Impact of water deficiency on the growth rate of wild-type and *cer10* mutant Arabidopsis *thaliana*

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ABSTRACT

We observed the effect of water deficiency on the growth rate of wild-type and *cer10* mutant *Arabidopsis thaliana* plants. Replicates consisted of 20 total pots, 10 containing two 14-day-old wild-type seedlings each and 10 containing two 14-day-old *cer10* mutant seedlings each. Five of each pot type were treated with 125 mL of water, and the other five received 50 mL when watered. Length of the longest rosette leaf for each plant was measured on days 1, 8, 13 and 19 and a two-way ANOVA was conducted on the growth rates between days 8 and 19. Our results indicate that reduced water availability decreases the growth rate of both the wild-type and *cer10* mutant *A. thaliana* ($p=0.007$). Furthermore, the effect of water availability on the growth rate appeared to be not significantly different for both the wild-type and *cer10* mutants ($p=0.502$). In addition, there is insufficient evidence to support that wild-type *A. thaliana* has a greater growth rate than the *cer10* mutant variety ($p=0.0831$).

INTRODUCTION

Arabidopsis thaliana has been widely used as a model organism because of its short life cycle and small genome (Koornneef and Meinke 2010). The mutant used in this experiment has a deletion mutation in the *ceriferum10* (*cer10*) gene that codes for the Enoyl-CoA reductase (ECR) enzyme. The ECR enzyme is responsible for the synthesis and elongation of the precursor to wax molecules, very-long-chain fatty-acids (Samuels *et al*. 2008). As a result of this mutation, *cer10* mutants have 60% less cuticular wax than the wild type (Samuels *et al.* 2008).

This experiment investigates the effects of water availability and plant type on the growth rate of *Arabidopsis thaliana*. The ecotype of *Arabidopsis thaliana* used in this experiment is Columbia. We aim to provide insights into the effectiveness of wax layers under various water stress, focusing on plant development and the extent to which wax and water amounts affect growth. This study is useful as it shows us how *A. thaliana,* as a representative of most plants, will respond during water supply shortages and drought conditions**.** Three sets of hypotheses are tested in this experiment:

Ho_1: Reduced water availability has no effect or increases the growth rate of *Arabidopsis thaliana*.

Ha_1: Reduced water availability decreases the growth rate of *Arabidopsis thaliana*.

Ho_2: The wild-type *Arabidopsis thaliana* has an equal or lower growth rate than the *cer10* mutant.

Ha_2: The wild-type *Arabidopsis thaliana* has a greater growth rate than the *cer10* mutant.

Ho_3: The effect of water availability on the growth rate of *Arabidopsis thaliana* is the same in the wild type and *cer10* mutant.

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

Water is an essential component in the development and maintenance of plants. A sufficient amount of water is important for plant cells to grow to an appropriate size as well as to trigger cell division (Bray 1997). When water supplies are insufficient, plant cells would be smaller and will take longer to reach the minimum size for division (Bray 1997). Thus water-deficient plants are generally expected to be smaller in size when compared to plants grown with sufficient water. The magnitude of growth rate difference due to differing water supplies is examined in the first set of hypotheses.

The wax layer of plants acts as a protection against various external stresses (Shepard and Griffiths 2006). One important function of the wax layer is to control the plant's rate of water loss (Shepard and Griffiths 2006). The hydrophobic nature of the wax determines the water permeability of the plant cuticle. It therefore follows that plants in dry locations typically have thicker cuticular wax layers (Shepard and Griffiths 2006). Accordingly, plants with greater wax accumulation should retain more water, making more available for cell development and

division. The relationship between growth rate and cuticular wax level is examined in the second set of hypotheses. The last set of hypotheses looks at the interactive effects of water availability and the amount of wax on the growth rate of *A. thaliana*.

METHODS

We had two control groups: the first control group consisted of wild-type *Arabidopsis thaliana* seedlings and the second consisted of *cer10* mutant seedlings. We gathered fourteen-day-old wild type and *cer10* mutant seedlings, dry potting soil, tweezers, calipers, 20 four-inch pots, two 125 mL flasks and tap water (as seen in Figure 1). We labelled the top of each pot with one of four assigned treatments: Treatment 1: Wild-type normal, Treatment 2: Mutant normal, Treatment 3: Wild-type dry and Treatment 4: Mutant dry. Five pots were assigned per treatment and then we placed two cups of dry potting soil in each pot.

