Effect of temperature on the germination and growth of thale cress (*Arabidopsis thaliana)*

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Abstract

Arabidopsis thaliana is a small flowering plant and a popular model organism that is distributed worldwide. This experiment aimed to investigate temperature conditions for the optimal growth of *Arabidopsis thaliana,* as measured by germination and stem length. Their germination and growth were monitored over 19 days by counting the number of sprouts and measuring the lengths of each stem under three temperature conditions of 17°C, 25°C, and 30°C. It was observed that with increasing temperature, there was a decrease in germination and an increase in the length of each stem. For germination, the results were not significantly different at 25°C or 30°C, but were found to be significantly greater at 17°C. Additionally, the different temperatures resulted in statistically significant differences in their growth. Increasing temperature increases the growth enzyme activity, and this is a potential cause for a more rapid growth that was observed at highest temperature, 30°C. Also, *Arabidopsis* is a winter annual plant, which germinated the most at the lowest temperature, 17°C. We concluded that the increase in temperature decreases germination and increases growth of *Arabidopsis*.

Introduction

Arabidopsis thaliana or commonly known as the thale cress is an ideal model organism for *in vivo* studies because of its fast growth rate, affordability, and ability to produce many seeds (Al-Shehbaz and O'Kane 2002). Furthermore, these plants are easy to cross-pollinate, and are able to self-pollinate in laboratory environments (Bechtold *et al.* 1993). Bennett *et al.* (2003) also states that *Arabidopsis* is good for genetic studies due to its small genome size. Since *Arabidopsis* is such a good model organism, it is used often in labs; therefore, it is important to know the ideal environmental factors for germination and growth, including the optimal temperature.

There are numerous published papers experimented on *Arabidopsis* plants but the temperatures used to grow them varied. For example, Trasenko *et al*. (2012) grew their *Arabidopsis* plants in 23 °C, whereas Vasseur *et al.* (2011) grew theirs in a combination of 30 °C for "day" temperature and 25 °C for "night" temperature. Due to these differences in

growth temperatures for *Arabidopsis* plants, we conducted our experiment to find the ideal temperature for *Arabidopsis* germination and growth. The two alternate hypotheses we tested were that increasing temperature will increase the germination of *Arabidopsis thaliana* and increasing temperature will increase the amount of growth of *Arabidopsis thaliana.* The null hypotheses were: increasing temperature will have no effect or will decrease the germination of *Arabidopsis thaliana*, and increasing temperature will have no effect or decrease the amount of growth of *Arabidopsis thaliana*.

Methods

We analyzed the effects of 3 different temperatures on the germination and growth of the wild type of *A. thaliana*. The three temperatures investigated were 17°C, 25°C and 30°C, referred to as treatments 1, 2 and 3, respectively. The control group was 25°C and the treatment groups were 17°C and 30°C. At 17°C, it added cold stress, and at 30°C, it added heat stress on the plants. For each temperature, we had 5 replicates, resulting in a total of 15 samples.

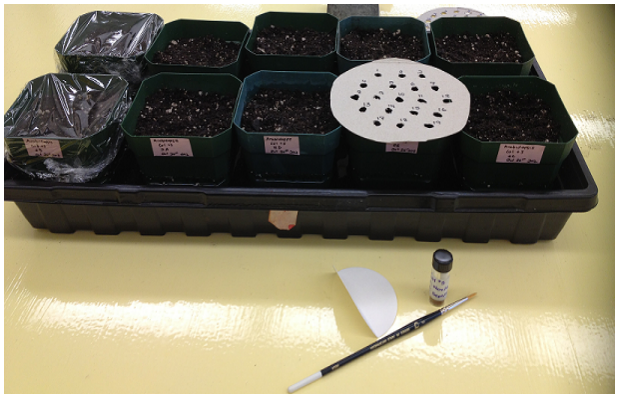

Figure 1. Preparation of samples and plantation of *Arabidopsis thaliana* seeds using the perforated planting board and paint brush.

