The effect of water deficit on the growth rate of *cer10 Arabidopsis thaliana* mutants

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Abstract

Arabidopsis thaliana (*A. thaliana*) is a vascular plant which can be found in a large range of habitats, partly due to its ability to regulate non-stomatal water loss using cuticular waxes. A mutation in the *cer10* gene shows a reduction in the cuticular wax load. The effect of this mutation on the water deficit tolerance of *A. thaliana* was tested by measuring the growth rate in response to changes in soil water content of four groups; watered (control) wild-type, watered mutant, water deficit (experimental) wild-type, and water deficit mutant, (0.791 cm^2/day (±0.0649), 0.255 cm^2/day (±0.124), 0.607 cm^2/day (±0.126), and 0.134 cm^2/day (±0.085), respectively). Plants were incubated at 25°C with eight-hour light and dark cycles. Watered and not-watered plants showed no significant differences in growth rate (p=0.116). Mutant plants in both experimental and control groups showed a significantly lower growth rate than the wild-type plants (p=4.79×10^{-5}), suggesting the mutant is more prone to water stress than the wild type. We observed no statistically significant interaction between water deficit and the presence of the mutation in the *cer10* gene on the growth rate of *A. thaliana* (p=0.734). The *cer10* mutant has been shown to exhibit increased non-stomatal water loss, and thus it is likely that the mutant seedlings had a more drastic decreased growth response when subject to water deficit.

Introduction

A. thaliana is a small, flowering weed in the mustard family, *Brassicaceae* (National Science Foundation 2013). *A. thaliana* is a photosynthetic vascular plant with a fast life cycle of approximately six weeks from germination to seed production (National Science Foundation 2013). It is easily grown, produces many seeds via self-pollination and requires minimal space and nutrients relative to other plants (National Science Foundation 2013).

Most aerial plant species excrete a waxy waterproof layer called the cuticle, which covers epidermal tissues of the shoot such as leaves and stems. This waxy layer helps to protect the plant from environmental stressors such as temperature, light intensity, pathogens and herbivory (Lee & Priestley 1924, Yang *et al.* 2011). Cuticular waxes are also used to control gas exchange and non-stomatal water loss to the environment (Lee *et al.* 2015). They are deposited onto the surface of the plant stem and leaves during development, and the

amount of wax produced can respond to changing soil water concentration and competition for nutrients (Lee *et al*. 2015).

A mutation in the enoyl-CoA reductase (ECR) gene, *cer10*, of *A. thaliana* has been shown to decrease wax accumulation in the cuticle (Zhang *et al.* 2005) and have an effect on the growth of *A. thaliana* (Zheng *et al*. 2005). Associated with the glossy phenotype produced by this mutation, *cer10* mutants' shoots were shorter and the leaves were smaller and more crinkled compared to the wild-type plant (Zheng *et al.* 2005).

The goal of our research is to determine if the mutation in the enoyl-CoA reductase (ECR) gene, *cer10,* has an effect on *A. thaliana* and its tolerance for water deficit conditions. Genes such as *cer10*, which are involved in the synthesis of cuticular wax, are important in identifying adaptations to water deficit conditions (Aharoni *et al.* 2004; Bourdenx *et al.* 2011). By conducting research on changes in water deficit tolerance in response to mutations, results may be applicable to other plants in the *Brassicaceae* family. These include plants that share analogous genes and general biochemical pathways, especially with regards to selection for drought-resistant crops and adaptations to climate change (Le Provost *et al.* 2013).

Our null and alternative hypotheses are:

H_{o1}: Water deficit will not have an effect on the growth rate of *A. thaliana* seedlings.

H_{a1}: Water deficit will have an effect on the growth rate of *A. thaliana* seedlings.

H_{o2}: The presence of the mutation in the *cer10* gene has no effect on the growth rate of *A. thaliana* seedlings.

H_{a2}: The presence of the mutation in the *cer10* gene has an effect on the growth rate of *A. thaliana* seedlings.

