Bio 102 Introductory Biology Lab

Module 2

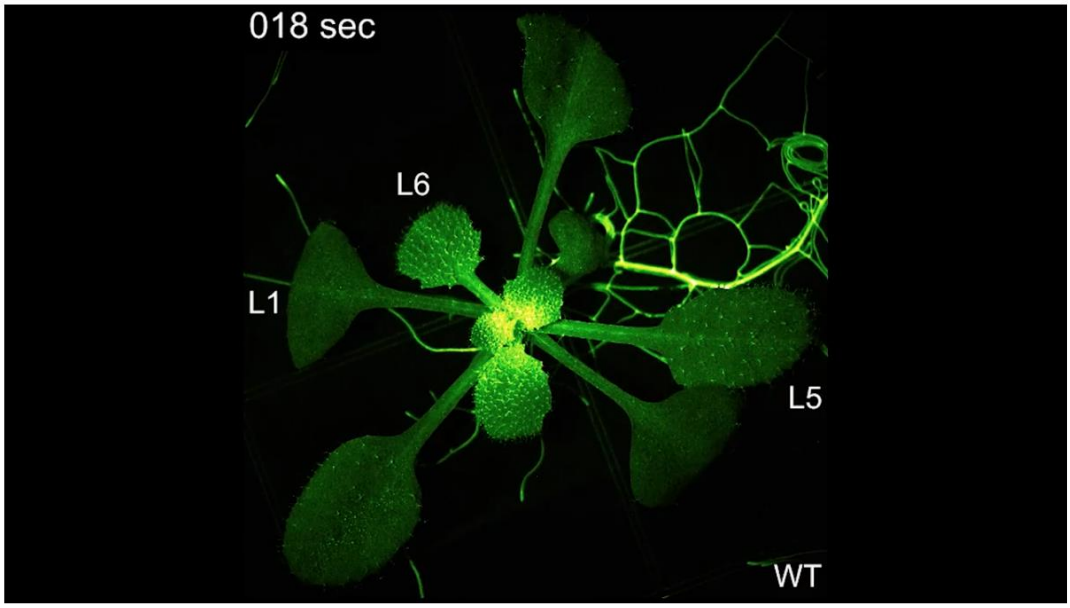


Table of Contents

Lab 7 - *Plant Tracer* 1

Name_____

BEFORE COMING TO LAB. **Complete the following checklist. This completed checklist must be turned in when you come to class.**

- ☐ **Download Lapse It from the App Store.**
- ☐ **Next, download *Plant Tracer* from the App Store (only works on iphones).**
- ☐ **Practice using Plant Tracer. First view the following YouTube video: https://www.youtube.com/watch?v=hj-gtbiS4N0. Also helpful is an on-line tutorial at http://planttracer.com to see the Plant Tracer tutorial**
- ☐ **Next conduct a practice analysis with the sample video in the gravitropism tab. Try to navigate through to the data page where you conduct a tracking analysis that generates a graph. If it's not clear, don't worry, your instructor will help you go through it in the lab**
- ☐ **Read the lab on the following pages.**
- ☐ **Bring your smartphone with its charger to lab. (iPhone is best if you have one)**

When you have completed these items turn in this check list with your name

---

Rationale:

For this lab meeting we take time to explore an innovative area of plant research: the genetics of plant behavior and signaling.

Objectives

1. Reconception: Study plants and their more dynamic signaling and movement qualities.
2. Learn how a genetic plant signaling pathway, auxin mediated gravity sensing, works.

Background

Plant Behavior

During the course of evolution, plants have developed sophisticated survival strategies. These strategies include sensing, signaling and behavioral systems that, although poorly understood, rival the sensing and signaling systems of animals. Here, one of the key features overlooked by much of the plant research world is the fact that plants are organisms with sophisticated behaviors. Plants may be tethered to the ground, but they are constantly on the move. Until recently time lapse was limited to professional photographers and scientists. With the advent of smartphone cameras, time lapse is available for you to explore. With this tool in hand, you can speed up the (relatively slow) world of plants so that their movement becomes visible. This is the first teaching lab designed for students to use time lapse from smartphones over a short lab interval. At the culmination of this lab, you should be able to see dramatic plant motions.

The intelligent plant

How does a cherry tree know when to bloom? How does a root recognize another root as a friend or foe? How does a plant reach toward the light? How does a plant calculate the number of days to bloom at the right time or what compounds need to be made and where to send these compounds to defend against a given pathogen? Taken together, all these questions bring up the most complex question of all: How do plants integrate all this information in such a way that the entire plant system reacts in a concerted manner? In short, we do not know. Moreover, if one looks at the complexity of the vascular system in plants, the phloem and the xylem, it has a resemblance to neural networks in animals. That is not to say that plants have nerves similar to those found in animals, but this does suggest that the vasculature would be the expected site of complex electrical signaling relay system is at work in plants as part of the mechanism behind plant function. One exciting frontier in this question of integrated plant signaling can be performed on coordinated movement, which you will do today using time lapse analysis with your cell phone or tablet.

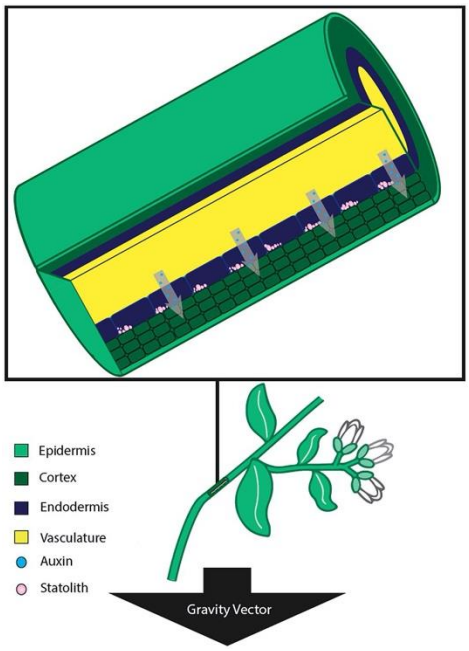
Arabidopsis: the genetic model plant

Here you will have the chance to view plant movement in Arabidopsis (Figure 1). Arabidopsis was chosen as the primary genetic model plant system and is now studied by thousands of labs, which explore various topics with examples including nitrogen sensing, root development, and plant behavior. Arabidopsis is easy to work with in the lab as it is small, grows quickly, produces copious amounts of seeds after only six weeks, and was the first plant to have its genome fully sequenced. You will conduct a genetic experiment on Arabidopsis to test the role of a gene to determine if it is involved in sensing gravity.

