The effect of light intensity on the hypocotyl length of *Arabidopsis thaliana*

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Abstract

The amount of light a plant receives during germination can greatly affect its growth and morphology. In this study, seeds of *Arabidopsis thaliana* were used to investigate the effects of light intensity on hypocotyl growth and length. Seeds of both the wild type and the mutant *cer10* of *Arabidopsis thaliana* were grown in petri dishes for a period of eleven days, under three varying light intensities. These were marked no light, low light and high light, averaging at 3 lux, 141 lux, and 6969 lux, respectively. It was found that for both the mutant and the wild type, seeds grown in a no light environment had a significantly longer hypocotyl (embryonic stem) length than those grown at high light intensity. For the wild type, at the end of the 11th day, those grown in the high light treatment had a mean hypocotyl length of 0.7±0.05mm, those grown in the low light treatment had a mean hypocotyl length of 4.0±0.8mm, and those grown in the no light treatment had a mean hypocotyl length of 10.5±1.3mm. For the mutant cer10, the high light treatment had a mean hypocotyl length of 1.0±0.2mm, the low light treatment had a mean hypocotyl length of 4.0±0.8mm, and the no light treatment had a mean hypocotyl length of 11.8±0.6mm. The results agree with current literature on the subject; however we fail to reject our initial null hypothesis for both the mutant and wild type.

Introduction

Arabidopsis thaliana, commonly known as thale cress, is part of the mustard family and is found throughout Asia, Europe, and North America (Meinke *et al.* 1998). According to Meinke *et al.* (1998), *A. thaliana* has a complete life cycle of six weeks from seed germination to the maturation of its first seeds. *A. thaliana* display relatively small features with mature plants reaching only 15 to 20 centimeters in height (Meinke *et al.* 1998). It is naturally capable of self-pollinating, but can also be cross-pollinated for laboratory purposes (Meinke *et al.* 1998). *A. thaliana* is an organism that is tolerant of varying environmental conditions such as shade, temperature and soil moisture (Rivero-Lepinckas *et al*. 2006).

Focusing on the condition of varying light intensities, the objective of this experiment was to determine the response of both the wild type and mutant (*cer10*) towards different light intensities. This investigation will study the responses that the wild-type and mutant strains have towards varying light conditions. These findings can help to further research in this area by providing insight on the ideal light conditions for optimal growth and the negative response towards other light conditions of *A. thaliana*. As *A. thaliana* is a model organism, the responses measured can also be used to predict the effect of light intensity on other plant species. To investigate this effect, three treatments of varying light intensities were used and four replicates were assigned to each treatment. These conditions were applied to both the wild type and the mutant.

Stem (hypocotyl) length is affected by light in that seeds exposed to low light levels will experience an elongation in the hypocotyl, otherwise known as skotomorphogenesis (Gendreau *et al.* 1997). High light levels promote the growth of leaves (cotyledons) and inhibit the growth of the hypocotyl (Gendreau *et al.* 1997)

