Germination

Summary: This module guides students through the process of investigating the germination response of various natural variants of Arabidopsis when exposed to different environmental stimuli. Through these activities students will learn about natural variation, adaptation, seed dormancy and germination.

Recommended Grade Level: Middle and high school

Duration: This module requires sixteen days for completion of all laboratory procedures and assignments.

Learning Objectives

Through this module students will:

- Plate and germinate five strains of Arabidopsis
- Compare phenotypes of seedlings grown in different environmental conditions
- Collect and analyze data
- Determine the effect of various light and cold treatments on different natural variants of Arabidopsis
- Define concepts and terms associated with genetics, growth and development including genotype, phenotype, adaptation, genetic variation, germination, dormancy, and stratification

Alignment with Next Generation Science Standards

NGSS	Middle & High School
Standards	-From Molecules to Organisms: Structures and Processes (MS-LS1-5 & MS-LS1-8) -Biological Evolution: Unity and Diversity (MS-LS4-4 & HS-LS4-4))
Science & Engineering Practices	-Constructing explanations and designing solutions
Disciplinary Core Idea	-Growth and development of organisms -Information processing -Adaptation
Crosscutting Concepts	-Cause and effect

Supporting Resources

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

- Germination student handout: Laboratory procedures & assignments
- Germination petri dish templates
- Germination grading rubric
- Growing Arabidopsis in the Classroom
- Greening the Classroom Terms & Concepts

Materials

Five strains of Arabidopsis seeds (See *Seed Strain Details*)

18 petri dishes (100x15mm)

72 pieces of filter paper (9 cm, grade 8, coarse)

Petri dish templates

18 strips of Parafilm

Distilled water

30 pieces of wax paper (approximately 10cmx10cm)

30 wood toothpicks

Aluminum foil

Magnifying glasses or dissecting microscope

Permanent markers (fine tip)

Rulers

Ball point pens

Lab notebooks

Refrigerator

Growth space with fluorescent lights

Growth space without light

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.

- **Fei-0** (Catalog # CS76412) – This natural variant was collected along a roadside in the village of Santa Maria da Feira, Portugal at X:-8.54, Y: 40.92, Z: 140. Observed flowering time for this strain is 21 days after planting.

    - **Santa Maria Da Feira, Portugal** is a coastal city with a mild, temperate climate. The average temperature is 49°F in the winter and 66°F in the summer. Average annual precipitation is approximately 39 inches with 77% humidity.

- **Kly-4** (Catalog # CS76384) – This natural variant was collected in a steppe in Kolyvan, Russia at X: 82.55, Y: 51.32, Z: 505. Observed flowering time for this strain is 31 days after planting.

    - **Kolyvan, Russia** is a rural village in the Kuryinsky district of Russia. Specific weather data for this small village is hard to find, so the following statistics are for larger cities in the same region of the country. The average temperature is 18°F in the winter and 76°F in the summer. In the winter there are fewer than four hours of sunshine per day. Average annual precipitation is 18 inches with 68% humidity.

- **Lag2-2** (Catalog #CS76390) – This natural variant was collected in a grazed pasture near a rock pile in Lagodechi, Georgia at X: 46.28, Y: 41.83, Z: 508. Observed flowering time for this strain exceeds 55 days after planting.

    - **Lagodechi, Georgia** is a small town located at the foot of the Greater Caucasus Mountains. The average temperature is 45°F in winter and 85°F in summer. Average annual precipitation is approximately 19.5 inches with 64% humidity.

- **Xan-1** (Catalog #CS76387) – This natural variant was collected along a partially shaded roadside and open pasture in Xanbulan, Azerbaijar at X: 48.80, Y: 38.65, Z: 37. Observed flowering time for this strain exceeds 55 days after planting.
    - **Xanbulan, Azerbaijan** is a small village within the Lankaran Rayon. The climate is sub-tropical, with average temperatures of 48°F in the winter and 85°F in the summer. Average annual precipitation is approximately 8 inches with 70% humidity.

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. Arabidopsis is native to many places all over the world and has adapted to live in a variety of environments. It is a member of the Brassicaceae family, and is related to a number of common food plants including cabbage, radish and cauliflower. Arabidopsis is a small, relatively easy to grow plant with a fast life cycle, going from seed to mature plant in six to eight weeks.

