HOW-TO-DO-IT

Light-Mediated Germination in Lettuce Seeds: Resurrection of a Classic Plant Physiology Lab Exercise

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Most students are taught that plants use light for photosynthetic energy conversion. However, many students do not realize that plants also use light as a source of information that is translated into changing growth and development. In 1952, H.A. Borthwick and colleagues demonstrated that light controls germination in certain varieties of lettuce seeds. They found that red light was most effective at inducing germination of imbibed Grand Rapids var. lettuce seeds whereas far-red light inhibited germination. They also discovered that when lettuce seeds were given multiple exposures of one light color followed by the other, it was the last light treatment that determined the germination response. This classic study demonstrated that red and far-red light act as an "on" and "off" switch to regulate seed germination (Borthwick et al., 1952). These observations ultimately led to the isolation and characterization of the red/far-red light plant photoreceptors called phytochromes (meaning "plant pigment").

Based on this publication, Grand Rapids var. lettuce seeds were used in plant physiology laboratory teaching exercises to demonstrate phytochrome mediated red\far-red control of seed germination. However, during the past 20 years, most Grand Rapids derived varieties no longer require red light to induce germination. Though having uniform seed germination is ideal from an agricultural point of view, these lettuce breeding efforts have also led to the abandonment of this laboratory exercise demonstrating how light can regulate seed germination in plants.

The purpose of this project is to identify which varieties derived from Grand Rapids lettuce still maintain a light-dependent germination response. With this information, this classic laboratory exercise can be resurrected. In addition, we present a new teaching tool that demonstrates the biological relevance of light-mediated seed germination and can be used in a lower budgeted class, such as in a public high school or college.

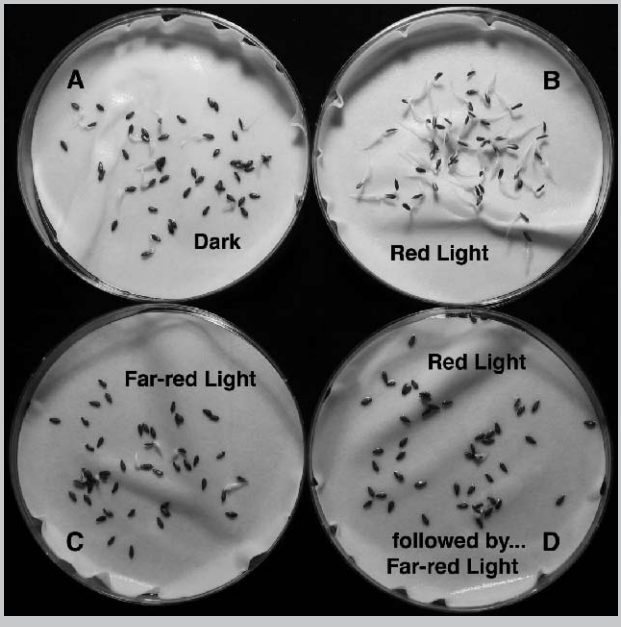
○ Germination Assay

Count out 50 seeds for each light treatment. The treatments will be: "dark", "FR" (far-red light), "R" (red light), and "R/FR" (red followed by far-red light). Place one or two sheets of Whatman (Maidstone, UK) #1 90 mm diameter filter paper (or some other absorbent paper) into four round, shallow petri dishes. Moisten the filter paper with distilled water so that they are damp, but not filled with standing water. In a darkroom lit by a green safety light (we use a 25A/TG, 25W, 120V, Transparent Green Party Bulb, A-19 Shape from General Electric, Fairfield, CT), place 50 seeds in the petri dish and label the dish with one of the light treatments. Wrap aluminum foil around each petri dish to create a light-tight seal (usually two layers) and let seeds imbibe (take up water) for at least one hour in the dark at room temperature. Be sure to re-label the plates after sealing with aluminum foil.

After at least one hour of imbibition, place both the red light treatment dishes and the red to far-red light treatment dishes in a red light treatment chamber. Expose seeds to red light for approximately five minutes. For our experiments described here, we use red light emitting diodes (LEDs) in an E30-LED chamber (Percival Scientific, http://www.percival-scientific.com). We have also used cool white fluorescent bulbs filtered through a sheet of red (#2423) acrylic for our red light treatment.

