How Can Plants Tell Which Way Is Up?

Laboratory Exercises To Introduce Gravitropism

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Many people think of plants as essentially sessile organisms that do not actively respond to their environment. What could be further from the truth! In fact, plants are capable of a variety of movements, including the dramatic nastic responses (such as Venus fly trap closure) and the less sensational tropisms. These latter movements are directed growth responses to some type of external stimulus such as gravity (gravitropism, formerly known as geotropism) or light (phototropism). This paper describes some interesting exercises that are derived from recent work, including research that has led to experiments performed on two Space Shuttle missions in 1997 (Kiss et al. 1998).

The study of tropisms can be a useful way to introduce students to plant biology in high school and introductory college courses. In our experience, students are fascinated by plant movements when they are presented in lectures and find laboratory experiences on this topic quite engaging. Laboratory work on plant tropisms can also be used to introduce important concepts in science such as hypothesis testing, quantitative analysis, and the use of statistics.

The laboratory exercises described in this paper involve the higher plant Arabidopsis thaliana, which has become an important organism in molecular biology research and is the focus of an international plant genome project. Based on the material presented here, a number of plant gravitropism laboratory exercises with Arabidopsis that are simple in terms of equipment/materials and procedures can be developed. These exercises are robust in that they work well even in the hands of introductory students, and they can be expanded according to the individual instructor’s needs. This paper describes two exercises that have been performed by beginning college students, and these exercises can easily be performed in biology classes in most high school settings.

Background

Gravitropism in plants can be divided into three temporal stages: perception, transduction/transmission, and response (i.e. the differential growth in a plant that leads to downward or upward curvature). These laboratory exercises are focused on the early events of plant gravitropism termed “gravity perception.” Gravitropism and other tropisms in plants have been extensively studied since late in the last century, and Charles Darwin and his son Francis published a book titled The Power of Movements in Plants in 1883. (In fact, the Darwins’ early experiments in phototropism are outlined in several introductory biology textbooks.)

Gravity perception in plants is hypothesized to be mediated by the interaction of dense organelles (called structures. In higher plants, a great deal of evidence suggests that amyloplasts (starch-filled plastids), which settle or sediment to the new lower cell wall following reorientation, function as statoliths. Thus, the idea to explain gravity perception in plants has been referred to as the “starch statolith hypothesis,” and the evidence supporting this hypothesis has been reviewed in a number of publications (e.g. Sack 1997), including a very accessible article in Scientific American (Evans et al. 1986).

Some of the strongest evidence that amyloplasts function as statoliths comes from research with mutants of Arabidopsis that lack starch (Kiss et al. 1997). These mutants are starchless since they are missing one of the enzymes (phosphoglucomutase or pgm) in the final steps of starch synthesis. Several studies have shown that the starchless mutants are much less sensitive to gravity, but these mutants still do respond to gravity (i.e. they are not agravitropic). The upshot of this work is that plastids (with starch in the case of the normal wild-type, WT, and without starch in the case of the mutant) can function in gravity perception. (It is important to note that both the WT and mutant plants have the same number of plastids, but the mutant has starchless plastids.) However, when starch is present, there is a greater plastid density and an increased total mass of these statoliths, and the plants do better in terms of gravity perception and response.

In the two related laboratory exercises that follow, students should be able to demonstrate that while both the WT and mutant respond to gravity, the WT has a greater response. One exercise focuses on gravitropism in the
flower stalks of mature plants, and the other involves a study of gravitropism in young seedlings. They can be performed independently or together, depending on the instructor and his or her course.

