Effect of different light wavelengths on the overall growth of *Arabidopsis thaliana* seedlings.

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Abstract

This project focused on the effect of different wavelengths of light on the overall growth rate of the *Arabidopsis thaliana* plant species during their germinating stage. As for any plant species, light is an important factor in the survival and growth of *A. thaliana*, it must therefore be able to adapt to the varied sources and wavelengths of light available to them in nature. To investigate this, seeds were grown in a controlled laboratory environment under red, green, and blue light, as well as unfiltered lighting (which, in this experiment, was fluorescent lighting with a clear filter), and in darkness, over a span of 11 days. Our alternate hypothesis was that increasing wavelength of light will have a significant positive impact on hypocotyl growth, and the null hypothesis was that increasing wavelengths of light will have no impact or will impede hypocotyl growth. We found that seeds grown in darkness (the positive control treatment) had grown most rapidly. For the coloured treatments, we found that red light had the most growth, followed by the green light, and blue light having the slowest growth rate. The clear plastic treatment was found to have growth similar to the red and green treatments. The results of an ANOVA test done on red, green, and blue light treatments produced a p-value of less than 0.00001, which led us to infer that there is a significant difference in the growth rates of the 3 treatments. This allowed us to reject our null hypothesis and provide support for our alternate hypothesis.

Introduction

Availability of light is one of the most important abiotic factors for growth and development of plants during germination and throughout their life cycle (Fankhauser and Chory, 1997). *Arabidopsis thaliana*, a small flowering plant from the Brassicaceae family, is no exception. The life cycle of *A. thaliana* is approximately six weeks, and is affected by the presence of light at every stage (Folta and Maruhnich, 2007). This plant can grow in a wide range of conditions, however its optimal environmental temperature is 16 to 25^{o}C and its optimal light intensity is 6480 to 8100 Lux (Weigel and Glazebrook, 2002).

The assimilation of light energy in plants is controlled by specialized cells called photoreceptors, the main ones in *A. thaliana* being cryptochromes, which mainly process blue light, and phytochromes, which mainly process red light (Fankhauser and Chory, 1997). It has been determined that *A. thaliana* has five different types of phytochrome receptors and two different types of cryptochrome receptors (Fankhauser and Chory, 1997). As land plants are stationary, they must be able to process different wavelengths of light depending on what light is available to them. For example, plants grown in underbrush may receive more green light that is being reflected off of the plants overhead, and less red and blue light, which are being absorbed by those plants (Folta and Maruhnich, 2007).

Cryptochromes and phytochromes both have effects on the growth of plants during initial seedling growth, following germination. Cryptochromes are known to inhibit hypocotyl (also known as the early plant stem) growth and phytochromes are known to be important for the major developmental transitions, including germination and the onset of flowering (Fankhauser and Chory, 1997; Lin et al., 1995). Furthermore, cryptochromes and phytochromes also are able to process green light, however, less effectively (Folta and Maruhnich, 2007).

It has been shown by Gendreau et al. (1997) that following germination, *A. thaliana* seeds grown in the dark will have a faster hypocotyl growth rate, and closed cotyledons, which are the first leaves. This pattern of growth is conducive to the seedling when grown in soil, as it allows it to reach the surface and find a light source as early as possible (Fankhauser and Chory, 1997). Plants grown in the light will have a slower hypocotyl growth rate, and their cotyledons will begin to open much sooner, as the presence of sufficient light will signal the onset of vegetative growth (Fankhauser and Chory, 1997).

In this study, the effect of wavelength of light on hypocotyl growth after germination in *Arabidopsis thaliana* was investigated. The null hypothesis was that an increase in wavelength will have no effect or will decrease the growth rate of the hypocotyl. The alternate hypothesis was that an increase in wavelength will increase the growth rate of the hypocotyl.

With human influences altering ecosystems around the world, plants need methods of adapting to different conditions. It is important to know how different light conditions, such as changes in wavelength, affect the germination and initial growth of plants. Previous studies have focused on the differences in growth in seedling grown in the dark and in the light (Gendreau et al, 1997). Our study expands on these results, by investigating hypocotyl growth under red light, blue light and green light, in addition to unfiltered light and darkness.

