The effect of different wavelengths on the germination time of *Arabidopsis thaliana* wild type and mutant type seeds

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Abstract

The main objective of this experiment was to investigate how different light wavelengths affect the seed germination of *Arabidopsis thaliana* mutant, cer10, and wild types. In this experiment, we covered trays of petri dishes that had wild-type and mutant seeds with different coloured acetate sheets: red, blue, green, and clear as control. The seeds were grown in a 17 degrees Celsius incubator, and we observed and recorded the growth of *Arabidopsis thaliana* using light microscopes for approximately one week. After 72 hours, it was observed that the mean number of germinated seeds was the highest (5.75 ± 2.387 for mutant and 5.25 ± 0.796 for wild type) for natural light compared to other colours even though the differences were not statistically significant. After 96 hours, red light had a significantly larger number of germinated seeds (9.5 ± 0.919) compared with other wavelengths of light for both mutant and wild types; red light correlated to faster seed germination. The difference was statistically significant between red and blue light, even though the trend was still present in the comparison of the red light with the other two wavelengths. We reject our null hypothesis; red wavelengths of light will slow or have no effect on the germination of *Arabidopsis thaliana*. Additionally, we tested another set of hypotheses regarding the difference in the number of germinated mutant cer10 and wild type seeds. According to the results, we fail to reject our null hypothesis; mutant type seeds germinate faster.

Introduction

Arabidopsis thaliana, also known as wall cress, is a commonly used species in plant biology. It is a member of the *Brassicaceae* family, but is not agriculturally important. Instead, *Arabidopsis* is important in biological and genetic research (National Institute of Health 2013). It has a small and fully sequenced genome, a life cycle of approximately six weeks from the time of seed germination, and a fast seed germination time of three to five days (National Institute of Health 2013). The objective of our research was to determine whether different wavelengths of light affect *Arabidopsis thaliana's* wild-type and mutant seed germination time.

Previous research showed a relationship between different wavelengths of light and *Arabidopsis thaliana's* wild-type and mutant-type percentage of germinated seeds. White light and red light are found to germinate all seeds, while variations such as far-red light decrease the percentage of mutant seeds germinated (Shinomura *et al*. 1994). The optimal wavelength of red light is 660 nanometers, and it corresponds to 100% of seeds germinating (Shropshire *et al*. 1961). Additionally, shorter wavelengths of light have similar effects on both mutant and wild-type seeds and do not result in 100% germination (Shinomura *et al*. 1998). These lower ranges correspond to violet and blue light (National Aeronautics and Space Administration 2011). Wavelengths of light varying between 400 and 690 nanometers result in 100% of mutant and wild-type seeds germinating, and wavelengths larger than 700 nanometers do not result in seed germination (Shinomura *et al.* 1998). These ranges correspond to green and red light (National Aeronautics and Space Administration 2011). Based on this information, there is a clear indication that light wavelengths longer than 400 nanometers and shorter than 700 nanometers are necessary for germinating 100% of *Arabidopsis thaliana's* seeds. Since longer wavelengths should result in larger percentages of germinated seeds, the order of light that will germinate the most seeds should be red, yellow, green and lastly blue.

Therefore our null hypothesis is that red wavelengths of light will slow or have no effect on the germination of *Arabidopsis thaliana* mutant cer10 strain. Our alternate hypothesis is that red wavelengths of light will lead to the fastest germination of *Arabidopsis thaliana* mutant cer10 strain. Furthermore, we

compared the seed germination of mutant-type seeds and wild-type seeds under the same wavelength. Our alternative hypothesis is that there is a decrease in the speed of *Arabidopsis thaliana* mutant type seed germination compared to the wild type. Our null hypothesis is *Arabidopsis thaliana* mutant type increases or has no effect on the speed of seed germination.