Figure 1. Measuring equipment (a), and amount of water given to the normal (125 mL) and dry (50 mL) treatments (b). Initial state of pots before planting seedlings (c, d and e). Wild-type (f) and *cer10* mutant (g) *A. thaliana* seedlings.

Next, two seedlings that were grown in a Conviron Controlled Environments incubator under 24-hour light at 20 °C were taken at random and planted into each pot equidistant from the

center and the edge, opposite one another. Before planting each seedling, we measured its longest rosette leaf and recorded those measurements (seedlings seen in Figure 2). The average of the two rosette leaves were used for our data analysis and were counted as a single replicate. We then added 125 mL of water to each of the 10 pots labeled "Wild-type normal" and "Mutant normal", which acted as control groups. Only 50 mL of water were added to each of the 10 pots labelled "Wild-type dry" and "Mutant dry", the experimental groups. The control groups were given 125mL of water in order to fully saturate the soil, replicating normal laboratory growing conditions (Mindy Chow, personal communication). The experimental groups were only given 50 mL of water, leaving their soil unsaturated.

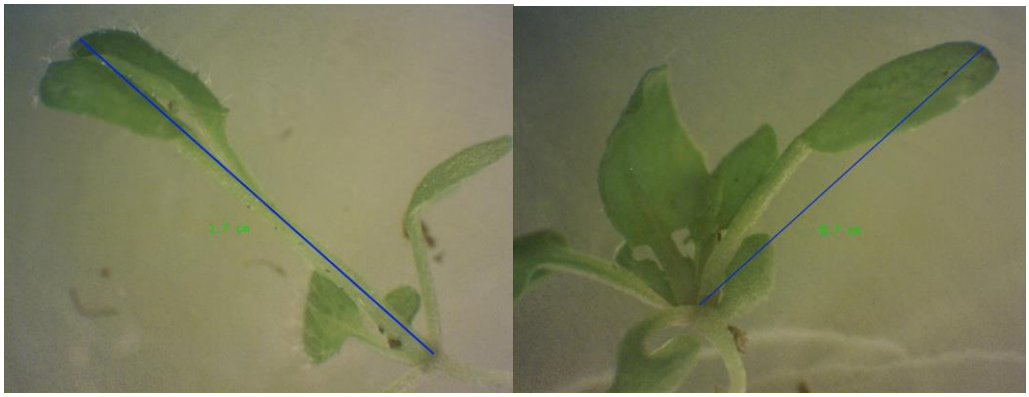

Figure 2. From left to right, Day 1 *A. thaliana:* WT, Mutant (Dinoscope image). Picture taken at magnification of 25x.

After watering each replicate, we placed the 10 "Normal" pots on one tray and the 10 "Dry" on another, keeping similar water treatment pots together to prevent water uptake from the bottom of the tray by the dry pots. The trays were then placed in the same Conviron incubator under the same conditions (24-hour light, 20°C) and left to grow undisturbed between measurements. We measured and watered the replicates once a week. The appearance and general health of each replicate was recoded as well (as seen in Figure 3).

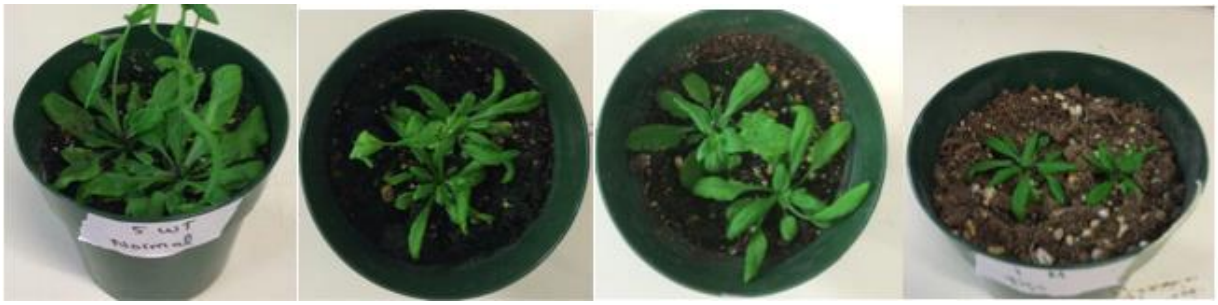

Figure 3. From left to right, day 19 *A. thaliana*: WT-Normal, Mut-Normal, WT-Dry, Mut-Dry

Leaf length was recorded on Days: 1, 8, 13 and 19. For our data analysis, in order to see the differences in lengths among treatments, we plotted the mean leaf length of each treatment. The statistical software R was used to perform a two-way ANOVA analysis on the selected data at the 95% significance level.

