The effect of temperature on the growth rate of *Arabidopsis thaliana* wild type and *cer10* mutant

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Abstract

Cuticular wax aids in regulating water loss in the small flowering plant, *Arabidopsis thaliana.* Unlike the wild type, the *eceriferum10 (cer10)* mutant lacks the ability to properly synthesize cuticular wax. This mutation may affect its growth rate when subjected to different temperature-stress environments. The purpose of this study was to determine whether different temperatures had an effect on the growth rate of the wild type or *cer10* mutation of *A. thaliana*. By measuring the diameter of the rosette from samples of wild-type and mutant seedlings grown in 20°C and 30°C incubators, growth rate was calculated over the course of ten days. A two-way ANOVA test was used on these average growth rates to determine if differences between mean growth rates were significant. The results indicated that temperature had no significant effect on the growth rate of *Arabidopsis thaliana* (p=0.23). Additionally, the presence of the *cer10* mutation had no significant effect on growth rate of *A. thaliana* (p=0.39). Moreover, the effect of temperature on the growth rate of *A. thaliana* is the same in wild type and *cer10* mutants (p=0.30).

Introduction

The small flowering plant *Arabidopsis thaliana*, commonly known as thale cress, was the first plant to have its complete genome sequenced (The Arabidopsis Genome Initiative 2000). *A. thaliana* is a member of the mustard family and has a relatively short life cycle (Meinke *et al,* 1998). The optimal temperature for growth is 22°C to 23°C (The Arabidopsis Biological Resource Center 2015).

This experiment examined both wild type and mutant forms of the Columbia ecotype of *A. thaliana*. The mutant plant had a deletion mutation in the *eceriferum10* (*cer10*) gene. The objective of this experiment was to measure the effect of two different temperatures on the growth rate of the wild-type *A. thaliana* and the *cer10* mutant. The *cer10* mutant differs from the wild type in that there is a severe disruption in the gene that codes for enoyl-CoA reductase (Zheng *et al.* 2005). Enoyl-CoA reductase, or ECR, is

required for the biosynthesis of very-long-chain fatty acids (VLCFAs) in *A. thaliana* (Gable *et al*. 2004). Absence of the ECR leads to a decrease in the production of all VLCFA-containing lipids – including epicuticular wax (Gable *et al*. 2004).

Due to this mutation, the *cer10* plants have significantly less cuticular wax than the wild type (Zheng *et al.* 2005). The wax layer is important as it decreases the amount of water that is lost when the plant is exposed to high temperatures (Jenks *et al.* 1995). Additionally, the wax layer acts as a barrier or shield and provides the plant with protection from the deleterious effects of environmental dangers (Jenks *et al.* 1995).

Considering the difference in wax layers, we aimed to examine the effects of temperature on growth rate in both mutant and wild-type *A. thaliana*. This study is important because processes observed in *A. thaliana,* which has had its entire genome sequenced, can be used as a foundation for additional eukaryotic research (The Arabidopsis Genome Initiative, 2000).

Shepard and Griffith (2006) found that plants that contain a greater amount of cuticular wax experience a decrease in the permeability of the epidermis. Therefore, we predicted that the growth rate of the wild type would be greater than that of the wax-less *cer10* mutant when both plants are exposed to higher than optimal temperatures for a period of time. The lack of cuticular wax was predicted to prohibit the mutant from retaining water and should consequently slow down its growth rate. For both the mutant and the wild type, we predicted that the temperature would have an effect on the growth rate and the rate would be highest at the optimal temperature. We proposed the following hypotheses:

Ho_1: Temperature has no effect on growth rate of *A. thaliana.*

Ha_1: Temperature has an effect on growth rate of *A. thaliana*.

Ho_2: Presence of the *cer10* mutation has no effect on growth rate of *A. thaliana.*

Ha_2: Presence of the *cer10* mutation has an effect on growth rate of *A. thaliana.*

Ho_3: The effect of temperature on the growth rate of *A. thaliana* is the same in wild type and the *cer10* mutation.