After the five-minute exposure, turn off the red light and, in the presence of the green safe light, cover the red light treatment dishes with enough aluminum foil to create a light-tight seal. Move the red to far-red light and the far-red light treatment dishes into a far-red light treatment chamber and expose for five minutes. For our experiments described here, we use far-red LEDs in an E30-LED chamber (Percival Scientific, Perry, IA, http://www.percival-scientific.com). Later we will describe an alternative method for supplying far-red light that is much cheaper than the ~$25,000 cost for an E30-LED chamber. After the five-minute exposure, turn off the far-red light and, in the presence of the green safe light, cover these dishes with enough aluminum foil to create a light-tight seal. After approximately 48 hours of incubation in the dark at room temperature, seed germination can be observed in normal room light (Figure 1).

Figure 1. Germination of Waldmann's Dark Green lettuce seeds after 48 hrs of imbibition and incubation in the dark **(A)**, or a five-minute red light treatment **(B)**, a five-minute far-red light treatment **(C)**, or five minutes of red light followed by five minutes or far-red light **(D)**.



○ Characterizing Different Lettuce Varieties

Using the above assay, we have shown that there are differences in 14 Grand Rapids derived varieties of lettuce purchased from Johnny's Selected Seeds (955 Benton Ave., Winslow, ME 04901: 1-877-564-6697). All results, including mean germination percent, standard deviation, and standard error, of these tests are expressed in Table 1.

Waldmann's Dark Green is the best variety for demonstrating light regulation of seed germination. Germination is relatively low in the dark (~50%) but is high in the red light treatment with a mean germination of 98.6%. When Waldmann's Dark Green is treated with far-red light or far-red after a red light exposure, germination is reduced to ~25% (Figure 2). Though almost all of the other varieties have dark germination rates over 90%, Two Star also has a lower dark germination than after a red light treatment. Marin and Black Seeded Simpson both have high germination rates in the dark but still show some inhibition of germination by far-red light. The rest of the Grand Rapids derived varieties show essentially no regulation of seed germination by light.

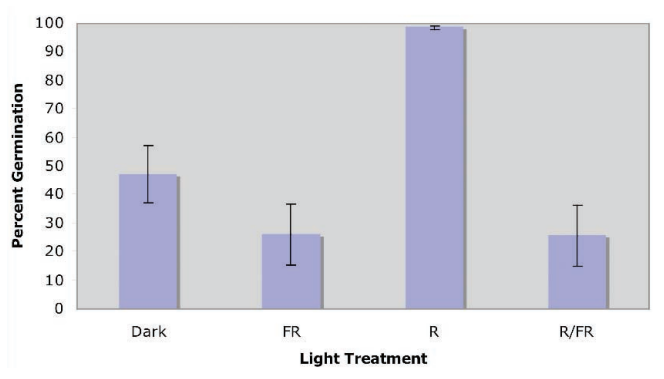
○ Using Lettuce Leaves as a Far-Red Filter

Although we have identified a Grand Rapids derived variety (Waldmann's Dark Green) that confers light-mediated control of seed germination, there are still some challenges with regard to performing this experiment outside a well-equipped lab. For example, far-red LED sources may not be readily available. In addition, some students may not understand the biological relevance of inhibiting seed germination with far-red light. With this in mind, we have developed a modified