A large amount of genetic variation is present among the many natural variants of Arabidopsis. A natural variant is a strain of Arabidopsis that was discovered in nature, as opposed to one that was generated in a laboratory. Natural variants are adapted to particular environments and exhibit variation in their light and temperature requirements for seed germination. Germination is the process by which a seed begins to sprout and grow into a seedling under favorable conditions. Variation in germination requirements lead to differences in seed dormancy.

Dormancy is the temporary inability of a viable seed to germinate despite the presence of favorable conditions. Many natural variants of Arabidopsis undergo a dormancy phase, and generally will not germinate until this dormancy is broken. Arabidopsis seed germination and dormancy are controlled by both environmental (external) and genetic (internal) factors. Seed dormancy in Arabidopsis can be broken by germination-promoting factors such as dry storage (after-ripening), light, and 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 between different genotypes of Arabidopsis. 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 at the most favorable time for mature plant establishment. Stratification is the process of subjecting seeds to short-term cold (2-5 °C) and moist conditions to simulate winter in non-tropical climates. The ideal time to stratify Arabidopsis seeds depends on the genotype and stratification temperature. Generally speaking, a stratification period of three to seven days is enough to promote uniform germination.

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. In agriculture, seed dormancy is crucial for grain production in 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.

Germination in Arabidopsis

The process of germination starts with the uptake of water by the seed, and its completion is marked by the appearance of the radicle through the seed coat. Because Arabidopsis seeds are so small, it can be difficult to detect the early stages of germination. A magnifying glass or microscope is needed to observe the tiny root tip as it emerges from the seed coat. Through the germination process, you will first observe an intact seed (Figure 1a), then the radicle (root tip) breaking through the seed coat (Figure 1b). In later stages of germination, you will notice the development of root hairs (Figure 1c), the hypocotyl (stem of a germinating seedling, Figure 1d), and the green cotyledons (seed leaves, Figure 1e-i) of the new seedling.

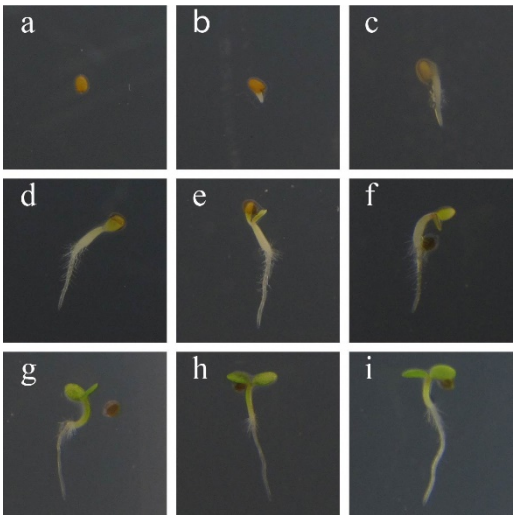


Figure 1. Stages of seed germination and seedling development in Arabidopsis. This image is reproduced as a courtesy and with permission from Dr. Annkatrin Rose, Department of Biology, Appalachian State University.

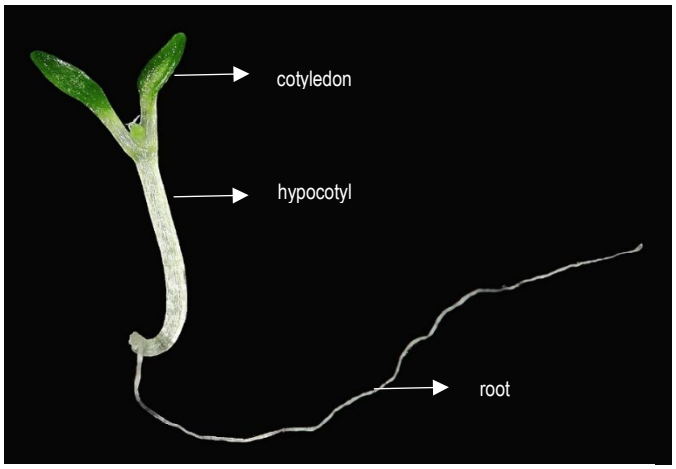


Figure 2. Anatomy of an Arabidopsis seedling.