Figure 1

The role of statoliths in gravisensing signaling using pgm (phosphoglucomutase)

One of the key pieces of evidence that statoliths activate gravitropic responses came from the experimental analysis of genetic mutants in starch biosynthesis. *pgm* (phosphoglucomutase) is an Arabidopsis mutant that has metabolically reduced levels of starch due to an impairment in an early biochemical step in starch biosynthesis. With less starch production, *pgm* mutants have reduced levels of statoliths. Loss of statoliths in *pgm* mutants is correlated with a decreased response to gravitropic stimulation in both stems and roots of Arabidopsis.



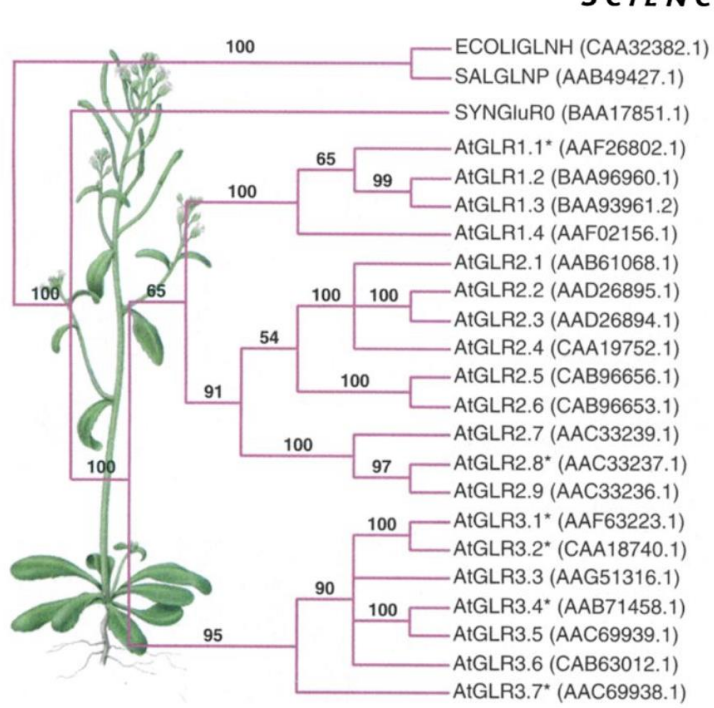
In general (but with some interesting exceptions), shoots are agravitropic and roots are gravitropic. It is believed that both roots and shoots sense gravity by the downward movement of “statoliths”, which are specialized starch crystals found in organelles known as amyloplasts. When a plant is changed in orientation, statoliths sediment to the “new” bottom of the cell. Statolith relocalization to the basal portion of the cell (Figure 2) then stimulates an increase of auxin at the new bottom of the cell, which in turn, stimulates growth at the new bottom of the cell. Hence, a localized increase in auxin concentration on the lower side of the stem induces asynchronous growth. The upper portion of the stem grows slower than the bottom. This causes the stem to bend upward, consequently lifting the apex. The curving process, itself, occurs by a progressive recurving along the length of the stem. The bending response is not like a hinge, but more like a sophisticated series of coordinated changes along the stem, much like a bending snake.

Figure 2.

- For review see "Comparing plant and animal glutamate receptors: common traits but different fates?" Michael M. Wudick, Erwan Michard, Custódio Oliveira Nunes and José A. Feijó., *Journal of Experimental Botany*, Vol. 69, No. 17 pp. 4151–4163, 2018)

One of the more unusual observations made when the sequence of the Arabidopsis transcriptome (mass sequencing of expressed RNAs) was first revealed, was the revelation that plants have glutamate-like receptor genes. In fact, there are twenty different glutamate receptor genes in Arabidopsis that evolved separately from the different clades of animal glutamate receptors. The origins of both animal and plant glutamate receptors are believed to come from a common ancestor; in photosynthetic bacteria there are glutamate receptors that function as potassium channels. Hence this is an ancient gene, that in the earliest prokaryotes originally functioned as amino acid permeases, which have been coopted over hundreds of millions of years of evolution to function as ligand gated ion channels that, in turn, evolved into receptors that transmit long distance electrical signals in the animal nervous system.

In plants the role of glutamate receptors is still obscure. There are 20 different glutamate receptors in Arabidopsis thaliana, the genetic model plant that are broken up into three clades (Figure 3).



Phylogenetic relationships within the Arabidopsis GLR gene family. The accession number is in parentheses. Asterisks, genes with an identified full-length cDNA. Amino acid sequences can be found at http://www.pasteur.fr/recherche/banques/LGIC/LGIC.html

Figure 3. Phylogenetic tree of Arabidopsis Glutamate Receptor Family

Because glutamate receptors function as an essential transmitter in the animal nervous systems, there is much interest to better understand the role of these genes in plants from a biomedical perspective. In plants there is no obvious "nervous system" - with interconnected neurons that interact in a neural pathway (although we cannot exclude this possibility, certainly not until scientists fully illuminate the structure and flow of the electrical signaling highway in plants). In fact, there is a long history of research tracking the electrical conductivity pathway in plants - with an effort to connect these signals to specific, measurable physiological responses.

Amidst the various attempts to define their function, it is believed that glutamate receptors in plants may be involved in a variety of roles. These roles include photosynthesis, immunity to pathogens, and long distance transmission of electrical signals (like a human nerve) in response to wounding. When a leaf is damaged by a hungry worm or a probing aphid) an electrical signal is sent through the vasculature to other leaves in the plant to activate an emergency defense response. This defense leads to the activation of protective compounds that are unpalatable (not tasty) to the worm.