RESULTS

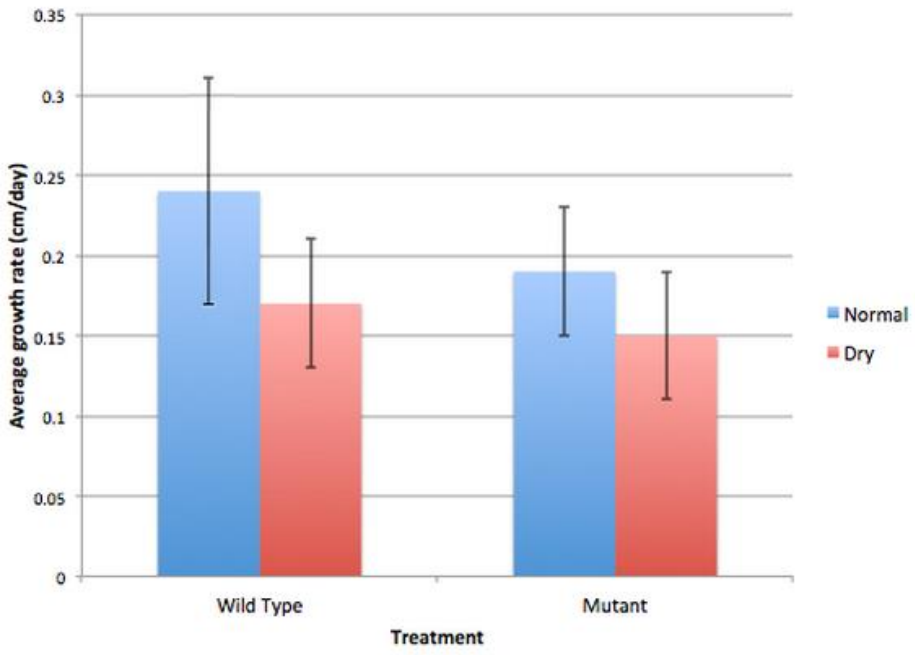

Figure 4. Average growth rate (cm/day) of the four treatments, n= 5. The error bars represent 95% Confidence Intervals.

After assuming that the seedlings required time to acclimate to their new environment after being transplanted on Day 1, we decided to use the measurements made from Day 8 to 19

in our analysis. Removing this first set of data points left us with four fairly constant growth rates.

Sample calculation of growth rate:

growth rate = (average length at day 19 - average length at day 8) cm / 11 days.

growth rate of WT normal replicate = (4.75 - 1.7) cm / 11 days

growth rate of WT normal replicate = 0.244 cm/day

Repeat the calculation for every replicate.

The values were then used for a two-way ANOVA test to calculate the p-values between plant types, between water availability, and between water availability and plant types. The F-value for the effect of water availability was 3.417. For the effect of the plant type it was 9.710, and the combined effect of the two factors was 0.471. The associated p-values were 0.0067 for the water treatments, 0.0831 for the plant types, and 0.5022 for the interaction of the two factors.

One-sample t-test was performed for each treatment group to obtain the 95% confidence interval with degrees of freedom of 4. The respective 95% confidence intervals for the wild-type normal, mutant normal, wild-type dry and mutant dry were 0.24±0.07 cm/day, 0.19±0.04 cm/day, 0.17±0.04 cm/day and 0.15± 0.04 cm/day and are illustrated in Figure 4. Generally, the plants in the normal treatment showed greater growth rates than those in the dry treatments. Also, the wild type plants had greater growth rates than the mutant plants under the same treatment (Figure 4). The amount of variation in growth rates was roughly equal for all groups except the wild type normal group with the widest 95% confidence intervals (Figure 4).

DISCUSSION

Based on a two-way ANOVA analysis, the p-value for the effect of water availability is 0.007, which is less than 0.05. We reject Ho_1 and provide support for the alternative hypothesis:

reduced water availability decreases the growth rate of *A. thaliana*. The amount of accessible water has a significant effect on the growth rate of *A. thaliana* regardless if wild-type or mutant. Our results are similar to past literature; according to a study by Weele (2000), *A. thaliana* seedling shoots did not grow at all under severe water deficit stress, but grew slowly at moderate stress. In another study, researchers examined the growth responses of *A. thaliana* seedlings to water deficit, and on average, water deficit was very detrimental for leaf production (Vile *et al.* 2011). Plants require water for structural support, productivity and growth. They use most of the absorbed water from soil for transpiration, which is a loss of water from the plant's stomata in vapour form (Hsiao 1973). However, only a small portion is used during photosynthesis for producing carbohydrates for plant's growth. A water deficit causes the closure of stomata and a decrease in transpiration and photosynthesis (Hsiao 1973). When a plant's stomata close, CO_2 is unable to enter the leaf and this causes photosynthesis to stop (Hsiao 1973). In addition, a reduction in transpiration causes an increase in the internal temperature of the plant and decreases the plant's growth (Hsiao 1973).