The importance of this investigation is that it has the ability to help researchers grow *Arabidopsis thaliana* under its optimal conditions and also aid researchers in determining the colour of light seeds should be germinating under. Previous research mainly investigated red light and white light, although some cover the entire light spectrum. In our biology lab, there are four different colours of light filters available: red, green, blue, and yellow. Our experiment compared these light colours and provided data on the germination of wild type *Arabidopsis thaliana* seeds, and the mutant *cer*10 seeds. *Arabidopsis thaliana's* seed germination is regulated by its photoreceptors which are pigments used by plants to detect different light (Liscum and Hangarter 1993). *Arabidopsis thaliana* has two types; they are phytochrome A, which absorbs red light strongly and allows early seed germination, and phytochrome B, which absorbs far-red light and is responsible for stem development (Reed *et al.* 1994). Different phytochromes will be activated by different wavelengths of light. Under wavelengths of 700 nanometers both phytochrome A and B will be active (Phytochrome 2013). However, phytochrome A will function optimally around 600 to 690 nanometers and at wavelengths larger than 700 nanometers it becomes inactivated, while phytochrome B remains active (Pytochrome 2013). Since, phytochrome A is necessary for the early steps involved

in seed germination, wavelengths around 660 nanometers will allow it to function best and promote the early germination of *Arabidopsis* seeds (Pytochrome 2013).

Methods

For this experiment, we used 32 petri dishes, 16 for wild-type seeds and 16 for mutants. We had a total of four replicate petri dishes for the four colours of acetate paper that were tested. We started the experiment by placing one filter paper into each petri dish. We then used a micropipette to pour one mL of tap water onto the centre of each filter paper, ensuring the entire filter paper was moistened and the tap water was evenly distributed. Using tweezers and paintbrushes, we loaded 10 wild-type seeds (per petri dish) onto the 16 petri dishes and 10 mutant seeds onto the other 16 petri dishes. Regarding the distribution of the seeds, we divided the area of the petri dish into two halves with an imaginary line and evenly placed five seeds on both sides of the petri dish. We prepared four trays, and each tray had four petri dishes with wild-type seeds and another four with mutant seeds. We labeled each petri dish with “WT” for wild type and “M” for mutant, and we also labeled them to keep track of their growth with numbers from one to four for each replicate. We divided the tray into halves with masking tape, placing the four wild type petri dishes together in one side and mutants in the other. On each tray, we placed acetate paper of different colors: clear, red, green, and blue. We then stored the trays in the 17 degrees Celsius incubator. To make the wavelength of light the only factor differing among the trays, we used a light meter and altered the height of

each tray with yogurt cases to make the light intensities reaching each petri dish equal.

For one week, we observed and recorded the growth of *Arabidopsis thaliana* under the light microscope on a daily basis. We made observations at a constant time of around one p.m. We added tap water whenever we thought the filter papers were getting too dry. In addition to the one mL of tap water we added in the beginning, we added 300 microlitres of water after 24 hours, none after 48 hours, 300 microlitres after 72 hours, and 100 microlitres after 144 hours.

We categorized the growth of *Arabidopsis thaliana* into five stages: not germinated, initial, intermediate, germinated, and post-germination. "Not germinated" indicated that the seeds had no projections. If the projection from the seed was longer than one quarter of the seed length, we counted them as "Initial." If the projection started to form sharp spikes around, we counted them as "intermediate." If the projection developed green leaves at the end, we counted them as "G" for germinated. Lastly, if the leaves clearly separated into two, we labeled them as "P", which denoted post-germinated seeds.

For calculations we used the number of seeds that had been germinated and post-germinated (G + P). We calculated 95% confidence intervals, performed F- and t-tests in our data analysis to see if the number of germinated seeds that had grown in different wavelengths of light differed significantly from one another.

Figure 1. The experimental set up of the experiment. Trays containing both mutant and wild type petri dishes covered with different colored acetate paper.

Results:

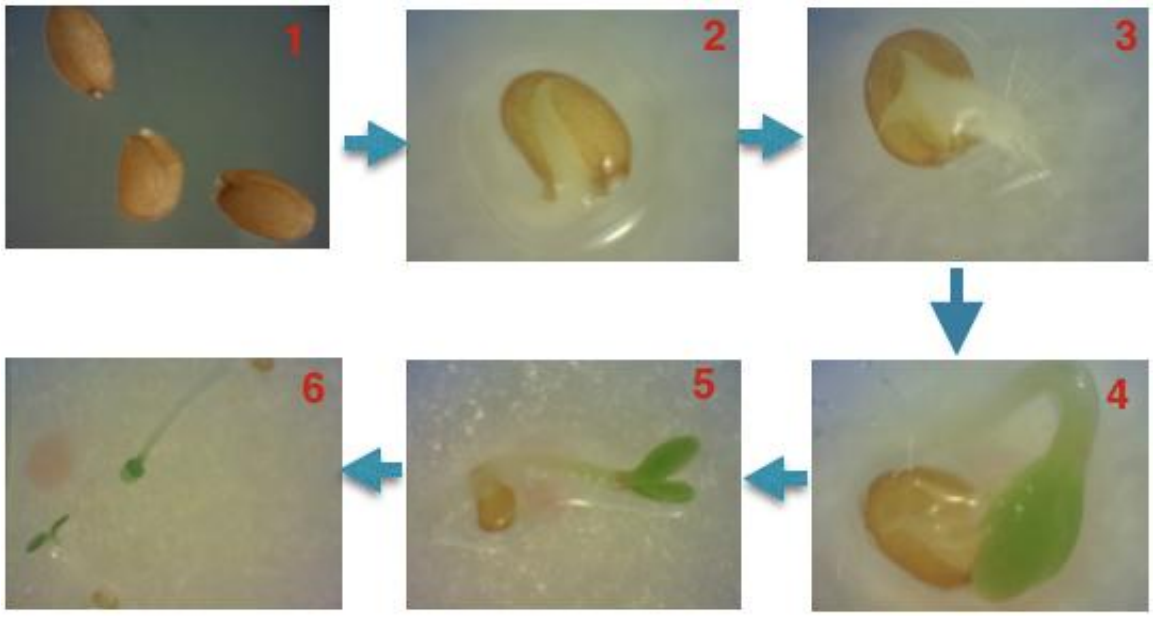

Figure 2. The germination process of *Arabidopsis thaliana* seeds.

As shown in the Figure 2, the seed went from initial germinated seed (2) to intermediate germinated seed (3) to germinated seed (4) to post-germinated seed (5,6). We used seeds shown in Figure 2 (4) as the starting point of germination.