Group Assignments

Group	Cold Treatment	Light Treatment
Group 1	No stratification	Light
Group 2	No stratification	Dark
Group 3	3 day stratification	Light
Group 4	3 day stratification	Dark
Group 5	7 day stratification	Light
Group 6	7 day stratification	Dark

Schedule of Procedures and Assignments

Day	Group(s)	Activity
Day 1 (Monday)	All groups	Procedure 1 – Prepare Plates
Day 2	All groups	Procedure 2 – Plate Seeds
	Group 1	Place in light growth area
	Group 2	Place in dark growth area
	Groups 3, 4, 5 & 6	Place in refrigerator for stratification
	All groups	Assignment 1 – Form Hypotheses
Day 3	Group 1	Assignment 2 - Collect data
Day 4	Group 1	Assignment 2 - Collect data
Day 5	Group 1	Assignment 2 - Collect data
	Group 3 & 4	Procedure 3 – Transfer Plates
Day 6 & 7	All groups	No activity – weekend
Day 8 (Monday)	Groups 1, 3	Assignment 2 - Collect data
Day 9	Groups 1, 3	Assignment 2 - Collect data
	Group 2	Assignment 2 - Collect data
	Group 5 & 6	Procedure 3 – Transfer Plates
Day 10	Groups 3 & 5	Assignment 2 - Collect data
Day 11	Groups 3 & 5	Assignment 2 - Collect data
Day 12	Group 3 & 5	Assignment 2 - Collect data
	Group 4	Assignment 2 - Collect data
Day 13 & 14	All groups	No activity – weekend
Day 15 (Monday)	Group 5	Assignment 2 - Collect data
Day 16	Group 5	Assignment 2 - Collect data
	Group 6	Assignment 2 - Collect data
	All Groups	Assignment 3 – Analyze data & Formulate Conclusions

NOTE: Procedure 1 (Day 1 above) can occur at any time before the start of the experiment. We recommend that Procedure 2 (Day 2 above) take place on a Tuesday. This schedule will allow each group to complete the protocols without any important activities falling on a weekend.

Laboratory Procedures & Assignments

PROCEDURE 1 – Prepare petri dishes

1. Divide the class into six groups. Assign each group a number and treatment type using the *Group Assignments* chart. Each group will prepare three petri dishes (plates) for their treatment.

2. Each group should prepare three pieces of labeled filter paper for their treatment using the provided templates, a ruler and a pen. The following details should be included on each labeled piece of filter paper: the date the seeds will be plated, treatment type, group number and replicate number.

3. Stack three blank pieces of filter paper on top of each other. Press this stack of papers into the bottom of an empty plate. Use a finger or the end of a capped pen to press the edges tightly into the corners of the plate.

4. Place one labeled piece of filter paper into the plate on top of the blank papers. Press around the edges for a smooth fit. A properly prepared plate will have a total of four pieces of filter paper (three blank, one labeled) firmly secured in the bottom of the plate.

PROCEDURE 2 – Plate seeds

For this procedure, each group will need 30 seeds each of the five genotypes used in the experiment. Each student within the group should work with only one strain of seeds at a time, and use new pieces of wax paper and toothpicks for each strain of seeds to prevent cross contamination. Plates grown in light conditions should be kept in 24 hour continuous light for the duration of the growth period.

1. Add enough distilled water (approximately 8-10 ml) to the plates to thoroughly moisten all four layers of filter paper.

2. Care should be taken not to over moisten the plate, as excess water could cause the seeds to shift from their designated location and/or promote the development of mold in the plate. Drain excess water into a waste container or sink.

3. Select one genotype of seed. Note the genotype with a permanent marker on the corner of a piece of wax paper. Place approximately 30 seeds of the selected genotype on the wax paper.

4. Thoroughly moisten the tip of a wood toothpick with water. Use the moistened tip of the toothpick to carefully pick up a single seed from the wax paper and place it in the corresponding space on the plate (see template for reference).

5. Repeat step 4 above until ten seeds have been plated in the appropriate space on all three plates. Dispose of the used wax paper and toothpick.

6. Repeat steps 3 – 5 with the remaining four genotypes until all five genotypes have been plated on all three plates for each group.

