Think Green

Summary: This module guides students through the process of investigating how genotype and natural variation influence plant responses to environmental conditions. Through these activities, students will subject ten strains of Arabidopsis to different stressors and observe the impact each has on growth, development and survival.

Recommended Grade Level: Middle and high school

Duration: This module includes six procedures and five assignments. It requires eight weeks for completion of all laboratory procedures. Student assignments can be completed over the course of nine class periods spread throughout the eight weeks.

Learning Objectives
Through this module students will:

- Plant and care for ten strains of Arabidopsis
- Make observations and compare phenotypes of natural accessions and mutant strains grown in different stress conditions
- Collect, display and interpret data
- Determine the effect of different environmental stressors on a selection of Arabidopsis strains
- Identify strains with higher resistance and susceptibility to individual stressors
- Define concepts and terms associated with genetics, growth and development

Alignment with Next Generation Science Standards

NGSS	
Standards	-Earth and Human Activity (MS-ESS3-3 & HS-ESS3-4) -From Molecules to Organisms: Structures and Processes (MS-LS1-5) -Ecosystems: Interactions, Energy and Dynamics (MS-LS2-1, MS-LS2-4 & HS-LS2-7) -Biological Evolution: Unity and Diversity (MS-LS4-4
Science & Engineering Practices	-Engaging in argument from evidence -Constructing explanations and designing solutions
Disciplinary Core Idea	-Human impacts on earth systems -Growth and development of organisms -Interdependent relationships in ecosystems -Ecosystems dynamics, functioning and resilience
Crosscutting Concepts	-Stability and change

Supporting Resources
The following supporting resources are available for download from the ABRC website:

- Think Green Module Video
- Student handout: Laboratory procedures & assignments
- Grading rubric
- Growing Arabidopsis in the Classroom
- Greening the Classroom Terms & Conditions

Materials

10 strains of Arabidopsis seeds (see *Seed Strain Details* below)

30 Weighing boats

Potting soil

14-14-14 Fertilizer (e.g. Osmocote®)

30 Plastic pots (Recommended size: 1 quart round pots, 4.7”d x 4.75”h)

8 solid trays (Suggested product - Hummert, Item #11-3050-1)

8 trays with holes for sub-irrigation (Suggested product - Hummert, Item #11-3000-1)

30 Disposable Pasteur pipettes

30 plastic bags (gallon, Ziploc) or paper envelops (letter size)

Plastic wrap (or 8 plastic domes)

Plastic teaspoons

Labeling tape

Fine-tip permanent marker

Table salt (sodium chloride)

Nickel sulfate (preferred) or copper sulfate (8mM solution)

Thermometer

Digital scale

Timer

Ruler

Scissors

Freezer

Watering can

Lab notebook

Growth space with fluorescent lights

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.

- ***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.

- ***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.

- ***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.

- **Wassilewskija** (Ws-2, Catalog # CS28828) – This strain is a natural accession of Arabidopsis that was originally collected in Wassilewskija, Russia (53˚ N, 30˚E). Weather data specific to Wassilewskija is not available, so the following weather trends are for Moscow (55˚ N, 37˚E). Average high temperatures are 78˚F in the warmest month (July) and 23˚F during the coldest month (January). Average low temperatures near the region are 59˚F in July and 14˚ in January. Moscow has an average of 82 days of measurable snowfall annually. This strain has shown a resistance to heavy metal contamination when compared to Columbia.

- **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.

- **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). Santa Clara has an average annual rainfall of 2.8”. The area does not receive any snowfall. Average high temperatures are 81˚ F in the warmest month (August) and 60˚ F during the coldest month (December). Average low temperatures for Santa Clara are 59˚ F in December and 42˚ in December. This strain accumulates significant quantities of nickel inside its cells.