To see the worm damage activated long distance electrical signal response watch this video:

- https://news.wisc.edu/blazes-of-light-reveal-how-plants-signal-danger-long-distances/.

This was the first molecular evidence that specific genes are involved in sending long distance action potentials in plants. In the above link a caterpillar can be seen chewing on a leaf where almost instantly an electrical impulse is sent from that leaf to a different leaf on a distant part of the plant. It has been shown that this signal then activates a downstream defensive response, which releases jasmonic acid, a defense alert gas that then stimulates the production of defensive proteins that will make future feeding caterpillars sick. The AtGLR genes shown to convey this electrical response are AtGLR3.1, 3.3, and 3.6. AtGLR3.3 and 3.6 appear to work in concert. AtGLR3.3 shows expression in the phloem and Atglr3.6 shows expression in the xylem. In xylem there are two types of cells: vessels, which are dead cells that transfer the solutes (kind of like pipes) and contact cells, which are believed to regulate somehow the materials that move into the dead vessels. It is in the contact cells that AtGLR 3.6 expression occurs. When either gene is knocked-out, the electrical wound signal is reduced. When both AtGLR 3.3/3.6 double knockouts are created there is an even stronger loss of electrical signaling, an indication that signaling is somehow coordinated between these two cell types in the vasculature.

Besides sensing wound signals, plant glutamate receptors have been implicated in pollen tube growth. Evidence indicates that these receptors function as calcium channels that when activated in the growing pollen tip create a flash of calcium that is followed by a wave of pollen tube growth. Every 15 minutes there is a flash of calcium followed by tube growth. Loss of these AtGLR receptors appear to retard the rate of pollen tube growth as seen in this figure:

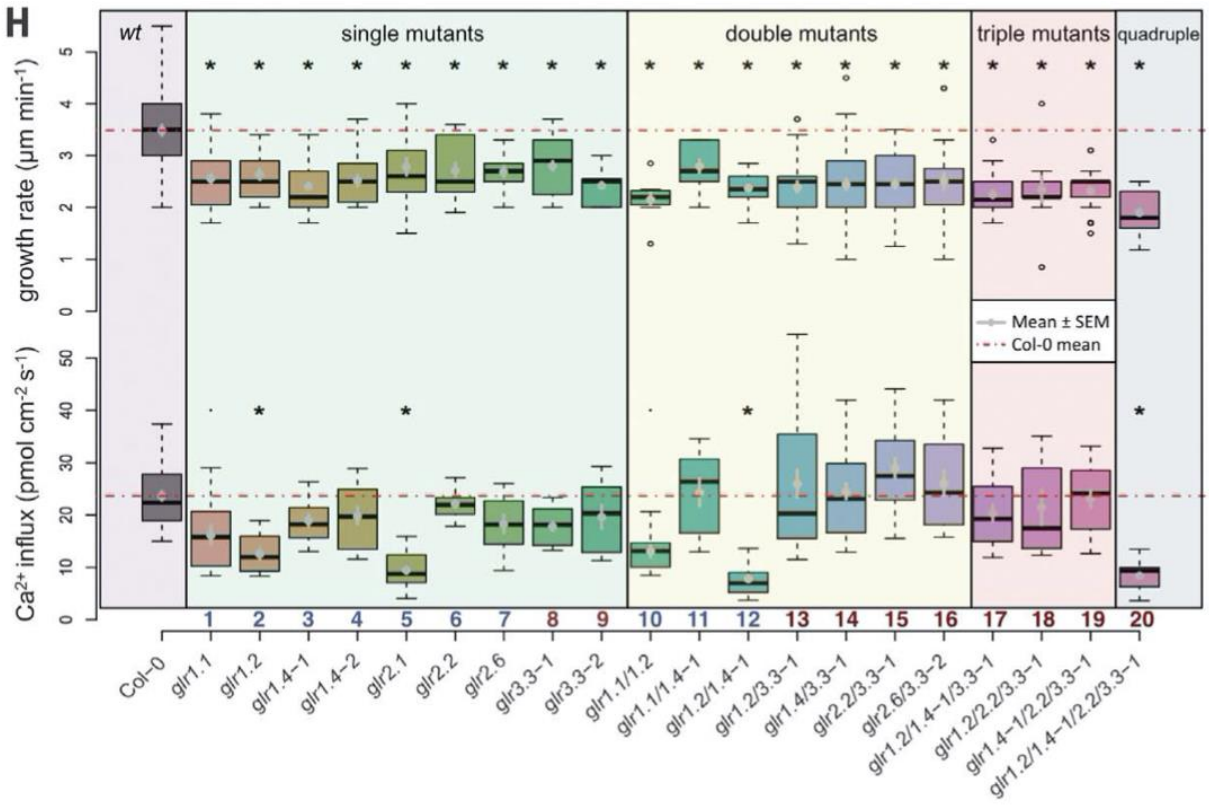


Figure 4

For this lab, some of these mutants are available for you to test for alterations in gravitropism. Because the glutamate receptor family is redundant in Arabidopsis, often the strongest phenotypes are seen when more than one gene is knocked out. For your tests you will have 2 different knockout lines, which in a previous study have been shown to have reduced growth in pollen tubes as show in figure 4. Never before have these mutants been tested for loss of gravitropic response in the flowering stems of Arabidopsis - novel data - novel experiments. The two quadruple knockout lines are *AtGLR*1.2/1.4-1/2.2/3.3-1 and *AtGLR*3.1/3.2/3.3/3.6, both shown in (Figure 4). If 3.3/3.6 double knockouts interfere with long distance electrical signaling, could these two genes, along with 3.1 and 3.2 be involved in gravitropic movement with flowering stems? Increasing the number of knocked out genes will increase the likelihood of seeing an altered phenotype in these kinds of tests.

