Effect of light and the *cer10* mutation on the growth rate of *Arabidopsis thaliana*

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Abstract

The presence of light and the presence of a mutation are two of several factors that influence the growth rate of *A. thaliana.* Light affects the rate of photosynthesis directly whereas the presence of the *cer10* mutation affects photosynthesis indirectly. In the case of the *cer10* mutation, there is an absence of epicuticular wax that is responsible for water retention. As such, we sought to investigate the factors of light and the *cer10* mutation on the growth rate of *A. thaliana* seedlings. We set up four different treatment groups: wild type with light, wild type with no light, mutant with light, and mutant with no light. The lengths of *A. thaliana* stems were measured five times during a two-week period. From this, the growth rates of each treatment group was calculated to be 0.20 ± 0.08 mm/day for the wild type light treatment, 0.07 ± 0.06 mm/day for the wild type no light treatment, 0.17 ± 0.08 mm/day for the mutant light treatment, and 0.05 ± 0.02 mm/day for the mutant no light treatment. Our results indicate that the presence of light increases the growth rate of both wild-type and mutant *A. thaliana* (two-way ANOVA, $p = 0.00124$); however the presence of the *cer10* mutation does not affect the growth rate of *A. thaliana*, and the effect of light is the same in the wild type and mutant ($p > 0.05$).

Introduction

Arabidopsis thaliana, commonly known as thale cress, is a model organism for the study of plants. *A. thaliana* is a plant from the mustard family and when fully grown can be 15 to 20 cm tall (Meinke *et al.* 1998). The mutant in our study – *cer10* – is an eceriferum mutant occurring due to a genetic deletion. *Cer10* mutants lack epicuticular wax, which is the wax normally present on the outermost cuticle of the plant.

The purpose of our experiment was to investigate the factors of light and mutation on *A. thaliana* growth rate. We chose these factors to gain more knowledge of how plant growth is affected by light and the importance of epicuticular wax in plant growth. Our hypotheses were:

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

Ha_1: Light has an effect on the 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 light on the growth rate of *A. thaliana* is the same in the wild type and mutant.

Ha_3: The effect of light on the growth rate of *A. thaliana* is not the same in the wild type and mutant.

For our first hypothesis, we predicted that light would affect the growth rate of *A. thaliana* due to photosynthesis because light provides *A. thaliana* with the energy it needs for electron transport and carbon fixation, which allow for growth (Dyson *et al.* 2015). Also, the amount of photosynthetic proteins, called calcium-sensing receptors (CaS), has been shown to increase with increasing light intensity, which provides regulation for photosynthesis (Vainonen *et al.* 2008). CaS allows *A. thaliana* to grow more as increased phosphorylation results in increased photosynthesis. For our second hypothesis, we predicted that the presence of the *cer10* mutation would affect the growth rate of *A. thaliana* as the *cer10* mutants lacked epicuticular wax. A cuticle is present on *A. thaliana* and acts as a permeability barrier of the cell wall; the cuticle is composed of soluble waxes in the matrix that are deposited onto the external surface (Reina-Pinto and Yephremov 2009). The cuticle and the waxes that are subsequently deposited on them, ultimately help to prevent water loss (Reina-Pinto and Yephremov 2009). For our third hypothesis, we predicted that the effect of light on the growth rate of *A. thaliana* would

be different in the wild type and mutant because more wax accumulates on plant leaves in the presence of light (Giese 1975). This wax prevents water loss, enhancing growth.

We propose that the presence of light affects both the wild-type and *cer10* mutant *A. thaliana.* Light increases plant growth, perhaps by causing the amount of CaS to increase, leading to an increase in photosynthesis. We also propose that for the wild type, the presence of light causes wax production to increase, allowing for less water loss and subsequently more photosynthesis, and therefore increased plant growth.

Methods

This experiment was designed to show the effects of light and the *cer10* mutation on the growth rate of *A. thaliana* over a two-week time period. We filled twenty pots to the brim with unfertilized soil. We then planted three seedlings of wild type *A. thaliana* in ten labeled wild-type pots and three seedlings of *cer10* mutant *A. thaliana* in the other ten labeled *cer10*-mutant pots (Figure 1). We placed five of the wild-type labeled pots and five of the *cer10* mutant-labeled pots in one tray and incubated them at 20°C under 24-hour light, with a light intensity of 5340 lux (Figure 2). At the same time, we placed the remaining five wild-type labeled pots and five *cer10* mutant-labeled pots in a second tray in the same 20°C incubator, but with no light as we placed another tray on top of it to block it from the light (Figure 2). Temperature and light intensity were constant throughout the experiment.

Figure 1. Photo taken on Day 0 of three *cer10* mutant seedlings that were planted.

Figure 2. Photo taken on Day 0 of both trays inside the 20°C incubator, in 24-hour light or dark.

