Effect of light intensity on the hypocotyl length of *Arabidopsis thaliana* during germination

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Abstract – In this experiment, seeds of the *Arabidopsis thaliana*, a model plant species, were subjected to different light intensities and the length of the hypocotyl was examined during germination. We hypothesized that *A. thaliana* seeds cultured under high light intensity would have longer hypocotyls than those cultured under low light intensity and no light. The seeds were grown in an incubator at 17 degrees Celsius at light intensities: 0 lux, 1396 lux, 7000 lux (optimal) and 12520 lux and the hypocotyl lengths were measured approximately every 24 hours for one week. Results did not support our hypothesis, and we found that *A. thaliana* cultured in the absence of light had the longest hypocotyls. More specifically, *A. thaliana* grew to a length of: 6.08 $\pm$2.00mm under no light, 1.50 $\pm$0.28mm at a light intensity of 1395 lux, 2.38 $\pm$0.42mm at 7000 lux, and 1.31 $\pm$0.25mm at 12520 lux, in a time frame of one week, or 168 hours. We concluded that in the absence of light, *A. thaliana* develops by skotomorphogenesis rather than photomorphogenesis, causing the length of the hypocotyl to elongate faster than under light conditions. In the light treatments, the hypocotyl length after one week was longest at the optimum light intensity and significantly shorter at both lower and higher light intensities.

Introduction

Plants provide food, fuel and medicine, and are a staple in modern life. In order to accommodate our fast growing population we must completely understand the plants that surround us. *Arabidopsis thaliana* is a flowering plant that has served as a base for our understanding of plant organisms for the last several decades. By conducting research on model plants, such as *A. thaliana*, and then inferring the results to biologically similar crop plants, we can attempt to increase food production. *A. thaliana* possesses several unique qualities allowing it to be a good model organism. It has a small genome, it requires few

nutrients for growth, and it produces many progeny, making it the perfect tool for understanding the basics of plant growth and development (Muller *et al.* 2010).

On the quest to learn more about plant development, many have studied how factors such as light intensity, nutrient availability and water consumption influence *A. thaliana's* growth. An important factor that has been heavily investigated is light intensity. To truly understand the importance of light intensity in plant growth, we must delve deeper into the underlying science. Plants grow by photosynthesis, where light energy is converted into chemical energy. Thinking logically, with more light, and thus a higher light intensity, one might expect photosynthesis to happen at a faster rate and for *A. thaliana* to grow taller. This seems to be the case as previous fieldwork shows that *A. thaliana* grown under high light intensity grow significantly taller than those grown under low light intensity. In fact, it was found that *A. thaliana* grew fastest at light intensity levels of 32400 lux, which is about the amount light you would expect in the summer at the Sub-Tropics (Bailey *et al*. 2001). Remarkably enough, 32400 lux of light intensity is about four times as much as the documented optimal, 7020-8100 lux, for *A. thaliana* growth (Rivero-Lepinckas *et al.* 2006). Although the previous statement may sound counterintuitive it does make sense as the optimum light intensity is designated not for the fastest growth but for the optimum, or "best" growth. As such, we see a difference in the literature between the optimum light intensity and the light intensity resulting in the fastest growth.

Several scientists have tried to understand how variations in light intensity influence *A. thaliana* growth; however, few have studied how light intensity influences the length of the hypocotyl, the stem of a germinating plant, during germination. According to the fundamentals of plant growth via photosynthesis, hypocotyl growth patterns are expected to be similar to those of plant stem growth. Bailey *et al.* (2001) found that stem growth was fastest at the highest light intensity. Thus, we expect the length of the hypocotyl to be the longest at the highest light intensity and shortest under no light. Based on this information, a model was proposed to examine variations of hypocotyl length under varying light intensities.

The null hypothesis states: Increased light intensity decreases or has no effect on hypocotyl length of *A. thaliana* during germination.

The alternative hypothesis states: Increased light intensity increases hypocotyl length in *A. thaliana* during germination.

Methods

For the initial setup of the experiment, we labelled four clear plastic Petri dishes for each of the four treatments with the corresponding treatment number and replicate number. The Petri dishes we used were 4 cm in diameter and we labelled the bottom of each dish on the outside.