When using time lapse analysis, the bending inflorescent stem shows sophisticated movement. It is not like a hinge that bends at one specific spot. The bending at what is called the inflection point is more fluid, somewhat curved. But what signals are causing this to happen? Is it solely statoliths mediating the auxin response? If that were the case, why in the *pgm* mutant, which has no statoliths, does bending still occur (albeit slower)? Hence, there is some other signal that induces, or conveys bending. Perhaps it is calcium signaling that occurs via *AtGLR* activation that then transmits a gravity response signal separate from statolith activated signaling? Or, perhaps *AtGLR* signaling may mediate a step between statolith movement and auxin that triggers the gravity response? In any case, here is an opportunity to see if these *AtGLR* genes are involved in the gravitropic response in the inflorescence of the flowering stem.

You are very fortunate to have these quadruple knock-out lines to examine for alterations in movement - novel data - novel experiments. Testing the role of glutamate receptors (repetitively) can be done using Arabidopsis lines where distinct glutamate receptor genes have been knocked out and comparing these lines to wild type. Which lines will you use? What will be your controls - both positive and negative?

Figure 5. Set up for gravitropic recording assay – wildtype vs. mutant

Plant Movement - Genetics

Step 1: Set up your positive vs. negative controls: The role of starch in gravitropism
In this first step of the experiment you will test two types of controls: a negative control, and a positive control. The negative control is wildtype (Arabidopsis strain, Columbia). The positive control will be the *pgm* mutant that lacks the statolith gravity sensors. Here you will conduct an experiment on Arabidopsis to determine whether the *pgm* mutant is retarded in gravitropic response and serves as a good positive control. To set up this experiment, choose two Arabidopsis plants with young, growing, flowering stems. One plant genotype is labeled wild-type and the other plant is labeled *pgm*. Place one of each of the plant genotypes resting on their sides with their foliar (flowering stem tissue "facing" towards each other (see Figure 2)). Make sure to have a black background, such as a black cardboard folder in the background. Make sure both plants, or at least the flowering stems, are in the camera viewer and then record as described in the "Guide to Using Lapse It" below.

Step 2: Set up your experiment to search for novel mutants in plant movement.
This is your opportunity to contribute new knowledge to the plant science community. Here you will be given an Arabidopsis strain that has a mutation in a known gene and you will determine if this mutation is altered in its response to gravity in the flowering stem using *Plant Tracer*. Once a gene of interest is selected, then a line (strain) of Arabidopsis with a mutation in this gene is located at the Arabidopsis Biological Resource Center (ABRC), which is a repository of various

research materials in Arabidopsis. The genes you will be exploring have a mutation in one or two of their glutamate receptors genes. Each mutant line has an accession number, which corresponds to its name where it is stored as seed at the ABRC.

To set up this experiment, choose two Arabidopsis plants with a young, single, growing, flowering stem. (Multiple stems may cross each other when moving, thus, throwing off the tracking capability of the App.) The plants are at least 5 weeks old and bolting (send out) Arabidopsis inflorescences (flowering shoots). Locate the flowering stem. Choose a wild-type plant for the control, and then a mutant plant for the test and MAKE SURE TO WRITE DOWN THE NAME. Place one of each of the plant genotypes resting on their sides with their foliar (flowering stem tissue "facing" towards each other (see Figure 5)). Your device should be resting horizontally. You can prop-up the device using a pair of brackets, binder clips or by using any method you construct. Make sure both plants, or at least the flowering stems, are in the camera viewer, as close to the camera as possible, and then record as described in the "Guide to Using Lapse It" below. Make sure that a metric black ruler with white writing is placed in the same focal plane of the inflorescences. Write down the names of your mutant and note if the apex is facing left or right. It's best if the names of the plant lines are visible in the camera view.

Guide to using Lapse It to set up a plant movement Time Lapse (go to Tutorial on http://www.planttracer.com)

1. If you haven't done so already, go to the App Store and download "Lapse It". An iPod, iPhone, iPad, or Android may be used, but Plant Tracer analysis can only be performed on an iPhone. Make sure to turn it on airplane mode so an unexpected call does not interrupt the recording.
2. Make sure the phone is charging to maintain power when making a time lapse movie. There are some iPod touches available to the lab (password 0605). They have been pre-loaded with Lapse It.
3. Open Lapse It. Tap the "Interval" button on the bottom, center. and change the Capture Interval to 2 Minutes (a picture is taken every 2 minutes). Leave all other settings on default
4. Place a solid-colored piece of black paper in the background.
5. Make sure your device is secure and that the subject (flowering shoot apex) of interest, and the genotype labels, is fully in the viewer. You may need to be creative to do this. Brackets, binders or test tube racks etc., can be used to support your device. You may need to use a little tape and/or paper towels to help prop up your device.
6. Once your device is supported, bring your camera as close as possible to the plant for good tracking and make sure the apices are in focus and the genotype (wildtype or mutant line) labels are fully visible (Fig. 2). You do not need the whole plant in the viewer, just the apices of the control and the test plant and the anticipated inflection point on the stem
7. Make sure a ruler with white increments and number with a black background is visible and placed in the focal plane with the apices
1. Hit the red capture button to begin recording.
2. DO NOT DISTURB the setup for the next ~1½ - 2 hours.
3. Stop the program.
4. View your video and make notes regarding the plant's behavior.
5. Tap Export, which will render the project.
6. Tap Save, and the movie will be sent to your video library. (You can upload to Facebook, send as an email, or directly download it to a computer.)

7. Go into your Photos and give the video a name that indicates what the subject is Is it a mutant strain? The rendered movie should be named with the name or accession number from the tag. An example would be: AtGLR3.3.