H_{o3}: The effect of water deficit on the growth rate of *A. thaliana* seedlings is the same in wild type and mutant.

H_{a3}: The effect of water deficit on the growth rate of *A. thaliana* seedlings is not the same in wild type and mutant.

In an experiment done by Zhang *et al*. (2005), it was shown that the wax accumulation in the cuticle prevents the evaporation of water from plant tissues. The mutation of the ECR gene, *cer10*, has been shown to result in a decreased wax accumulation in the cuticle (Zheng *et al*. 2005). Signs of desiccation include increased dryness of plant tissues and yellowing of the leaves (Pucholt *et al.* 2015). Wax accumulation in the cuticle layer of plants plays an important role in inhibiting water loss, therefore decreasing dehydration (Al-Abdallat *et al*. 2014). We predict that due to a decreased wax accumulation caused by the *cer10* mutation, mutant *A. thaliana* will have decreased water deficit tolerance, and will show signs of desiccation sooner than the wild-type.

Methods

We tested our prediction of the effects of water deficit in *A. thaliana* with four groups; wild-type, mutant, wild-type control and mutant control. Plants in the control groups had a regular watering schedule of 300 mL every two to three days, we withheld water from non-control groups to stimulate water deficit, and temperature was kept constant at 25°C. We used the control group to simulate "normal" growing conditions of *A. thaliana* as a comparison for our experimental (water deficit) group.

We began our experiment by preparing the soil for seedling transplantation by pouring two litres of water into trays containing the experimental and control pots. Before the start of our experiment seeds were germinated in an eight-hour light and dark cycle at 20°C, and watered once a week. We randomly chose seedlings two-week old seedling and repotted them into their respective groups - with five pots per group and three seedlings in each pot (Figure 1).

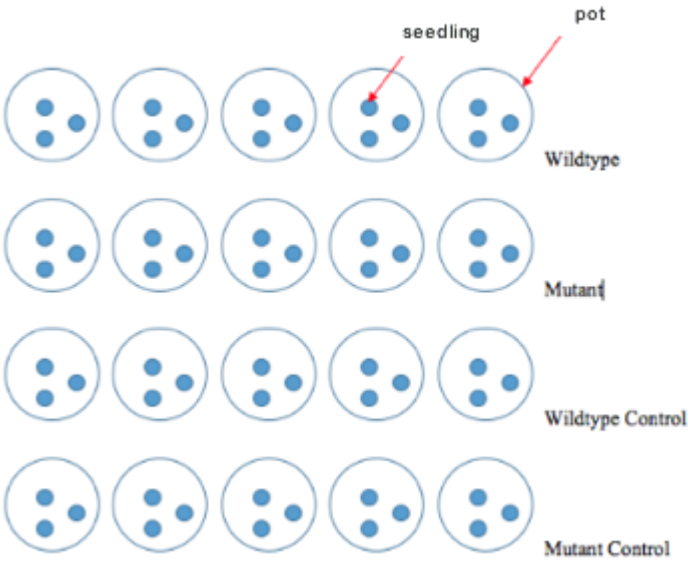

Figure 1. The experimental layout summary includes four groups, each with five replicate pots containing three pseudoreplicate seedlings.

After repotting the plants, we took photographs of each pot alongside a ruler for scale calibration in ImageJ (Figure 2). We held the camera 7.4 cm away from the plant for each photo in order to compare the relative leaf sizes. We took soil measurements for each pot using a Rapitest® moisture meter prior to watering. The incubator was kept at 25°C on an eight-hour light and dark cycle.

Figure 2. The experimental set-up used to analyze the leaf surface area of each replicate of *A. thaliana*. On the left is the experimental wild-type replicate five on day one, and on the right is the same replicate pictured on day 15.

We took measurements of soil moisture content and leaf surface area every Monday, Wednesday and Friday for two weeks. We calculated the leaf surface area using the computer program, ImageJ and traced around the perimeter of each leaf to find the area based on the calibration of the ruler in each photograph. We observed and recorded qualitative data, such as leaf colour and shape. To control for any variation in light or water availability to the plants in the trays, we rotated the pots one position in the tray each day we took measurements. When we saw signs of desiccation such as wilting/falling leaves, we took note of the day and severity of drying.