First, we put the soil into the pots (Figure 1), filled the bottom tray with water, and let it sit for a day at room temperature in the trays, so that the soil was saturated with water. The following day, we used a perforated planting board to plant 10 seeds in each pot. We used a small paint brush to accurately get one seed per hole in the board. Then, we labeled

each pot with its identification such as the name of the organism, wild type, the treatment number and the date of plantation. After plantation, since the seeds did not need as much oxygen but required carbon dioxide for germination, we wrapped each pot with plastic wrap and held it tight with an elastic band**.** Next, we added water in each tray to the height of 2.5 cm to all 3 trays containing the 5 replicates to eliminate any possibilities of water becoming a limiting factor. Also, for each incubator, we measured the light intensity to keep all the treatments under the same and constant light. We then placed the 3 treatment trays into the corresponding incubators at temperatures 17°C, 25°C and 30°C. As soon as we saw germination, we poked holes into the plastic wrap, and then removed it completely the following day. Throughout the experiment, we measured the water level for excess water supply (Figure 2).

We observed the germination and the growth of the plants in each treatment for 19 days. We counted the number of seedlings for germination, and measured the stem lengths using a 30cm ruler for growth. For our data collection, we observed the plants 3 times per week, collecting 7 data points per treatment.

For statistical data analysis, we calculated the 95% confidence interval (C.I.) to determine the significance of various temperatures on the germination and growth of *Arabidopsis*.

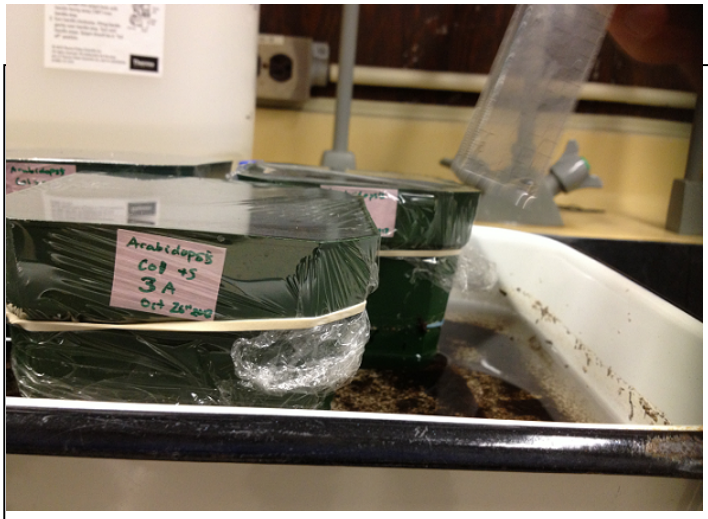

Figure 2. Checking the height of water during every data collection.