Methods

Our experiment looked at the germination and initial growth of seeds under five different treatments of light: red light (λ=680nm), green light (λ=520nm), blue light (λ=520nm), unfiltered light, and darkness. The controls chosen for this experiment were the clear, or unfiltered treatment, and the black or dark treatment, because we were able to predict how the seedlings grow in these conditions based on the research of Fankhauser and Chory (1997). For each treatment, we prepared 5 replicates containing 4 seeds each, which totalled to 100 seeds. The mean growth of the 4 seeds was calculated for each replicate. Petri dishes were labeled from one to twenty five to identify which replicate they were and each dish had every corner labeled from one to four to keep track of each seed, as seen in Figure 1. Each dish was then prepared by lining the bottom with a single layer of filter paper for a growth medium and watering. A single

seed was then carefully placed on each labeled corner. Using a micropipette, we then evenly dampened the filter paper with 500µl of water and closed the dish.

Figure 1 - (left) Seed placement and numbering of dish and seed. The center number represented replicate number while the corners represented seed number within replicate. (right) Petri dish placement on tray for storage/light exposure.

Upon preparing the Petri dishes, we obtained 5 trays and placed 5 dishes, or replicates, onto each one. Each tray was then covered; one with blue (420nm) acetate paper, one with green (520nm) acetate paper, one with red (680nm) acetate paper, one with clear acetate paper, and one with a black garbage bag. The trays were then taken to a 17^{o}C incubation chamber and placed under lighting, as seen in Figure 2. Light intensities were measured using a Lux meter placed under the coverings. To control for light intensity, the trays were lifted or lowered closer to the light source until the light intensities measured in at a reading of 110 +/- 10 Lux (excluding the darkness treatment which read 3 Lux).

Figure 2 - (left) Experiment setup in 17^{o}C chamber for Green, Red, Blue treatments. (right) Setup for dark and clear treatments.

Each day we measured the light intensities of each tray and took them out of the chamber to measure the samples. They were measured daily from the base of the stem to the base of the leaf. Measuring was done using a dissecting microscope, the Dinoscope attachment, and the ImageJ program. The Dinoscope was used to take a picture of each seedling along with a picture of a ruler in millimeters. The ruler was then calibrated in ImageJ which allowed us to measure each seedling by using the program's trace feature.

When finished with our measurements, we re-watered the Petri dishes with 200µl of water whenever we felt the filter papers were dry. The trays were then taken back downstairs into the 17^{o}C incubation chamber. We once again placed them accordingly on the shelves until a reading of 110 +/- 10 was shown on the Lux meter (excluding the darkness treatment which read 3). This process was repeated every day over a period of 11 days, Mon. to Fri.

Statistical analysis was needed to determine if there were significant differences among the treatments. The mean growth of each replicate was calculated by taking the average of the four seeds in each replicate. The mean growth for each treatment was then determined by taking

an average of the 5 replicates. The growth rate was calculated in mm/day for each time interval, and an overall mean growth rate was obtained. A one-way Analysis of Variance (ANOVA) test was performed on the growth rates all five treatments, and a second one-way ANOVA test was performed on the growth rates of the blue light, green light and red light treatments. 95% confidence intervals were calculated for each mean growth rate for each treatment.

Results

The mean of each replicate was determined by averaging the four seeds within each Petri dish. The averages of the replicates were then used to analyse the data and from the calculated means, determine the overall average for each treatment, standard deviations, and 95% confidence intervals.

The seeds did not show signs of growth until the second day of observation on day 4 (97 hours), when most had shown signs of sprouting and already lengthening of the stem. The red treatment (680nm) showed the most growth in the first four days, but the darkness treatment began to show faster growth on day 5 (116 hours) and continued to do so for the remaining days as seen in Figure 3. The other treatments continued to grow less rapidly and to smaller lengths than the samples in darkness (black; λ=0nm).