Quantifying movement using Plant Tracer. To see *Plant Tracer* in use go to:

https://www.youtube.com/watch?v=hj-gtbiS4N0

1. Open Plant Tracer
2. Choose GRAVITROPISM
3. Upload a Video by tapping on the camera icon
4. Trim the video length using the sliders to remove the logo at the end or unnecessary footage. While trimming, bring the apex back to the point in time where it has just moved an approximate 90 degree angle from the axis.
5. From Lapse It enter FRAME RATE (default value 2 gives a frame of 0.5 frames/minute)
6. Set the scale. Click “Draw Line” and tap on two spots on the margin of the ruler. If the line is not parallel with the ruler markings, click “Undo” and then “Draw Line” again until you are sure it is straight and that an accurate span can be measured.
7. MARK by tapping to center the box over the organ (usually the apex) for tracking.
8. Set the inflection point by tapping a square of maximum bending on the axis (REPLAY the video if needed.
9. To identify the precise inflection point location). DRAW LINE to connect the boxes and calibrate the line distance into the tracer.
10. Tap TRACKING to trace the apex and SHOW RESULT to see X and Y graphs and amplitude, rate and angle.
11. Screenshot saves a picture of the data into the photo library. Next copy your data into the worksheet. Enter Max Amplitude (the distance that the apex moved), the Rate and the Angle. Enter the plant strain (WT or mutant).

Name _____ Lab Partner(s)_____

Plant Movement and Signaling
1. View your time-lapse video. Describe your observations. Note any "behaviors" from your plants. Describe your results.

Expected results are wild-type plants show a faster gravitropic reorientation than *pgm*. Also, WT shoots may exhibit a much sharper angle of bending than *pgm*. Other behaviors may include circumnutation in shoots that are done bending or are already oriented up and down. Mutant plants are less likely to show noticeable differences compared to wildtype. However, expect there to be considerable "stochastic" variation. Approximately 1/5 times, *pgm* curves as fast as wildtype. This is a preliminary study. Potentially positive results will be tested by future students and/or scientists.

2. Run Plant Tracer on both plants in your movie and list data regarding your experiment testing Arabidopsis movement:

Control strain Columbia

Movement Rate mm/min _____ Amplitude _____ Angle degrees_____

Mutant strain/Test Strain (ABRC number) (pgm or knock-out line) _____

Name of mutated gene_____

Movement Rate mm/min _____ Amplitude _____ Angle degrees_____

X and Y graph: (Paste screen shot of graphs below)

3. Explain how gravisensing works in plants. Draw out the steps.

Gravisensing is initiated when the plant organ has been turned on its side. Statoliths will settle to the (new) bottom of the cell. This stimulates auxin to also move to the (new) bottom of the cell.

Auxin induces expansion of the bottom cell wall, which causes the entire shoot to begin to curve in such a way that the shoot is now oriented vertically again (in the stem region above the auxin induced expansion zone).

4. How does the *pgm* mutant impair gravitropic signaling?

5. Did you see a difference between the mutant and control(s)? Speculate what the role of your gene might have on plant growth and development, or if you have positive data - plant movement (there is no correct answer for this)?

Cells that cause plants organs to move are called "Motor Cells". For rapid closing leaves, such as mimosa, or for jointed leaves, such as Venus flytrap, swelling of the cells at the joints forces the leaves or leaflets to close. The mechanics of circumnutation are simple but fascinating. In Arabidopsis it has been shown that cells swell on one side of the stem while cells on the other side of the cell contract. This swelling and contracting occurs in a cyclical fashion causing the growing portion of the stem to rotate and/or swivel.

Send your data sheets to your professor.

Exercise 2. The Molecular Genetics of Gravitropism (Plant Tracer 1 trial)

Statoliths can be easily viewed by staining with Lugol's reagent, which is simply a solution of iodine. Iodine binds starch. Because statoliths are made of starch, Lugol's solution will indicate the presence of statoliths (Figure 6), which are located near the root cap. Recall that the Arabidopsis pgm mutant is impaired in the synthesis of starch. Would you expect to see statolith staining in the pgm mutant? To answer this question, perform the following exercise below.

Lugols Staining of Arabidopsis root tip statoliths

1. Using forceps, gently place both some Columbia (wildtype) and some *pgm* seedlings onto a microscope slide so that they are lying side by side. To avoid damaging the seedlings during the transfer, gently hook the green cotyledons. Lay the seeds flat on the slide.
2. Quickly! (the root tips can dry out quickly) - apply a small drop of Lugol's reagent to the root tip and let it incubate for 1 minute.
3. Using a Kimwipe, slowly wick up the Lugol's. Placed the used Kimwipe in the medical waste.
4. Now add water to the root tip and allow it to site for 1 minute. Next wick up the water with a Kimwipe.
5. Place a drop of 50% glycerin over the root tip
6. Place a cover slip over the root time.
7. Using a compound microscope compare WT vs. pgm root tips. Look carefully. Do you see a difference? What would happen to gravitropic responses if the statoliths are missing?

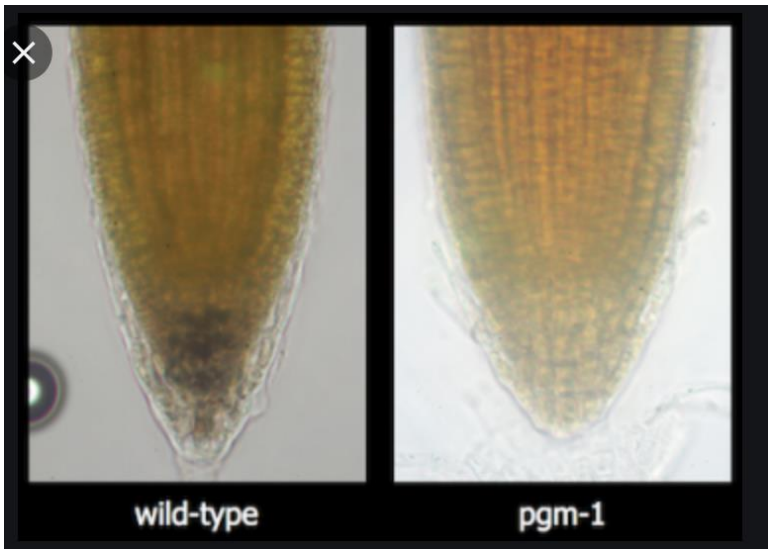


Figure 6. Statolith staining in Arabidopsis root tips. Left, wildtype. Right *pgm-1*, a mutant that is lacking statoliths and hence poorly sensing gravity.