Next, as seen in Figure 1, for each replicate we placed a filter paper at the bottom to cover the entire dish base. Once the filter paper was set, we pipetted 1 mL of water into each replicate in order to dampen the filter paper without having excess water droplets. Then, we placed ten *Arabidopsis thaliana* seeds into each Petri dish using a paint brush, ensuring that they were equally distributed on the filter paper.

Figure 1. Experimental setup for treatment grown in optimal light. Four Petri dishes each with a damped filter paper and ten *Arabidopsis thaliana* seeds.

Upon completion of the initial setup, we brought all the replicates into a 17 degree Celsius incubator where we applied a different light intensity to each treatment. The incubator had fixed light sources at each shelf. As shown in Figure 2, we placed each treatment on the shelf, at a different distance from the light source in order to obtain the desired light intensity.

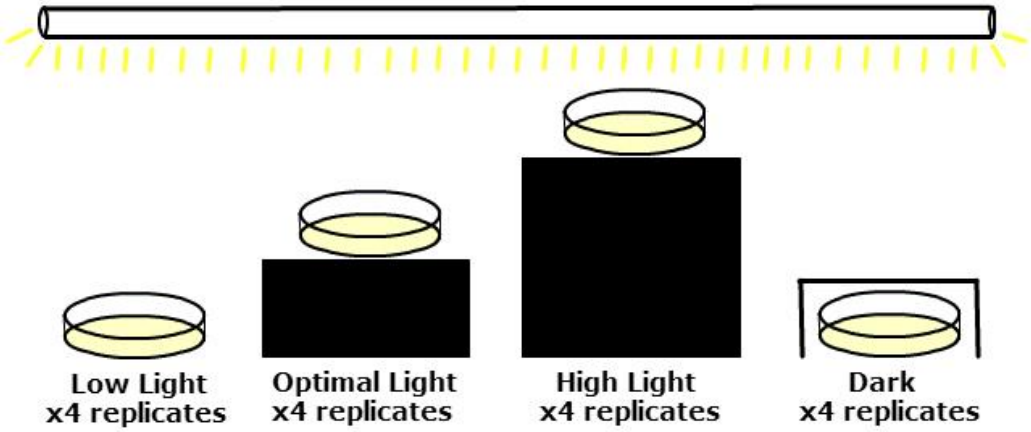


Figure 2. Experimental setup of four treatments: low light, optimal light, high light, and dark, at different distances from the light source.

First, for the high light treatment we used a light meter to measure an area where the light intensity was approximately 50% greater than the optimal light intensity range of 7020 lux to 8100 lux (Rivero-Lepinckas *et al.* 2006). As seen in Figure 2, we had to place the high light treatment closest to the light source compared to the other treatments. In order to elevate the Petri dishes closer to the light source to obtain such a high light intensity, we placed our replicates on an inverted basket. The light intensity measured at such distance from the light source was 12520 lux.

Next, for the optimal light treatment, we found an area within the incubator where the light intensity was a close match with the optimal light intensity range of 7020 lux to 8100 lux (Rivero-Lepinckas *et al*. 2006). We elevated the Petri dishes on inverted containers, as seen in Figure 1, to an intermediate distance from the light source in order to obtain the light intensity (see Figure 2). With a light meter, we measured the light intensity at this distance from the

light source to be 7000 lux. Although this is not within the optimum range mentioned by Rivero-Lepinckas *et al.* (2006), it was relatively close and therefore, was considered to be our "optimal" light treatment.

To simulate the low light treatment we used a light meter to measure where the light intensity was approximately 50% less than the optimal light intensity mentioned above. As seen in Figure 2, we placed the low light treatment furthest away from the light source by placing the replicates directly on a shelf. We measured the light intensity to be 1395 lux.

Finally, for the dark treatment we covered the replicates with a cardboard box (see Figure 2). We placed the dark treatment replicates directly on the shelf alongside the low light treatment. We measured the light intensity under the cardboard box to be 0 lux.

