The Effect of Temperature and *cer10* Mutation on the Stem Growth Rate of *Arabidopsis thaliana*

Ashley Dolman, Rubeen Sangha and Henry Yeh

Abstract
Cuticular wax in the small, flowering plant *Arabidopsis thaliana* serves a variety of functions. In particular, the wax cuticle helps to regulate water loss in different temperature-stress environments. The *cer10* mutation in *A. thaliana* results in an inability to properly synthesize cuticular wax. The lack of a cuticular wax may impact the growth of mutant *A. thaliana* under temperature stress. Our study compared the stem growth of mutant and wild type *A. thaliana* grown in 10^0C, 20^0C, and 30^0C incubators. Using a two-way ANOVA, it was found that mutant *A. thaliana* plants grow significantly shorter stems than wild-type plants. We also discovered that the high-temperature stress environment of the 30^0C incubator produced a significant increase in stem growth of both wild-type and mutant *A. thaliana*. This may be linked to increased indole-3-acetic acid (IAA) production, which is released to promote stem elongation under high temperature conditions.

Introduction

Arabidopsis thaliana, commonly known as thale cress, is a flowering plant naturally distributed across Europe, Asia and North America. It has a simple root structure, in which a single primary root grows vertically until it diverges into smaller lateral roots (Meinke *et al.* 1998). The central stem grows above the basal rosette leaves and reaches heights of 20-25 cm at maturity. *A. thaliana* also produces small white flowers on their tips after about three weeks (Dorn *et al.* 2000).

The epidermis of *A. thaliana* is coated with a wax layer that is mainly composed of very-long-chain fatty acids (VLCFA) (Zheng *et al*. 2005) (Figure 1a). However, *Arabidopsis thaliana* with mutations in genes responsible for the production of cuticular wax may reduce wax loads or completely abolish wax production. Knockout mutations in the *cer10*, a gene responsible for the production of Enoyl-CoA Reductase (ECR) which is essential for the synthesis of VLCFA, have reduced cuticular wax deposition and generally appear glossier

and darker than the wild type (Rashotte *et al.* 2001) (Figure 1b). This decreased wax load alters the reflection of light (Koonrnneef *et al.* 1989).

Figure 1a. Image of a wild-type *A. thaliana* stem. The outside layer is covered with epicuticular wax giving it a matte appearance.

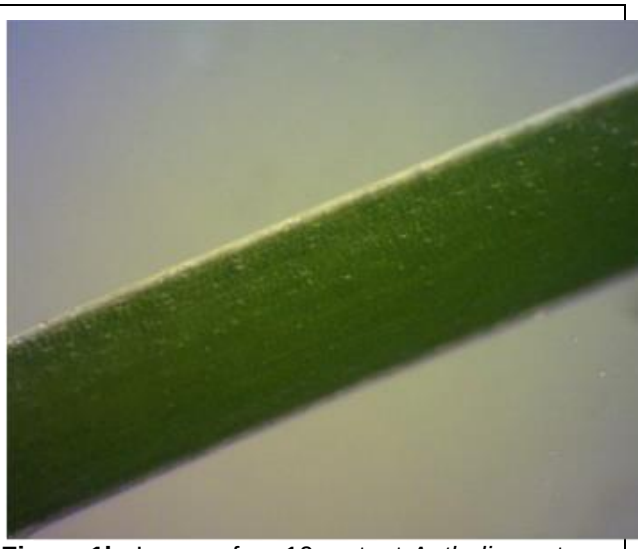

Figure 1b. Image of *cer*10 mutant *A. thaliana* stem. It is glossier and darker than the wild type, due to the lack of epicuticular wax.

The epicuticular wax layer plays an important role in the survival of *Arabidopsis thaliana*. It protects the plant from various environmental stresses, such as freezing temperatures and harmful pathogens (Jenks *et al.* 1995). Moreover, the primary function of the wax layer is to reduce water loss under high temperatures or intense radiation (Aarts *et al.* 1995; Jenks *et al.* 1995).

Although the wax layer provides some protection against environmental stressors, *A. thaliana* can still suffer from cellular water deficit under prolonged exposure to high temperatures (Bray 1997). This often results in disruption of membrane rigidity, change in cell volume or shape, and even death by desiccation (Bray 1997).