Variety	Treatment	Mean (% Germination)	SD	SE
Waldmann's Dark Green	Dark	47.2	31.51	9.96
Data from 10 trials	FR	26.2	33.58	10.62
	R	98.6	2.32	0.73
	R/FR	25.6	33.53	10.6
Two Star	Dark	70.4	28.78	9.1
Data from 10 trials	FR	59.8	33.18	10.49
	R	97.8	5.03	1.59
	R/FR	58.4	29.5	9.33
Marin	Dark	89	6.22	3.11
Data from 4 trials	FR	60	20.07	10.03
	R	99.5	1	0.5
	R/FR	64.5	12.79	6.4
Black Seeded Simpson	Dark	90.4	10.7	3.38
Data from 10 trials	FR	82.4	24.01	7.59
	R	92.2	14.59	4.61
	R/FR	79.4	26.52	8.38
Baronet	Dark	94.6	8.22	2.74
Data from 10 trials	FR	95.6	5.4	1.8
	R	97.8	4.37	1.46
	R/FR	94	8	2.67
Tropicana	Dark	95.6	6.59	2.08
Data from 10 trials	FR	93.2	10.5	3.32
	R	87	25.55	8.08
	R/FR	93.6	7.76	2.45
Concept	Dark	96.8	5.35	1.69
Data from 10 trials	FR	91	21.48	6.79
	R	97.6	5.4	1.71
	R/FR	89.2	27.34	8.65
New Red Fire	Dark	98.25	2.25	0.8
Data from 8 trials	FR	100	0	0
	R	99.25	1.04	0.36
	R/FR	94.75	7.48	2.64
Red Sails	Dark	90.4	26.33	8.32
Data from 10 trials	FR	99	1.41	0.45
	R	92.8	21.37	6.76
	R/FR	91.6	22.5	7.12
Vulcan	Dark	99.2	1.4	0.44
Data from 10 trials	FR	99	1.7	0.54
	R	99.5	1.35	0.43
	R/FR	95.8	5.69	1.8
Galactic	Dark	98.2	2.2	0.7
Data from 10 trials	FR	98.2	2.74	0.87
	R	99.4	1.35	0.43
	R/FR	99.2	1.93	0.61
Blackjack	Dark	98.8	1.93	0.61
Data from 10 trials	FR	99.6	0.84	0.27
	R	99.6	1.26	0.4
	R/FR	98.2	2.57	0.81
Firecracker	Dark	99.2	1.4	0.44
Data from 10 trials	FR	99.6	1.26	0.4
	R	100	0	0
	R/FR	99.2	1.4	0.44
Simpson Elite	Dark	95.2	6.12	1.94
Data from 10 trials	FR	87.4	10.16	3.21
	R	98.6	3.27	1.03
	R/FR	98	3.78	1.19

Table 1. Compiled results from all Grand Rapids derived lettuce varieties tested. Most varieties were tested 10 separate times. The number of tests for each variety was divided equally between two different students and performed on different days. R = red light. FR = far-red light. R/FR = red light followed by far-red light. SD = standard deviation. SE = standard error.

Figure 2. Germination of Waldmann's Dark Green lettuce seeds 48 hours after treatment. R = red light. FR = far-red light. R/FR = red light followed by far-red light. n = 10 independent trials. Error bars = standard error of the mean.



version of this experiment using lettuce leaves as an inexpensive far-red filter.

A picture of the light treatment apparatus can be seen in Figure 3. Take a Pyrex dish and place electrical tape along all edges and sides, so light is only able to pass through the bottom which, when inverted, will be on the top. After inverting the Pyrex dish, cover the exposed glass with enough lettuce leaves to create a two- to three-leaf layer. Take a second Pyrex dish of the same size and place it on top of the lettuce leaves such that all light passing through the glass must be filtered through the lettuce leaves. Fill this second Pyrex dish with $^1/_2$ to 1 inch of water. Place an incandescent light bulb approximately 6 inches above the water surface. In this experiment we used a 150 Watt flood lamp.

The lettuce germination assay is performed as described previously using this apparatus as the far-red light filter. Be sure to keep the light bulb off when unwrapping the imbibed lettuce leaves. Place the far-red light treatment plates under the lower Pyrex dish being careful not to spill the water in the upper dish. We slide the whole apparatus to the edge of the lab bench without lifting. When enough space is available, the lettuce seed plates can be slid under the lower Pyrex dish and then the apparatus can be slid back onto the bench such that the lettuce seeds are only exposed to light that has passed through the lettuce leaves. Results from experiments using lettuce leaves as far-red filters are shown in Figure 4.