Ha_3: The effect of temperature on the growth rate of *Arabidopsis thaliana* is not the same in wild type and the *cer10* mutation.

Methods

The experiment was conducted using two different incubators set at temperatures of 20°C and 30°C. The 20°C treatment served as the control for this experiment, since 22-23°C is the ideal temperature for growth of *A. thaliana* (Rivero *et al.* 2014). The 30°C represented a high-temperature stress environment. Each incubator contained a tray holding eight pots of plants. The tray held four wild-type replicates and four mutant replicates (Figure 1). Four seedlings were planted in each pot. The placements of the mutant and wild-type replicates within each tray were assigned randomly using a number of cards. Water levels were replenished and kept at a constant level in each tray. A horizontal line along the inside of the both trays was drawn at approximately three quarters of a centimeter above the bottom of the tray and was used to ensure equal soil moisture within all the treatments. Both trays were placed in the center of their respective incubators to maintain light intensity at approximately 3000 lux. Width and height measurements were recorded four times a week (all weekdays excluding Tuesday) between 11:00 am and 2:00 pm. When measuring width, we used calipers and recorded

the widest diameter of the rosette from an aerial view. When measuring height, we used a ruler and measured the highest point of the seedling from the base of the plant. Measurements of both these variables were rounded to the nearest millimeter and general observations on the water and moisture levels were recorded. We also made note of each seedling's health, as it was likely that some would die throughout the experiment. Mean growth rates for each replicate were calculated by finding the average of all surviving plants in each pot. A two-way ANOVA was performed on these average growth rates to determine if differences between mean growth rates were significant.

Figure 1. Experimental setup for replicates at 20°C and 30°C. Four pots with wild type and four pots with mutant plants were randomly arranged in each tray.

Results

We observed a steady growth rate from the first day to the last day the measurements were taken. We used Equation 1 listed below to calculate growth rate in centimeters per day.

Equation 1.

$$\frac{\textbf{plant width at day 10} - \textbf{plant width at day 0}}{\textbf{10 days}} = \textbf{Growth rate } (cm \cdot day^{-1})$$

After obtaining growth rates for all four replicates a mean was taken to get the average growth rate over a period of ten days. A plot of the average growth rates for the four replicates can be found in Figure 2.