With this in mind, we wanted to test the effect of different temperatures on the growth rate of wild-type *A. thaliana.* We expected to see highest stem growth rate from the plants incubated in the optimal temperature range because temperatures higher and lower than the ideal temperature have been known to cause injury effects (Went 1953).

Furthermore, we suspected that the presence or absence of the *cer10* mutation could lead to differential growth under varying temperature regimes. The focus was on growth of the plant stem because we considered it to be a sufficient representation for overall growth. This experiment was conducted under three sets of hypotheses:

H_{a1}: Temperatures above or below the optimal temperature will decrease the stem growth rate of *A. thaliana.*

H_{01}: Temperatures above or below the optimal temperature will increase or have no effect on the stem growth rate of *A. thaliana.*

H_{a2}: Presence of the *cer10* mutation will decrease the stem growth rate of *A. thaliana.*

H_{02}: Presence of the *cer10* mutation will increase or have no effect on the stem growth rate of *A. thaliana*.

H_{a3}: Increased temperatures will have a different effect on the stem growth rate of *A. thaliana* for wild-type and mutant plants.

H_{03}: Increased temperatures will have the same effect on the stem growth rate of *A. thaliana* for wild-type and mutant plants.

Methods

The experiment was conducted using three different incubators set at temperatures of 11^0C, 20^0C, and 30^0C. The 20^0C treatment served as the control for this experiment, since it is closest to the ideal temperature for growth of *A. thaliana* (22-23^0C) (Rivero *et al.* 2014*)*. The 11^0C treatment represented a low-temperature stress environment, while the 30^0C represented a high-temperature stress environment. Each incubator contained a tray holding ten pots of plants: five wild-type replicates and five mutant replicates (Figure 2a). The placements of the mutant and wild-type replicates within each tray were assigned using

a random number generator. This was done to avoid grouping all the plants of one type together, as plants on one side of the tray may experience slightly different conditions compared to the other side. Three seedlings were planted in each pot, and the outside of the pot was labeled “A”, “B”, and “C” to distinguish the seedlings from one another for measuring purposes (Figure 2b).

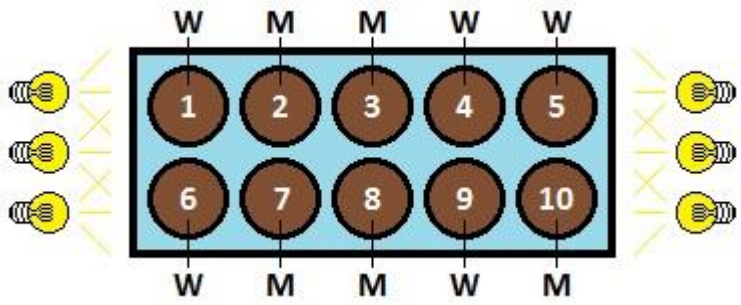

Figure 2a. Schematic of the experimental setup for each tray. Five wild-type and five mutant plants were randomly arranged in each tray, using a random number generator. Water level in the tray was held constant throughout the experiment. W= wild type and M = mutant.

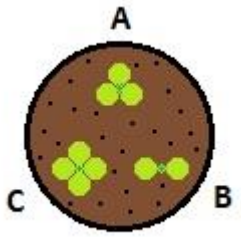

Figure 2b. Representation of a single replicate. It contains three wild-type or three mutant *A. thaliana* seedlings that were labelled A, B or C.

Efforts were made to minimize the influence of abiotic differences among the incubators. Cheesecloth covers were used to cover brighter lights in order to maintain the light intensity in all three treatments at approximately 3000 lux. Water levels were held constant in each tray (as opposed to giving each pot plants a set volume of water), approximately a centimeter above the bottom of the pots, ensuring that soil moisture content would be the same across all treatments.

Measurements of the stem length (to the nearest mm) and general observations on the growth patterns of each seedling were taken daily (Monday to Friday), using calipers, for 16 days between 12:00 and 1:00 PM. Mean growth rates over the observation period for

each replicate were calculated by taking the average of all surviving plants in each pot. A two-way ANOVA was performed on these average growth rates to find differences between the mean growth rates, and a post hoc Tukey test was used where applicable to determine which groups were distinct from the others.

