The impact of mutation at the At3g55360 locus (cer10) results in a reduction in the cell length of *Arabidopsis thaliana.*

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Arabidopsis thaliana has been widely used for genetic analysis in plants due to the vast amount of information available to researchers. The impact of mutations at the At3g55360 locus of *A. thaliana* genome has been explored by many researchers. More specifically the cer 10 mutant has a transcriptional knockout mutation that prevents the synthesis of Enoyl-CoA Reductase, which is vital for facilitating cell elongation. This study explored the morphological impacts of the cer10 mutation on the cell length of *A. thaliana*. Results were collected for cell and leaf lengths across 3 wild and mutant type *A. thaliana* groups with different planting dates. The means, 95% confidence intervals and t-tests were calculated for samples with $n \geq 3$. The average cell lengths ranged from 57.9μm to 68.9μm in wild type cells and 36.2μm to 51.9μm in mutant type cells. Significant differences in average cell length between wild and mutant types were observed at day 28, 49 and 56. Based on the significant differences in the 95% confidence intervals between genotypes of *A. thaliana* along with the t-test analysis from days 28, 49, and 56 after planting, we can reject the null hypothesis and lend support to the alternate hypothesis that average length of mutant type leaf cells is less than the average cell lengths observed in wild type *Arabidopsis thaliana* of the same age.

INTRODUCTION

Arabidopsis thaliana, common name thale-cress, is a small winter annual with a widespread native distribution spanning much of Europe, Asia and Northwestern Africa (GRIN unknown). The plants are small, growing to an approximate height of 20cm, and produce 20-30 seeds per flower (Zheng et al. 2005) *Arabidopsis thaliana* is utilized as a model organism in molecular genetic research due to its various natural attributes. These include its small size, short generation time, ability to self-fertilize, large progeny production, and finally small diploid genome all contribute to create a species that can be easily studied genetically (Haughn and Kunst 2010).

A number of studies have been conducted on *Arabidopsis thaliana* at a genetic level involving the impact of mutations. One such mutation is the cer10 mutant (specifically an At3g55360 locus

mutation). It is a transcriptional knockout that results in a disruption in the production of Enoyl-CoA Reductase (ECR), an essential enzyme in very-long-chain fatty acid (VLCFA) synthesis (Zheng et al. 2005). These VLCFAs are necessary for sphingolipid metabolism, cuticular wax formation and the production of storage lipids. The cer10 mutant has also been shown to have an impact upon endocytosis of cells (Zheng et al. 2005). The loss of ECR leads to an imbalance in the endocytic membrane traffic in the mutant types, likely a result of the changes in sphingolipid levels due to the lack of VLCFA production. Golgi bodies were also found to be larger and clustered in ring-like patterns compared to the wild types (Zheng et al. 2005) with reduced activity compared to wild type cells.

The cer10 mutant cells have been observed to be approximately one-third of the size of wild type cells (TAIR 2003) due to locus cer10's role in sphingolipid formation; a vital part of cellular membrane formation in both the Golgi apparatus and on plasma membranes themselves (Zheng 2005). The stem height in the cer10 mutants has been likened to semi-dwarf varieties (TAIR 2003), an impact of ECR not being present to facilitate cell elongation. Other impacts on plant formation include stem and leaf glossiness and abnormal organ morphology (TAIR 2003). The change in morphology is believed to result in the reduced fertility of the mutant plant due to altered wax and sphingolipid levels on the surface of pollen grains (Preuss 1993). Research on ECR is vital in determining impacts of such genes on cellular processes as well as highlighting potential genes of interest in other plant species.

Our null hypothesis (H_O) for this experiment states that the length of cer10 mutant *Arabidopsis thaliana* leaf cells will be greater than or equal to the cell lengths observed in wild type *Arabidopsis thaliana* leaf cells of the same age.

Our alternate hypothesis (H_A) states that the length of cer10 mutant *Arabidopsis*

thaliana leaf cells will be less than the length of wild type *Arabidopsis thaliana* leaf cells of the same age.