Lab 8 - *Plant Tracer* 2

Good science requires repetition. Repeat your experiment from last week. However, if you cannot find the same genotypes, or, if your plants are not quite at the right developmental stage for this analysis, then try a different genotype for your analysis if necessary. While you experiment is running, investigate the unusual plants that are available. These plants exhibit rapid motion when touched.

Exercise 3. Plant Movement Detection with the Naked Eye (Plant Tracer 2 Trial)

Plants on the hunt

The fastest animals are carnivorous. Imagine a cheetah hunting a gazelle. The fastest plants are also carnivorous. Below are two examples of "fast" carnivorous plants:

Venus Flytrap (*Dioneae muscipula*)

Observe a Venus flytrap. This well-known insectivorous plant captures its prey in a fast-closing leaf. The Venus flytrap typically grow in nitrogen poor bogs. The nitrogen collected from its prey is incorporated into the plant's proteins, DNA and other structures. Capturing animal prey gives these plants a selective advantage over non-carnivorous plants that grow in wet bogs. Venus flytrap closure is activated by a long distance electrical potential (Figure 7).

What to examine: Venus flytrap

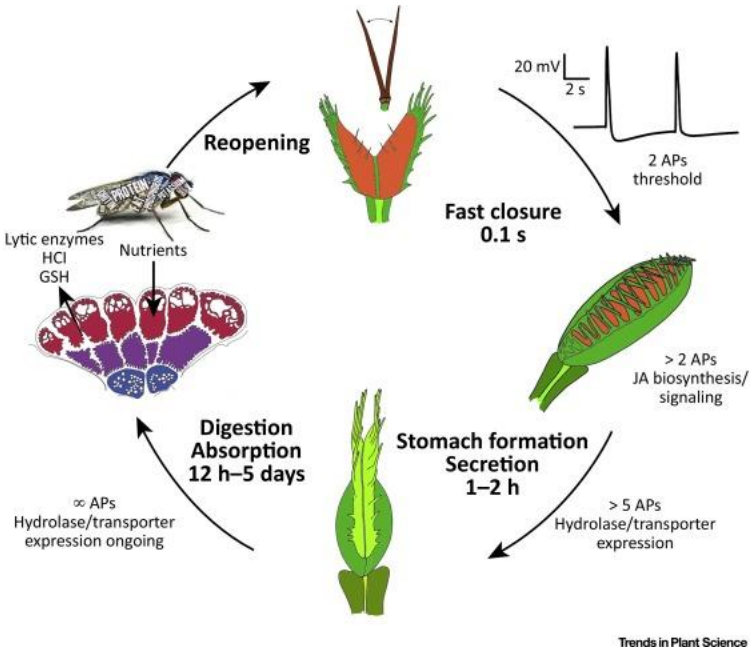


Figure 7. Process of Stimulation, electrical conduction and leaving closing in Venus fly trap

Sensitive plant (*Mimosa pudica)*

Touch a sensitive plant leaf and watch it close. Similar to the Venus fly trap, mimosa leaves fold by a touch activated electrical signal (action potential) that causes loss of turgor pressure in motor cells at the base of the leaf (Figure 8) and leaflet, the minor leaf-like structures along the central axis of the leaf. What might be the purpose of this response?

What to examine: Sensitive plant

Touch the leaves and leaflets of this plant. Note their successive closing pattern.

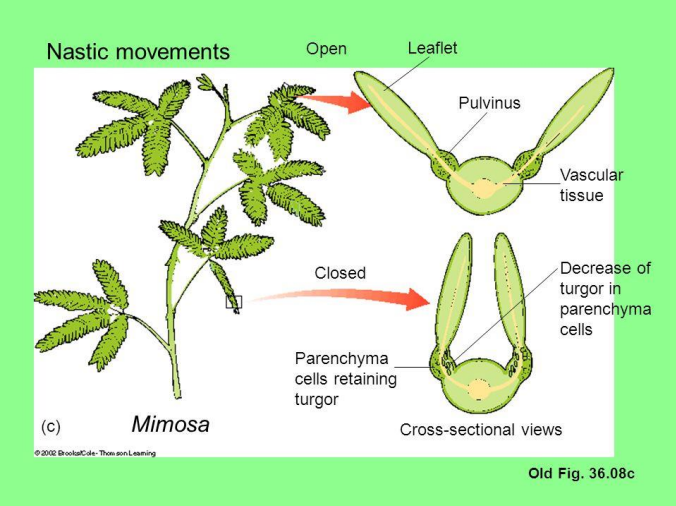
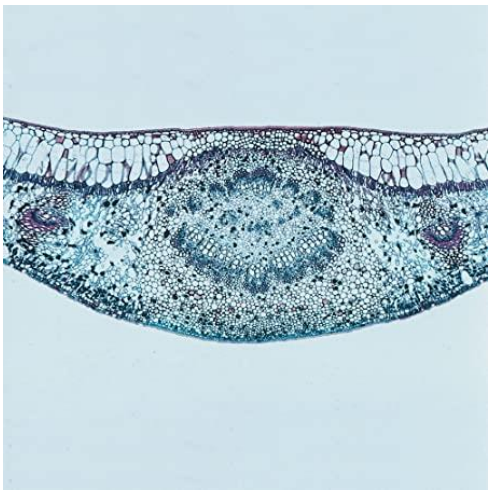


Figure 8. Diagram of *Mimosa* pulvinae



Slides

If available, examine a slide Venus Fly Trap leaves showing a cross section. There is a structure called pulvinae (Figure 9). This is a round mass of cells at the base of the leaf stem. When triggered, the pulvinae cells swell with water forcing the trap to close. What elicits closure? In the Venus fly trap two hairs on the leaf need to be tapped in sequence within a 30 second interval for the trap to close. If only one hair is tapped, then the trap will not close. This is an example of plant memory. Remarkably, when the second hair is tapped, and electrical current passes through the leaf activating the closing process.