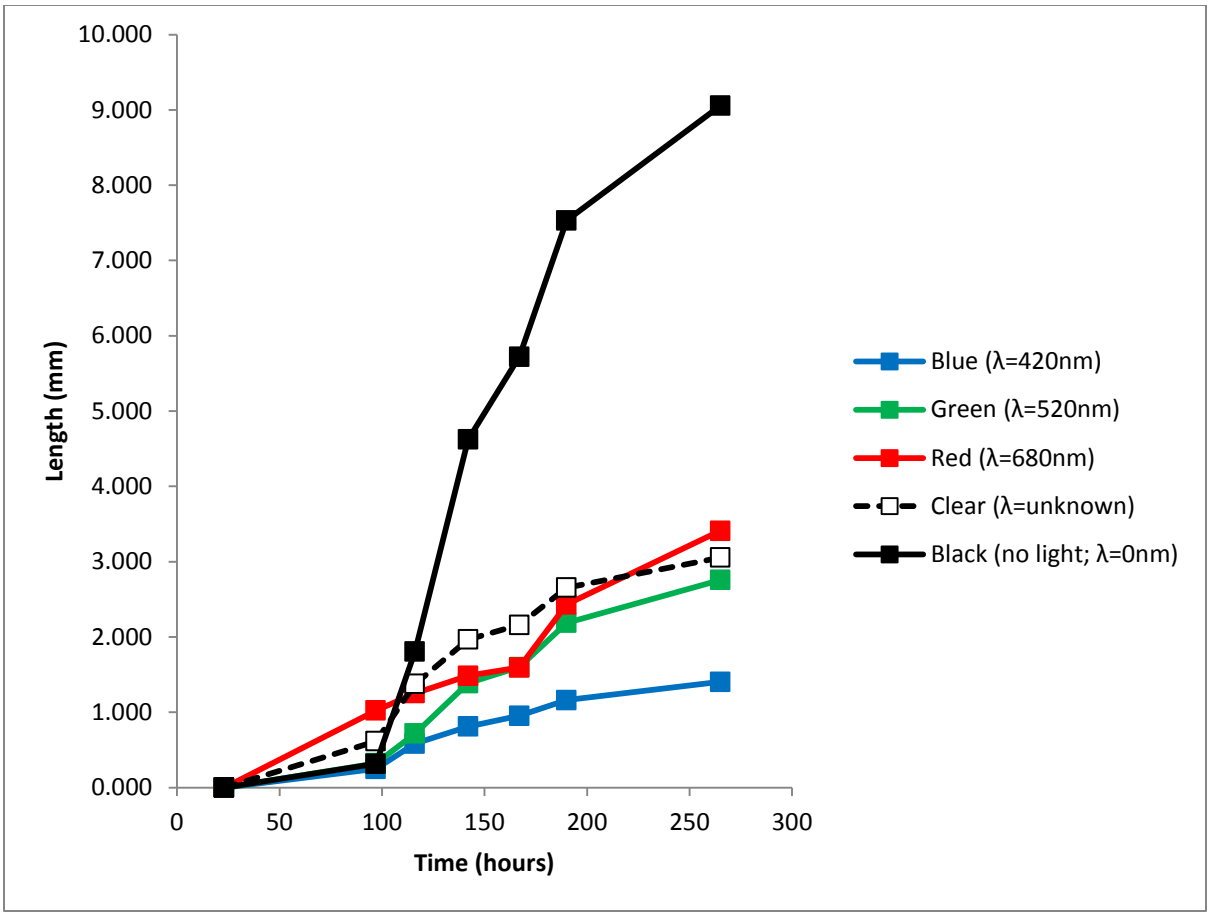

Figure 3 - The average length of stem (mm) during seed germination at 23, 97, 116, 142, 167, 190, and 265 hours under different light wavelengths.

Due to such rapid growth and longer lengths, the darkness control had the highest growth rate (mm/hr) out of all the treatments, including controls, as shown on Figure 4. Out of the tested, known wavelengths, excluding controls, red (680nm) had the highest growth rate. The green (520nm) had the second highest with the slowest growth rate belonging to the blue treatment (420nm). The unfiltered (clear) light treatment had growth rates closer to that of the red or green treatment.