Every day for four consecutive days after the seeds had been planted, we took the treatments out of the incubator at approximately the same time ($\pm$ 4 hours) and took pictures of each of the ten seeds in each of the four replicates for all four treatments. We took the pictures using a dissecting microscope connected with a DinoXcope. Every second day where pictures of the seeds were taken, we watered each replicated by pipetting 300 µL of water onto the filter paper. After one week, on the eighth day of the experiment, we took pictures of each seed one last time as our final measurement.

After all the pictures had been taken, we measured the hypocotyl (stem of germinating seedling) length in every picture we had taken over the span of the experiment using a

program called Image-J. To make accurate measurements using Image-J, the program was first calibrated by drawing a line over a known distance on a picture of a ruler taken at the same magnification as the pictures of seedlings to be measured. After Image-J was calibrated, we measured each *Arabidopsis thaliana* hypocotyl length by tracing the hypocotyl including the root with the line tool as shown in Figure 3. We then calculated the average length of the hypocotyls for all replicates of each treatment group for every measurement day. Furthermore, we calculated the standard deviation and 95% confidence intervals for each treatment for all measurement days.

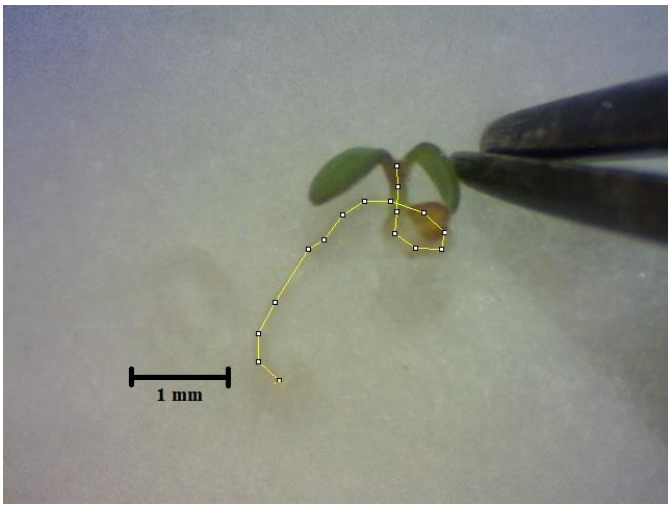


Figure 3. A sample measurement of the hypocotyl length using a yellow segmented line tracing along the entire length of the hypocotyl, from the tip where the cotyledons split, to the end of the root, using Image-J.

Results

As shown in Figure 4, all four treatments started to germinate after 52 hours of the experiment. The mean hypocotyl lengths became significantly different from each other at

approximately 73 hours after the seeds had been planted with the exception that the high and low light treatments were not significantly different from each other. The treatment grown in the dark had the most rapid change in mean hypocotyl length, followed by the treatment grown in optimal light intensity. Treatments grown in low light and high light experienced the least change in mean hypocotyl lengths, and showed no significant difference from each other after one week, 168 hours, of germination. However, changes in mean hypocotyl length in all four treatments slowed down and leveled-off after 98 hours of the experiment.

One major trend was observed from the three treatments grown in the presence of light. As shown in Figure 5, total growth in hypocotyl length after one week was 1.5$\pm$0.28 mm in the low light treatment. As light intensity increased from 1395 lux to 7000 lux, the optimal value, total hypocotyl growth peaked at 2.38$\pm$2.00 mm. As light intensity continued to rise from 7000 lux to 12520 lux, total growth in hypocotyl length declined to 1.41$\pm$0.25 mm. The high and low light treatments had no significant difference in their mean hypocotyl lengths while the optimum treatment was significantly was significantly longer than both.

When comparing the differences between light-grown and dark-grown treatments, Figure 6 showed that dark-grown plants had a significantly greater hypocotyl length after one week at 6.08$\pm$2.00 mm, than light-grown plants at 1.76$\pm$0.61 mm. As shown in Figure 7, the cotyledons in replicates grown in the absence of light appeared to be closed, while the replicates grown in the presence of light had open cotyledons.