Figure 9. Stained cross section of Venus fly trap pulvinae (below)

.

Plant Tracer Worksheet 7

Name _____¨ Lab Partner(s)_____

Plant Movement and Signaling
1. View your time-lapse video. Describe your observations. Note any “behaviors” from your plants. Describe your results.

2. Run Plant Tracer on both plants in your movie and list data regarding your experiment testing Arabidopsis movement:

Control strain Columbia

Movement Rate mm/min _____ Amplitude _____ Angle degrees_____

Mutant strain/Test Strain (ABRC number) (pgm or knock-out line) _____

Name of mutated gene_____

Movement Rate mm/min _____ Amplitude _____ Angle degrees_____

X and Y graph: (Paste screen shot of graphs below)

3. The Venus fly trap catches insects for a meal.

   A. Why?

   B. How does it move (consider electrical signals and swelling tissues)?

4. *Mimosa pudica* is also known as the sensitive plant.

   A. Why?

B. How does it move (consider electrical signals and swelling tissues)?

5. Observe this movie of *Arabidopsis* circumnutating. This is a universal movement found in a large variety of plants where plant organs wave back and forth. In trellising (climbing) plants circumnutation proceeds until the stem finds a support to climb. In roots, circumnutation has been shown to function as a motion to help the growing tip drill into the ground. But many plants like *Arabidopsis* circumnutate for no particular known reason (*Arabidopsis* is not known to climb on other plants for support). Speculate as to the purpose of *Arabidopsis* circumnutation shown in this video: https://youtu.be/_t8o9KqoDXY

The Significance of Plants

Plants are essential?

Big changes are happening in the plant research and technology community. We note here four new trends in research in plant biology that are rocking the research community. Choose one of the topics below and write a little synopsis for Worksheet 8 (at least 500 words) how plant research in that area is progressing. What kind of careers are available that involve plant biology?

A. **Plant Meat.** Plants have always been important for survival of all life on the planet. Yet as the human population continues to climb, we need to ensure a secure food source for humanity. Recently tremendous improvements have occurred in making substitute meals that taste incredibly like real meat. Enter Impossible Foods and similar companies making novel synthetic animal products. Explain how this new technology has entered the food industry and the science behind it - and specifically for this assignment, how it is leading to exciting new careers.

For more information see: https://www.cnet.com/how-to/impossible-burger-everything-you-need-to-know/ https://impossiblefoods.com/heme/

B. **Ecosystems Services.** The complex web of ecology on earth depends on a constant input of energy from the sun, captured by plants. Human overconsumption is straining the biosphere to the breaking point. Climate change, land conversion, invasive weeds, pollution and many other anthropogenic events are making life systems tenuous in the world. Biologists are sounding the alarm and teachers are spreading the message. Describe an ecological problem and a concerned individual that is trying to do something to help the planet protect plants. How does knowledge about plants help this individual to sound the alarm and/or mitigate this ecological problem?

C. **Natural Products.** Most of the medicines we use today originally were discovered in plants. Aspirin came from willow tree. The chemotherapeutic agent taxol, which treats ovarian cancer, came from the Pacific Yew tree that grows in California. Even today bioprospecting continues to detect new medicines from plants. Meanwhile, herbal treatments are a popular method for home remedies. herbs, teas and coffee beverages are important in social interactions. Drinks from new plants continue to find their way into the market, Acai, Kombocha, and coconut water are just a few. Give an example of a new medicine or plant based product that has become important in our lives.

https://untamedscience.com/biology/plants/

D. Genetics. Plants were the first organism used to prove genetic theory beginning with Gregor Mendel revealing independent assortment from peas. Barbara McClinton used maize to detect the first extrachormosomal heredity units known as transposons. Today plants still continue to serve as excellent genetic study systems as well as genomics. Plant genomic research continues to offer approaches to improve crop yields and a variety of novel plant products.

https://www.youtube.com/channel/UCLdgn7_1J6n1Hk6bqNL_Xag
https://www.youtube.com/watch?v=ER3t5J5RpJE

Below are two links to career sites involving plants. Often people do not think of looking for a career that somehow involves plants, particularly if you live in an urban area. However, there are new job possibilities in this field due to advances in technology and ecological changes.

https://cms.botany.org/home/careers-jobs/careers-in- botany/careers_in_botany_profiles.html

https://jobs.plantae.org/jobs/browse
https://community.plantae.org/video/4722514355696961121/plantae-seminar- agricultural-r-amp-d-in-today-s-industry-opportunities-and-navigating-a-career

Name _____

Find a plant related profession or hobby that you think is interesting and write a 250 word description on why this might be interesting and meaningful.

Present to the class your results of your *Plant Tracer* experiment.
Presentations should include X number of slides

Title slide

- Short descriptive title of experiment
- Names of individuals in your testing group
- Date

Introduction (1-2 slides)

Background (make sure to reference the literature)

- Relevant information on Animal GLRs
- What is known about AtGLRs that is relevant to your analysis
- What is not known about AtGLRs that is relevant to your analysis
- What is the rational of your experiment? What is the gap in knowledge?
- What is your question/hypothesis?

Results Slide (1-2 slides)

- Movie of one or more plants movie with your interpretation
- Graph of your data
- Analysis of other student's data in your class looking at the same lines
    - Determine average value parameters (rates, distance, angle for combined data from each genotype

Discussion Slide (1 slide) (make sure to reference the literature)?

- Reflect back on the issue(s) raised in the introduction - rational - importance.
- Restate the findings in the context of the question/hypothesis
- What is the relevance of the findings
- Follow up work?

For next year?