We measured the stem length in millimeters with a ruler and measured from the top of the soil to the tallest stem of the *A. thaliana*. We measured all three seedlings in each pot and calculated an average from seedlings that survived. On Day 0, we measured the stem length for each replicate before we placed the trays in the incubator, and we took subsequent measurements on Day 4, Day 7, Day 10, and Day 14. We monitored each tray on the measurement dates to regulate the amount of water needed by filling the trays up to a water level of about 1.2 cm from the bottom of each tray. We made observations on leaf color, the number of leaves present and glossiness.

After two weeks, we calculated the average growth rate from Day 0 to Day 14 of each replicate by subtracting the Day 0 average stem length from Day 14 and then dividing by 14 days. We then ran a two-way ANOVA test to determine the statistical significance of our results.

Results

The mean of each replicate was calculated by averaging the stem length of each of the three seedlings within each pot. Then, the average of all five replicates was calculated

for each of the four treatments, to deduce the average stem length for each day of measurement (Day 0, Day 3, Day 7, Day 10 and Day 14). The average growth rate for each treatment group was calculated by looking at the difference in stem lengths on Day 14, compared to Day 0.

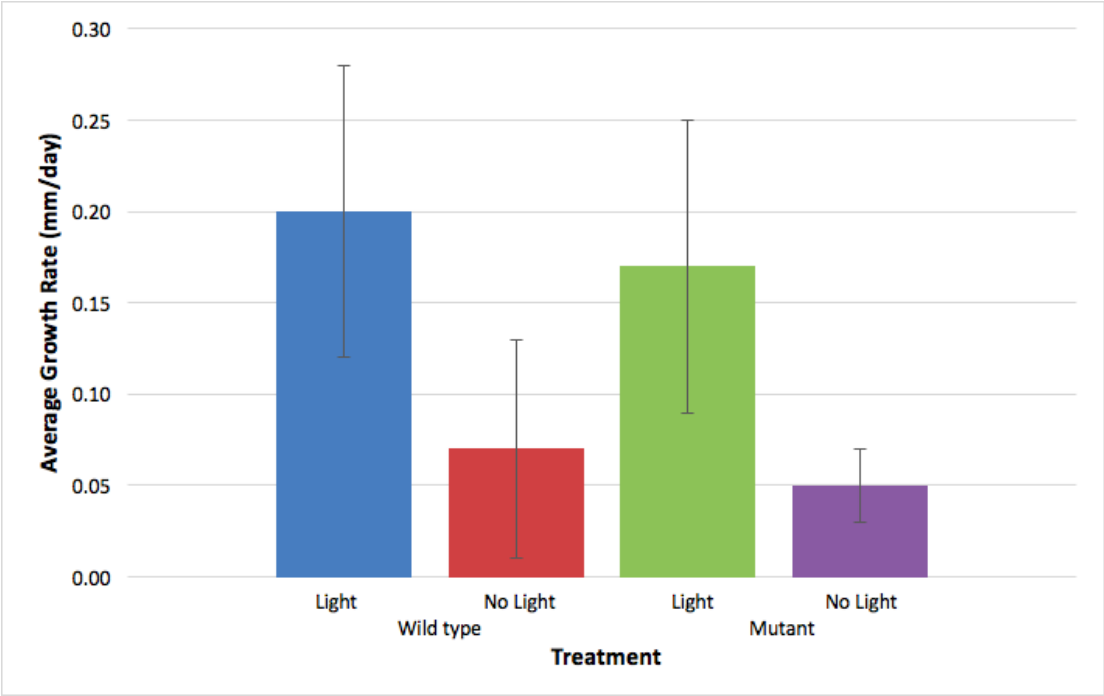

Figure 3. The average growth rate of *A. thaliana* over 14 days for four treatment groups (wild type light, wild type no light, mutant light and mutant no light). Bars represent 95% confidence intervals. n = 5.

The mean growth rate and standard deviation were calculated for each of the four treatment groups to determine the 95% confidence interval. The average growth rates for wild type light, wild type no light, mutant light and mutant no light were 0.20 ± 0.08 mm/day, 0.07 ± 0.06 mm/day, 0.17 ± 0.08 mm/day and 0.05 ± 0.02 mm/day, respectively, as shown in Figure 4.

A two-way ANOVA was performed on all four treatment groups to calculate the *p*-values for the three hypotheses. We obtained a *p*-value of 0.00124 for the first hypothesis, 0.51732 for the second hypothesis 0.85897 for the third hypothesis.

The plants in the light treatment showed significantly faster growth rates than those in the no light treatment, for both wild-type and mutant *A. thaliana* (Figure 3). However, the wild type plants did not show significantly greater growth rates than the mutant plants under the same treatments, as the 95% confidence intervals of the means for wild type light and mutant light and for wild type and mutant dark overlap, (Figure 3).