During data analysis, we took an average of the total leaf surface area for each pot. We calculated the average pot leaf surface area for each day we took measurements, and plotted this against the number of days past for each of the four groups (wild-type water deficit, mutant deficit, wild-type watered, and mutant watered). We used regression lines to obtain the growth rate for each replicate, represented by the slope.

Results

Figure 3 illustrates that in watered conditions, the wild-type plants grew an average of 0.791 cm^2/day (±0.0649), while the mutant plants only grew 0.255 cm^2/day (±0.124). Similarly, in the water deficit condition, the wild-type plants grew an average of 0.607 cm^2/day (±0.126), while the mutant plants grew only 0.134 cm^2/day (±0.085). We conducted a two-way ANOVA test to determine if there is a significant difference between watered vs. water deficit conditions on growth rate and we found a p-value = 0.116. The mutant showed a slower growth rate than the wild-type in both watered and water deficit conditions (p-value = 4.79×10^{-5}). Furthermore, there was a lack of overlap of the 95% confidence intervals

(C.I.) between wild-type and mutant plants. The two-way ANOVA also tested the statistical significance of the interaction between water deficit and presence of the mutation in the *cer10* gene on the growth rate of *A. thaliana*, and we found a *p*-value = 0.734.

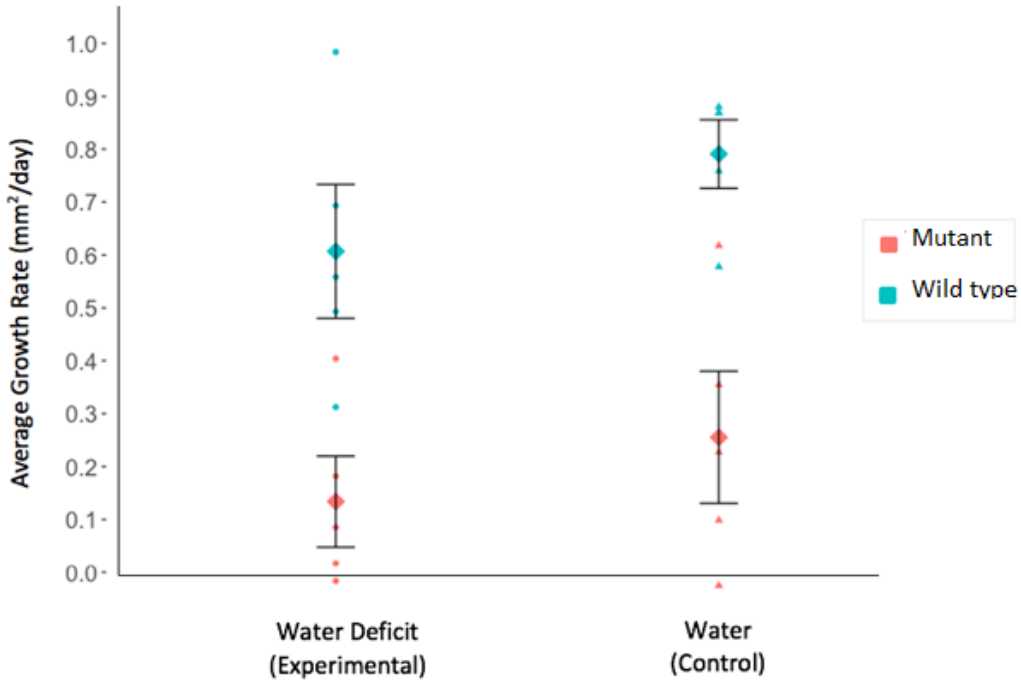

Figure 3. Average growth rates of *A. thaliana* in water (control) and water deficit (experimental) conditions for both wild-type and mutant seedlings. Plants exposed to eight-hour light and dark cycles in 25°C incubator. Mutant replicates n=5; Wild-type replicates n=5. Error bars represent 95% C.I.s. *P*-value for the condition (watered or water deficit) is calculated to be p=0.116. *P*-value for the plant phenotype (either mutant or wild-type) is calculated to be $p=4.79\text{x}10^{-5}$. *P*-value for the interaction between water deficit and presence of the mutation in the *cer10* gene on growth rate is calculated to be p=0.734.