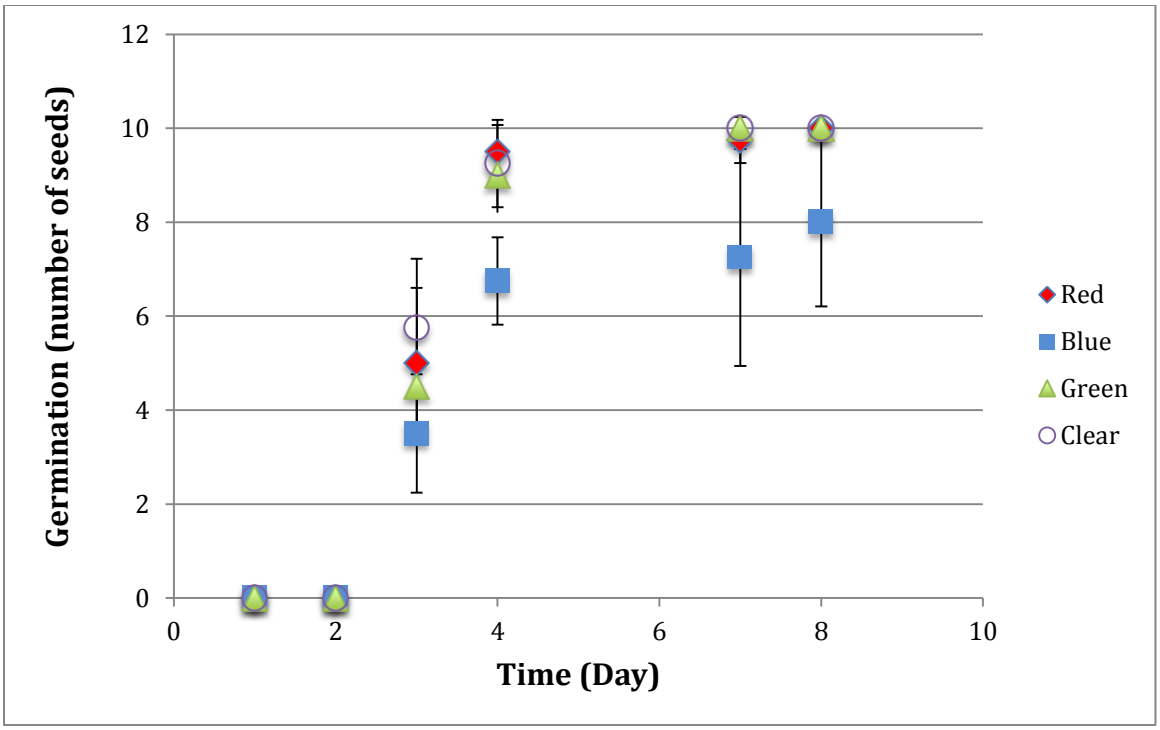

Figure 3. The germination of mutant type *Arabidopsis thaliana* with 95% confidence interval under different light wavelengths (n=4).

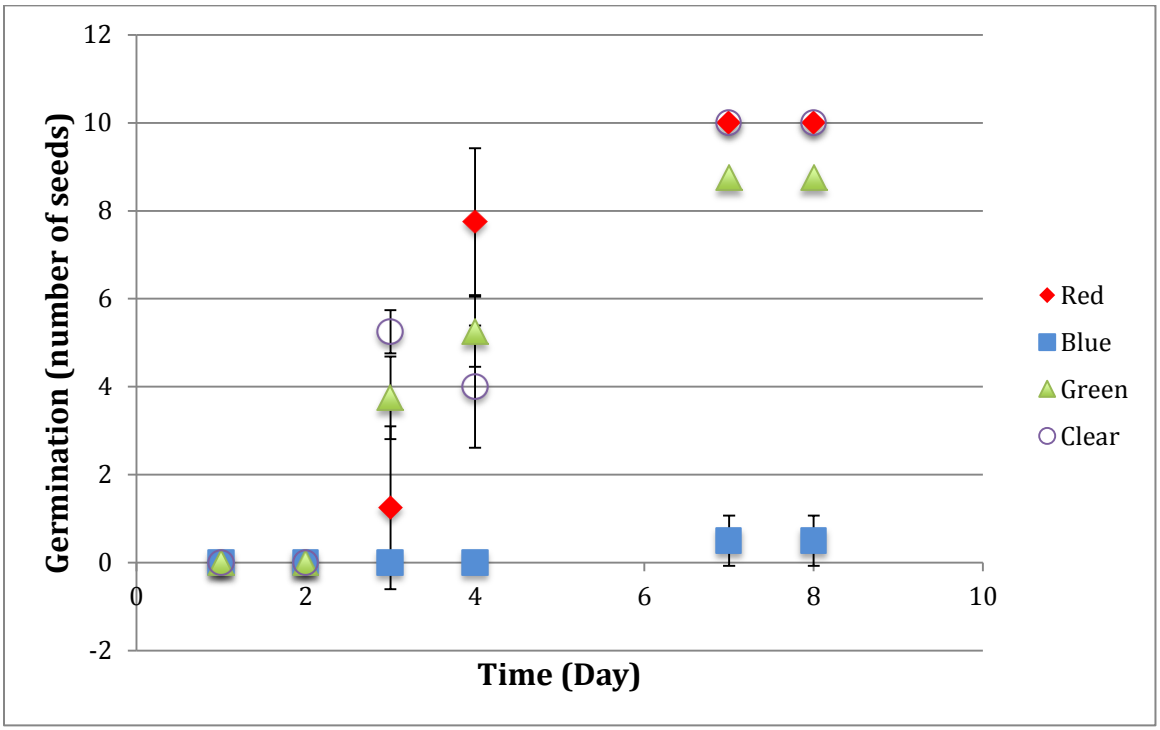

Figure 4. The germination of wild type *Arabidopsis thaliana* with 95% confidence interval under different light wavelengths (n=4).

Figure 3 and Figure 4 have shown that none of the seeds germinated in the first two days for both mutant and wild type. After 72 hours, the clear acetate filter had the most germinated seeds compared with other wavelengths for both mutant and wild type. After 96 hours, red light had the highest number of germinated seeds for both mutant and wild type, i.e. seeds took a shorter amount of time to germinate under red light compared to other wavelengths. There is a significant difference between red light and blue light for both wild type and mutant type. However, the difference is not significantly different between red and green, or red and white light.