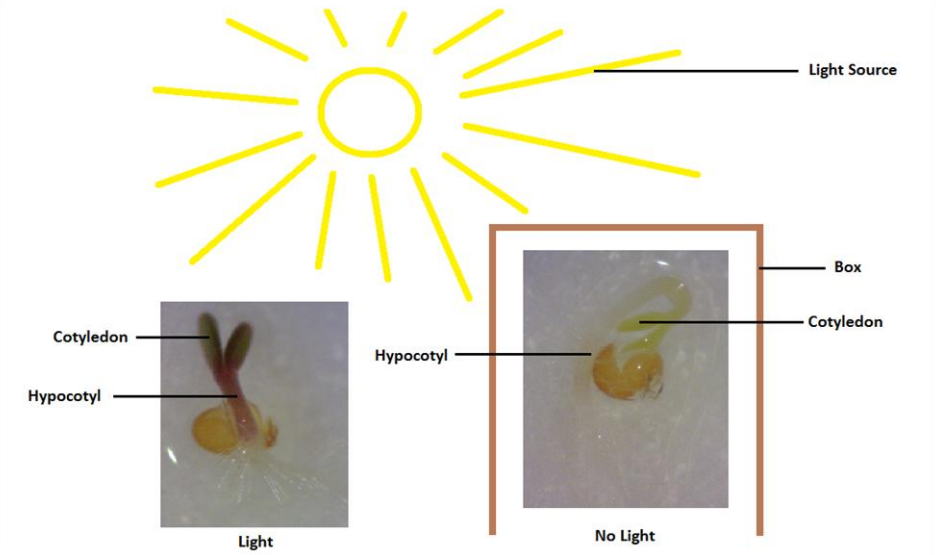

Figure 1. Illustration of hypocotyl elongation in response to light exposure versus no light exposure, total magnification = 10.

As Figure 1 indicates, the specimen exposed to light reacted by concentrating its growth on the cotyledon and inhibiting the growth of the hypocotyl. Conversely, the specimen that was not exposed to any light responded by demonstrating hypocotyl elongation.

The null hypothesis for this investigation is that an increase in light intensity will decrease or have no effect on the hypocotyl length of the mutant and wild-type *Arabidopsis thaliana*. The alternate hypothesis is that an increase in light intensity will cause an increase in hypocotyl length of both the mutant and the wild-type *Arabidopsis thaliana.* According to Rivero-Lepinckas *et al.* (2006), the optimum temperature range for growth is 23°C to 25°C. The higher light intensity would increase temperatures from 17°C and based on this information, we expected both the wild type and mutant hypocotyl lengths to increase. As plant species are autotrophs, this means that they convert inorganic light energy to organic energy – a process known as photosynthesis. Therefore, we assumed that there would be increased hypocotyl growth under high light intensity.

Methods

To test our hypotheses, the hypocotyl lengths of the mutant and wild type *A. thaliana* were measured. We placed a piece of filter paper on a 60 mm diameter, 15 mm deep polystyrene petri dish. We then placed 8 mutant seeds on the filter paper. This was repeated four times for each treatment at the high, low and no light level. We then did the same using the wild-type seeds for a total of four replicates for each treatment. The filter papers were then saturated with tap water. We had one tray for each treatment level, and placed four mutant and four wild type petri dishes on each tray (Figure 2). The trays were then transferred into a growth chamber. One tray was placed under a bright fluorescent

tube lamp with average light intensity of 6969 lux for the high light treatment, another under a dim fluorescent tube lamp with average light intensity of 141 lux for the low light treatment, and the last tray was covered by a cardboard box with average light intensity of 3 lux for no light treatment (Figure 3). The tray under the cardboard box was our treatment control because the mutant and wild-type *A. thaliana* seeds received no light which allowed for comparison to the mutant and wild type seeds that received light.

Figure 2. The layout of the petri dishes on the tray for each treatment.