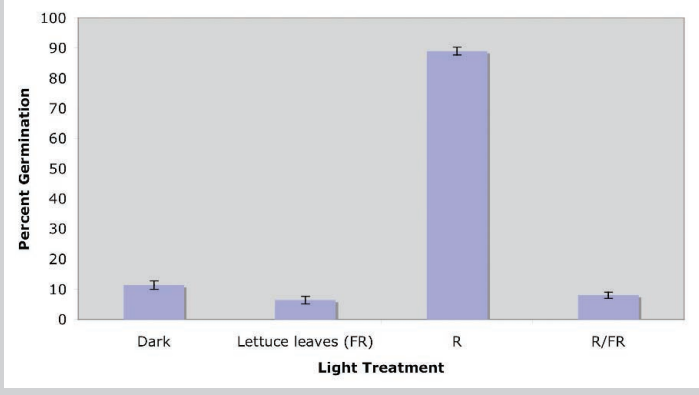
○ Discussion

This lab activity is a great way for students to see how light affects seed germination. One of the major questions likely to come from your students is: Why does light filtered through a leaf inhibit seed germination? Sunlight includes both red and far-red light. Leaves absorb red light for photosynthesis but not far-red light. Thus, direct sunlight is enriched in red light whereas light under the shade of a canopy of leaves is enriched in far-red light. In the wild, a lettuce seed in direct sunlight will be induced to germinate due to the presence of red light. However, if the lettuce seed is in the shade of another plant (such as the parent lettuce plant), the enriched far-red light will inhibit germination. When the shading leaves are removed, for example by a passing deer, the seed will be exposed to red light, inducing germination.

Figure 3. Our apparatus using lettuce leaves as a far-red light filter. Green leaf lettuce was used in this experiment. We have also successfully used red leaf and iceberg lettuce as well as collard greens as a far-red light filter.

Another important point of discussion relates to whether germination in all types of seeds is regulated by light. In general, only small-seeded, sun-loving plants have a mechanism for using light as a germination cue. These seeds have a small energy reserve and therefore must germinate under ideal growth conditions. Larger-seeded plants such as beans and peas use soil temperature and moisture as a cue for inducing germination. These seeds have a large energy reserve allowing stem elongation under suboptimal light conditions until the young leaves can reach optimal light. One modification for these experiments would be to use other readily available small seeded plants such as radish, beets, grass, and numerous weed seeds that can be collected by the students. For example, germination of seeds from the weeds *Amaranthus* (common names: Amaranth or pigweed) and *Sinapis arvensis* (common

Figure 4. Germination of Waldmann's Dark Green lettuce seeds 48 hours after treatment. R = red light. R/FR = red light followed by far-red light filtered through lettuce leaves as in Figure 3. n = 10 independent trials. Error bars = standard error of the mean.



names: wild mustard or charlock) has been shown to be regulated by red and far-red light (Kendrick & Frankland, 1968; Frankland, 1976).

There are also modifications of this experiment that can be performed using Waldmann's Dark Green lettuce seeds. For example, a greater number of plates can be used where: one receives only red light, the second: red, then far-red, the third: red/far-red then red, the fourth: red/far-red/red then far-red, etc. This experiment demonstrates that for many repeated exposures, only the last color of light controls the germination response. In another version, the exposure time between the inductive red-light treatment and the inhibitory far-red light treatment can be varied. This allows the students to examine the escape time sufficient to prevent far-red light from inhibiting germination. Students can also use different leaves as a far-red light source. Dried, non-photosynthetic leaves can also be tested to examine their ability to inhibit seed germination.

By including different lettuce varieties in addition to Waldmann's Dark Green, the students can examine the efforts by breeders to remove the requirement of light for inducing germination. Discussions that may follow include addressing why breeders and farmers would not want light to regulate seed germination. Since every packet of seeds include the results of in-house germination assays, it can be pointed out that farmers want all of the seeds that they purchase to germinate. In addition, many varieties of lettuce seeds are coated with clay to increase the handling size of the seeds. This allows small lettuce seeds to be planted with conventional seeding equipment designed for larger seeded plants. Since this clay coating prevents light from reaching the planted seeds, pelleted varieties of lettuce must not require light to induce germination.

The *National Science Education Standards* (National Research Council, 1996) outline in their Life Sciences Content Standard C, that students in grades 9-12 should develop an understanding of behavior of organisms. Though the Standards tend to focus on behavior in animals, they do mention, on page 187, that "plants also respond to stimuli." The American Society of Plant Biology (www.aspb.org) has developed 12 key principles of plant biology as a tool for providing important concepts for science education at the K-12 levels (http://www.aspb.org/education/foundation/principles.cfm; accessed 11/1/08). The 11th principle states: "Plant growth and development are under the control of hormones and can be affected by external signals such as light, gravity, touch, or environmental stresses." This lab exercise clearly demonstrates plant growth responses to light, which complements standard lessons on the use of light for energy via photosynthesis.

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