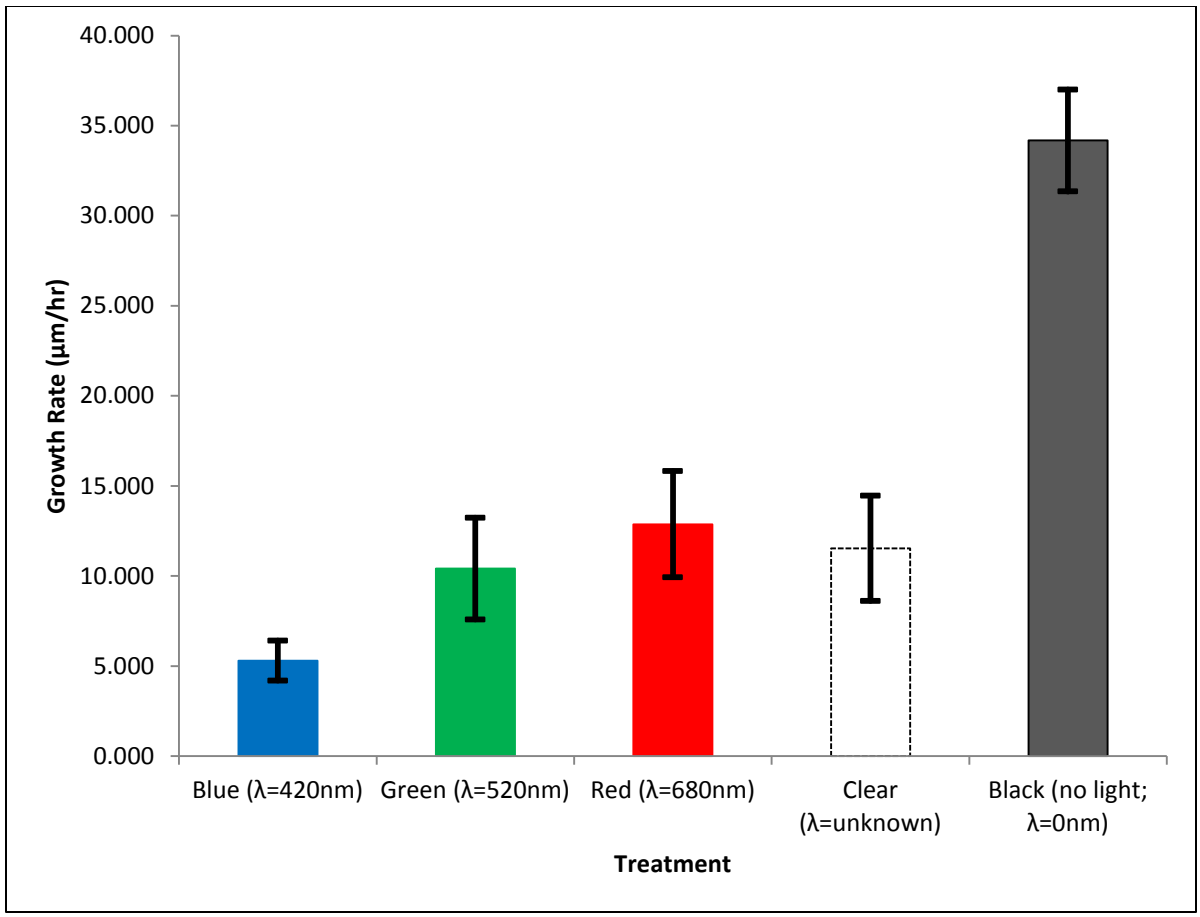

Figure 4 - The average growth rate of each treatment over the course of 11 days. The black bars on each treatment represent the 95% confidence intervals.

The 95% confidence intervals of the means of each treatment are shown in Figure 4. The confidence interval for the blue replicates was 4.19 to 6.41µm/hour, for green replicates, it was 7.58 to 13.2µm/hour, and the interval for red replicates was 9.92 to 15.8µm/hour. For the two controls, the clear treatment had a confidence interval of 8.62 to 14.4µm/hour and for the black treatment; it was 31.3 to 37.0µm/hour. The only treatments with significantly different means of were blue and the black control treatments as neither of their confidence intervals overlap.