Results

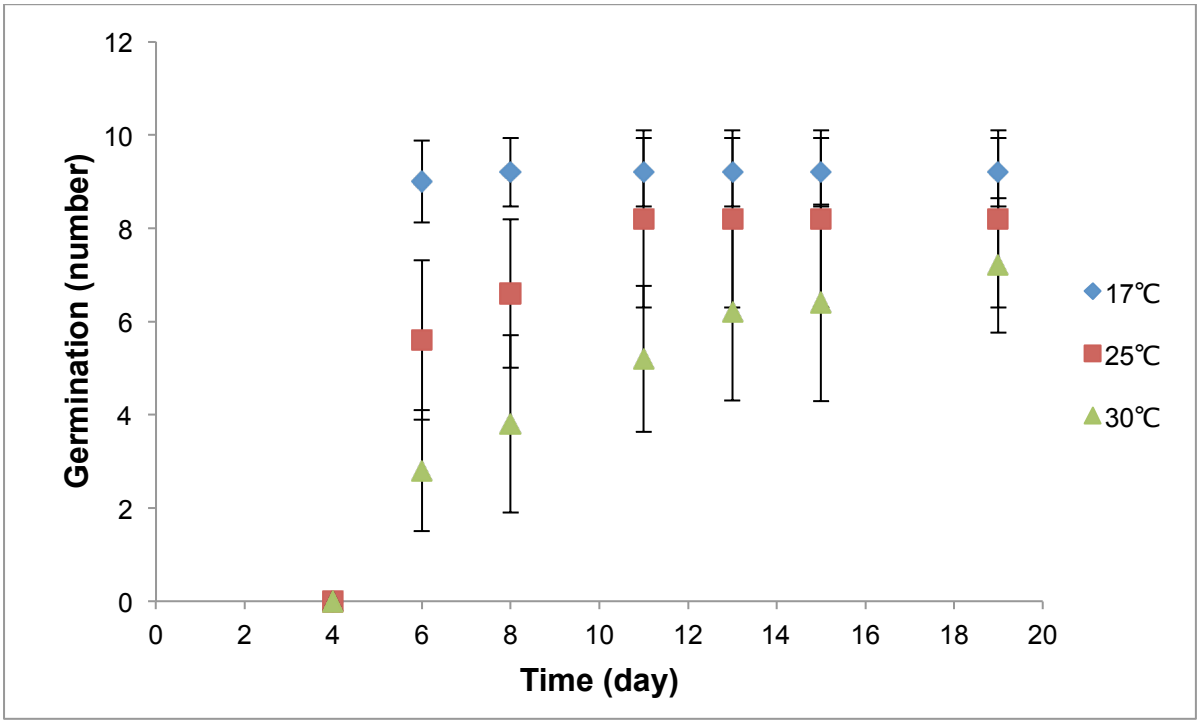

Figure 3. The germination of *Arabidopsis thaliana* with 95% confidence interval. The average germination with the 95% C.I. at 17°C, 25°C, and 30°C are 7.9, 6.4, and 4.5, respectively. These plants germinated better at lower temperatures. The plants at 25°C and 30°C are not significantly different, but plants at 17°C are significantly different from both 25°C and 30°C until day 11, where all three treatment groups' 95% C.I. overlaps, and there is no difference between the treatment groups, n = 15.