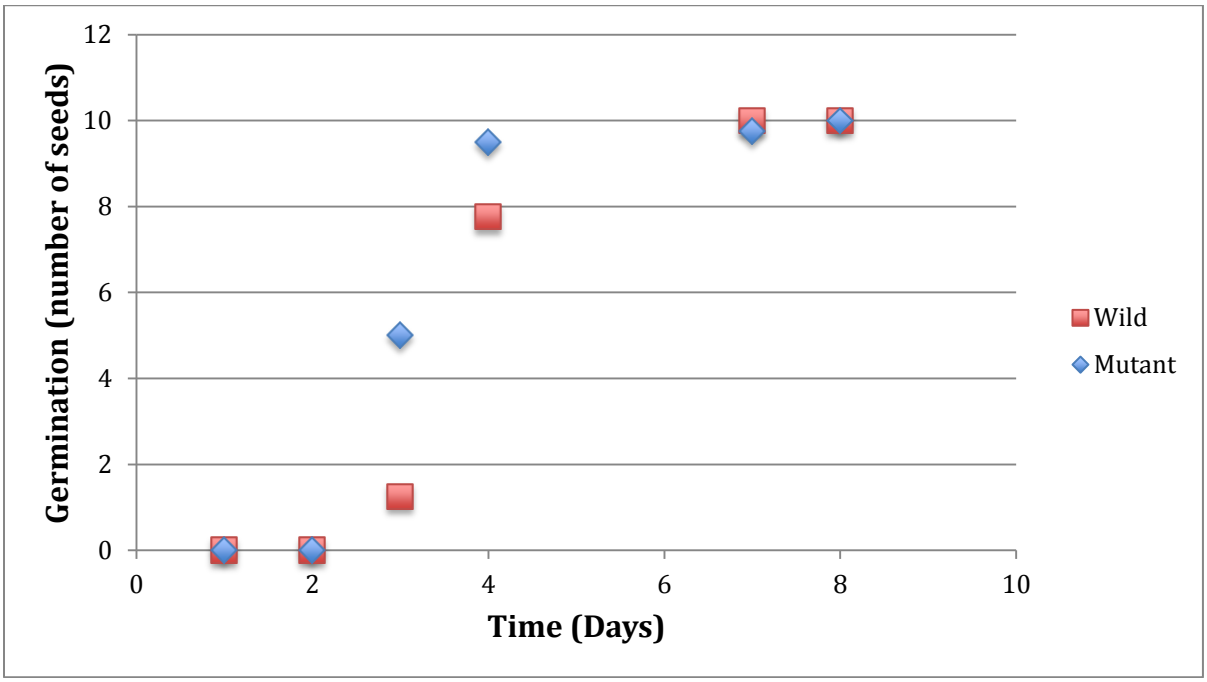

Figure 5. The germination of *Arabidopsis thaliana* seeds of mutant and wild type under the red light (n=4).