**Exercise #1 - Flower Stalks from Mature Plants**

**Materials Needed**

Seeds of normal WT *Arabidopsis* and a starchless mutant are available free of charge (or in some cases, for a small fee) to educators through the *Arabidopsis* Biological Resource Center (ABRC) at Ohio State University. Orders may be placed through the Internet (http://aims.cps.msu.edu/aims/), by e-mail (arabidopsis+@osu.edu), or by standard mail (ABRC, 309 B & Z Building, 1735 Neil Ave., Columbus, OH 43210; telephone: 614-292-9371; fax: 614-292-0603). The starchless mutant is pgm-1 (stock no. CS210), and the WT strain to use is Columbia (Col-2, stock number CS907). The ABRC will not send many seeds, but you can grow more plants for your own seed stock (i.e., plants self-pollinate), and each plant will produce thousands of seeds. Other materials needed for this exercise include soil, pots, a light bank (fluorescent "shop" lights work well), a light-tight box (e.g., photocopy paper boxes or large shoe boxes), and pro-

**Preparation**

You will need to grow plants in pots until they start forming flower stalks. There are many ways to do this, but we will provide highlights of one general method here, and the reader is referred to the above ABRC site to learn more details (if needed) and alternatives in growing these plants. The good news is that *Arabidopsis* can be grown in a variety of containers and in many ways, including in growth chambers, greenhouses, on window ledges, and outdoors (after all, it is a real weed).

The first thing to realize is that *Arabi-

dopsis* seeds are very small (0.4 mm in diameter), and one has to be careful in handling them. About one to two dozen seeds are sown by sprinkling them in four-inch plastic pots in a commercial potting soil such as Metromix 350 or Peter's potting soil. Seeds should not be covered with additional soil because they require light for germination. Several pots can be placed in larger plastic containers that can be covered with a plastic wrap in order to provide a humid atmosphere to stimulate germination. At this point, the pots are placed under continuous illumination from 40-watt fluorescent bulbs in a "shop" light fixture. Remarkably, the starchless mutant and WT are identical in morphology and size if they are maintained together under continuous illumination. If plants are grown on a light/dark cycle, then the mutant may be smaller and develop at a slower rate compared to the WT. However, we have found that the growth rate, in continuous light, of the WT and mutant flower stalks is almost identical; thus potential differences in growth are not significant when evaluating gravitropism.

An alternate to growing the plants in a soil mixture is to use a synthetic substrate, such as Rockwool cubes or Oasis floral foam cubes, purchased from a greenhouse or gardening supply store. The advantage of this method is that there is no potential problem with soil spilling when the plants are reoriented. In any case, soil moisture should be carefully maintained until the seeds germinate, which should be in about three to five days after sowing. Under conditions of low humidity, it may take a few more days for germination. Seeds will germinate at room temperature (20 to 22°C), but temperatures above 25°C should be avoided. Following germination, plants should be watered as needed, but too much watering may lead to fungal growth. *Arabidopsis* is in the mustard family, and the plants will form rosettes of leaves. Once the plants are at the rosette stage, they may need to be thinned to avoid overcrowding in the pots. About three to four weeks after sowing of seeds, the rosettes will start to form flower stalks or inflorescences. The gravitropism experiments work best on flower stalks that are 2 to 6 cm in length.

**Procedures**

After the above preparation, the experiment itself is quite simple. Both WT and mutant plants are reoriented 90 degrees in the dark, and this beginning of the experiment is referred to as time 0. We use photocopy paper boxes for the dark environment, and reoriented plants are carefully removed from the boxes at several time intervals (e.g., 0.5 hour, 1.0 hour, 2.0 hours, 3.0 hours). Do not worry about the time the plants are exposed to light since the phototropic response is relatively weak. (Instructors can excise flower stalks at the end of the experiment, and if the plants are left in favorable conditions, they will continue to produce additional stalks that can be used for other experiments.)

Curvature is measured at each time interval with a protractor, and the plants then are returned to the box. Sample data are shown in Table 1. Students can observe that WT flower stalks curve faster compared to the mutant flower stalks, even though the growth rates of both strains are similar. The data can be presented in table format or values can be used to draw a graph. Instructors can also have the students perform some simple statistical analyses (such as a Mest) to determine if the curvature values are significantly different between the WT and the mutant flower stalks.