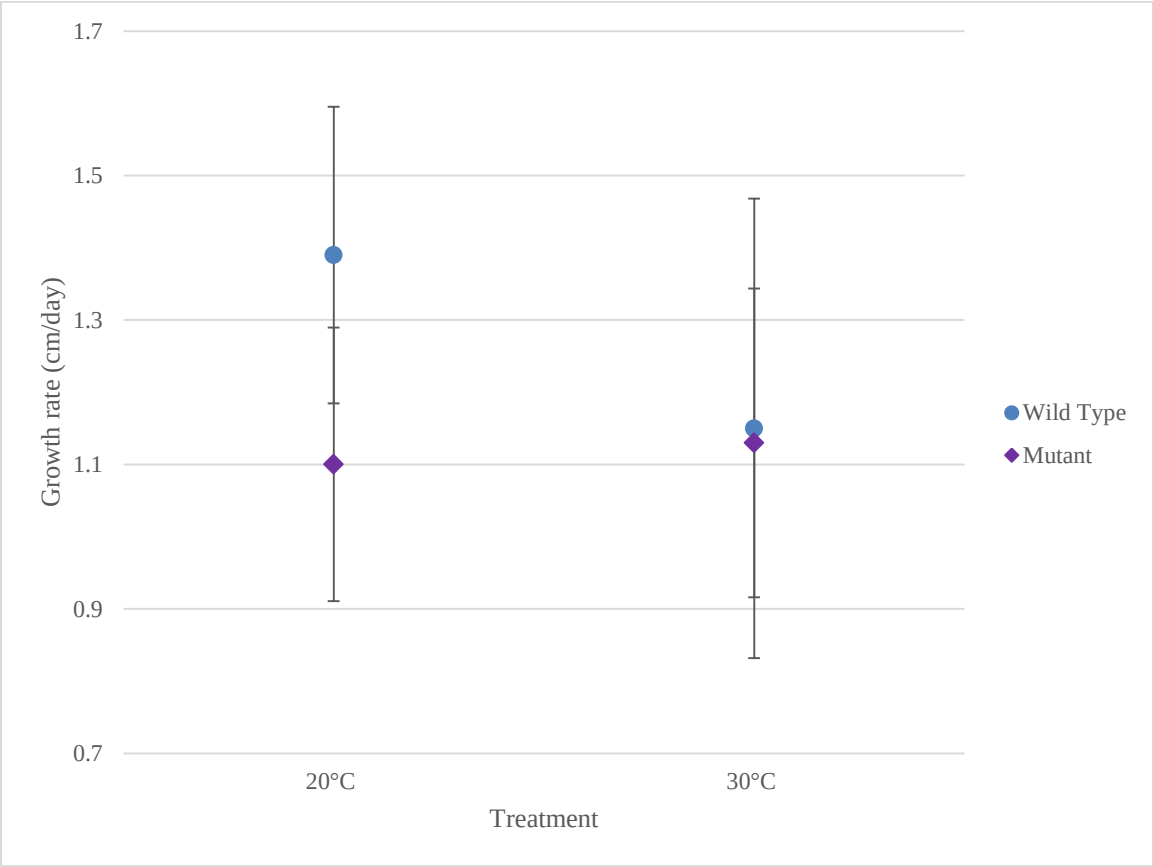

Figure 2. Average growth rate (cm/day) of *A. thaliana* wild type and *cer10* mutant at 20°C and 30°C incubation with 95% confidence intervals.

At 20°C it was calculated that the wild type has an average growth rate of 1.39±0.21 cm/day while the *cer10* mutant has a lower average growth rate of 1.11±0.19 cm/day. At 30°C the wild type has an average growth rate of 1.15±0.32 cm/day while the *cer10* mutant has a lower average growth rate of 1.13±0.21 cm/day. It was found that at 20°C the wild type has highest growth rate of 1.39±0.21 cm/day and the *cer10* mutant at 20°C was found to have the lowest growth rate of 1.11±0.19 cm/day. From Figure 2 it can be

seen that there is overlap between the 95% confidence intervals for the wild type and *cer10* mutant in the 30°C and 20°C treatment. The wild type at 30°C has the largest confidence interval of 0.31 cm/day; the mutant in the 20°C treatment has the smallest confidence interval of 0.19 cm/day.

A two-way ANOVA was then done to obtain *p*-values to test for significance. A *p*-value of 0.23 was obtained for Ho_1 which looked at the growth rate between strains. A *p*-value of 0.39 was obtained for Ho_2 which looked at the growth rate between treatments. Lastly we obtained a *p*-value of 0.30 for Ho_3 which looked at the effect of strain on growth rate in different treatments.

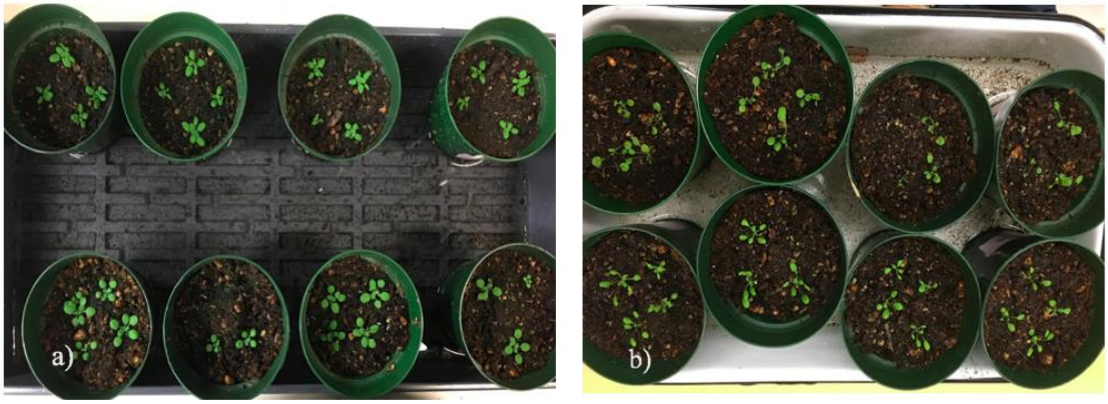

Figure 3. The image on the left (Figure 3a) is *A. thaliana* wild type and *cer10* mutant at day ten at 20°C incubation. The image on the right (Figure 3b) is *A. thaliana* wild type and *cer10* mutant at day 10 at 30°C incubation.