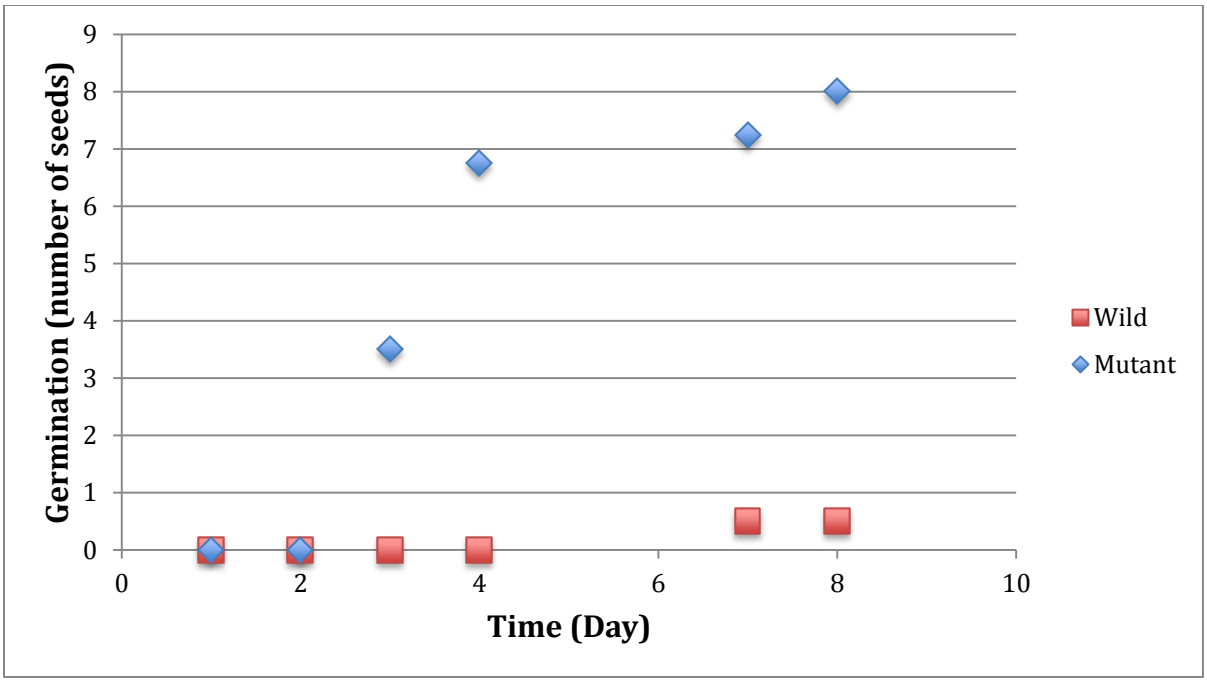

Figure 6. The germination of *Arabidopsis thaliana* seeds of mutant and wild type under the blue light (n=4).

Figures 5 and 6 compare the germination of *Arabidopsis thaliana* seeds under the same wavelength of light for wild type and mutant. By conducting F-test and t-test, we found out that after 78 hours, the number of germinated seeds in mutant and wild type was significantly different for red and blue light. After 96 hours, the number of germinated seeds in mutant type and wild type was significantly different at all wavelengths. In both cases, mutant seeds germinated faster than wild-type seeds. Table 1 below shows the t-values of number of germinated seeds under different light wavelengths.

78 hours after				
	Red	Blue	Green	Clear
F-value	0.81	N/A	0.94	0.104
t-value	0.012	8.00E-04	0.16	0.28
96 hours after				

	Red	Blue	Green	Clear
F-value	0.11	N/A	0.2	0.54
t-value	0.05	4.00E-06	0.005	0.0004

Table 1. The t-values and F-values of germinated seeds under different light wavelengths.

Sample Calculation:
72 hours after:

$$average = \frac{5+7+5+3}{4} = 5$$

$$C.I. = 1.96 * \frac{\sqrt{\frac{(5-5)^2+(7-5)^2+(5-5)^2+(3-5)^2}{3}}}{2} = 1.60$$

$$F_{calc} = \frac{s_1^2}{s_2^2} = \frac{1.63}{1.89} = 0.86$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s\sqrt{\frac{1}{n_1}+\frac{1}{n_2}}} \quad \text{where } s^2 = \frac{\sum(x-\bar{x}_1)^2 + \sum(x-\bar{x}_2)^2}{n_1+n_2-2}$$

For the mutant:

$$\sum (x-\bar{x}_1)^2 = (5-5)^2 + \ldots + (3-5)^2 = 8$$

$$\sum (x-\bar{x}_2)^2 = (0-1.25)^2 + \ldots + (1-1.25)^2 = 10.75$$

$$s^2 = \frac{8+10.75}{6} = 3.125$$

$$t = \frac{5-1.25}{1.77 * \sqrt{\frac{1}{2}}} = 3$$

The t-test value at 3 degrees of freedom corresponds to a probability of 0.012, and since it's smaller than 0.05, we can say that the number of germinated seeds is significantly different between wild type and mutant.

Discussion

The data show a significant difference between red and blue wavelengths of light, but not between the other wavelengths. Therefore, we reject our null

hypothesis and provide support for the alternative hypothesis. This means that red wavelengths of light do result in faster germination times of *Arabidopsis thaliana* seeds.

