The effect of temperature on the germination of *Arabidopsis thaliana* seeds

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Abstract

Arabidopsis thaliana is a model organism widely used by researchers to study many different plant traits. To understand how rapid climate change and rising global temperatures could affect flowering plant growth, crop production and agriculture, *A. thaliana* has been used to observe the progression of germination of cotyledons and hypocotyls at different temperatures. Three incubation temperature treatments were selected: one at optimal condition for growth at 20°C, one lower than optimal condition, 11°C, and one higher than optimal condition, 30 °C. Four replicates, each containing 10 *A. thaliana* seeds, were incubated at each temperature. Cotyledon and hypocotyl growth were observed under a dissecting microscope daily and measured using ImageJ image processing software. On day two, the lengths of cotyledons and hypocotyls were very similar as germination had not occurred yet. On day five, the mean germinated length of the hypocotyls and cotyledons for the 11°C and 30 °C conditions were similar, at 0.1784mm and 0.2435mm respectively. In contrast, *A. thaliana* grown at 20°C had significantly more growth, measuring 2.031mm on average. On day eight, similar patterns were observed, with mean growth lengths of 1.2627mm, 3.2599mm and 1.0644mm for the 11°C, 20°C and 30°C conditions respectively. We conclude that non-optimal growth temperatures inhibit the germination of *Arabidopsis thaliana* as shown by the reduced cotyledon and hypocotyl length.

Introduction

Arabidopsis thaliana is a plant native to Europe and Asia. It has a short life cycle of about six to eight weeks from germination to seed maturation (Blamey and Grey-Wilson 1989). Its ability to be cultured in restrictive spaces makes it very desirable for researchers to use for crop production studies (Thomas 2001). It is also the first plant to have had its entire genome sequenced (Bennet *et al* 2003). *A. thaliana* is a popular organism to use for understanding many developmental plant traits such as flower development (Alvarez-Buylla 2010), leaf growth (Tsukaya 2013) and light sensing (Casal 2012). *A. thaliana* can grow up to 25cm tall, forming a dense rosette and white flowers at the end of its stem under optimal conditions. Under optimal conditions, seed germination and cotyledon/hypocotyl growth (Figure 1) can be observed in as few as three days (Tsukaya 2013).

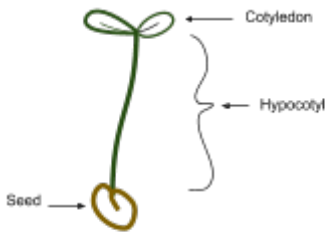

Figure 1. Cotyledon and hypocotyl growth from a seed.

This study aims to observe the effect of three different incubation temperatures on the magnitude of cotyledon/hypocotyl growth of *A. thaliana*. The three incubation temperatures, 11°C, 20°C and 30 °C, were chosen to represent a colder-than-optimal, optimal and warmer-than-optimal growing environment for the *A. thaliana*. The optimal temperature range for *A. thaliana* growth is 16-25°C (ABRC 2013), therefore, 20°C was chosen as a control condition.

Previous studies that looked at the effect of sunlight and shade on *A. thaliana* showed that when it was grown in less optimal conditions, such as in the shade, the plant grew to be significantly shorter with fewer true leaves (Casal 2012). Changes in temperature also have an impact on the ability of *A. thaliana* to photosynthesize and on its protein functions (Bunce 2008). Therefore, we hypothesize that temperature will have an effect on *A. thaliana* cotyledon and hypocotyl length (Figure 2). Additionally, we predict that temperatures outside the optimal growth temperature range have an inhibiting effect on the cotyledon/hypocotyl growth. Our null hypothesis is that temperature has no effect on the germination of *A. thaliana*.