On day ten we observed that at 20°C both *A. thaliana* wild type and *cer10* mutant have a healthier appearance as seen in Figure 3a compared to *A. thaliana* wild type and *cer10* mutant seen in Figure 3b at 30°C incubation. It was also noted that regardless of the strain *A. thaliana* leaves appear to be larger at 20°C (Figure 3a).

Discussion

Based on our two-way ANOVA, we fail to reject all three null hypotheses (Ho_1, Ho_2, and Ho3) and the results fail to support all three of our alternate hypotheses (Ha_1,

$Ha_{2,}$ Ha_3). The *p*-values obtained from the two-way ANOVA are 0.23, 0.39, and 0.30, respectively, and all three *p*-values are larger than the critical *p*-value of 0.05.

In our experiment, temperature had no significant effect on the growth rate of *A. thaliana*. This contradicted our prediction which was that a higher than optimal temperature of 30°C would decrease the growth rate of *A. thaliana* compared to those growing in an optimal temperature of 20°C. According to Gray *et al*. (1998), the plant hormone indole-3-acetic acid (IAA or auxin) is responsible for the elongation of cells in *A. thaliana* at higher temperature (29°C). We observed similar results to Gray *et al.* (1998); the plants in 30°C grew longer vertically while growing wider horizontally, whereas the plants in 20°C grew mainly horizontally. However, our method focused on the horizontal plant growth during the earlier stages of plant development, and therefore, the vertical growth was not incorporated in our results and this may have been the reason our results caused us to fail to reject the null hypothesis Ho_1.

The presence of the *cer10* mutation had no significant effect on the growth rate of *A. thaliana* in our experiment. Based on previous research, we predicted that the lack of water retention in mutants due to a deletion in the gene coding for enoyl-CoA reductase (ECR), an enzyme required for very-long-chain fatty acid (VLCFA) synthesis and increase in cuticular wax load, would significantly decrease the growth rate of *A. thaliana* (Jenks and Ashworth 1999, Zheng *et al.* 2005). However, as contradictory research has shown, the lack of cuticular wax is not always associated with significant water loss (Johnson *et al*. 1983, Premachandra *et al.* 1992, and Jefferson *et al*. 1998). Our research agrees that the water retention from cuticular wax is not a significant factor in plant growth. Moreover, the relatively small surface area of the plant body could have been

another reason the lack of cuticular wax from the presence of the *cer10* mutation had no significant effect on the growth rate of *A. thaliana.*

The effect of temperature on the growth rate of *A. thaliana* was the same in both the wild type and *cer10* mutants in our experiment. Koornneef *et al.* (1989) found dwarfism, the reduction of plant size of up to one-third of wild-type height was observed for *cer10* mutants, thus we predicted the effect of temperature on the growth rate of wild type and mutant would be different. However, the dwarfism was not significant for the *cer10* mutants and this may have also been due to the relatively earlier stage of plant development as opposed to fully-grown plants used in the research of Koornneef *et al.* (1989).

In our experiment, our seedlings showed slower growth than expected. According to Meinke *et al.* (1998), the entire life cycle of *A. thaliana* is known to be six weeks, but our seedlings did not grow more than 2.5 cm in three weeks. The slower growth may have been due to damaging the roots of the seedlings while transplanting, or making a larger hole in the soil than needed. Moreover, despite our efforts to control the soil moisture, there could have been minor sources of error from the different incubators used for 20°C and 30°C. The trays used in the two different incubators had different sizes so both the water level and the amount of water could not be precisely controlled. After the weekends and Remembrance Day we observed depletion of water in our trays, which could have limited the water supply required for the optimal growth of our plants. Another source of variation would include that measurements were not consistently done by the same person.

