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

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Abstract

The objective of this study was to determine whether dehydration treatment had a differentiated effect on the growth rate of wild-type and *cer10* mutant *Arabidopsis thaliana*. We treated half of the mutant and wild-type replicates to dehydration conditions, which were watered up to 0.5 cm from the bottom of the tray weekly, and the other half with control conditions, which were watered up to 2.5 cm three times weekly. We measured the height of all the plants each week for four weeks. We then calculated the mean growth rates for the control wild type (5.31 ± 1.44 cm/week), control mutant (1.34 ± 0.82 cm/week), dehydration wild type (3.11 ± 1.88 cm/week), and dehydration mutant (2.80 ± 0.51 cm/week). We analyzed these data using a two-way ANOVA and found that dehydration did not have a statistically significant effect on the growth rate, while the presence of the *cer10* mutation, which negatively affects very long chain fatty acid (VLCFA) synthesis resulting in waxless mutants, had a significant effect on the growth rate. Moreover, dehydration treatment slowed the growth rate of wild-type *A. thaliana* significantly more than the *cer10* mutant. This was unexpected as it was assumed due to the lower cuticular wax load on mutant stems would increase water loss, which was predicted to cause the growth rate of the dehydration group mutants to be much lower than wild type.

Introduction

Arabidopsis thaliana is a small flowering plant, with a short life cycle requiring only six weeks from the time of germination to seed production (TAIR). Under normal circumstances, *A. thaliana* usually self-pollinates, however, it is possible to cross-pollinate it experimentally (Meinke *et al.* 1998). It was the first plant to be genetically sequenced (Arabidopsis Genome Initiative 2000). Since then, the plant has been used extensively in multiple studies such as flower development and morphogenesis (TAIR). At the University of British Columbia (UBC), *A. thaliana* is currently being used by Dr. Ljerka Kunst to study plant fatty acid and lipid metabolism.

This research project aims to evaluate the impact of dehydration treatment on wild-type *A. thaliana* and a waxless mutant (*cer10*) and to test specifically whether dehydration has differentiated or similar impacts on mutant and wild-type *A. thaliana*. This is important for the purposes of establishing whether mutant plants are better equipped to withstand this extreme condition.

Plant height is a good indicator of the total development of plants. Engelmann and Schlichting (2005) found that plants grown in high water levels had more leaves and branches and had taller stems on average than plants grown in low water levels. We were interested in measuring the stem height for a set period of time in order to approximate the average growth rate of the *cer10* mutant and wild-type *A. thaliana*. Another study evaluated the responses of wild-type *A. thaliana* to water deficit and high temperatures and found that plant growth was significantly reduced under both stresses (Vile *et al*. 2012). However, the study also recorded that some traits responded differently. For instance, in response to water deficit, *A. thaliana* increased root allocation.

Wild-type *A. thaliana* from the Columbia ecotype (wax coated) and *cer10* mutant (waxless) were used as replicates. We found evidence suggesting that the Columbia ecotype *A. thaliana* (wild type) had a higher drought tolerance due to a variety of mechanisms such as the assignment of biomass to vegetative organs in comparison to other ecotypes (Meyre *et al.* 2001). The *cer10* mutation causes a defect in the enoyl-CoA reductase (ECR) protein that interrupts the synthesis of very long chain fatty acids (VLCFA) yielding a waxless phenotype (Zheng *et al.* 2005). Rashotte *et al.* (2000) studied several different (*cer)* mutants and state that the stem epicuticular wax load of the *cer10* mutant is significantly lower than on the wild-type stem. Furthermore, Bernard and

Joubès (2013) note that there are many genes responsible for wax production in *A. thaliana* and the biosynthesis and secretion of these plant waxes can differ (Kunst and Samuels, 2003). We expected the wax-coated wild type would thrive better in response to the dehydration treatment, because the mutant does not have the wax coating that plants need to retain water (Jenks and Ashworth 1999, Dylan *et al.*, 2009). This led to the development of the following hypotheses for this study:

Ho_1: Dehydration has no effect on growth rate of *A. thaliana*.
Ha_1: Dehydration has an effect on growth rate of *A. thaliana*.

Ho_2: Presence of the *cer10* mutation has no effect on the growth rate of *A. thaliana*.
Ha_2: Presence of the *cer10* mutation has an effect on the growth rate of *A. thaliana*.