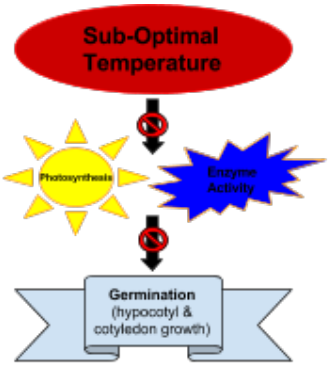

Figure 2. Model demonstrating the hypothesised effect of temperature on seed germination.

Studying the response of *A. thaliana* germination at different temperatures may help us to better our understanding of the effects of climate change on plant growth and develop agricultural practices that increase and optimize crop production.

Methods

To observe differences in growth during the germination period, *A. thaliana* seeds were grown at three different incubation temperatures of 11°C, 20°C and 30 °C. Each day, the seeds were viewed under a dissecting microscope to look for any cotyledon/hypocotyl growth. The cotyledon/hypocotyl length was measured to look for any differences in length of growth between the optimal-temperature growth samples and the sub-optimal growth temperature samples.

Preparing the samples

For this experiment, we prepared three treatments for *Arabidopsis thaliana* in 11ºC, 20ºC (control) and 30ºC incubators. As shown in Figure 3, each temperature treatment had one tray of four petri dishes (replicates) with 10 seeds (pseudoreplicates) placed on filter paper in each petri dish. We labelled the petri dishes from I to IV in each treatment. At the beginning of the experiment, we added 400Lof tap water to moisten the filter paper completely. We arranged the seeds into three groups on the filter paper, illustrated in Figure 3, to allow for efficient data collection with the microscope as it allowed us to locate and capture images of the seeds with

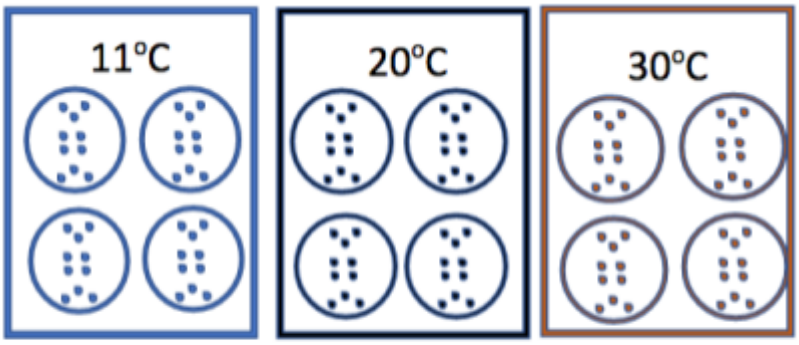

Figure 3. Illustration of seed and replicate distribution within the three treatments (11ºC, 20ºC, 30ºC).

more ease. We maintained the light intensities in the 11ºC, 20ºC and 30ºC incubators at 265 lux, 278 lux, and 273 lux respectively. After observing which treatments required more or less water due to evaporation caused by the incubation temperature, we adjusted the amount of water added daily, at the sampler's discretion, to ensure the filter paper was completely moist at all times. On each day of sampling, we gave all replicates between 200 and 1000mL of tap water, depending on the relative moisture of the filter paper. As displayed in Figure 4, we looked for a bubble of water around each seed, as well as a fully damp filter paper as an indicator to represent a sufficiently moistened growing environment.

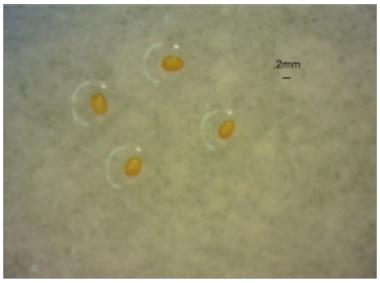

Figure 4. *A. thaliana* seedlings showing water bubble, dissecting microscope.
Total magnification: 9.6x

Measuring the samples

We watered and took pictures of the seeds every day for eight days between 11:00 am and noon. We removed each tray of petri dishes from their respective incubator for a maximum of 30 minutes. Using the dissecting microscopes and the Dinoscope, we took pictures of all the seeds and measured the length of the hypocotyl and cotyledon for each seed on ImageJ. For analysis on ImageJ, the length we measured included the length of the white hypocotyl protruding from the seed and any leaf growth, as outlined in Figure 5.