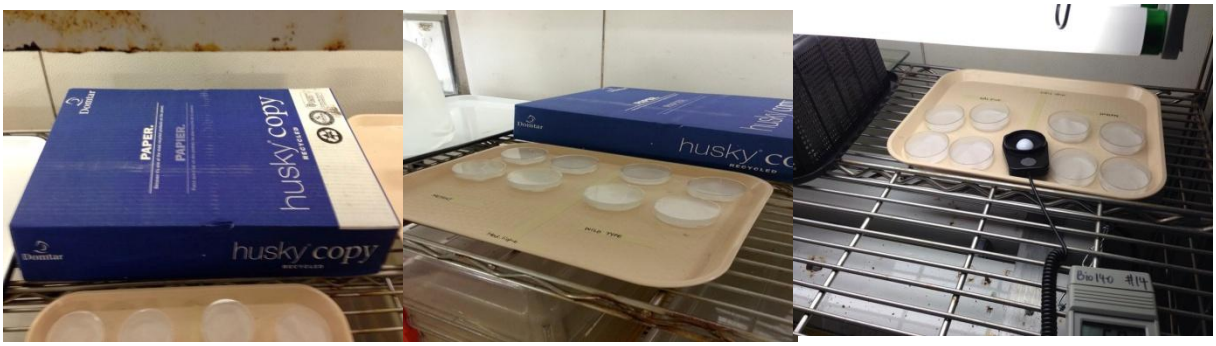

Figure 3. The setup of the trays for no light (left), low light (middle), and high light (right) treatments in the growth chamber.

The experiment was conducted over 11 days. We took pictures of a sprout picked at random from each petri dish, and made observations of the germination every day of the

week except for Day 5 and Day 6. We measured hypocotyl length on Day 4, Day 8 and Day 11. For both the mutant and wild-type *A. thaliana*, one sprout from each replicate was randomly chosen and the hypocotyl length of that sprout was then measured. Therefore, every time we measured the hypocotyl length, we had a total of 4 measurements from the mutant and a total of 4 measurements from the wild type for each treatment. To randomize the selection of sprouts, we measured the hypocotyl length of the first sprout we could find under a microscope from each replicate.

The extraneous abiotic factors that could have had an impact on our results were temperature, and the type of water used. Temperature in the growth chamber was kept constant at 17 degrees Celsius. The light in the growth chamber was on for 14 hours and off for 10 hours each day. We used tap water to water the plants, and kept the water at room temperature so it remained constant throughout. The same bottle of water was also used throughout the experiment. Furthermore, we watered the plants whenever the filter paper looked dried. Additionally, light intensity was measured each time we put the trays back into the growing room to make sure that it did not fluctuate too much for each treatment. If it did, we would move the tray so that the light intensity for each treatment was consistent with previous days.

All pictures of germination were taken using a Dinoscope, and hypocotyl length was measured using a program called ImageJ and recorded in millimetres. The observations were made based on the differences we could see both macroscopically and microscopically. The light intensity from each day was averaged and recorded in lux. For our statistical analysis, the means of the four hypocotyl lengths from each treatment on Day

4, Day 8 and Day 11 were calculated for the mutant and wild type *A. thaliana.* The corresponding 95% confidence intervals were also calculated and graphed.

Results

After observing hypocotyl length of both mutant and wild type *Arabidopsis thaliana* under different light intensity treatments, the hypocotyl lengths were measured to be longest when grown under no light and shortest when grown under high light intensity. This was consistent for both the mutant and wild type, as seen in Figure 4 and Figure 5, respectively.

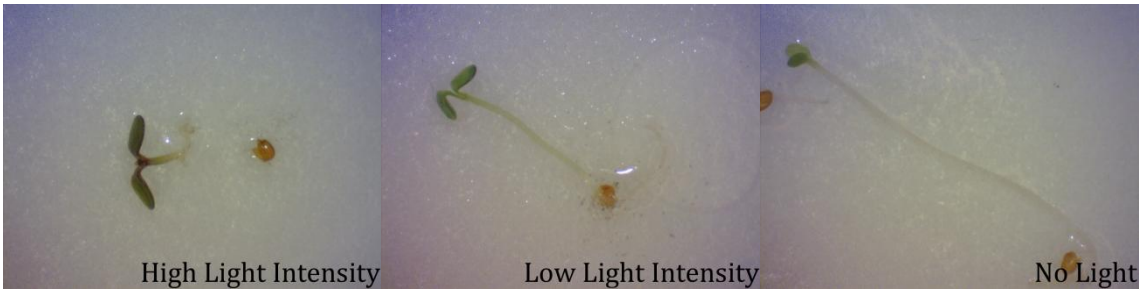

Figure 4. Images of mutant *Arabidopsis thaliana* on day 11 from seeds germinated in high light intensity, low light intensity and no light, total magnification = 7.