Ho_3: The effect of dehydration on the growth rate of *A. thaliana* is the same or greater in wild type than in *cer10* mutant.
Ha_3: The effect of dehydration on the growth rate of *A. thaliana* is less in wild type than in *cer10* mutant.

Methods

The *A. thaliana* seeds that were provided to us were planted on September 30th and October 1st by multiple groups of students including us, and we transferred the seedlings on October 20th. We transferred the seedlings to pots filled with wet soil containing 100 ml of water for each pot. We used two types of plants: 40 wild type and 40 *cer10* mutants, with two treatments: control which had normal water levels, and dehydration. Each pot consisted of four seedlings, and we took the average of their

growth (taken from estimates of stem height) to give us one replicate per pot. Five pots (replicates) were assigned for each condition: control wild type, control mutant, dehydration wild type, and dehydration mutant. Then we divided those pots into two trays, one for control and one for dehydration (Figure 1).

Figure 1. Set-up of the control tray. Ten replicates were used, five for *cer10* mutant and five for wild-type *Arabidopsis*. Each pot contained four seedlings.

We watered the control tray three times a week for four weeks, keeping the water level at 2.5 cm from the bottom of the tray, which we measured with a standard ruler. We

decided on this water level according to the recommendations of the online *Arabidopsis* growing manual for Centre for Plant Lipid Research at University of North Texas (Cotter 2005). For the dehydration treatment, we watered the tray once per week and refilled the water level to only 0.5 cm from the bottom of the tray. All the replicates were watered at around the same time of day, 1:00-2:00 PM. We used tap water throughout the entire experiment. When we were finished with watering, we stored both trays in the same incubator where temperature was held at a constant 20ºC and light intensity was kept at a constant 6380 lux.

We collected the data on Wednesdays at 2:30 PM for four weeks. We measured the height of the stems in centimeters using a ruler, from the soil level to the tip of plants. We then calculated the growth rate in centimeters per week for each replicate using the slope of the best-fit line. We then used the mean growth rate values for ANOVA analysis. Finally, we calculated the total mean growth rates in cm/week and graphed it along with the 95% confidence intervals.

Results

We calculated mean growth rate for control plants to be 1.71 cm/week and plants that underwent dehydration to be 1.55 cm/week, with 95% confidence intervals of ± 1.12 cm/week and ± 0.69 cm/week respectively (Figure 2). Upon applying the two-way ANOVA test, we obtained a p-value of 0.63 for our first set of hypotheses.