Background Information

Arabidopsis thaliana (Arabidopsis) was the first plant to have its genome completely sequenced. Although technically a weed, this plant has been transformed into an important model system for plant research, and a useful tool in teaching a variety of science concepts in K-12 and college level instruction. Arabidopsis is member of the Brassicaceae family and is related to a number of common crop plants including cabbage, radish and cauliflower. It is a small, relatively easy to grow plant with a fast life cycle, going from seed to mature plant in six to eight weeks. With this module, students will grow three natural accessions, two reference strains, and five mutants of Arabidopsis. This module will introduce the concept of adaptations and survival in a changing environment.

An adaptation is a structural, physiological or behavioral characteristic that makes an organism better suited to its environment, and therefore increases the organism’s chances of survival. An adaptation can occur at the individual or group (e.g. species) level. The process of adaptation is a response to environmental change. It occurs slowly over the course of generations. Organisms that do not have the necessary adaptations to survive in a specific habitat must seek out a more favorable environment in order to survive and reproduce.

Because plants are sessile, they lack the ability to move to a new habitat when faced with environmental changes and must respond to adverse conditions or risk death. As such, many plant populations incorporate enough genetic variation within the population to allow at least a portion of the population to survive a dramatic change in environmental conditions. In addition to genetic variation, plasticity in individual plant growth in response to environmental conditions is another means by which plants can survive in a changing environment. Throughout its lifetime, a plant senses, integrates and responds to multiple environmental cues. These cues result in a range of responses including determining which way is up, and responding to different conditions such as nutrient levels, water availability, seasonal change and soil contamination. Scientists study plant responses to environmental stimuli to learn about the factors controlling plant growth in general, and to study the potential effects of environmental change on wild and cultivated species.

Arabidopsis is well suited to this type of investigation. Many natural accessions of Arabidopsis have been collected from different habitats throughout its range. These natural accessions are adapted to specific environmental conditions particular to the locations where they were collected. In addition, many mutants of Arabidopsis are available, which allows scientists to investigate the genetic basis for differences in environmental response. Through this experiment, students will explore the physiological differences in response to a variety of environmental stimuli in ten different strains of Arabidopsis. By comparing the response of natural accessions and mutant strains to those of a reference strain with a fully sequenced genome, researchers can learn about the underlying genetic mechanisms behind observed differences.

These types of experiments enhance our understanding of plant development, and improve our ability to predict how plants will survive in a changing environment. This research can lead to exciting advances in the development of new varieties of crops that will be of vital importance to ensuring an adequate food supply for future generations.

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.

- **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.

- **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.

- **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.

Group Assignments & Seed Strain List

Group	**Treatment**	**Seed Strain List**
Group 1	Temperature stress	*chs1-2* (CS6256), Col-0 (CS70000), Lov-1(CS22574), Ler-0 (CS20)
Group 2	Temperature control	*chs1-2* (CS6256), Col-0 (CS70000), Lov-1(CS22574), Ler-0 (CS20)
Group 3	Drought stress	*aba1-1* (CS21), *abi1-1* (CS22), Ler-0 (CS20)
Group 4	Drought control	*aba1-1* (CS21), *abi1-1* (CS22), Ler-0 (CS20)
Group 5	Salt stress	*sos1-1* (CS3862), *abi3-1* (CS24), Col-0 (CS70000), Ler-0 (CS20)
Group 6	Salt control	*sos1-1* (CS3862), *abi3-1* (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)