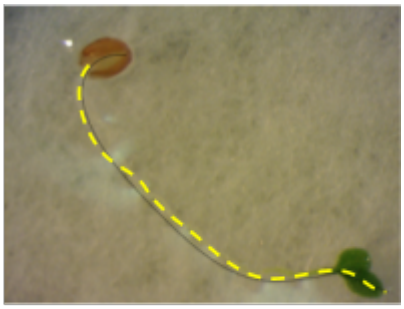

Figure 5. Hypocotyl and cotyledon growth on Day 8 at 30ºC. Dashed yellow line illustrates the length measured by ImageJ.
Total magnification: 24x

Analysis

We calculated the means and used a one-way ANOVA test to determine if the differences in growth were statistically significant at the different temperatures. For each temperature treatment we calculated the mean cotyledon/hypocotyl lengths for day two, five, and eight. Using an ANOVA calculator, we calculated the F-value, which can be used to compare the variability of data and determine whether the true means of the treatment groups are significantly different, or rather due to chance (Biology 342 Resources). We then calculated p-values using the differences in means at the 5% significance level for our data from days two, five and eight.

Results

The results of our experiment showed that *A. thaliana* grew the most in the 20ºC treatment, with a mean cotyledon length of 3.26mm on day eight compared to the 11ºC and 30ºC treatments, where there was only 1.26mm and 1.06mm of growth respectively (Figure 6). Our data showed that by day five, germination was observed in 90% of the 20ºC seeds, 47.5% of the 11ºC seeds, and only 22.5% of the 30ºC seeds. By day eight, germination was recorded in 92.5% of the 20ºC seeds, 100% of the 11ºC seeds, and 25% of the 30ºC seeds. We ran a one-way ANOVA test on the mean cotyledon/hypocotyl lengths and found p-values of 0.58, 5.2×10^{-20} and

$8.6x10^{-9}$ for day two, five and eight respectively. We calculated the 95% CI of each mean, which can be used to further support the statistical significance of the data at day five and eight, as the intervals do not overlap.