7. Care should be taken not to tip the plates sideways once the seeds have been plated. Prepared plates should be transported and stored horizontally to avoid displacing seeds from their designated areas.

8. Place the lid on each plate and seal edges with Parafilm to prevent the filter paper from drying out.

9. Each group should handle the plates based on their treatment type as outlined in the table below.

Group #	Group Protocols
Group 1	1. Place plates in the light growth area
Group 2	1. Stack plates and wrap with aluminum foil. 2. Label the outside of the foil with your group number. 3. Place in the dark growth area (e.g. drawer or cabinet within the same room as the light growth area)
Group 3 Group 4 Group 5 Group 6	1. Stack plates and wrap with aluminum foil. 2. Label the outside of the foil with your group number. 3. Place the plates in a refrigerator at approximately 40°F.

ASSIGNMENT 1 – Form Hypotheses

Review the geographical and climate information available about each genotype's collection location. If time allows, do additional research about each location. Consider what you know about seed dormancy, germination and what plants need to grow and survive.

1. Define key terms related to genetics, growth and development:

   Genotype, phenotype, natural variant, germination, dormancy, radicle, cotyledon, hypocotyl

2. Form a hypothesis for each prompt below. Be sure to explain your reasoning to support each hypotheses.

   a. How will the germination rate of each genotype be affected by each of the six treatments?

   b. Which genotype will have the highest and lowest germination rate for each treatment?

   c. Which treatment will result in the highest germination rate for the largest number of genotypes?

   d. Which treatment will result in the earliest germination for each of the six treatments?

PROCEDURE 3 – Transfer Plates

The timing and details of this procedure vary slightly from group to group. The section below outlines general protocols but does not address timing specific to each group's unique treatment. Refer to the *Schedule of Procedures and Assignments* earlier in this document and the *Student Handout* for each group for a comprehensive day-by-day list of activities.

1. For treatments that require stratification and light conditions, remove the plates from the refrigerator after the appropriate number of days have passed. Unwrap the plates and place them under a fluorescent light on a 24 hour light cycle.

2. For treatments that require stratification and dark conditions, remove the plates from the refrigerator after the appropriate number of days have passed. Do not unwrap the plates. Place them in a dark location (e.g. drawer or cabinet) in the same room as the light growth area.

ASSIGNMENT 2 – Collect Data

The specific days of data collection vary for each group. The section below outlines general data collection protocols but does not outline the collection schedule for each group. Refer to the *Schedule of Procedures and Assignments* earlier in this document and the *Student Handout* for each group for a comprehensive day-by-day list of activities.

1. Each group should record the date they placed their plates in the light or dark growth area.

2. For light treatments, use a magnifying glass or microscope to observe the seedlings on a daily basis for seven days after placing them in the light growth area.

    a. Count and record the number of germinated seeds per genotype daily.

        i. Count a seed as germinated when you observe a broken seed coat and the radicle has emerged from the seed (Figure 1b).

        ii. If condensation on the lid of the plate makes data collection difficult, the lid can be removed. Be sure to replace and reseal the lid after each data collection session.

3. For dark treatments, collect no data for the first six days after placing the plates in the dark growth area. On day seven, remove the foil from the plates and observe the seedlings.

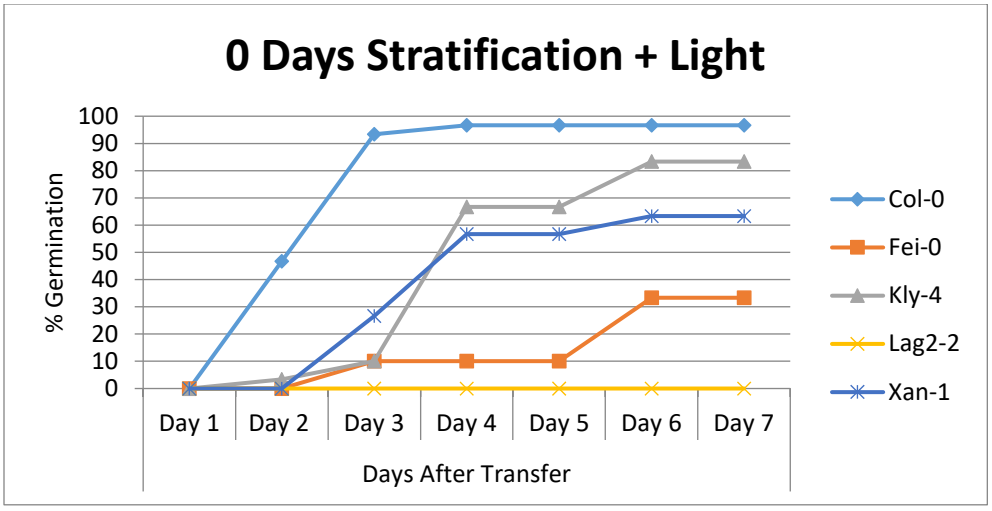
    a. Count and record the number of germinated seeds per genotype, following the directions provided in 2ai and 2aii above.