Results

The 2-way ANOVA showed that there was a significant difference among the three temperature treatments ($F=22.6097$, $df=2$, $p<0.05$). However, a Tukey test showed that the stem growth in the 30^0C treatment was significantly higher than the growth in both the 20^0C and 11^0C treatments ($p<0.05$), not lower as we initially hypothesized. Growth rates were higher in the 20^0C treatment compared to the 11^0C treatment, but this was not statistically significant ($p>0.05$).

Comparing the stem growth rates for wild-type plants versus mutant plants, there is a significant difference between the two ($F=5.1244$, $df=1$, $p<0.05$). However, the combined effect of temperature and presence/absence of the *cer10* mutation did not have a significant effect ($F=1.6454$, $df= 2$, $p>0.05$). A summary of the mean daily growth rates for each treatment can be found in Figure 3.

Qualitatively, most of the growth was observed in the third week of data collection. In the 11^0C treatment, stems remained very small, barely emerging above the rosette. In the 20^0C treatment, all leaves in the rosette grew to be quite large – several centimeters in length. Stems grew fairly thick, and some small white flowers could be seen at the tops of some wild type plants. In contrast, although stems grew very long in the 30^0C treatment, they were much thinner, and none of them produced flowers by the end of the experiment. Leaves of these thin-stemmed plants were small as well, often even smaller than the leaves

of the small plants in the 11^0C treatments. A total of five seedlings in the 30^0C treatment did not survive; all other seedlings survived in the other two treatments (Figure 4 a and b).