Plant Tracer Presentations
Rubric and guidelines (presentation is equal to worksheet 9)

Slide 1: Title (1 slide)

- **Clear informative title.**
- **Full names of the team that worked on the project.**
- **Date**

Slide 2: Background (1-2 slides)

- **Something relevant about gravitropism in plants**
- **What is known/important/relevant about gravitropism in Arabidopsis**
- **Something relevant about the genes and the related mutants that are being used in this study?**
- **What is not known about gravitropism in Arabidopsis or plants?**

Slide 3: Set up (1 slide)

- **How was the experiment performed [this should be quick since all students are doing a similar study]. Images?**

Slide 4: Results (1-2 slides)

- **Movie(s)**
- **Graphs (can also use data from other students experiments)**
- **Movement parameters - rates, distance, angle**

Slide 5: Discussion (1 slide)

- **Implications of the data, inconsistencies**
- **Future experiments**

General:
Be informative. Speak clearly. Talk should come across as well practiced. Passing from slide to slide should be fluent. Explanations about gravitropism, the role (or relevant question) of the chosen glutamate receptors in plants should be well defined. The experimental outcome should be discussed.

Writing Assignment 2: Overview

Write a scientific paper describing your experiment with *Plant Tracer*. Write using scientific terms and phrases. Keep your sentences simple and straightforward. Write in formal scientific English. Science writing should be concise. Make sure to use your examples and cite your references throughout your paper.

The Title

Your title should capture the essence of your work. It should include the key terms from your hypothesis, so that readers immediately understand your topic and what you found. Be concise and to the point! For example, "The effect of ozone on the rate of pine root tip growth".

Introduction (25 points)

The Introduction briefs the reader on the topic. It describes **what** is known, and **why** this information is relevant to the story. The introduction then narrows its scope to the central question or hypothesis being tested. It also states why it is important to answer that question—giving significance towards finding the answer. The shape of an introduction is like an inverted triangle with the general scientific picture at the broad end and then **focusing** on the question of inquiry at the apex. Make sure to cite your references.

1. Discuss (briefly) something important about the role of glutamate receptors in animals and plants.
2. What is known about gravitropic movement in plants and/or more specifically Arabidopsis?
3. What is known about gravitropic movement in stems or more specifically Arabidopsis stems?
4. Discuss (briefly) something important about the role of glutamate receptors in plants.
5. Now using this background, describe a "gap" in our understanding of the role of glutamate receptors in plants. From this description in the scientific gap in knowledge, derive your hypothesis. Remember to write your hypothesis as a statement that could be proved right or wrong.

Write in past tense when referring to previous work. Write in present tense when discussion existing facts. Write in future tense when discussing future work that may be done.

Materials and Methods (25 points)

This section is the nuts-and-bolts of your paper. Another scientist reading this section should be able to use this information to recreate your experiment. Assume that the reader has basic familiarity with laboratory technique so there is no need for you to present basic facts such as "the sample was placed in a microfuge tube". Manipulations need to be explained in such a way that a scientist could repeat each experimental step. Omit details that are unessential. List the identity of the plant material you are using or the computer programs used in your analysis. Describe the format of the output of your computer programs Make sentences brief. If certain technical steps are repeated, then streamline your method by writing, for example, "the experiment was repeated over a certain period of time".

Describe the materials used and how you did your experiment

Report what materials were used, including biological materials (sources, species, strains, etc.), chemicals, and apparatus used (name, description, source company). List the materials as their use comes up in the description.

Detail how the experiment was done in a way that other scientists could replicate your experiment:

- Describe the steps sequentially
- State specific quantities
- Name the materials and apparatus.
- Do not use bullet points or numbering. Write in paragraph format.
- Write in past tense. Be comprehensive but concise.
- DO NOT cut and paste the protocol from the manual or internet or other sources (that would be plagiarism). Use your own words.

Results (25 points)

In the Results section, you explain your findings – including your observations and any technical analysis. Here you could include images of your plant before and after the analysis, a link to your movie on YouTube, and images from the *Plant Tracer* experiment as well as your graph data and your specific movement parameters (angle, rate and distance).
Design this section so that it has a logical flow. Briefly restate the objective of the experiment in the first sentence. Next describe your findings. Only mention experimental results that that you would like to highlight that are relevant to your observations.
Describe your data:

- Compare controls vs. mutants
- What is worth noting about your results? Were the results consistent between different experiments?

Conclusion (25 points)

The conclusion is where you explain the relevance of your work. What is informative and interesting about your time lapse observations? Do you think your results are correct or informative? What about your sample size? Was it large enough? What steps would you take to repeat your results? If you had difficulties getting results, what may have been the problem? If you were to repeat the experiment what changes might you make? What future experiments might you want to conduct in light of your findings? How might your findings be important to others? Relate your conclusion back to your Introduction, while considering your hypothesis and experimental objectives. What are your thoughts regarding the possibility that plant glutamate receptors may be involved in plant movement, or not? What is known about plant gravitropic movement that could touch on your experiment?

Writing Assignment 2 Information

Formatting: Double-space your work and use an 11-point font. Maximum length: Four pages, double-spaced.

- Hardcopy: Assignment must be handed in lab on the due date as a hard copy.
- E-copy: An e-copy must be uploaded to Brightspace on the due date before lab.
    - Submit only one attachment.
    - Only use file types: Word, PDF, HTML, RTF, or plain text.
    - Always include file extension.

Good luck and please let your instructor know, Dr. Brenner, or visit the Pace Learning Center know if you have any questions

For this writing assignment and all others, please see the syllabus for the policy on plagiarism.

In short, plagiarism is not tolerated. A simple act of cutting and pasting a phrase from the internet or a fellow student's paper, or all other forms of plagiarism will be dealt with in accordance with Pace policy. For your own sake, do no plagiarize.

Rubric Writing Assignment 2

Section	**Points**
Introduction	
Background information (relevance, thorough)	5
Question and Hypothesis	5
Writing Style - (fluid, formal scientific)	5
Proper use of tense, species names, grammar	5
Materials and Methods	
Clear description of materials and manipulations	20
Proper use of tense, species names, grammar	5
Results	
Clear explanation on data collected, comparisons	15
Figures with captions, Movies accessible	5
Proper use of tense, species names, grammar	5
Discussion	
Significance of results	15
Future studies	5
Proper use of tense, species names, grammar	5
Overall	
Use of informative sub-captions	
Writing quality (concise, clear, importance, interest)	10
Total points	100