Figure 5. Images of wild type *Arabidopsis thaliana* on day 11 from seeds germinated in high light intensity, low light intensity and no light, total magnification = 7.

The hypocotyl lengths of the *cer10* mutant of *Arabidopsis thaliana* on day 1, 4, 8 and 11 can be seen in Figure 6 below.

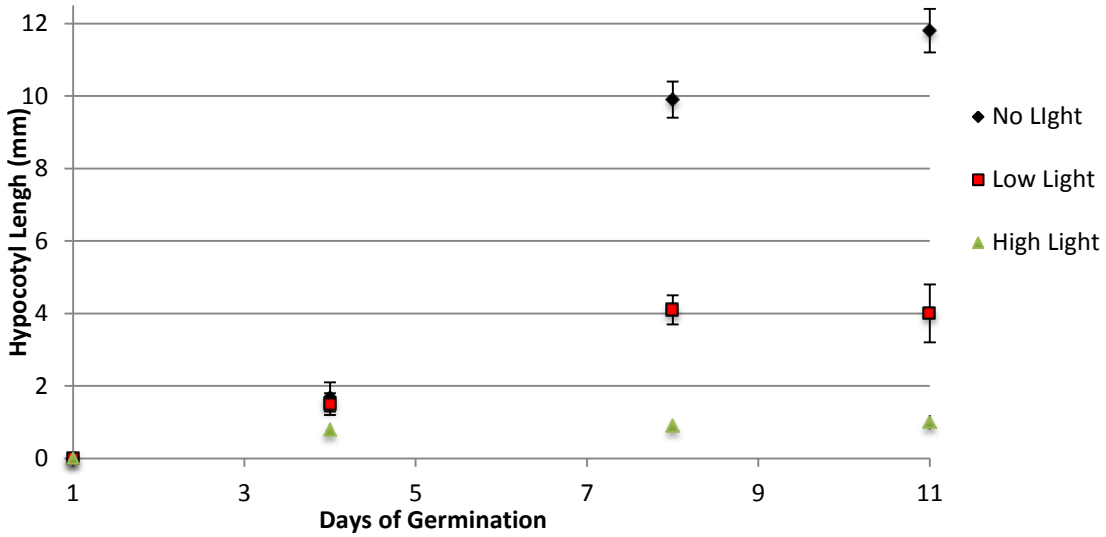

Figure 6. Hypocotyl of *cer10* mutant *Arabidopsis thaliana* over 11 days under no light, low light and high light intensities with 95% confidence intervals, n=4.

The hypocotyl length of the mutant *Arabidopsis thaliana* germinated with no light had a mean hypocotyl length of 11.8±0.6mm on the 11th day of germination. In contrast, the plants that were germinated in high light intensity had a mean hypocotyl length of 1.0±0.2mm on the 11th day of germination. By the end of the 11th day, mutant *A. thaliana* that were germinated in low light intensity had a mean hypocotyl length of 4.0±0.8mm. The hypocotyl lengths of all three light levels were significantly different by day 8 and day 11 because the 95% confidence intervals did not overlap. However, there was no significant difference between the hypocotyl length of the seeds in high light intensity and low light intensity on and before day 4 because the confidence intervals overlapped.

The mean hypocotyl length of wild type *Arabidopsis thaliana* grown in no light, low light intensity and high light intensity on days 1, 4, 8, and 11 are graphed in Figure 7. The mean hypocotyl length for seeds germinated with no light was 10.5±1.3mm on the 11th day, 4.0±0.8mm with low light intensity and 0.7±0.05mm for high light intensity. The hypocotyl

lengths in all three light intensities were significantly different because the 95% confidence intervals did not overlap.