Looking at differences in variation across the treatments, we can see from Figure 4 that the dark treatment has the most variation over the light-grown treatments. Of the three light grown treatments the variation is roughly similar as seen in Figure 5.

Qualitatively, we observed that the filter papers from the optimal light and high light treatments, although still damp, appeared to be drier than the ones from the low light and dark treatments.

Sample Calculations

Mean hypocotyl length of dark treatment plants after 168 hours:

$$\bar{x}=\frac{x_1+x_2+x_3+x_4}{4}=\frac{6.9336+7.4606+6.9091+3.0398}{4}=6.08 \text{ mm}$$

Standard deviation of hypocotyl length of dark treatment plants after 168 hours:

$$S=\sqrt{\frac{\sum (x_i-\bar{x})^2}{n-1}}=\sqrt{\frac{(6.9336-6.08)^2+(7.4606-6.08)^2+(6.9091-6.08)^2+(3.0398-6.08)^2}{4-1}}=2.05 \text{ mm}$$

95% Confidence interval (95% CI) of mean hypocotyl length of dark treatment plants after 168 hours:

$$6.0858 \pm 1.96 \times \frac{s}{\sqrt{n}} = 1.96 \times \frac{2.05}{\sqrt{4}} = 6.08 \pm 2.01 \text{mm}$$

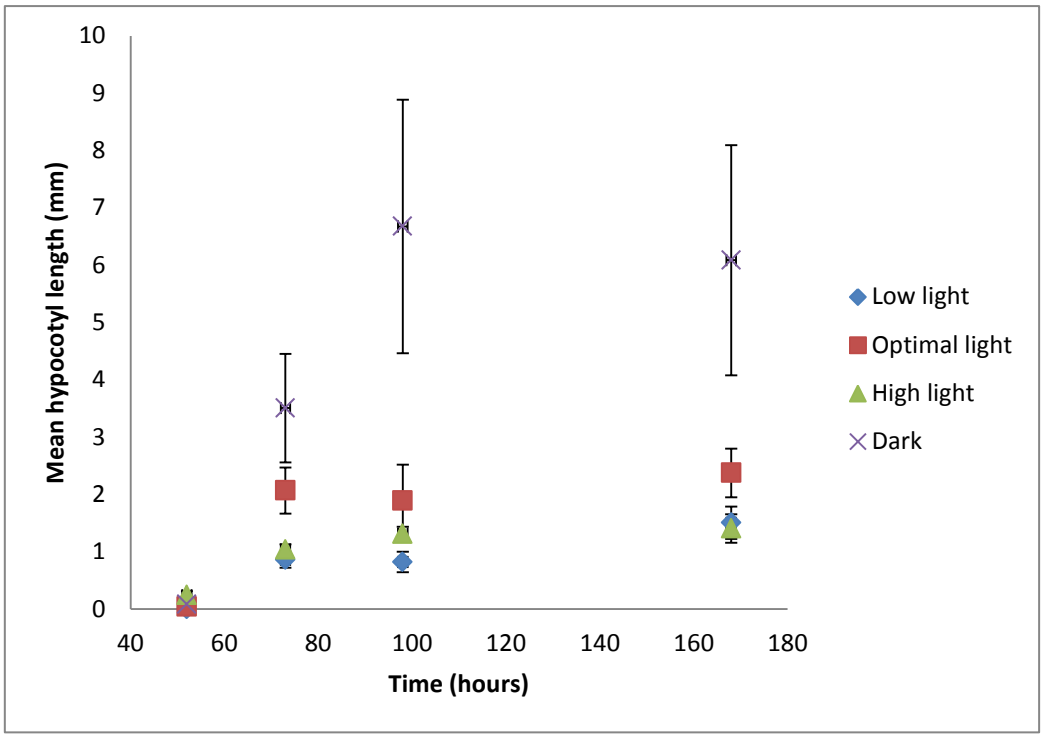


Figure 4. Change in mean (±95% CI) *Arabidopsis thaliana* hypocotyl length of 4 experimental treatments with different light intensities over a course of one week, 168 hours. Low light (n=4) at 1395 lux, optimal light (n=4) at 7000 lux, high light (n=4) at 12520 lux, and dark (n=4) at 0 lux. Germination started after 52 hours of experiment, data prior to this were not included.