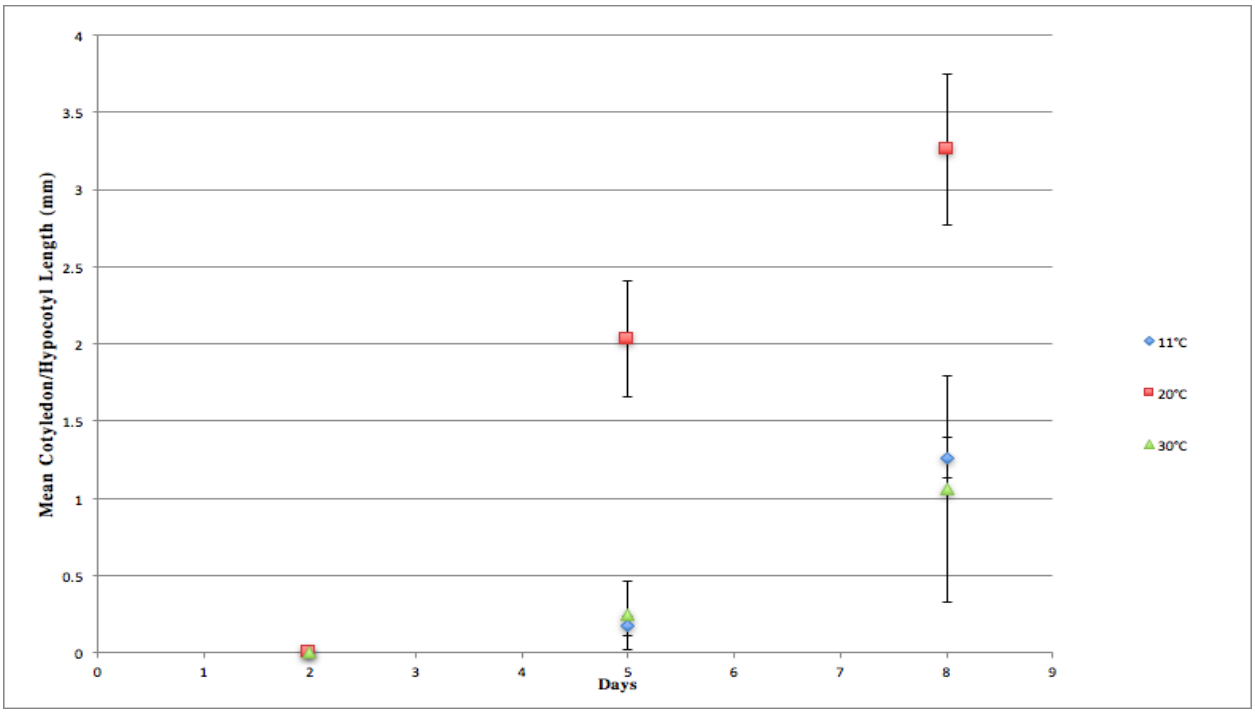

Figure 6. Mean cotyledon/hypocotyl length of *A. thaliana* seeds grown in 11ºC, 20ºC and 30ºC temperature treatments over an eight day germination period. Error bars represent 95% CI.

Discussion

Based on our statistical analysis, the p-value was less than than 0.05, indicating that we can reject our null hypothesis, which states that temperature has no effect on cotyledon and hypocotyl growth, and provide support for our alternate hypothesis, which states that temperature has an effect on mean cotyledon and hypocotyl growth. On day two, it does not appear that temperature affected *Arabidopsis* hypocotyl and cotyledon growth. Antoun and Ouellet (2013) observed that *Arabidopsis* cotyledon or hypocotyl lengths between 11ºC and 30ºC treatments were highly variable during the first three days of germination. This result agrees with the results we found in our experiment.

From day five onward, the difference in growth temperature from the optimal temperature affects the growth of *Arabidopsis thaliana*. Our results also confirm our prediction that the 30ºC and 11ºC treatments inhibit germination and result in lower mean cotyledon/hypocotyl lengths compared to the control. Furthermore, our confidence intervals between the control and our treatments do not overlap, indicating a difference in mean cotyledon length between our experimental treatments and our control treatment. We observed that all 40 seeds in the 11ºC sample had germinated by day eight. This was similar to the results of the final control germination percentage of 92.5%. Additionally, the cotyledon lengths of the 11ºC samples were relatively similar to each other, with a lower variation between lengths. Conversely, the 30ºC sample had a much lower percentage of seeds that had germinated by the end of the experiment, but the individual cotyledon lengths of the few germinated seeds in this treatment were extremely long. The more gradual germination rate and the shorter cotyledon/hypocotyl lengths observed in the 11ºC sample could be due to cold-stress, which delays germination time (Martinez-Penalver *et. al.* 2011). As seen in Figure 7a, long root hairs were seen in the 11ºC sample by day eight. This corresponds to a stress response detailed by Sun *et al.* (2015) in *Arabidopsis* seedlings. Martinez-Penalver *et. al.* (2011) also stated that photosynthetic ability was the most inhibited at cold temperatures, with a slowing of metabolic function. Additionally, Knight (2002) remarked that in order for *Arabidopsis* to tolerate colder temperatures, the organism increases calcium uptake in order to regulate various signal conduction pathways that assist with cold tolerance. Thus, energy is being allocated to tolerance and survival of the seed rather than growth. This combination of seedlings under cold-stress, inhibited photosynthetic ability, and additional energy used to take up calcium, can explain the

slower rate of hypocotyl growth and the longer duration of time required for the seeds to germinate.