Conclusions

Based on our results, we failed to reject Ho_1 and failed to support Ha_1 finding that under our experimental conditions, temperature has no effect on the growth rate of *A. thaliana*. We found the presence of the *cer10* mutation to have no effect on growth rate so we failed to reject Ho_2 and failed to support Ha_2. This was not as predicted; we expected that temperature would have an effect on the growth rate in both wild type and mutant and the rate would be highest at the optimal temperature. We also found no significant difference between growth rate when comparing the effect of temperature on wild type and mutant and thus failed to reject Ho_3 and failed to support Ha_3. This was contrary to our prediction that the growth rate of the wild-type would be greater than that of the *cer10* mutant when both plants were exposed to higher than optimal temperatures for the same period of time.

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Literature Cited

Arabidopsis Genome Initiative and Ar Gen In. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature, **408**(6814): 796-815.

Arabidopsis Biological Resource Center. 2015. The Ohio State University. Retrieved from https://abrc.osu.edu/seed-handling.

Gable, K., Garton, S., Napier, J. A., and Dunn, T. M. 2004. Functional characterisation of the *Arabidopsis thaliana* ortholog of Tsc13p, the enoyl reductase of the yeast microsomal fatty acid elongating system. J. Exp. Bot., **55**: 543–545.

Gray, W. M., Ostin, A., Sandberg, G., Romano, C. P., Estelle, M. 1998. High temperature promotes auxin-mediated hypocotyl elongation in *Arabidopsis*. Proceedings of the National Academy of Sciences of the United States of America **95**(12): 7197-202.

Jefferson, P. G., Johnson, D. A., Rumbaugh, M. D., and Asay, K. H. 1989. Water stress and genotypic effects on epicuticular wax production of alfalfa and crested wheatgrass in relation to yield and excised leaf water loss rate. Canadian J. Plant Sci. **69**: 481-490.

Jenks, M. A., and Ashworth, E. N.1999. Plant epicuticular waxes: function, production, and genetics. John Wiley & Sons, Inc., West Lafayette, Indiana.

Jenks M. A., Tuttle H. A., Eigenbrode S. D., and Feldmann K. A. 1995. Leaf epicuticular waxes of the eceriferum mutants in Arabidopsis. Plant Physiology. **1081**(1): 369–377

Johnson, D. A., Richards, R. A., and Turner, N. C. 1983. Yield, water relations, gas exchange, and surface reflectances of near-isogenic wheat lines differing in glaucousness. Crop Sci. **23**: 318-325.

Koornneef M., Hanhart, C. J., and Thiel, F. 1989. A genetic and phenotypic description of eceriferum mutants in *Arabidopsis thaliana*. Journal of Heredity, **80**(2): 118-122.

Meinke, D. W., Cherry, J. M., Dean, C., Rounsley, S. D., and Koornneef, M. 1998. *Arabidopsis thaliana*: A model plant for genome analysis. Science, **282(**5389): 662-682.

Premachandra, G. S., Saneoka, H., Fujita, K., and Ogata, S. 1992. Leaf water relations, osmotic adjustment, cell membrane stability, epicuticular wax load and growth as affected by increasing water deficits in sorghum. J. Expt. Bot. **43**: 1569-1576.

Rivero, L., Scholl, R., Holomuzki, N., Crist, D., Grotewold, E., and Brkljacic, J. 2014. Handling *Arabidopsis* plants: growth, preservation of seeds, transformation, and genetic crosses. Methods in Molecular Biology, **1062**: 3-25.

Shepherd, T., and Griffiths, W. D. 2006. The effects of stress on plant cuticular waxes. New Phytologist, **171**(3): 469-499.

Zheng, H., Rowland, O., and Kunst, L., 2005. Disruptions of the *Arabidopsis* enoyl-CoA reductase gene reveal an essential role for very-long-chain fatty acid synthesis in cell expansion during plant morphogenesis. The Plant Cell, **17**(5): 1467-1481.