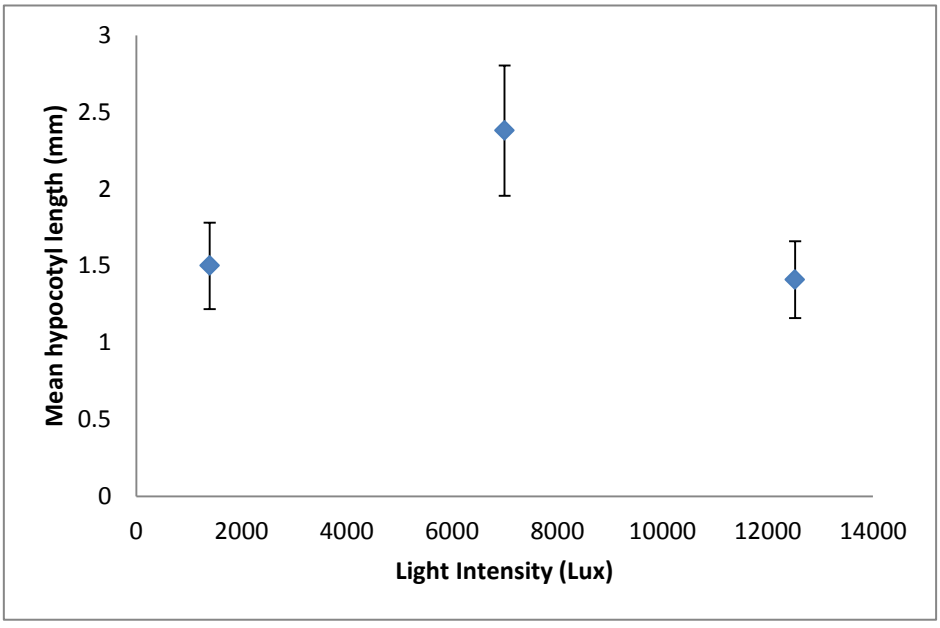


Figure 5. Mean (±95% CI) *Arabidopsis thaliana* hypocotyl length of 3 treatments grown in the presence of light: low light (n=4) at 1395 lux, optimal light (n=4) at 7000 lux, and high light (n=4) at 12520 lux. Data were collected after one week, 168 hours, of experiment.

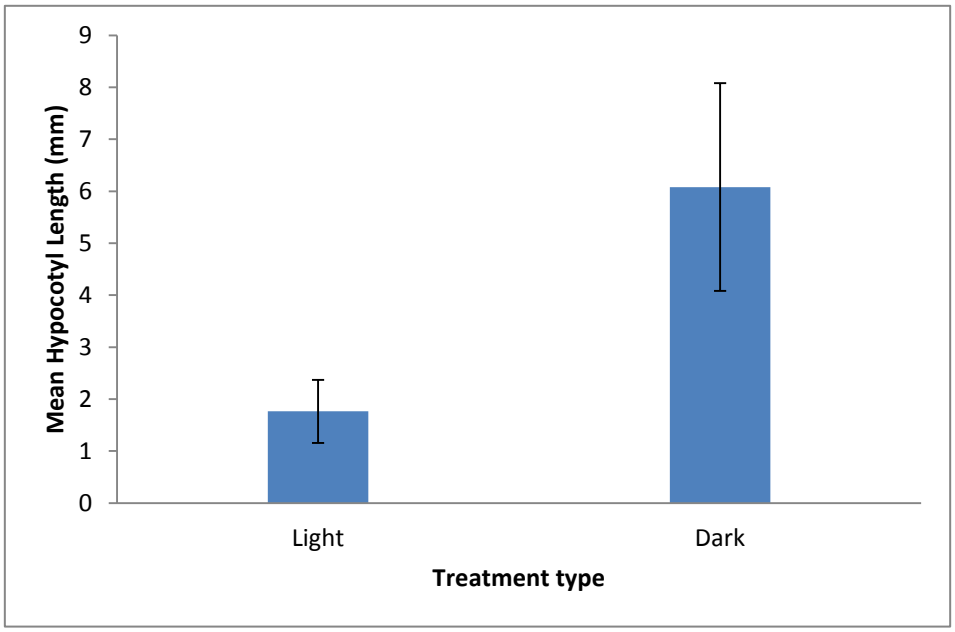
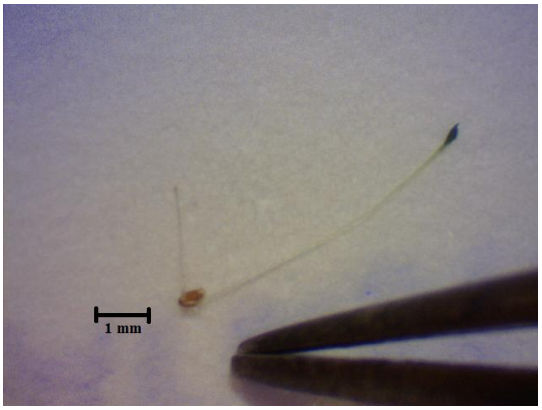