Discussion

We reject Ho_1 and provide support for Ha_1 because our calculated *p*-value (0.00124) is less than 0.05. This result supports our prediction that light will have an effect on the growth rate of *A. thaliana*, regardless if it is the mutant or wild type. Light may have had an effect on the growth rate of *A. thaliana* because it is one of the main requirements for photosynthesis. According to our proposed mechanism, the CaS molecules located in thylakoid membranes of *A. thaliana* increase in the presence of light; this increases photosynthesis and therefore, plant growth. Vainonen *et al.* (2008) found that an insertion mutation for CaS showed reduced growth in *A. thaliana*. Therefore, we suggest that the *A. thaliana,* in the presence of light, grew because of increased CaS protein levels, while the *A. thaliana* under no light exhibited reduced growth because of lower CaS protein levels. Furthermore, CaS has been noted to play a role in closing stomata of *A. thaliana* (Zhao *et al.* 2015). The closing of stomata is important because most water is lost through stomatal pores (Xu *et al.,* 2015). Thus, in addition to our proposed mechanism, we suggest that the increased amount of CaS in the presence of light also helps limit water from being lost, effectively helping the plant to grow.

On the other hand, we fail to reject Ho_2 because our calculated *p*-value (0.51732) is greater than 0.05. This result does not support our prediction that the *cer10* mutant will have an effect on the growth rate of *A. thaliana*. The *cer10* mutant may not have had an effect on the growth rate of *A. thaliana* because our study may not have been long enough for there to be a significant difference in wax accumulation. Although it was found that the *cer10* mutant had a 60% reduction in total stem cuticular wax, the *A. thaliana* used in that study were about five to seven weeks old (Zheng *et al.,* 2005). It was also found that *cer10* mutants were still able to produce some wax despite not having a fully functional gene for the synthesis of wax (Zheng *et al.,* 2005). Therefore, our results suggest that two weeks may not have been long enough for the growth rates of the wild type and mutant to differ significantly. Furthermore, wax mutants exhibit a glossy appearance, which is associated with a decreased epicuticular wax load (Jenks *et al.,* 1996). However, our qualitative observations suggest that the wax load in the mutant and wild type may have been similar because we were unable to see any differences in glossiness. Thus, we suggest that both the wild type and mutant were able to retain the same amount of water needed for photosynthesis and plant growth.

As for our third hypothesis, we also fail to reject Ho_3 because our calculated *p*-value (0.85897) is greater than 0.05. This result does not support our prediction that the effect of light on the mutant and wild-type growth rates of *A. thaliana* is different. According to our proposed mechanism, we expected the wild type to have a higher growth rate because light was found to increase the wax content on plant leaves (Giese 1975). On the other hand, since the *cer10* mutant did not have the enoyl-CoA reductase needed for wax synthesis, we expected it to have decreased wax even in the presence of

light. Light may have had similar effects on the mutant and wild type growth rates of *A. thaliana* because the amount of wax deposited may have been the same. Light was found to be essential in the transcription of *cer6*, a condensing enzyme that provides precursors for wax composition (Hooker *et al.*, 2002). The overexpression of *cer6* resulted in increased wax accumulation, thus the responsiveness of *cer6* to light plays a huge role in the amount of wax deposited (Hooker *et al.*, 2002). Since *cer6* is not disrupted in both the mutant and wild type, we suggest that light increased transcription of *cer6* allowing a similar amount of wax to accumulate in the mutant and wild type. We also suggest that *cer10* is not light regulated because there was no further wax accumulation in the wild type.

Although we tried to minimize as many potential errors and sources of variation in our study, there are a few that may have impacted our data. We watered the plants based on our three and four day intervals. However, after each interval in the light treatments, we noticed that the water added was already gone by the next interval. For example, on the third day, the plants were watered, but by seventh day, the water in the light treatments had already been used up and the soil was dry. Because we were unsure when the water had run out between intervals, we do not know when our plants were put under water stress. This could have affected how the wax accumulated in each replicate under light conditions because water deficiency was shown to increase the amount of wax (Kosma *et al.*, 2009). To improve this design, we suggest monitoring the water level every day to see if it has run out. Furthermore, some seedlings in each pot died or we could not find them anymore. Since we put three seedlings in each pot, we encountered some situations where two died, making us only measure the stem length of one seedling.

As a result, our data may not have been very accurate because we had to rely on only one or two measurements in some replicates. To get more accurate results, we suggest planting more seedlings in each pot.

Conclusion

We reject Ho_1 and provide support for Ha_1 that the presence of light on *A. thaliana* results in a faster growth rate compared to *A. thaliana* that receive no light, as predicted. However, we failed to reject Ho_2 and Ho_3, indicating the presence of the *cer10* mutation has no effect on the growth rate of *A. thaliana*, and that the effect of light on the growth rate of *A. thaliana* is the same in wild type and mutant, in contrast to our prediction.

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