In this study we aimed to compare the wild type average cell lengths to the mutant type average cell lengths to determine the impact of the cer10 mutation on *A. thaliana* observed under laboratory conditions.

METHODS

To test our hypotheses, treatments of wild type and mutant type forms of *A. thaliana* cell lengths were measured. To increase the pool of data, all plants that were available for us to measure were utilized in the experiment. All wild type *A. thaliana* plants that were pre-planted were used as a control to allow for comparison to the mutant type *Arabidopsis thaliana* data.

The experiment was conducted over two weeks, which consisted of two days of data collection where both collection days were spaced one week apart. Week one results were taken on the 30th of October and week two results were taken on the 6th of November. For wild type *A. thaliana*, pots planted on September 4th, September 11th and October 2nd were investigated. For mutant type *A. thaliana*, pots planted on September 4th, September 11th and Oct 2nd, 3rd, and 4th were used. Mutant seedlings showed reduced germination rates so early October seedlings were grouped to provide an adequate number of plants. It is assumed that the three day spread of planting will not have a substantial effect on the cell length of *A. thaliana.*

From each time frame, a total of 4 leaves were sampled. The leaf length was measured using a ruler and recorded in centimeters. To randomize the selection of leaves, a randomizer was created out of the rolled tape provided as seen in Figure 1. This tape was spun and a leaf was chosen from the region containing the star. Each individual leaf's length was measured and

recorded. From each leaf a sample of 5 cells were measured. This gave us a total of 6 replicates, however a total of 8 plants were sampled.

Figure 1. This figure shows the randomizing device that was used to select leaves at random. The device was spun and leaves were selected from the quadrant containing the star. These leaf samples were used to analyze leaf length and cell length of *Arabidopsis thaliana*.

For time frames that contained more than one pot of *A. thaliana*, the pots were aligned so that the distributions of the plants from the separate pots were clustered. The randomizer was spun and then would be placed on top of the area of the clustered distribution. For example, this was done for October 2nd, 3rd and 4th mutants of *A. thaliana* due to the lack of individuals germinating for any one specific date.

To determine the cell length of *A. thaliana*, sectioning of the leaf was necessary. To create these sections (one cell thick preferable), the center of the leaf was sandwiched between two glass microscope slides as seen in Figure 2. A single edged razor blade was used to take a transverse section of the leaf. The razor tip was dipped in a glass dish with distilled water releasing the thin sections from the blade. A small paintbrush was then used to aid in handling of the sections to allow preparation of a wet mount. After each group member calibrated their compound microscope, the leaf sections were viewed so that at least 5 cells were visible in the center of the microscope's field of view at a magnification of 400x (10x ocular, 40x objective). From a single field of view 5 cells were selected for measurement in micrometers as seen in Figure 3.

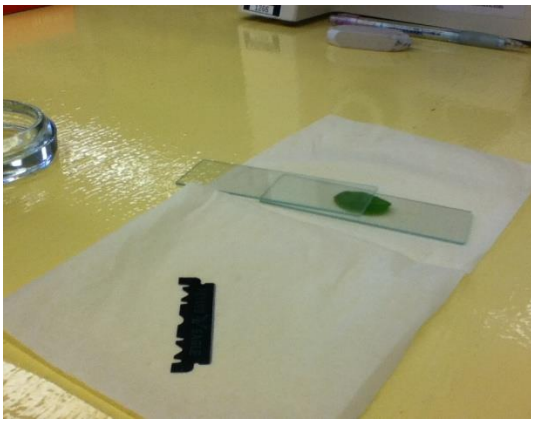

Figure 2. This image displays the equipment and setup used to section leaves. The leaf was placed between two staggered glass microscope slides and the cuts were made against the side of the top slide and cut until the bottom slide.