Schedule of Procedures and Assignments

Week/Day	Activity	
Week 1	Group	Activity
Monday	All	Procedure 1 – Assign Groups, Prepare Pots & Plant
Any day	All	Assignment 1 – Define Key Terms Assignment 2 – Experimental Design
Week 2	Group	Activity
Monday	All	Assignment 3 – Weekly Observations & Measurements
M,W,F	All	Procedure 1 – Water* *Only if seedlings are visible & plastic wrap has been removed
Week 3	Group	Activity
Monday	All	Assignment 3 – Weekly Observations & Measurements
M,W,F	All	Procedure 1 – Water
Week 4	Group	Activity
Monday	All	Assignment 3 – Weekly Observations & Measurements
	Group 1	Procedure 3 – Temperature Stress
M-F	Group 3	Procedure 4 – Drought Stress
M,W,F	Groups 1, 2, 4, 6, 8	Procedure 2 – Water
	Group 5	Procedure 5 – Salt Stress
	Group 7	Procedure 6 – Heavy Metal Stress
Week 5	Group	Activity
Monday	All	Assignment 3 – Weekly Observations & Measurements
M-F	Group 3	Procedure 4 – Drought Stress
M, W	Group 7	Procedure 6 – Heavy Metal Stress
M,W,F	Groups 1, 2, 4, 6, 8	Procedure 2 – Water
	Group 5	Procedure 5 – Salt Stress
Friday	Group 7	Procedure 2 – Water
Week 6	Group	Activity
Monday	All	Assignment 3 – Weekly Observations & Measurements
M-F	Group 3	Procedure 4 – Drought Stress
M,W,F	Groups 1, 2, 4, 6, 7, 8	Procedure 2 – Water
	Group 5	Procedure 5 – Salt Stress
Week 7	Group	Activity
Monday	All	Assignment 3 – Weekly Observations & Measurements
M-F	Group 3	Procedure 4 – Drought Stress
M,W,F	Groups 1, 2, 4, 6, 7, 8	Procedure 2 – Water
	Group 5	Procedure 5 – Salt Stress
Week 7	Group	Activity
Monday	All	Assignment 3 – Weekly Observations & Measurements Assignment 4 - Measure Aboveground Fresh Weight
Any day	All	Assignment 5 – Display & Interpret Data

Laboratory Procedures & Assignments

PROCEDURE 1 – Assign Groups, Prepare Pots & Plant

1. Divide the class into eight groups. Assign each group a number and treatment based on the *Group Assignment & Seed Strain List*. Each group will prepare one paired tray. Each group will prepare one pot for each seed strain indicated for the assigned treatment. (e.g. Group 1, temperature stress, will prepare one paired tray and four pots).

2. Prepare the paired trays – 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.

3. Prepare tray labels - Using labeling tape and a fine-tip permanent marker, label each tray with the group number, treatment type and date (see example below).

Group 1 – Temp. stress
Date

4. Prepare pot labels - Label one pot for each seed strain listed for each treatment in the *Group Assignment & Seed Strain List* (e.g. Group 1, temperature stress, will make four different pot labels). Each pot label should include the group number, treatment, and strain type (see example below).

Group 1 – Temp. stress
chs1-2

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 a portion of the seeds of one stock 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 stock 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.

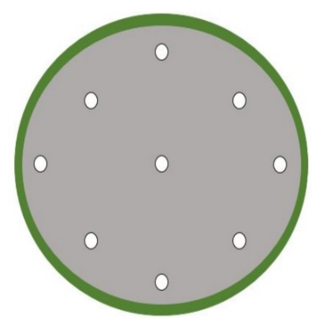

Figure 1. Placement of 9 seeds on soil surface (Price *et al.*, 2018).

9. Once the planting is complete, each group should place all of their pots (3-4 depending on group) in the same tray. If plastic tray domes are available, place a dome securely over the tray. If a dome is not available, wrap each tray tightly with plastic wrap to maintain moisture levels during germination.

10. Optional – If space is available, place all of the trays 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 the trays under fluorescent lights (see Growing Arabidopsis in the Classroom for lighting suggestions). If the soil was prepared with adequate moisture, you should not need to water your pots while they are covered with plastic wrap.