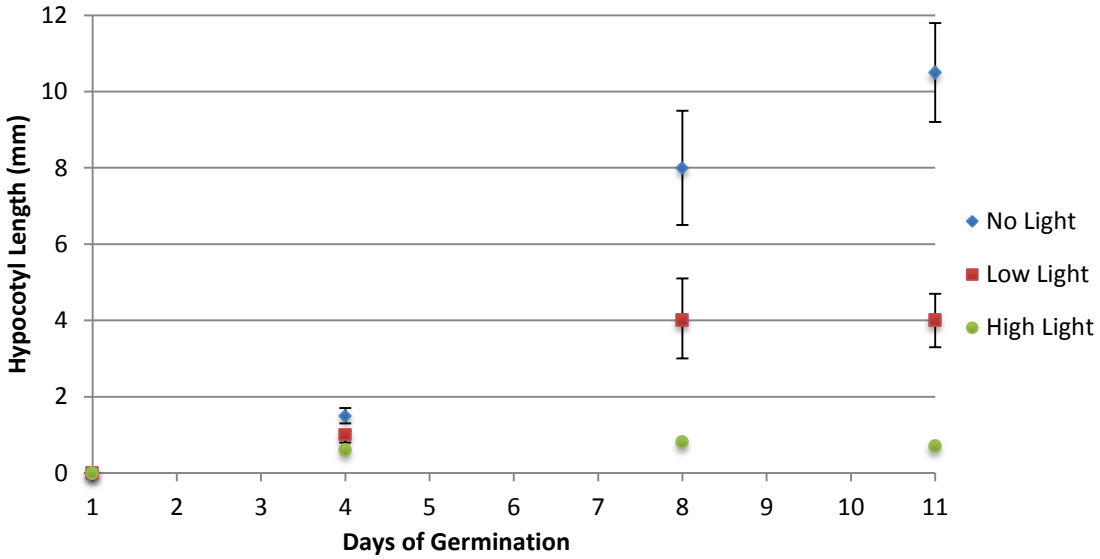

Figure 7. Hypocotyl length of wild-type *Arabidopsis thaliana* over 14 days in no light, low light and high light intensities with 95% confidence intervals, n= 4.

A common trend was observed for both mutant and wild type. The hypocotyl lengths for the seeds across all the light levels grew constantly between day 4 and day 8. The hypocotyl length for the seeds germinated in no light continued to grow constantly until day 11, but those grown in low light and high light intensities seemed to have plateaued starting on day 8. These trends can be observed in Figure 6. and Figure 7.

Sample Calculations

1. Mean Hypocotyl length (Calculated in Excel): $\bar{x} = \frac{\sum_i^n x_i}{n}$

$$\bar{x} = \frac{11.8 + 12.7 + 11.5 + 11.2}{4} = 11.8\, mm$$

2. Standard deviation (Calculated on excel): $sd = \sqrt{\frac{\sum(x-\bar{x})^2}{n-1}}$

$$sd = \sqrt{\frac{(11.8-11.8)^2 + (12.7-11.8)^2 + (11.5-11.8)^2 + (11.2-11.8)^2}{4-1}} = 0.6288$$

3. 95% Confidence Interval (Calculated on excel): 95% $CI = 1.96 \times (\frac{sd}{\sqrt{n}})$

$$95\%\ CI = 1.96\ \times \left(\frac{0.6288}{\sqrt{4}}\right) =\ 0.6$$

Discussion

Based on our results, we failed to reject the null hypothesis that an increase in light intensity will decrease or have no effect on the hypocotyl length of the mutant and wild type specimens of *Arabidopsis thaliana*. This is because the longest hypocotyl length was produced in the no light treatment. The hypocotyl length was longest in seeds germinated in no light surroundings and shortest from seeds germinated in high light intensities. Although the hypocotyl lengths of the mutant seeds germinated in high light and low light intensities were not significantly different on day 4, by the end of the experimental period, the 95% confidence intervals of the hypocotyl length did not overlap for any of the light treatments. The 95% confidence intervals for wild type hypocotyl lengths did not show overlap across any of the light treatment levels from the first hypocotyl measurement until the last experimental day. This means that the hypocotyl lengths are significantly different across all treatment levels for both mutant and wild type *Arabidopsis*. The results thus did not provide support for our alternate hypothesis, which stated that an increase in light intensity will increase the hypocotyl length of the mutant and wild type of *Arabidopsis*. As

the results showed that the hypocotyl lengths were significantly different when grown in varying light intensities, a negative relationship between light intensity and sprout growth during germination is suggested.