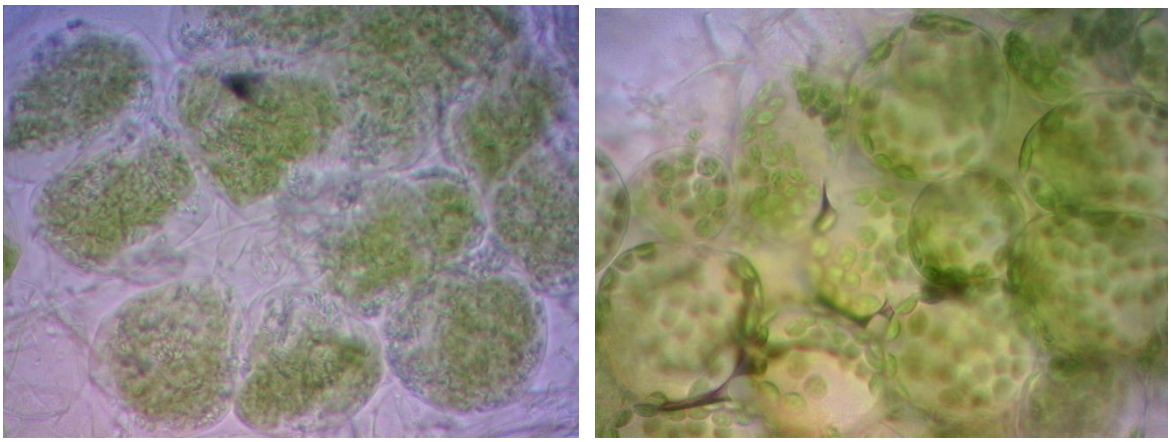

Figure 3. This image displays photos taken on October 30th 2012 using a microscope camera (Dino-Lite digital microscope). The *Arabidopsis thaliana* cells were viewed under a total magnification of 400X in a single field of view. The October 2nd planted wild type (left) and the October 4th mutant type (right) are examples of the images used to measure cell length.

For all treatments, both *A. thaliana* wild type and mutant plants were exposed to the same environmental conditions in a plant incubator where light intensity, temperature, water and nutrients were constant between the mutant and wild type plants.

Cell length of *A. thaliana* wild type and mutant were measured using the ocular micrometer within the compound microscope eyepiece. The average cell lengths, corresponding 95% confidence intervals, and a t-test were calculated and results collated graphically.