12. Remove the plastic wrap once you see seedlings emerge from the soil (approximately seven days after planting).

ASSIGNMENT 1 – Define Key Terms

Define key terms related to genetics, plant development and anatomy:

- Natural accession, mutant (strain), reference strain, genome, genome sequence, genotype, phenotype, adaptation, susceptible, resistant

- Stratification, germination, bolting, senescence

- Cotyledon, rosette, inflorescence, silique

ASSIGNMENT 2 -Experimental Design

Review the background information for your group's assigned environmental stressor and seed strains. Review the experimental protocols related to your group's stress treatment. Consider what you know about what plants need to grow and survive. Based on this information, respond to the prompts below. Be sure to explain your reasoning. When responding about a specific stress treatment, only consider the genotypes assigned to that treatment for your answer.

1. Identify the variable and the control.

2. Formulate a hypothesis - How will the stress treatment affect the plants in general?

3. Formulate a hypothesis - Which genotype will be the most susceptible to the environmental stressor?

4. Formulate a hypothesis - Which genotype will be the most resistant to the environmental stressor?

PROCEDURE 2 – Water

1. Once the plastic wrap has been removed, 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 tray to a depth of ½ inch and allow pots to soak. After 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

Once you remove the plastic wrap from the pots begin weekly observations. 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 (days after planting) and stage of each plant (seedling, rosette, bolting, flowering, or setting seed).

3. 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.

4. Once the plants develop true leaves, use a ruler to measure the diameter of the rosette weekly.

5. 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.

6. Once the first flower opens, use a ruler to measure the height of the inflorescence weekly.

PROCEDURE 3 – Temperature Stress (Group 1)

1. Place each pot in the freezer (temperature should be approximately 0° F) for five minutes.

2. Return the pots to the growth area and continue to water according to *Procedure 2*.

3. 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 Group 3 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)

1. Prepare salt-water solution – Mix 8 grams of table salt (sodium chloride) for every 1 liter of water.

2. Begin watering Group 5 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)

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

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

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.

References – Need to format

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

Knee, E.M., Rivero, L., Crist, D., Grotewold, E., and Scholl, R. (2009). Germplasm and molecular resources. In: Genetics and Genomics of the Brassicaceae. Jinnie Kim, Senior Editor in revision.

Koornneef, M. & Meinke, D. (2010). The development of Arabidopsis as a model plant. *The Plant Journal*, *61(6),* 909-921.

Latittude.to v1.54 beta. (2020). *Latitude and longitutde of Gävleborg County in Sweden.* Retried 3/18/20, from https://latitude.to/articles-by-country/se/sweden/34223/gavleborg-county.

Miura, K. & Furumoto, T. (2013). Cold Signaling and Cold Response in Plants. *International Journal of Molecular Sciences, 14(3),* 5312-5337. doi: 10.3390/ijms14035312

New World Encyclopedia. (2019). *Adaptation*. Retrieved 3/18/20, from https://www.newworldencyclopedia.org/entry/Adaptation.

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. *et al.* (2016). 50 years of Arabidopsis research: Highlights and future directions. *New Phytologist*, *209(3),* 921-944.

Rasool, S., Hameed, A., Azooz, M. M., Muneed-u-Rehman, M., Siddiqi, T. O., Ahmad, P. (2013). Salt stress: Causes, types and reponses of plants. In: Ahmad P., Azzoz M., Prasad M. (eds) Ecophysiology and Responses of Plants under Stress. Springer, New York, NY

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.

Time and Date AS. (2020). *World temperatures – Weather around the world*. Retrieved 3/18/20, from https://www.timeanddate.com/weather.

UC Davis Global Soil Health Portal. (2019). *Salinity*. Retrieved 3/18/20, from http://soilhealth.ucdavis.edu/soil-challenges/salinity.

Wuana, R. A. & Okieimen, F. E. (2011). Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation. *International Scholarly Research Notice,* https://doi.org/10.5402/2011/402647

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.

Xu, Z., Zhou, G. & Shimizu, H. (2010). Plant responses to drought and rewatering. *Plant Signaling and Behavior, 5(6)*, 649-654. doi: 10.4161/psb.5.6.11398