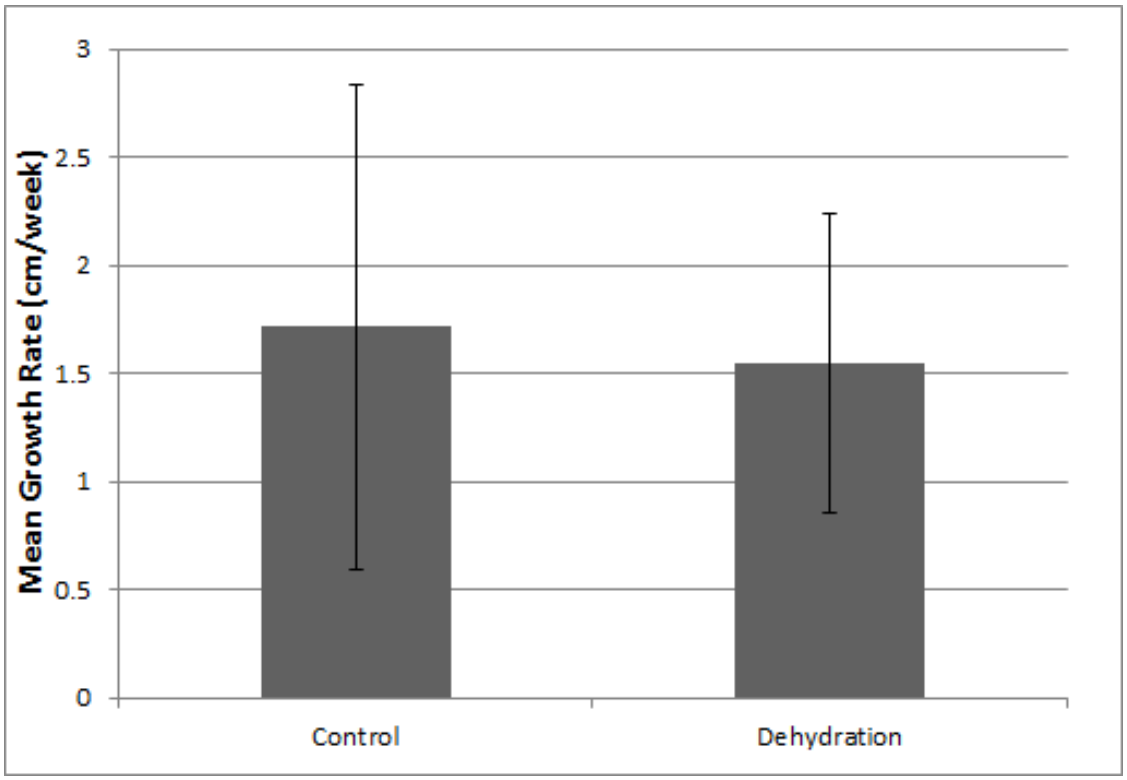

Figure 2. Growth rate of *Arabidopsis thaliana* in centimetres per week under control condition (watered up to 2.5 cm from the bottom of tray, three times a week. n=10), and dehydration condition (watered 0.5 cm from the bottom of tray, every Monday, n=10). 95% confidence intervals are represented with vertical error bars.

We calculated the mean growth rate for wild-type plants to be 2.19 cm/week and mutant plants to be 1.07 cm/week, with 95% confidence intervals of ± 0.98 cm/week and ± 0.50 cm/week respectively (Figure 3). The calculated p-value for the second set of hypotheses was 0.0050. The calculated mean growth rate for control wild type was 2.74 cm/week, control mutant was 0.69 cm/week, dehydration wild type was 1.64 cm/week whereas the *cer10* mutant was 1.45 cm/week, with 95% confidence intervals of mean ± 0.78 cm/week, ± 0.44 cm/week, ± 0.99 cm/week and ± 0.27 cm/week respectively (Figure 4). The p-value for the third set of hypotheses was 0.016.

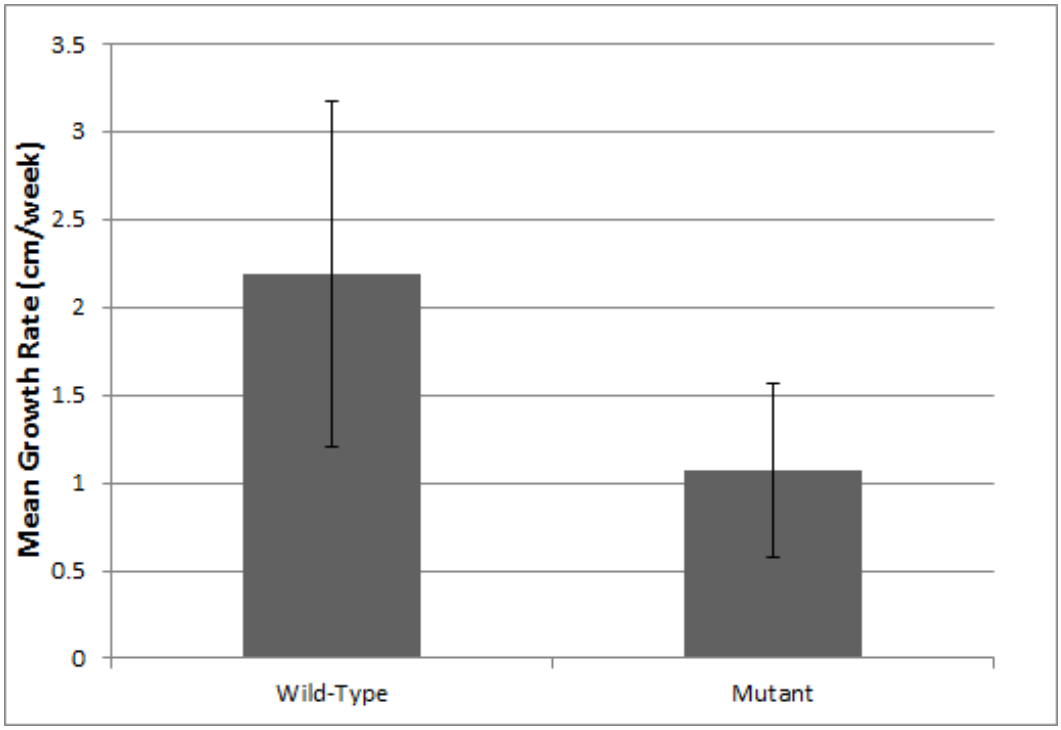

Figure 3. Growth rate of *Arabidopsis thaliana* in centimetres per week for wild-type plants (n=10), and *cer10* mutant plants (n=10). 95% confidence intervals are represented by the vertical bars.