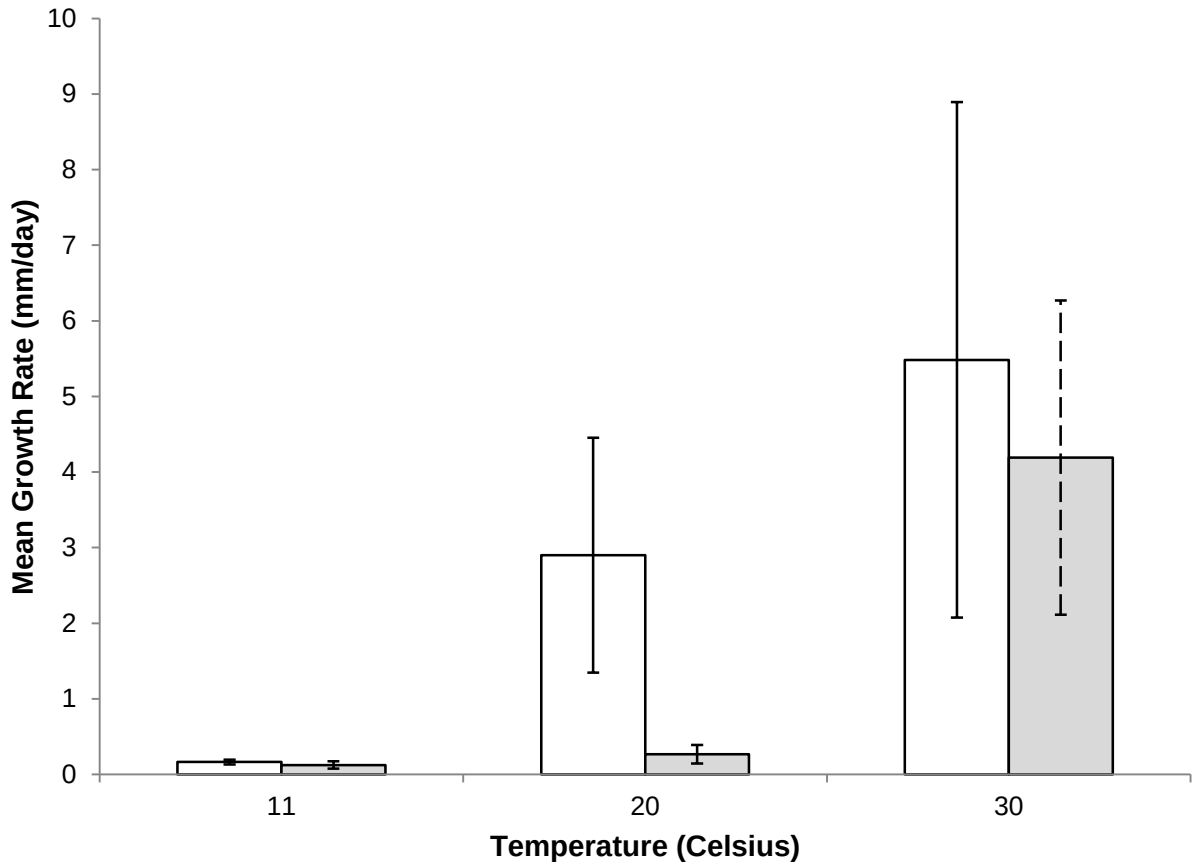

Figure 3. Mean growth rates for each treatment over the 16-day observation period. Final stem heights of all replicates in each treatment (11^0C, 20^0C, and 30^0C crossed with presence/absence of the *cer10* mutation) were averaged and divided by 16 for the mean daily growth rates. White boxes with solid lines represent wild type plants and grey boxes with dashed lines represent *cer10* mutants. Error bars represent 95% confidence intervals (n=5 per treatment).

Figure 4a. Wild-type *Arabidopsis thaliana* grown in a 30^0C incubator (photo taken on day 15 of observations). Stems are relatively straight and erect.

Figure 4b. *Arabidopsis thaliana cer*10 mutant grown in a 30^0C incubator (photo taken on day 15 of observations). Note that compared to wild type, these stems are not as straight and erect.

Discussion

At the end of the experiment, *A. thaliana* seedlings grown in the 30^0C incubator displayed the highest amount of growth with many stems reaching lengths in excess of 15cm. In contrast, stems of *A. thaliana* cultivated in 11^0C and 20^0C incubators did not reach

these lengths. Increased temperature appears to significantly increase the growth rate of *A. thaliana*. These results do not support H_{a1} and as a result, we did not reject H_{o1}. We originally predicted that a high-temperature environment would make it difficult for *A. thaliana* to retain its internal moisture levels. This lack of moisture should have made it difficult for *A. thaliana* to maintain turgidity and grow. Overall, this was not the case, but it is certainly possible that desiccation was still a factor for plants grown at this temperature, as some of the seedlings did not survive in the 30^0C treatment. The larger error bars in the 30^0C treatment compared to the 11^0C treatment can be attributed to the fact that plants were simply larger in the 30^0C treatment therefore biological variation within the plants could manifest itself on a larger scale.

The observed difference in stem growth could be due to the amount of indole-3-acetic acid, or auxin (IAA) found in *A. thaliana*. IAA is a compound that is involved in cell elongation and its response pathway is regulated by temperature (Collett *et al.* 2000). Gray *et al*. (1998) found that high temperatures (~29^0C) can increase the level of IAA output, which promotes stem elongation for *A*. *thaliana.* Our results are consistent with the findings of this study, where the seedlings grown at high temperature (30^0C) exhibited dramatic stem elongation compared to the seedlings grown at 11^0C and 20^0C. Although Gray *et al*. (1998) do not provide a definitive explanation for why this physiological adaptation occurs, they suggest that an elongated stem may help to promote cooling of the plant by increasing the surface area exposed to moving air.

In all three temperature treatments, mutant *A. thaliana* stem lengths were shorter than wild type in each treatment. This effect was significant at the 95% level, so we can reject H_{02} and accept H_{a2}. In addition, the mutant stems were typically crooked and bent while the wild-type stems were straight and upright (Figure 4a,b). This is possibly indicative of difficulty maintaining turgidity, as the lack of wax reduces the amount of water that can be

held in the plant (Zheng *et al.* 2005). According to Zheng *et al.* (2005), the *cer10* mutation causes a reduction in size of aerial organs such as leaves, flowers and stems because the lack of a complete wax layer makes them more sensitive or vulnerable to environmental stresses due to the aforementioned process. Similarly, a study conducted by Koornneef *et al*. (1989), showed that *A. thaliana* with the *cer10* mutation only grew to a third of the height of wild type plants.