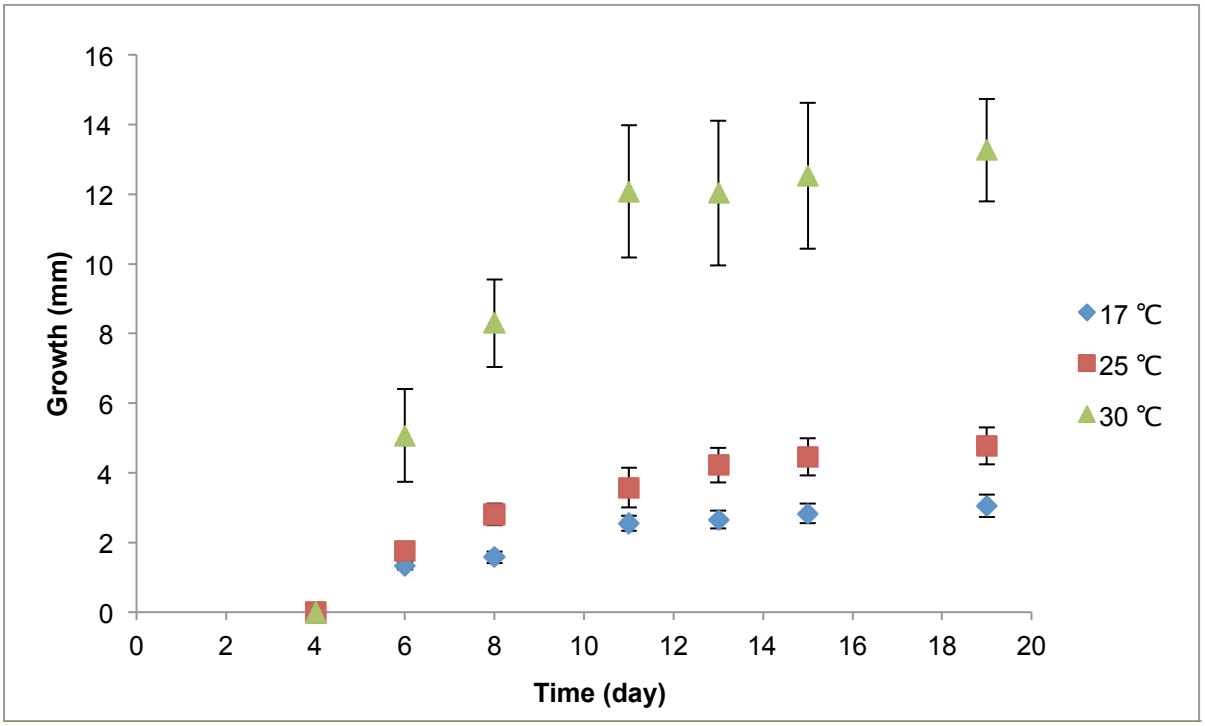

Figure 4. The growth of *Arabidopsis thaliana* at three treatment groups, 17°C, 25°C, and 30°C over the duration of 19 days, n= 15. There was optimal growth at 30°C and minimal growth at 17°C. Although the amount of growth of the plants at 17°C and 25°C are similar compared to the ones at 30°C, which show steep increase in growth around days 6 to 11, the 95% C.I. on the data shows that there are significant differences in the growth of the plants due to the varying temperatures.

Sample calculation of germination of seedlings using data from November 13 for 30°C:

$$\text{Mean} = \bar{x} = \frac{\sum x}{n} = \frac{8+6+6+9+5}{5} = 7$$

$$\text{Variance} = s^2 = \frac{\sum(x-\bar{x})^2}{n-1} = 2.7$$

$$\text{C.I.} = \bar{x} \pm 1.96\frac{s}{\sqrt{n}} = 3$$

Sample calculation for growth (mm) using data from November 13 for 30°C:

$$\text{Mean} = \bar{x} = \frac{\sum \text{average}}{n} = \frac{14.375+13.5+11.5+14.667+10.8}{5} = 13$$

$$\text{Variance} = s^2 = \frac{\sum(x-\bar{x})^2}{n-1} = 20.26114$$

$$\text{C.I.} = \bar{x} \pm 1.96\frac{s}{\sqrt{n}} = 1$$

Clear trends were observed in both the germination and the growth of *Arabidopsis* at different temperatures. In Figure 3, more germination was apparent at 17°C than at 25°C and 30°C. For all three treatment groups, we saw a sharp rise in germination around day 6. However, as time further passed, the differences started to decrease between the three treatment groups. Germination at 17°C reached steady-state around day 6 and in treatment groups 2 and 3, germination slowly increased over the duration. In Figure 4, growth at 30°C was more explicitly shown than growth at 17°C and 25°C. There was a significant difference in the growth rate between 17°C and 25°C because the 95% C.I. did not overlap. Figure 4 showed very little variation at 17°C, where the variances ranged from 0 to 1, which meant little differences in the lengths of individual plants. Meanwhile, at 25°C, we saw a more varied set of data, which ranged from 0 to 3, and accordingly, we saw a wider range for growth compared to the plants at 17°C. Lastly, for the plants grown at 30°C, the variation was large, and ranged from 6 to 37; hence the big gaps in the data points from day to day.

Over the duration of the experiment, we observed that *Arabidopsis thaliana* in treatments 1 and 2 the plants were generally shorter in comparison to the ones in treatment 3. However, we observed that even though plants at 30°C were taller, they were frail and withered compared to plants at 17°C and 25°C. The leaves of the plants in these two treatments were greener and larger than the leaves in treatment 3 (Figure 5a, 5b, and 5c).

Figure 5a. **Figure 5b.** **Figure 5c.**

Figure 5. Pictures of *Arabidopsis thaliana* at the 3 treatments on the last day of our data collection. **Figure 5a**. Replicate A at 17°C with bigger and greener leaves. **Figure 5b.** Replicate A at 25°C with smaller and thinner leaves. **Figure 5c**. Replicate A at 30°C with smaller and thinner leaves.