The total surface area of wild-type plants was significantly larger than that of mutant plants. Plant desiccation started to appear on day ten of the experiment. Although none of the plants lost any leaves, the plants appeared to be dead as their leaves had dried, shrunk and turned yellow.

Discussion

The results of a two-way ANOVA analysis indicated that there is no significant difference between the growth rates of *A. thaliana* in watered and water deficit conditions, as

the *p*-value was greater than our α of 0.05 (p=0.116). Thus, we failed to reject our null hypothesis that water deficit do not have an effect on the growth rate of *A. thaliana.*

Although these results did not support our prediction, they are consistent with the observation that very few of the experimental plants showed severe signs of desiccation. The majority of the plants were still photosynthesizing and taking up water from the partially wet soil, which never gave a moisture reading of zero, even by the end of our experiment. Previous experiments have shown complete desiccation of *A. thaliana* three days after a 14-day drought was induced (Pandey *et al.* 2013). As our experiment was conducted only for 14 days, our results may not reflect the true water deficit response of *A. thaliana* under severe water stress. This may be explained by our assumption that desiccation was a linear process, and we would see the water deficit plants slowly desiccating at constant rates throughout our experiment. Contrary to our assumption, previous research on drought tolerance of *A. thaliana* suggests that the rate of water loss can increase over time of water deficit (Aharoni *et al.* 2004). In other words, it is possible that desiccation occurs in a more abrupt fashion than we anticipated, where the plant is able to retain enough water to survive early in water deficit conditions, but loses water more quickly near the end of its stress tolerance. *A. thaliana* are aerial plants that have short-term adaptations to help tolerate water deficit, such as closing their stomata, which are small epidermal pores that expose the inner tissues of the plant to the environment (Samuels *et al.* 2008). Closing of stomata reduces transpiration, the process in which water is transported from the roots up through the plant, and released as water vapour through these epidermal pores. Thus, reducing transpiration allows the plant to retain water that would otherwise be evaporating out into the atmosphere. However, transpiration is necessary for plant survival as it facilitates the transfer of dissolved nutrients from the roots to the shoot tissues, and thus stomata cannot be closed indefinitely (Kirkham 2004). As *A. thaliana* is eventually forced to reopen its stomata in order to photosynthesize,

water is expected to evaporate faster near the end of its water stress period, likely just following a two-week drought period (Aharoni *et al.* 2004). This evidence suggests that it is possible we would have seen a difference between the leaf surface area of watered and dehydrated plants given another few days, after the soil water content had been completely depreciated, and the plant groups were facing maximum water stress.

Although the results did not support our first null hypothesis, they did show a significant difference between the growth rates of the wild-type and mutant *A. thaliana*, as the *p*-value was less than $\alpha = 0.05$ ($p = 4.79 \times 10^{-5}$). Thus, we can reject our second null hypothesis that the presence of the mutation in the *cer10* gene has no effect on the growth rate of *A. thaliana* seedlings, and provide support for our second alternate hypothesis.