Statistical analysis of the data through a one way ANOVA for all treatments revealed that the mean for the growth rate of the blue treatment (420nm) was 5.03µm/hour, 10.4µm/hour for the green treatment (520nm), 12.9µm/hour for the red (680nm), 11.5µm/hour for the clear

control, and 34.2μm/hour for the darkness control. The standard deviation for the blue treatment was 1.27μm/hour, for the green treatment it was 1.73μm/hour, 0.902μm/hour for the red treatment, for the clear treatment it was 0.882μm/hour, and finally for the black treatment, the standard deviation was 4.68μm/hour. The ANOVA reported that the calculated F value was 110.9967 and the F critical value was 2.87 giving a p-value of less than 0.00001. A second one-way ANOVA was performed on only three of the treatments: red, green, and blue. The results of the ANOVA excluding the unfiltered and dark controls were very similar to the ANOVA of all five treatments. The second ANOVA test had a critical F value of 6.94 and a calculated F value of 41.3557 resulting in a p-value for the second test to be less than 0.00001.

Discussion

A one-way ANOVA test done on all five treatments resulted in a p-value of less than 0.0001, which showed that there is a significant difference in the growth rates of at least one of the treatments. We can see from the 95% confidence interval bars in Figure 4 that the dark treatment is significantly different from the other four treatments, as the confidence intervals do not overlap with any of the others and was the mean of the dark treatment was much higher than the other growth rate averages. This was expected, as results from a study done by Gendreau et al (1997) determined that plants that are allowed to germinate and start their growth in the dark will have faster growth rates than plants grown in the light.

A one-way ANOVA test done on the treatments at three different wavelengths (red light at 680nm, green light at 520nm, and blue light at 420nm) resulted in a p-value of less than 0.00001, which showed that there is a significant difference in the growth rates of at least one of the treatments. The 95% confidence interval bars in Figure 4 showed that the blue light treatment

is significantly different from the other two treatments, as the confidence intervals do not overlap with any of the others.

As our p-values for both ANOVA tests were less than our α of 0.05, we were able to reject our null hypothesis, which stated that an increase in wavelength will have no effect or will decrease the growth rate of the hypocotyl in *Arabidopsis thaliana.* Thus, we were able to provide support for our alternate hypothesis, which stated that an increase in wavelength will increase the growth rate of the hypocotyl in *Arabidopsis thaliana*. The mean growth rates for red light, green light and blue light were 12.9μm/hour, 10.4μm/hour, and 5.03μm/hour, respectively. This provided further support for our alternate hypothesis as the seedlings grown under red light, which at 680nm was the longest wavelength that was used, had the fastest mean growth rate, and seedlings grown under blue light, which at 420nm was the shortest wavelength that was used, had the slowest mean growth rate. The seedlings grown under green light, which at 520nm was the intermediate wavelength, had a mean growth rate in between the other two treatments. Therefore, we determined that plants responded differently to light of different wavelengths, and grew faster under longer wavelengths.

These results are consistent with the literature, which suggests that seedlings grown under darkness will have the fastest hypocotyl growth rate and plants grown in the light will exhibit stunted hypocotyl growth rates. Further, Fankhauser and Chory (1997) suggests that cryptochromes, the blue light receptors, inhibit hypocotyl growth. This was observed in our study by the slowest hypocotyl growth rate of the plants grown under blue light. In addition, Fankhauser and Chory (1997) determined that phytochromes, the red light receptors, are needed for the induction of growth and for development, which was observed in our study by the fastest hypocotyl growth rate of the plants grown under red light. One of the developmental stages that

took place in the early stages of growth is de-etoliation, which includes an increase in hypocotyl growth rate, corresponding to the fast growth rate observed in the seedlings grown under red light. Green light is processed by phytochromes and cryptochromes (Folta and Maruhnich, 2007), and therefore it is intuitive that plants grown under these wavelengths would have a growth rate intermediate to the other 2 treatments. Folta et al (2007) also suggest that green light reverses the effects that blue light have on the growth, such as hypocotyl growth inhibition. Therefore, the growth rate of seedlings grown under green light should be faster than that of seedlings under blue light, as was observed in our experiment.