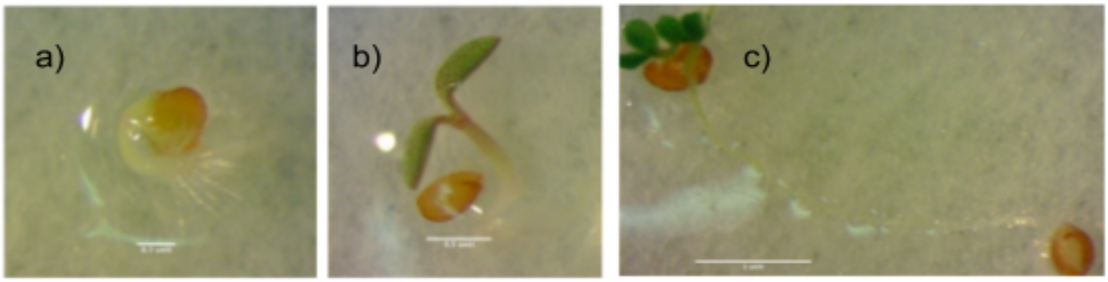

Figure 7. Germinated seeds at Day 8; units are in mm. 5a: Seed from Replicate I of the 11°C treatment. Germinated length = 1.237mm. Long, fibrous root hairs and wide hypocotyl observed. 5b: Control seed from Replicate I of the 20°C samples. Germinated length = 1.821mm. 5c: Seed from Replicate I of the 30°C treatment. Germinated length = 5.141mm. Long, thin hypocotyl observed with darker leaves than the control sample.

In our 30ºC sample, we observed a large growth spurt of a few *Arabidopsis* individuals while the other seeds did not germinate at all throughout the eight day period. We observed that the filter paper was drying out during the first days of measurement. We subsequently increased the amount of water that the 30ºC samples received to 1000mL per day. Afterward, we observed an increased percentage of germination and long outgrowth from a few individual seeds. These outgrowths had a thinner, twisting hypocotyl and smaller, darker green leaves at the ends. The initial low amount of germination can be attributed to a low water potential in the earlier days of the experiment, which inhibits germination and induces dormancy (Edwards *et. al.* 2016). Martinez-Penalver *et. al.* (2011) noted that the inhibitory effects of heat on photosynthesis increased significantly later in *Arabidopsis* development. Higher temperatures were also found to induce growth (Balasubramanian *et. al.* 2006). Although Burghardt *et. al*. (2016) mention that higher temperatures should provide more *Arabidopsis* germination than lower temperatures, our results can be attributed to the lower water potential at the beginning of the experiment placing

some seeds into dormancy. The seeds that received sufficient water based on petri dish placement could potentially then receive the growth benefits of a higher temperature, resulting in the observed, longer phenotype.

The variation observed in our experiment could occur from our seed placement, as some seeds were closer to other seeds than others, thus potentially leading them to compete for resources. Additionally, insufficient amounts of water were given to the 30ºC sample during the first few experimental days. The water itself may have not been distributed evenly among the seeds, depending on the orientation of the filter paper with the petri dish. Additionally, minute disparities, such as slightly over- or under- drawing lines when measuring lengths through ImageJ could have also slightly altered the results.

Conclusion

In our experiment testing the factor of temperature on the cotyledon and hypocotyl growth over eight days, we were able to reject the null hypothesis and provide support for our alternate hypothesis. Seeds at 20ºC grew the most, while seeds at 30ºC grew the least. Our results supported the alternate hypothesis and predictions stating that temperatures above or below *A. thaliana*'s optimal growth temperature affected the growth cycle and hypocotyl/cotyledon emergence from the seed; in this experiment the growth had been inhibited. Although temperatures are fluctuating globally *A. thaliana* will still be able to grow at different rates, but growth temperatures must be optimized to maximize crop production.

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