4. Observe and note the phenotypes of the seedlings for each genotype in each treatment. Pay attention to the shape and size of the seedling, as well as the color of the hypocotyl and cotyledon.

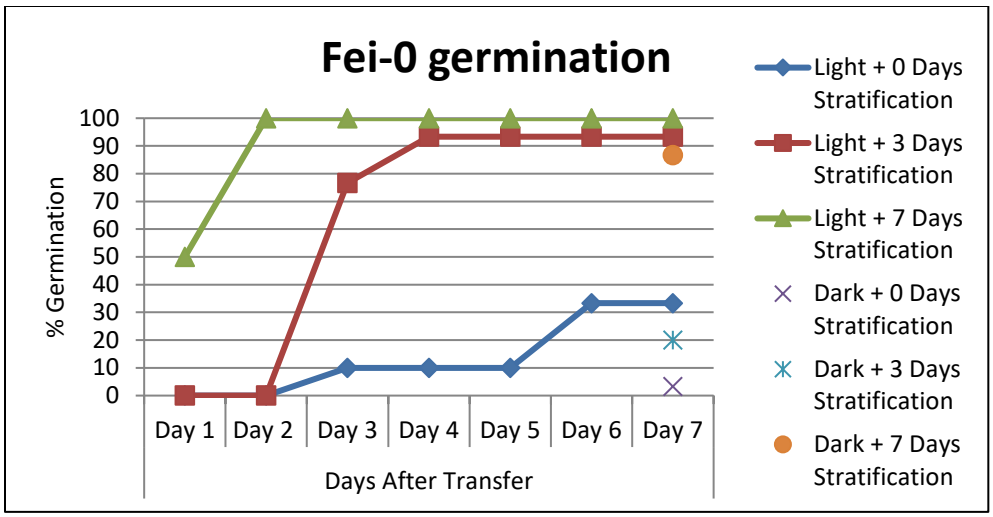
5. As a class, prepare an excel data sheet (or use the pre-made excel sheet provided) to record the daily germination for all three replicates of each of the six treatments.