Initial growth rates were the highest for unfiltered light and red light treatments, and lowest for the blue light, green light and dark treatments, as seen in Figure 3. This initial surge in growth for red and unfiltered lights could be due to the fact that red light is processed by phytochromes which are responsible for initial growth of the hypocotyl. Since unfiltered light includes all wavelengths of visible light, this response would be present in the unfiltered light and red light grown plants.

For the seeds grown in the dark, the initial growth rate is slow. However, at 100 hours, we see an extreme spike in growth rate that continues throughout the 11 days of observation. The germination of these plants grown in the dark has no light energy to power the process and therefore it is slower initially. However, as soon as the seed has germinated, the plant needs to find light in order to begin photosynthesis, which is why the hypocotyl growth rates spikes. In a plant grown in the dark, it would normally be under the soil, so this spike in growth rate allows it to reach the top of the soil, where it will encounter light, in the most efficient way (Gendreau et al, 2007).

A significant difference in the way the plants grown under dark conditions compared to the other four treatments was observed. Plants grown in the dark not only had a faster growth rate, but also had their cotyledons closed throughout the 11 days of growth that we observed. However, the plants growth under unfiltered light, red light, green light and blue light all had open cotyledons from the moment they sprouted. This observation is consistent with Fankhauser and Chory (1997), who described how the plants that germinate and begin growing in the dark will have more hypocotyl elongation, but closed cotyledons, whereas plants that germinate and begin growing in the light will have less hypocotyl elongation, and open cotyledons. We also observed that the plants grown in the dark were transparent, whereas plants grown under other conditions were green. This is due to the fact that as soon as plants get access to light, their light receptors signal physiological changes, such as the formation of chloroplasts and the opening of the cotyledons, to provide more favourable conditions to begin photosynthesis. The seedlings grown in the dark therefore had not yet begun to form chloroplasts.

While we minimized sources of error as much as possible, there were several factors, apart from the treatments, that possibly influenced the results. The most prominent source of error was likely measuring the hypocotyl length, as the seedlings were very small and even slight deviations in measurement had the capacity to affect the results. In addition, the positioning of the seedlings as they grew was not uniform, as some grew vertically which made it difficult to get accurate measurements under the microscope. A related problem was the fact that measuring the plants using ImageJ required a steady hand, and often one measurement would be quite different from the next. Another source of error was variation in time of germination. In our green light treatment, we had some seeds that did not germinate, or were very slow to do so. In total, there were three seeds where germination was not observed until the 11^{th} day and two seeds

that did not germinate at all. In addition, one seed in the blue light treatment did not germinate at all. The non-germination and late germination of seeds was likely due to it not being viable, or due to natural variation. This also hinted at the overall biological variation of the seedlings, which we tried to control for by using four seeds per replicate, and five true replicates. Lastly, it is possible that some Petri dishes were opened during measurement and exposed to mould and fungi, which could have impeded its growth.

Conclusion

In this experiment, we rejected our null hypothesis and were able to provide support for our alternate hypothesis was supported by the data. The growth rate of seed germination of *Arabidopsis thaliana* was found to be dependent on the wavelengths of light they are exposed to, with longer wavelengths promoting a faster growth rate. Blue light, with the shortest wavelength, was shown to slow hypocotyl growth. Conversely, the red light was shown to increase growth rate of the hypocotyl, due to its involvement in the promotion of developmental stages. The control plants in the dark treatment seedlings' growth rate was the fastest, as expected.

Acknowledgements

We would like to thank the University of British Columbia for funding, providing equipment and laboratory space, and for giving us the opportunity to conduct this experiment and to take the course, Biology 342. We would also like to express our gratitude to our professor, Dr. Carol Pollock, our lab technician, Mindy Chow, our teaching assistant, Katelyn Tovey, and our peer tutor, Kathleen Cruz for providing us with continued guidance, feedback and encouragement.

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