Our experimental data show a large difference between the amount of time it takes wild-type *Arabidopsis thaliana* seeds to germinate under red and blue wavelengths of light. Previous research indicated that seeds do not optimally germinate at wavelengths of light near the shorter wavelength portion of the light spectrum. Wavelengths near 400 nanometers are not sufficient for germinating wild type *Arabidopsis thaliana* seeds (Shinomura *et al*. 1998). Since blue light is 475 nanometers and violet light is 400 nanometers, the wavelength of the blue acetate sheet is between 400 and 475 nanometers (National Aeronautics and Space Administration 2011). This is lower than the optimal wavelengths needed to germinate wild type seeds. However, the red light has a wavelength of 650 nanometers, and the wild-type seeds growing under the red acetate sheets had optimal wavelengths of light for seed germination (Shinomura *et al.* 1998).

Until day 3, none of the wild-type seeds had enough time and light to begin germination. However, after day three the difference between the wild type under red and blue light became evident. The wild-type seeds growing under red light were germinating; Figure 5 shows that by day seven all of the wild-type seeds had germinated. On the contrary, Figure 6 shows the wild-type seeds growing under blue light did not germinate.

The lack of seed germination in the wild-type seeds growing under the blue acetate paper may be primarily due to the inactivation of the phytochrome A

pigment (Reed *et al.* 1994). This photoreceptor, which is necessary for early seed germination, is primarily active near wavelengths between 550 and 700 nanometers (Liscum and Hangarter 1993). At around 660 nanometers, red light provided the optimal light wavelengths for phytochrome A to become activated and cause seed germination. Hence, wild type seeds fully germinated under red light. The wild-type seeds growing under blue light did not germinate because phytochrome A remained inactive in the shorter wavelengths of blue light and this prevented seed germination (Reed *et al.* 1994).

Furthermore, the number of seeds germinated differs between the mutant and wild-type strains. The pattern we observed was contrary to what we expected. The mutant seeds germinated faster than the wild-type seeds. Consequently, we failed to reject our null hypothesis.

Wild-type *Arabidopsis thaliana* seeds of most ecotypes require light for efficient germination and action of phytochrome A is considered the primary event in seed germination (Koornneef and Karssen 1994). Light exposure and changes in temperature affect seeds breaking dormancy to germinate (Finch-Savage *et al.* 2006). Our mutant *cer*10 lacks the *ECR* gene, which regulates very long chain fatty acid (VLCFA). Furthermore, *ECR* is required for normal shoot development and cell expansion (Zheng *et al.* 2005). From our results we observed that mutant seeds germinated faster than the wild type seeds. One possible explanation for this could be that the light intensity required for wild type seed germination at 24° Celsius is around 2000 Lux. In our experiment, due to equipment limitations, the light intensity for all the treatments was around 75-80 Lux, which is a lot lower than the

required light intensity. This results in longer wild type seed germination times. Miller *et al.* (1956) found that cytokinin in the dark could replace red light to induce seed germination, and lower light intensities might affect the secretion of cytokinin. Mutant seeds may have a lower threshold of light intensity for seed germination because of the lack of cytokinin receptors; therefore they germinate faster under lower light intensity.

There were a few sources of error that occurred during our experiment. One major problem was ensuring all of our samples had equal light intensities. In the incubator, we had to alter the heights of the sample trays to attempt to get the most consistent light intensities. On day 7, our blue sample tray was moved from its normal location, which changed the light intensity to which it was exposed. Another source of error we encountered was in counting our germinating seeds. The seeds were changing on a daily basis and reaching new germination stages, hence some of our tallying was inaccurate and we deemed seeds as being germinated too late. This resulted in our data being slightly inaccurate because seeds should have been labelled "G" for germinated earlier than when we noted them as being germinated.

Conclusion

In conclusion, we reject our null hypothesis and provide support for our alternative hypothesis that red light increases the speed of *Arabidopsis thaliana* seed germination. We also failed to reject our second null hypothesis. Our data show that there is a significant difference in the number of wild-type and mutant seeds germinated; the mutant seeds have faster germination times.

Acknowledgements

We would like to thank the laboratory teaching asistant, Haley Kenyon and technician, Mindy Chow, for their time and dedication towards our experiment and always answering our questions. We would also like to thank Dr. Pollock for her assistance throughout the duration of our experiment. Finally, we would like to thank the University of British Columbia providing us with the opportunity to take the Biology 342 laboratory course.

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