RESULTS

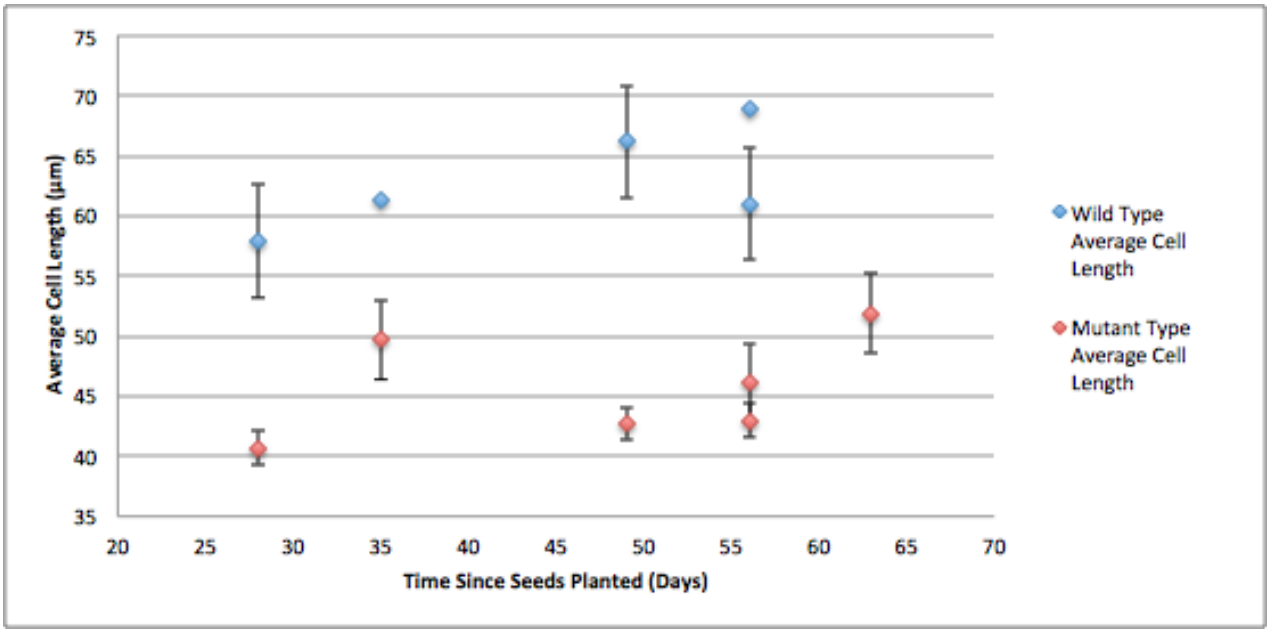

Figure 4. The effect of the cer10 mutation on average cell length in *Arabidopsis thaliana* between 0 days and 63 days after planting. Bars represent 95% confidence intervals, n=3. Two data points are without confidence intervals as those samples had n < 3. One data point is therefore missing from the graph. The graphs axes do not begin at zero, as the error bars are only visible at a smaller range of values

As depicted in Figure 4, the average cell lengths of 6 plants, 3 wild type and 3 mutant type, were collected at two time points and plotted along a time scale reflecting the average cell lengths throughout the plants life cycle. Figure 4 shows that wild type average cell lengths are greater than mutant type average cell lengths at all time points. 95% confidence intervals were calculated for all samples with $n \geq 3$. One wild type sample was not recorded, as cells were not available from replicate of that sample also making calculation of 95% confidence intervals impossible.

	average wild type cell length (x_1)	average mutant type cell length (x_2)	combined standard deviation	t-test value
Day 28	57.88	40.73	13.36	4.06
Day 49	66.20	42.71	14.20	5.23
Day 56	61.05	43.05	15.77	3.61

Table 1. Data table summarizing the values used in the calculation of probability using t-test for 3 dates (Day 28, Day 49, and Day 56). These days were used in calculating whether average cell lengths for wild type and mutant type *Arabidopsis thaliana* were significantly different.

Table 1 displays the steps used to determine the calculated t-test value of 4.06 for day 28, 5.23 for day 49 and 3.61 for day 56. When these t-test values are compared with the theoretical values from a two-sided t-test table (Changing Minds 2012), the probabilities for all the days calculated are smaller than 0.001.

	Average Leaf Length	**Average Leaf Length**
	Wild Type	**Mutant Type**
Oct. 30th Data Collection		
Sample 1	3.3 cm	2.22 cm
Sample 2	3.8 cm	2.8 cm
Sample 3	2.4 cm	1.1 cm
Nov. 6th Data Collection		
Sample 1	N.A.	2.2 cm
Sample 2	3.3 cm	2.8 cm
Sample 3	2.7 cm	2.0 cm

Table 2. A comparison of average leaf length in mutant type and wild type *Arabidopsis thaliana.* The first six samples were sampled on Oct. 30th 2012 and the second six samples were sampled on Nov. 6th 2012. The wild type sample 1 for the Nov. 6th 2012 data was not available for testing as the plant had reached maturity and leaf death.

Table 2 portrays secondary data collected, comparing the average leaf length of wild type and mutant type samples. The maximum average leaf length was from a wild type sample measured at 3.8 cm. The minimum average leaf length was from a mutant type sample was measured at 1.1 cm. Average leaf lengths for wild type samples ranged from 2.4 cm to 3.8 cm and average leaf lengths for wild type samples ranged from 1.1 cm to 2.8 cm. Once again, the data for wild type sample from data collection on Nov. 6 was not available for sampling.

A t-test value of 3.55 corresponds to a probability of 0.001. Therefore any t-test value that is larger than 3.55 corresponds to an even smaller probability. Looking at our data for day 28, 49, and 56 we can see that all t-test values are larger than 3.55 therefore have a probability of less than 0.001.