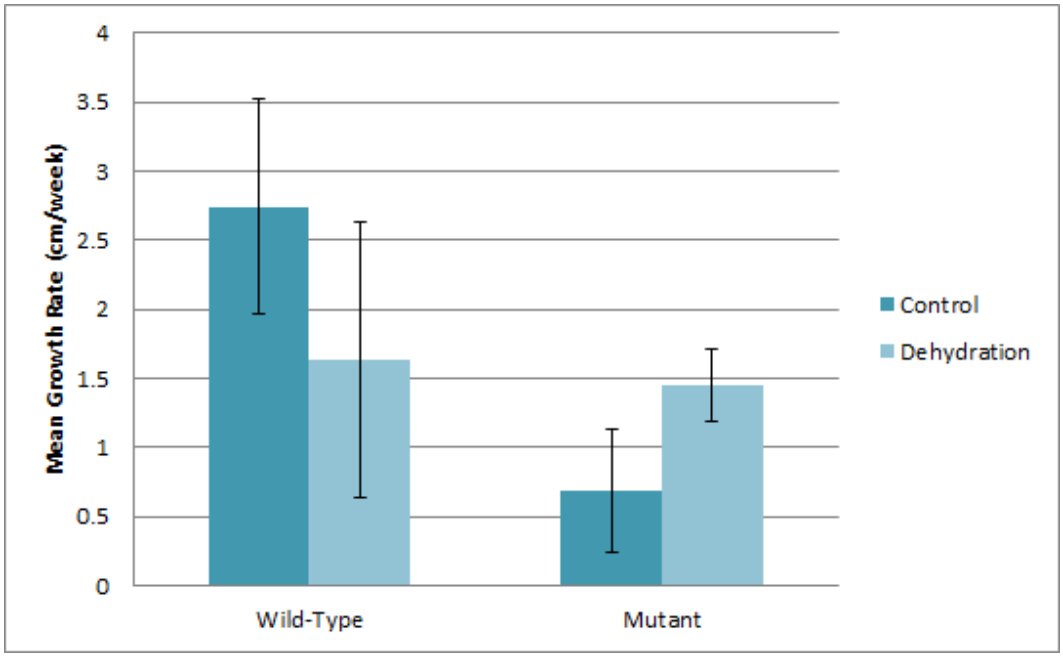

Figure 4. Mean growth rate of wild-type and mutant *Arabidopsis thaliana* in centimetres per week for control and dehydration conditions (n=5 for each group). Plants in the control treatment were watered 2.5 cm from the bottom of tray, three times a week whereas plants in the dehydration treatment were watered 0.5 cm from the bottom of tray, once a week. 95% confidence intervals are represented by vertical bars.

Qualitative Observations

We first observed *A. thaliana* flowers budding in week three for the wild type, and week four for the *cer10* mutants. The first stem observation occurred during the second week of data collection. Moss was observed on the control group pots during the fourth week.

Discussion

Data Analysis

The results were analysed statistically using a two-way ANOVA test.
For our first set of hypotheses we obtained a p-value of 0.63. As this is greater than the 95% significance level of 0.05, we fail to reject Ho_1 and therefore, fail to support H_{A1}: Dehydration has an effect on growth rate of *A. thaliana.*

Our results support that dehydration treatment has no effect on either the mutant or wild-type plants. This was unexpected when compared to previous research done which indicated that a deficiency in water retards plant growth (Bray 2004). The experiment conducted by Bray (2004) on *A. thaliana* plants suggest that three sets of genes were most commonly repressed under water stress and are stated as follows along with the protein: *EXGT-A1* (xyloglucan endotransglycosylase), *AtGER1* (germin-like protein) and *AtGER3* (germin-like protein). The expression of these genes promotes cell expansion, therefore, when these genes were repressed, we should observe reduced plant growth. The reason we failed to reject H_{O1} was probably because we could not prevent the expression of the three genes due to excessive watering. Therefore, there was no significant difference in the growth compared to the control plants that were watered

abundantly. Furthermore, the mutant plants of the control group grew much slower than all the other groups, due to the seed quality. The mutant control seedlings were noticeably weaker when we transferred them reducing the average result for all the replicates in the control group in comparison to the dehydration group.

