. A roadside along a major highway where salt treatments are used during winter conditions
 - d. Near the site of a mine where the soil is contaminated with heavy metals
6. Imagine you are going to continue researching the effect of environmental stress on plants. What stress conditions not included in this experiment could you investigate? Develop a research question and hypothesis for this new experiment.
7. Imagine you are a scientist working on a research project to enhance survival of crop plants in adverse environmental conditions. Which mutant strain of Arabidopsis would you select for your experiment, and which environmental stressor would you study? Explain your reasoning.