Figure 6. Mean (±95% CI) *Arabidopsis thaliana* hypocotyl length after one week, 168 hours, categorized by 2 treatments, light and dark. Mean hypocotyl length of the light treatment was calculated by taking the average of 3 treatments grown in the presence of light: low light (n=4), optimal light (n=4), and high light (n=4). Mean hypocotyl length of the dark treatment was calculated from treatment (n=4) grown in the absence of light, 0 lux.

(a) 

(b) 

Figure 7 (a-b). Germination of *Arabidopsis thaliana* observed after one week, 168 hours. (a) Plant from the dark treatment, grown in the absence of light. Picture taken at magnification of 10. (b) Plant from the optimal light treatment, grown in the presence of light at 7000 lux. Picture taken at magnification of 15x.

Discussion

From our results, we fail to reject our null hypothesis and so we are unable to provide support for our alternate hypothesis that increased light intensity increases hypocotyl length in

Arabidopsis thaliana. Although the hypocotyl length was significantly longer after one week at the optimum light intensity than at the low light intensity, it was significantly shorter at the high light than the optimum. Additionally, the hypocotyl length was significantly longer in the dark-grown than in the three light-grown treatments.

Looking at Figure 5 of the three light treatments, we see that the hypocotyl length increases with increasing light intensity up to the optimum after which the length decreases with further increases in light intensity. Germination can take place either through photomorphogenesis, which occurs in light, or skotomorphogenesis, which occurs in the dark (Wei *et al*. 1994). These three light treatments all must develop through photomorphogenesis as they all were grown under some amount of light. The optimum light intensity treatment of 7000 lux, which is approximately that found by Rivero-Lepinckas *et al.* (2006), must be around the light intensity range at which hypocotyl lengths grow the longest through photomorphogenesis. This is because the hypocotyl lengths found at the optimum are significantly higher than that found in both the high and low light treatment conditions (see Figure 5). As well, the optimum is roughly halfway in between the high and low light treatments, which have hypocotyl lengths that are not significantly different from each other after seven days. Perhaps if we had more experimental points at different light intensities in Figure 5, a bell-curve trend would become evident. Future experiments could observe hypocotyl lengths at more light intensities to explore

the photomorphogenic trend further and find a more precise light intensity that produces the longest hypocotyl growth.

We see this trend under photomorphogenesis as an increase in light intensity must increase the rate of photosynthesis and therefore increase the growth rate of the plant up to this optimum range. An increase in growth rate leads to a longer hypocotyl. This explains why we see the hypocotyl length increase from the low light to the optimum light treatment in Figure 5. Past the optimum, another factor must be decreasing the growth rate of germinating seeds as we see shorter hypocotyl lengths at light intensities past 7000 lux. Strong visible and ultraviolet radiation can harm the photosynthetic apparatus of plants and reduce the rate of photosynthesis (Powles 1984). This is called photoinhibition. As well, strong radiation can harm cells themselves (Yokawa *et al*. 2013). Therefore, at the high light intensity treatment, the radiation from the light must be causing photoinhibition and harm to cells, leading to shorter hypocotyl lengths than the optimum.