A possible explanation for this may be seen in other water deficit studies of *A. thaliana* seedlings, in which plants were shown to be resilient when subjected to moderate water deficit but were unable to tolerate severe water deficit (Weele *et al.* 2000). Water stress is initially sensed in the roots, and a biochemical signal is transmitted up the plant (Hsaio 1973, Schulze & Hall 1982). In response to this, the plant closes its stomata (Samuels *et al.* 2008). Stomata, as described above, facilitate transpiration and nutrient uptake, but also allow for gas exchange with the environment needed to support photosynthesis (Hsaio 1973). Therefore, upon closing stomata in response to water stress, it reduces gas exchange, thus reducing photosynthesis rates. A decrease in photosynthesis results in a slower growth rate (Hsaio 1973). As our mutant has been shown to exhibit increased non-stomatal water loss due to a lack of wax in the cuticle (Le Provost *et al.* 2013), it is likely that this plant responds more negatively to water stress by closing its stomata sooner than the wild type, which does not lose as much water to the environment and can keep its stomata open and continue to photosynthesize. This explanation is consistent with our study when comparing the mutant

and wild-type plants because it supports the trend that the mutants had an average slower growth rate than the wild-type.

Additionally, the mutant shows a smaller average leaf surface area and fewer leaves than the wild type in both watered and drought conditions. This may be explained by the damage to leaf production associated with severe water deficit to seedlings (Vile *et al.* 2011). Specifically, under conditions of water stress, especially during early development, a greater proportion of resources is allocated to the root system in attempt to take up more water (Jackson *et al.* 2000, Schulze 1986). As a result, there are fewer resources available to grow leaves higher up in the foliage of the plant. This explanation is consistent with our observations of smaller mutant plants with fewer leaves. Water is also essential for absorbing nutrients from the soil up through the rest of the plant body (Kirkham 2004). A lack of water provided to the roots could result in inadequate nutrition reaching the leaf cells located at the top of the plant. This insufficient nutrition may also explain the stunted size of the mutant plants.

Finally, the results of the two-way ANOVA showed that we failed to reject our third null hypothesis that the effect of water deficit on the growth rate of *A. thaliana* seedlings is the same in wild-type and mutant, as the *p*-value was greater than $\alpha = 0.05$ (p=0.734). These results did not agree with our predictions that the mutant would have a decreased water deficit tolerance and would show signs of desiccation sooner than the wild-type.

Sources of error should be considered while discussing the implications of this experiment. While great effort was taken to minimize error, some sources of error may have influenced our results. For example, while conducting the experiment, we measured leaf surface area as accurate as possible by using the program ImageJ. While this program was helpful in speeding up the data analysis, it was difficult to trace the exact surface area of each leaf. This would cause a minor misrepresentation of the relative surface area of each leaf

(from day 1 to 15). Another source of error is the seedlings and how they interact with the other seedlings in the pot. By planting three seedlings in one pot we are assuming that they do not have an effect on each other's growth. However, if there is a negative relationship between neighbouring plants, then the effect of water deficit conditions cannot be directly associated with the results of our experiment.

Furthermore, sources of uncertainty may have misled our interpretations of the data. While we had identified the *cer10* mutation in half of the plants, both wild type and mutants could have possessed other mutations that were not accounted for. There have been many other mutations identified in *A. thaliana* that impact water deficit tolerance or development, and the presence of any number of these mutations could have resulted in misleading data (Aharoni *et al.* 2004). It is also important to note that mutation reflects a gradient, and choosing seedlings based on phenotype is not ideal because not all mutants may show the phenotype to the same severity or degree. For instance, the amount of wax accumulation may not only be different between mutant and wild type but also between individuals of the same group, simply due to genetic variation. Since our sample size is small (n=5), it may not have been representative of the trend we would observe in the true population size.

Conclusion

Our results and statistical analysis indicate that water deficit has no significant effect on the growth rate of *A. thaliana.* However, the presence of the mutation in the *cer10* gene has a significant effect on the growth rate of *A. thaliana*. Moreover, the effect of water deficit on the growth rate of *A. thaliana* seedlings is same in the wild-type and mutant plants. Further long-term research could be conducted on the *cer10* mutant in order to establish a temporal progression of the entire desiccation process, and how it may differ from the wild type.

Acknowledgements

We would like to acknowledge Dr. Carol Pollock, Dr. Celeste Leander, Jordan Hamden, and Mindy Chow for their help and expertise in conducting this experiment. We would also like to acknowledge the University of British Columbia for the opportunity to take this course.

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