Like any other plant, *A. thaliana* requires light to grow. However, the amount of light that a plant receives during germination will affect the way it grows, morphologically (Bentsink and Koornneef 2008). In this experiment, it was noted that the longest hypocotyls, in both wild type and mutant (*cer1*0) of *A. thaliana*, belonged to those under the no light condition. This is because seedlings subject to no light will use more energy to grow their hypocotyl, to make sure that the apex of the plant reaches the surface of the soil before the seed reserves are all used up (Arsovski *et al.* 2012). The hypocotyl is the embryonic stem of a plant, while the apex is the top of the growing plant, or the terminal bud (Arsovski *et al.* 2012). They will also have a shorter root system compared to those grown in higher light condition (Arsovski. *et al.* 2012). On the other hand, seedlings subject to light will have shorter hypocotyls because they are able to capture more light by expanding its cotyledons (Arsovski. *et al.* 2012).

Walters and Horton (1994) did a study on how *A. thaliana* adapt to changes in its light environment - similar to our investigation. They planted seeds of *A. thaliana* and placed them in four different light conditions to show how their development and morphology differed (Walters and Horton 1994). They were able to conclude that *A. thaliana* grown under low light resulted in more emphasis on hypocotyl growth, whereas *A. thaliana* grown under high light showed expanded cotyledons and extensive root growth (Walters and Horton 1994). Their results are consistent with our results, which showed

that both the mutant and wild type *A. thaliana* had hypocotyl lengths that were measured to be the longest under no light and shortest when grown under high light intensity.

In this investigation, the mean hypocotyl lengths were calculated using one measurement from each replicate. It was expected that hypocotyl length would increase with an increase in light intensity. Conversely, hypocotyl length decreased with an increase in light intensity. The no light treatment, averaging at 3 lux, garnered the longest hypocotyls among the three treatments. The high light treatment, averaging at 6969 lux, produced the shortest hypocotyls.

Despite the significant results recorded, experimental error may still have been introduced. For example, the variability in measurements could have been due to discrepancies in measuring judgments. We decided that hypocotyl length would be measured from just under the cotyledon, down to the loss of green color near the root. However, different researchers saw differently where the color ended and where the cotyledon began, which would lead to differences in measuring techniques. Measuring techniques for curved hypocotyls could have introduced another source of variability in the results. The program ImageJ was calibrated and used to measure hypocotyl length. However, for curved hypocotyls, separate lines were drawn to match the curve as closely as possible during measurements. The point at which one drew the first line may not have matched the point at which the next line was drawn. This would lead to increased hypocotyl lengths being measured as the next line was often drawn at a point which had already been measured.

Another possible source of error to the investigation was in the dehydration of the high light treatment on Day 7. This occurred as the following two days during the weekend

were not taken into consideration, thus the treatments were not prepared for the two day drought. The high light treatment dehydrated the quickest as they were subject to the harshest light condition which would promote the evaporation of water. However, Figure 6 and Figure 7 show the plateau of growth after Day 7. This plateau also occurred in the adequately watered, low light treatment which shows that the dehydration may not have had as large an effect as previously thought.

Conclusion

Based on our results, we fail to reject our null hypothesis that seeds grown in high light conditions would have the longest hypocotyl growth, compared to those grown in lower light conditions. Though we fail to reject the null hypothesis, our results are consistent with current literature on the subject.

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