While we were able to obtain statistically significant results, sources of error may have still been present. Despite our efforts to hold soil moisture constant, it may not have been kept constant during the weekends when we were not taking measurements and could not replenish the water supply. This could have led to the dehydration of some of the plants that died in the 30^0C incubator. Similarly, we tried to maintain a constant light intensity, but due to differences between the various incubators, plants in the 11^0C treatment experienced slightly lower light intensity (2960 lux) and plants in the 20^0C treatment were subject to a slightly higher light intensity (3096 lux). Although these differences are small, they still represent a confounding variable that should be parsed out of future experiments (Bailey *et al.* 2001). Furthermore, as a systematic error, the temperature of 11^0C incubator occasionally fluctuated between 10^0C and 13^0C, possibly affecting the growth the plants.

Future experimentation on this subject could focus on the two elements of our study that produced the clearest results: firstly, plants grew much longer stems in the 30^0C treatment compared to cooler temperatures and secondly, that mutant plants grew shorter stems than their wild type counterparts under similar conditions. The gap between 20^0C and 30^0C is very large and a more gradual series investigating intermediate temperatures would help to specify the conditions under which the stem elongation response begins to occur. Furthermore, our experiment set out to quantify overall growth using stem height as a

surrogate, but other measurements that we were unable to take might be more telling about overall growth, such as biomass or O_2/CO_2 concentrations. Plants grown in the 20^0C incubator appeared to be healthier and more robust than plants in other treatments, so using one of these other metrics could reveal other physiological effects that would otherwise not be indicated by measuring the length of stems alone.

Conclusion

Based on our results, we failed to support H_{a1} and failed to reject H_{01}. On the contrary, we found that increased temperature led to an increase stem growth rate of *A. thaliana.* We were, however, able to reject the H_{02} and provide support for H_{a2}; the presence of the *cer10* mutation did have a significant effect on the average stem length of *A. thaliana* when compared to wild type. Lastly, we failed to reject H_{03} and determined that the combined effect of increased temperatures and the presence or absence of the *cer10* mutation did not significantly affect stem length.

Acknowledgements

We would like to thank Dr. Carol Pollock, Dr. Maryam Moussavi, Shannan May-McNally, Katelyn Tovey, and Mindy Chow for all of their guidance throughout our experiment. They provided invaluable suggestions for improving our experimental design and procedures. We would also like to thank Mindy Chow for allowing us to access the incubators every weekday. Thank you to UBC for giving us the opportunity to take this course.

Literature Cited

Aarts, M. G. M., Keijzer, C. J., Stiekema, W. J., and Pereira, A. 1995. Molecular characterization of the CER1 gene of *Arabidopsis* involved in epicuticular wax biosynthesis and pollen fertility. The Plant Cell, **7** (12): 2115-2127.

Bray, E. A. 1997. Plant responses to water deficit. Trends in Plant Science, **2** (2): 48-54.

Collett, C. E., Harberd, N. P., and Leyser, O. 2000. Hormonal interactions in the control of *Arabidopsis* hypocotyl elongation. Plant Physiology, **124** (2): 553-562.

Dorn, L. A., Pyle, E. H., and Schmitt, J. 2000. Plasticity to light cues and resources in *Arabidopsis thaliana*: testing for adaptive value and costs. Evolution, **54** (6): 1982-1994.

Gray, W. M., Östin, A., Sandberg, G., Romano, C. P., and Estelle, M. 1998. High temperature promotes auxin-mediated hypocotyl elongation in *Arabidopsis*. Proceedings of the National Academy of Sciences, **95** (12): 7197-7202.

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, **108** (1): 369-377.

Koornneef, M., Hanhart, C. J., and Thiel, F. 1989. A genetic and phenotypic description of *eceriferum* (*cer*) mutants in *Arabidopsis thaliana*. The 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.

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.

Unseld, M., Marienfeld, J., Brandt, P., and Brennicke, A. 1997. The mitochondrial genome of *Arabidopsis thaliana* contains 57 genes in 366,924 nucleotides. Nature Genetics, **15** (1): 57-61.

Zheng, H., Rowland, L., and Kunst, L. 2005. Disruption 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.