If a light-tight box is not practical to implement, it is possible to do this experiment in general room light since the phototropic response is much weaker than the gravitropic response in these flower stalks. Another variation is simply to do the experiment with the WT only, and this exercise still allows students to appreciate the phenomenon in plants and to do some data analysis. The upward curvature of WT flower stalks is illustrated in Figure 1. This exercise can be enhanced by demonstrating the differing phenotypes of the WT and starchless mutant. Leaves from the plants are excised and left overnight in ethanol to remove the chlorophyll. The leaves then are placed in an iodine solution (i.e., 2.0 g potassium iodide and 0.2 g iodine are added to 100 mL of water), and the WT will stain intensely black while there will be no reaction in the starchless mutant.

**Exercise #2 - Young Seedlings**

**Materials Needed**

Seeds of normal WT *Arabidopsis* and a starchless mutant are available through the ABRC as indicated above. Other materials needed include: agar, disposable petri dishes (9 cm diameter), Parafilm”, commercial bleach, disposable Pasteur pipets, small vials, fluorescent "shop" lights, aluminum foil, and protractors.

**Preparation**

An agar mixture, 1.0 to 1.2% (w/v), is autoclaved and prepared by standard microbiological methods. At the same time, some water is autoclaved (or sterile water can be purchased). The agar solution is poured into petri dishes, which are filled to about one third of their height. The petri dishes
Table 1. Mean upward curvature in degrees (± standard error) of Arabidopsis flower stalks following a 90-degree reorientation in the dark. Curvature in the normal wild-type is compared to that of a starchless mutant (97 < n < 100).

<table>
<thead>
<tr>
<th>Time after reorientation (hours)</th>
<th>(WT)</th>
<th>(Mutant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>7.3 ± 1.3</td>
<td>2.8 ± 1.1</td>
</tr>
<tr>
<td>1.0</td>
<td>40.6 ± 2.7</td>
<td>24.9 ± 1.5</td>
</tr>
<tr>
<td>2.0</td>
<td>78.4 ± 3.5</td>
<td>48.8 ± 1.9</td>
</tr>
<tr>
<td>3.0</td>
<td>92.2 ± 3.1</td>
<td>62.9 ± 2.4</td>
</tr>
</tbody>
</table>

then are left to cool until the agar solidifies.

Prepared agar plates or ready-topour agar that only needs to be heated in a microwave oven can be purchased (e.g. Wards Natural Science Inc., Box 92912, Rochester, NY 14692-9012) if an autoclave is not available. A nutrient agar can also be used and is preferable; recipes can be found at the ARBC Web site listed above or in Kiss et al. (1996). Arabidopsis seeds are surface sterilized in a 30% (v/v) solution of Clorox® for 15 minutes in a vial, and then are rinsed four times (10 minutes for each step) in sterile water. It helps to place a drop of a detergent such as Triton X-100 or Tween (or even dishwashing liquid) into the Clorox solution and rinse water. Seeds are placed onto the surface of the agar with a Pasteur pipet and are placed approximately 1 cm apart. In the petri dishes, two rows of seeds can be accommodated. Petri dishes are sealed with Parafilm™ and placed on edge in some type of rack so that the surface of the agar is vertical, and an arrow is drawn on the dish to indicate the direction of gravity (Figure 2). At this point, people walking into your lab will realize something new (and maybe strange) is happening since petri dishes are typically placed flat on the bench!

The dishes are placed under a fluorescent "shop" light for 24 hours. Even though this is a "dark-grown" experiment, the seeds have a light requirement for germination. After the 24-hour light treatment, the dishes are wrapped in aluminum foil and placed in a dark environment such as a shoe box or in a drawer. It also helps to make an arrow on the foil as well as the petri dish itself to indicate the direction of gravity. Seedlings that are 1.0 to 1.5 cm in length should develop in about three days following the wrapping of the petri dishes with foil.