Discussion

The purpose of our study was to examine developmental change of *A. thaliana*, as measured by germination and growth under different temperatures. The results of our first hypothesis contradicted our experiment, where the data show an increase in temperature actually decreases the number germinating. There were significant differences in the number germinating only for the first three days of the experiment and the trend showed a decrease in the number germinating with increase in temperature (Figure 3). Therefore, our first alternate hypothesis, which states that an increase of temperature increases the number germinating, was not supported, and thus we failed to reject our first null hypothesis, stating that an increase of temperature has no effect or decreases the number germinating. Although we failed to support our first alternate hypothesis, our data was consistent with previous findings on the effects of high temperature on germination in *Arabidopsis* (Chiu *et al*. 2012). That is, a high temperature would delay the germination controlled by a temperature-sensitive-regulator, FUS3. In agreement with this article, our seeds germinated quickly at 17°C, with some delay at 25°C, and with a strong delay at 30°C. Also, most of our seeds germinated by day 16. Therefore, we observed significantly higher germination at a lower temperature on early days and delayed germination at a higher temperature (Figure 3). Moreover, a possible explanation for high number of germination at low temperature may be that *Arabidopsis* is a winter annual plant. They usually germinate more and do not grow very tall during autumn or winter when temperature is low, because they are about to die in cold temperature, and this is consistent with the study by Al-Shehbaz and O'Kane (2002) which states that *Arabidopsis* was primarily abundant in cold temperature regions such as Europe.

In our second hypothesis, the alternate hypothesis was supported, which stated that an increase in temperature would increase the amount of growth in *Arabidopsis*. Also, we rejected the second null hypothesis, which states that an increase in temperature would have

no effect or decrease the amount of growth in *Arabidopsis*. According to the results (Figure 4), the amount of growth varied significantly at all three different temperatures starting from Day 6, and there was increasing trend in growth with increasing temperature. This finding was consistent with high temperature effect on growth of *Arabidopsis* (Gray *et al*. 1998). That is, in a condition at temperature of 29°C, *Arabidopsis* shows a dramatic increase in growth. Corresponding to our experiment, the growth of our plants at 30°C was significantly greater than at any other temperatures. In general, we observed significantly higher growth at temperature of 25°C and 30°C. A possible explanation for this finding could be that a high temperature increases the rate of metabolism in plants. As temperature increases, the rate of growth enzymes and their substrates move faster, increasing the chance of binding. Therefore, the growth of *Arabidopsis* increases with increasing temperature.

During the experiment, there were major limitations and errors that may have affected our results. We tried to keep every possible factor constant other than the temperature, the variable of interest. However, there were still limitations and errors such as light intensity, level of water, and measurements of stems.

We had inconsistent light intensities in all three growth incubators. Light intensities were measured to be approximately 4950 lux, 4890 lux, and 4930 lux respective to 17°C, 25°C, and 30°C. If better incubators and light meters were available, similar light intensities would be possible.

Unfortunately, the light bulbs in 25°C incubator stopped working, dropping the light intensity by half (4890 lux to 2340 lux) on November 5, 2012. Therefore, we adjusted the light intensities in 17°C and 30°C incubators to 2550 lux and 3750 lux respectively to closely match the light intensity of 2340 lux in 25°C incubator. However, this malfunction only spanned an interval of two days, and an obvious change in data was not observed.

There was no way to maintain the same amount of water for every replicate, because

the rate of evaporation was different, and there was no device for adding water automatically. Therefore, water was constantly refilled to be kept in excess, so water was not a limiting factor.

The measurement of growth was done by measuring the stem length, which was defined as being the visible part of the stem above the soil to the first node of the branches. However, there was further growth in branches and leaves above the first node. Thus, the result in growth might be different if the whole plant was measured, including stem, branch, and leaf.

There was no suitable equipment to measure the length of stems when plants had just sprouted, because the rulers had blank spaces at both ends, which was measured to be 8 mm, so plants that were shorter than 8 mm could not be measured accurately. Therefore, newly sprouted plants were measured by eyes, which may have lead to measurement errors. In order to minimize the error, one designated member of the group measured all the plants to keep the values consistent.

Conclusion

We observed the germination and growth of *Arabidopsis thaliana* in 17°C, 25°C and 30°C incubators for 19 days after plantation to study the relationship between germination and temperature, and growth and temperature. We could not support our first alternate hypothesis, that germination would increase as temperature increased, and failed to reject the null hypothesis because germination of *Arabidopsis* plants was significantly higher in the 17°C incubators compared to the plants kept in the 30°C incubator. Our second alternate hypothesis, growth of *Arabidopsis* plants would increase with temperature, was supported and the null hypothesis was rejected. Although many experimenters grow *Arabidopsis*

thaliana at 25°C, we found in our study that *Arabidopsis* plants grow faster in higher temperatures but germinate slower.

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