We fail to reject Ho_2 based on the two-way ANOVA analysis. The calculated p-value is 0.083 and is greater than 0.05. This indicates that plant type does not have a significant effect on the growth rate of *A. thaliana*. We fail to support Ha_2 as wild-type *Arabidopsis* does not have a higher growth rate than the *cer10* mutated variety. We did not expect this, as both the literature and our own observations of mature plants indicated that the wild type grew both taller stems and longer rosette leaves than the *cer10* mutant in the same amount of time (Zheng *et al.* 2005*).* From our initial measurements it is apparent that wild-type *A. thaliana* seeds grow at a faster rate than *cer10* mutant seeds under normal lab conditions. At the time of transplantation (seedlings = 14 days old), mutant seedlings were a full centimeter shorter than their wild-type counterparts.

So although the wild-type plants reached a greater final length, the rate of growth was not significantly different between the two types of *Arabidopsis* over our selected period of study.

Finally, we fail to reject Ho_3 based on the p-value 0.502 being greater than 0.05, so we cannot support Ha_3. This indicates that the effect of water availability on growth rate of *Arabidopsis* is the same in both the wild-type and *cer10* mutant varieties. This was our last hypothesis and we wanted to see if the mutant variety would be less able to cope with restricted water conditions than the wild-type due to the decreased amount of wax in mutants. It appears that restricted water conditions have the same effect on the growth rate of both wild-type and *cer10* mutant *A. thaliana*.

Our observations showed the wild-type replicates having broader and longer rosette leaves than the mutants from day 8 to 19. This was due to the fact that at the time of transplantation, wild-type replicates started out larger on average (Figure 2). As the experiment progressed, even with restricted water conditions, both wild type and mutants grew at similar rates. By the end of day 19, the mutant replicates were still smaller than the wild-type, but only by the same initial amount. Our finding that water availability had the same effect on the growth rate of both the wild-type and *cer10* mutant is opposite to that of Wen (2009), which stated that the defect in the mutant's wax composition should have hindered the plant's cuticle function of preventing water loss. This discrepancy may be a result of adding too much water (50 mL) to the "Dry" replicates, therefore not restricting them to a level that would make a difference in coping ability and limit growth.

In order to minimize error due to biological variation, we used five replicates for each treatment, each having two seedlings for us to take an average of. Even so, the mutant plants started at shorter lengths than wild type (0.7 cm vs. 1.7 cm respectively, after 14 days). Our

experiment was also susceptible to human measurement error, as different individuals measured different plants each time. Another challenge we faced when measuring the leaf length of *A. thaliana* was that some of the rosette leaves were growing sideways or curling down rather than growing in the plane of the pot. Therefore, it was difficult for us to measure the true length of the rosette leaves. We also had to use our judgement when choosing the rosette leaves that seemed the longest. As a result, it may not have been the same leaf each time that we measured. For example, with different rosette leaves potentially growing at different rates, a shorter leaf may have overtaken the previous week's "longest leaf", leading to a smaller amount of growth measured and an underrepresentation of the true growth rate. Additionally, though approximately equal volumes of soil were added to each pot, the presence of clumps in the soil may have altered the density of each pot, affecting the water absorption efficiency of *A. thaliana*. We believe that a key error may have been the amount of water being added under dry conditions (50 mL) being too great. By potentially not stressing the plants coping ability, water loss was not significantly greater in the mutant plants than the wild-type, leading to the failure to reject Ho_3. It is recommended that future studies use less water (e.g. 0-25 mL) for the dry replicates in order to obtain significant differences in growth rate.

CONCLUSION

Our results indicate that reduced water availability negatively impacts the growth rate of both wild-type and *cer10* mutant *Arabidopsis thaliana* seedlings. Plant type had no significant impact on the growth rate of *A. thaliana*. Although wild-type *A. thaliana* had the longest rosette leaves on average, there is not sufficient evidence to support wild-type *A. thaliana* having a greater growth rate than the *cer10* mutant variety.

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