**Procedures**

Petri dishes with four-day-old seedlings are removed from the dark, and the aluminum foil is unwrapped. Students should easily see that the WT seedlings are oriented relative to gravity while the mutant seedlings are dis-oriented (Figure 3). This deviation can be quantified by measuring with a protractor the angles that hypocotyls are deviated from the vertical gravity vector. If the vertical gravity vector is defined as 0 degrees, the mean angle for the WT should be about 0 degrees, and the mean angle for the mutant...
hypocotyl curvature is shown in Table 2 with a protractor. Sample data for curvature of the hypocotyl is measured dark at these time intervals, and hours. Seedlings are removed from the time points for observation might than is observed in the flower stalks, so response to reorientation is much slower presented 90 degrees in the dark. The Petri dishes with seedlings are reoriented above in the flower stalk experiment. The best way to convey the excitement of the study of plant movements is by illustrating the process with motion pictures. To this end, an excellent Internet resource with time lapse movies of tropisms and other plant movements has been developed by Dr. Roger Hangarter of Indiana University (http://sunflower.bio.indiana.edu/~hangart/plantmotion/). These movies clearly demonstrate the dramatic nature of various plant movements and feature *Arabidopsis* as well as other plants. The topics include seed germination, gravitropism, phototropism, "sleep movements," mutation, and flower opening. Particularly relevant to the laboratory exercises described in this paper is the fact that gravitropism in *Arabidopsis* flower stalks is part of the movie collection.

### Table 2. Mean upward curvature in degrees (± standard error) of the stem-like hypocotyls of *Arabidopsis* following a 90-degree reorientation in the dark. Curvature in the normal wild-type is compared to that of a starchless mutant (94 < n < 251).

<table>
<thead>
<tr>
<th>Time after reorientation (hours)</th>
<th>WT</th>
<th>Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33.0 ± 1.0</td>
<td>1.8 ± 1.1</td>
</tr>
<tr>
<td>3</td>
<td>37.6 ± 1.2</td>
<td>1.8 ± 0.9</td>
</tr>
<tr>
<td>8</td>
<td>52.3 ± 1.1</td>
<td>6.4 ± 1.2</td>
</tr>
<tr>
<td>24</td>
<td>57.7 ± 0.9</td>
<td>13.5 ± 1.4</td>
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Another useful supplement to these laboratories is a two-page cartoon in the August 1997 issue of *Discover* magazine. Plant gravitropism is introduced in a humorous, engaging (yet accurate) manner in a cartoon titled “Starch wars” (Gonick 1997), which has been used in an educational outreach program for high school teachers by North Carolina State University.

### Conclusions & Perspectives

In terms of scientific conclusions, the important point is that while both the WT and mutant respond to gravity, the response (i.e. curvature) is greater and more rapid in the WT. Based on their data, students can make arguments for and against the starch statolith theory for plant gravity perception. While it is clear that starch *per se* is not needed for gravitropism, a full complement of starch (as in the WT) provides a better gravitropic response. Thus, the results are still consistent with the essence of a statolith model (i.e. dense particles participating in gravity perception). Perhaps the idea should be called the "plastid statolith hypothesis" since it appears that plastids (with starch in the case of the WT, and without starch in the case of the mutant) can function in gravity perception. Instructors will find a more detailed discussion of these ideas in Salisbury (1993) and Sack (1997).

From the perspective of teaching this material, we have found that students in introductory college biology courses enjoy the laboratory exercises outlined.
in this article. They like the idea of performing experiments that are based on current research and the relationship of the exercises to the space program. After all, space has been and continues to be a useful way to promote and encourage interest in the natural sciences. Students also value the opportunity to gather "real" data that can be used to evaluate a scientific hypothesis.

While we do not have experience with these experiments in a high school setting, they seem to be appropriate and quite feasible for biology classes at the secondary level. The two exercises are very flexible in that they can be performed independently or coupled together as part of a larger project. They also can be performed with fairly basic equipment and entirely on a macroscopic scale if needed. Appropriate add-on portions for each exercise have been discussed for the instructor who has the need and time for more complex laboratory experiences. Finally, we believe that these laboratory exercises will stimulate students to think carefully about their results and to develop critical thinking skills that will be useful to them throughout their educational careers.

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References