The test for the second pair of hypotheses had a p-value of 0.005, which is smaller than the significance level of 0.05 leading us to reject the Ho_2 and therefore, accept Ha_2: Presence of the *cer10* mutation has an effect on growth rate of *A. thaliana*.
This result suggests that the presence of the *cer10* mutation has an adverse effect on the growth rate of these plants in comparison to the wild-type replicates. This mutation causes a disruption in gene coding for the protein enoyl-CoA reductase (ECR). ECR is necessary in very long chain fatty acid (VLCFA) elongation, and is essential for cuticular wax production. This leads to delayed development and slower growth rate of the mutant plants (Zheng *et al.* 2005). Since the wild-type plants did not have this deficiency, they likely experienced uninterrupted growth rate.

The test for the third pair of hypotheses yielded a p-value of 0.016, which is less than the significance level of 0.05. However, we could not reject Ho_3, because the two-way ANOVA analysis is non-directional, but our hypothesis is directional. Since the direction was the opposite of what we expected, we failed to reject H_{O3}: The effect of dehydration on the growth rate of *A. thaliana* is greater in wild type than in the *cer*10 mutant. Although these results were unexpected, we found a similar phenomenon in the literature. Aharoni *et al*. (2004) experimented with wild-type and a *shine* mutant *A. thaliana*, which produced six-fold more cuticular wax than the wild type. The overexpressed mutant, however, had increased cuticle permeability, probably due to

changes in its ultrastructure. This study found that *shine* mutants, even though they had higher water permeability, which is similar to the *cer10* mutant in our study, showed significant drought tolerance. This could be due to reduced stomatal density, a factor we did not take into account. This may be an explanation for the result we obtained.

Uncertainty and variation

There was some systematic uncertainty associated with the ruler, which we quantified as ± 0.5 mm. Since we did not use the same ruler for each measurement, this might have contributed to the variations in our data as well. There were other limitations such as human errors that contributed to the uncertainty of our results. During data collection, we took turns measuring the height of *A. thaliana*, which could have led to a variation in the data collected.

In addition, biological variation could have also played a factor in the way our results turned out. Some of the *A. thaliana* plants we used were not planted by us, but by another group that placed hundreds of seeds in one pot. When we transferred those seedlings to our own pots, it was difficult to separate their roots without damaging other roots, which may have led to the weakening of some seedlings. Consequently, these pseudo-replicates showed little to no growth. In some cases, it was hard to decide if a plant should be considered dead or not, so we decided to exclude all plants that showed a final height of less than 1 cm in our final week of data collection.

Procedural uncertainty could be another reason why we failed to reject our Ho_1, supporting that dehydration has no effect on the growth rate of *A. thaliana.* For our dehydration treatment, watering the plants up to 0.5 cm from the bottom of the tray once

a week might have been more than enough for the plants' survival. Furthermore, our decision to water the control group up to 2.5 cm from the bottom of the tray three times a week might have been excessive, indicated by moss growth on the soil.

Conclusion

We established that the growth rate is not the same in wild-type and *cer10* mutant plants using different water levels, but not in the direction we expected. Therefore, we failed to reject Ho_3, meaning the effect of dehydration on the growth rate of *A. thaliana* is greater in wild type than in the *cer10* mutant. Furthermore, we found that dehydration has no effect on the growth of *A.thaliana* and rejected our hypothesis that a mutation in the cer10 gene has an effect on the growth of *A. thaliana*. We concluded that *cer10* mutant plants survived better than the wild-type plants under drought conditions even though they had stunted growth.

Acknowledgements

We would like to thank Dr. Carol Pollock, our teaching assistant Shannan May-McNally, and our lab technician Mindy Chow for providing us information about *A. thaliana* and the materials to conduct our experiments. Also thank you to the other *A. thaliana* groups who donated their seedlings to this study. Lastly, we would also like to thank UBC for funding this project.

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