What was most surprising from our results was the relatively long hypocotyls in the no light condition (see Figure 6). Nevertheless, these results can still be explained by the literature. As mentioned above, previous research has determined that cells grown in the dark develop by skotomorphogenesis rather than photomorphogenesis (Wei *et al*. 1994). In skotomorphogenesis, plants expend more energy on rapid elongation of the hypocotyls, and less energy on having bigger, open cotyledons (Josse and Halliday 2008). Thus, they generally

have small, closed cotyledons and longer and thinner hypocotyls than plants developing through photomorphogenesis. Plants developing in the dark eventually need light, so their resources go towards rapid hypocotyl elongation to efficiently find light as soon as possible (Josse and Halliday 2008). From Figure 7, we can see that the *A. thaliana* plants in our study follow this trend as the seed grown in the dark has a longer hypocotyl and closed cotyledons, while the plant from a light treatment has a shorter hypocotyl and larger, open cotyledons.

Wei *et al*. (1994) found that cellular and morphological differentiation pathways for *Arabidopsis* seeds germinating in dark and light conditions were similar for the first two days. After the second day the light and dark conditions began to develop much differently. Our findings match those of Wei *et al.* (1994); from Figure 4 we can see that at 52 hours there is no significant difference between the hypocotyl length of the light and dark treatments. However, when the next measurements were taken at 73 hours, there was a significant difference between the hypocotyl lengths in the light and dark treatments. The fact our timing for the divergence of developmental pathways aligned with the literature helps us to be more confident that the seedlings in the dark are in fact germinating through skotomorphogenesis. Therefore the increased hypocotyl lengths in the dark treatment must be due to *A. thaliana*'s skotomorphogenic developmental pathway.

The experiment was subject to several different errors and variations. The main qualitative error and variation induced on our experiment was the fact that our team had not

taken into account that with higher light intensities, water from the filter papers were subjected to more evaporation. Thus, the seeds in the Petri dishes with higher light intensities did not have the same water supply as the seeds with lower or zero light intensity, which experienced less water loss by evaporation. This error was evident when observing the filter papers upon measurement of the seeds because the filter papers on the light induced treatments were dry whilst the filter papers on the dark treatment were still moist. This variation may have fueled the seeds in the dark to grow more rapidly due to the increased water supply. It would have been a possibility to measure the water differences and account for this difference when watering our seeds. This would have equalized the net amount of water that was available to our *Arabidopsis thaliana* seeds.

The second error, which was more quantitative, was the fact that we encountered difficulties when measuring the *Arabidopsis thaliana* hypocotyls upon the filter paper as they exhibited similar colours. The similarities in colour challenged us to determine where exactly the hypocotyls started and ended on the filter paper. In addition, we were unable to distinguish between the hypocotyl of the germinating seed and the root. So our measurements of hypocotyl length include the root. This leads us to get more variance as the root lengths across the treatments were likely different. Furthermore, the ruler was not photographed with every measurement day under the microscope. The error caused by this mistake would be minimal but nonetheless it would have introduced slight variations in our measurement of the seeds.

Another challenge we faced when measuring the hypocotyl length of *Arabidopsis thaliana* was that some of the stems were growing upwards rather than growing in the plane of the Petri dish. This made it extremely difficult for us to measure the accurate length of the hypocotyls. We had not prepared ourselves to measure three dimensionally. Perhaps stretching the hypocotyls out into a straight line with tweezers may have been the solution, but other implications such as stress to the cells may have emerged from such methods, possibly decreasing growth.

Conclusion

We failed to reject our null hypothesis and so we are unable to provide support for our alternate hypothesis that increased light intensity increases hypocotyl length in *Arabidopsis thaliana*. We did, however, find that *A. thaliana* germinating in the dark had longer hypocotyl lengths after one week than those in light, as they developed through skotomorphogenesis. Of the treatments germinating through photomorphogenesis, in the light, the optimum light intensity had significantly longer hypocotyl lengths than the lower and higher light intensities.

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