ASSIGNMENT 3 – Analyze Data & Formulate Conclusions

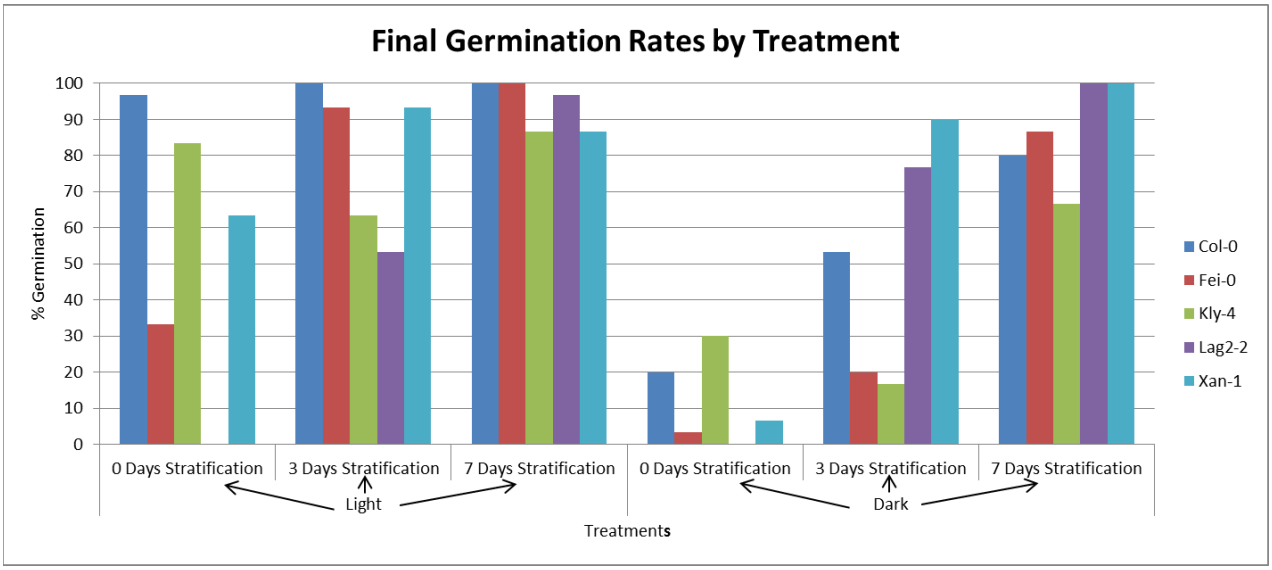
There are a number of ways that students can analyze the data from this experiment: a) compare the effects of each treatment on the germination rate of different genotypes (Example 1), b) compare the performance of a single genotype under different conditions (Example 2), c) compare the final rates for all genotypes under each treatment (Example 3), d) compare treatments in general, e.g. stratification versus no stratification in the light or in the dark for each genotype.



Example 1. Germination in light conditions with no stratification



Example 2. Germination rate of Fei-0 in six different environmental conditions



Example 3. Final germination rates for all five genotypes in all six environmental conditions

1. Each group should calculate the daily germination rate of each genotype for each replicate.

2. Calculate the mean and standard deviation of the three replicates, or use the *Data Analysis Spreadsheet* for data entry and calculations.

3. Use the means and standard deviations to generate a scatter plot to compare the germination rate among the five genotypes within each treatment, or use provided spreadsheet to automatically generate graphs. Create this type of scatterplot for all six treatments.

    a. In this plot, the Y axis will represent the germination percentage and the X axis will represent the days of evaluation after transfer to the growth area (Example 1).

4. Next, use the same data to create a new scatterplot comparing the effect of the six treatments on the germination rate of each genotype.

    a. In this plot, the Y axis will represent the germination percentage and the X axis will represent the days of evaluation (Example 2).

5. Compare the final germination rates of different genotypes under different conditions (Example 3).

6. Review the data about the shape, size and color of the seedlings. Does this data give you any insights into the effect each treatment has on the overall health of the developing plant?

7. Answer the following questions:

    a. Do different variants have different requirements for light and stratification in order to germinate?

    b. How would you summarize the light and stratification requirements of each of the five genotypes?

    c. Do certain treatments increase the speed of germination?

    d. Are some genotypes affected more than others by the extended cold treatment?

    e. Which genotypes have more dormancy?

    f. How many days of stratification are sufficient to break dormancy and attain near 100% germination in each genotype?

    g. Were there dark-germinating genotypes?

    h. Discuss the effects of the possible interactions between genotype, stratification and light on seed germination.

    i. Based on what you observed, predict which genotypes will be more likely to survive under winter conditions.

    j. How could seed dormancy give plants an adaptive advantage over other plants in different harsh environmental conditions?

    k. Why is seed dormancy an important economic trait for agricultural production?

8. Revisit your original hypotheses. Does the data you collected support or disprove your hypotheses?

References

https://weather-and-climate.com
https://en.wikipedia.org/wiki/Lagodekhi
https://en.wikipedia.org/wiki/Santa_Maria_da_Feira
https://en.wikipedia.org/wiki/Xanbulan
https://en.wikipedia.org/wiki/Kolyvan,_Altai_Krai

Additional Reading

Picó, F.X., Ménedez-Vigo, B., Martinez-Zapater, J. M., & Alonso-Blanco, C. (2008). Natural genetic variation of *Arabidopsis thaliana* is geographically structured in the Iberian Peninsula. *Genetics*, *180*(2): 1009-1021.