DISCUSSION

Based on the results obtained from this experiment we were able to reject our null hypothesis (H_O) and support our alternate hypothesis (H_A) that the average cell length of cer10 mutant type *Arabidopsis thaliana* is shorter than the average cell length of the wild type of the same age. Our results support previous research by Zheng et al. (2005), which proposes that a mutation at the cer10 locus, the coding region for enoyl-CoA Reductase (ECR), affects the length of cells.

From the samples depicted in Figure 4, a significant difference was observed between the average cell length of wild and mutant type cells on days 28, 49, and 56 based on non-overlapping 95% confidence intervals. Although we were unable to calculate standard deviations for all cell length data, a trend towards reduced cell length was observed across all data: wild type *A. thaliana* appear to have longer leaf cells compared to mutant type plants. For wild type plants the average cell length appears to peak at around day 56 in its life cycle. According to Boyes et al. (2001), this date corresponds to the end of the plant's life, after it has already undergone flowering. We can hypothesize that the gentle rise to a peak in average cell length is due to the plant growth as it progresses through germination (day 0-7), leaf production (day 7-25), rosette growth (day 19–33), inflorescence emergence (day 28), flowering (day 32-49) to reach its mature state (Boyes et al. 2001). From Table 2, it was observed that for all time points the average leaf length the wild type plants contain longer leaves on average compared with the mutant type plants. This agrees with our results on average cell length because as the individual cells increase in length they will cause an elongation the overall leaf length.

A t-test was utilized to determine whether the data generated from two types of *Arabidopsis thaliana* differ significantly. The t-test values calculated in this experiment as seen in

Table 1 for day 28, 49, and 56 are 4.06, 5.23, and 3.6 respectively. All of these t-test values are greater than the theoretical t-test value of 2.725 when a degree of freedom of 38 is used. Since all the t-test values are larger than 2.725, this indicates a probability value of less than 0.001. This demonstrates a significant difference between each treatment's average cell lengths at day 28, 49, and 56. A probability of 0.001 shows that if we assume that the mutant type *Arabidopsis thaliana* shows no change or increase in cell length compared to the wild type, there is a 0.1% chance that our observed results is due to random chance or variation. This statistical test along with the 95% confidence intervals ensured that we could reject the null hypothesis and support the alternate hypothesis.

Overall, mutant cell size tended to be reduced in length relative to the wild type cells. Our findings support Zheng et al. (2005) whose study results concluded that cer10 mutant affects cell elongation. Zheng et al. (2005) found that this mutation results in enoyl-CoA-reductase(ECR) not being synthesised. ECR is an important enzyme that allows for synthesis of VLCFA, which is an important component in the production of complex lipid and waxes. There are four biochemical reactions involved in elongating fatty acids: condensation of malonyl-CoA with the acyl-CoA substrate, 3-ketoacyl-CoA reduction, 3-hydroxyacyl-CoA dehydration and enoyl-CoA reduction. (Cinti et al. 1992). These processes allow cells to use VLCFAs to facilitate cell elongation. Hence, in this experiment, shorter leaf cell lengths were observed in the mutant types, where ECR was not present, compared to the wild type plants.

Although there was a significant difference in cell lengths found in the study, there are several weaknesses in the study design that could have influenced the results. The lifespan of *A. thaliana*, which has generally been observed at 6 weeks (Meinke et al. 1998), was reduced in our wild type plants. This led to a loss of data for individuals planted on September 4th due to full

maturity, and accompanying leaf death, at approximately 4 weeks as seen in Figure 5.

Sources of error for our experiment include a sampling bias and variation in cell length within each individual leaf and plant. Firstly, sampling bias is introduced when each member sections and judges the cell lengths under their microscope. Each person's sectioning style varied due to level of experience and this lead to some cell samples being more easily visible under the microscope than others. These samples may have yielded more accurate cell length measurements. Each group member tested a leaf from each of our 6 plant samples and then combined the results in an attempt to equally bias each sample.

Variation within and between leaves posed a potential weakness within the experimental design. To counteract variation within plants a randomizing system was used, as explained in our methods section. Sections were taken from the measured center of the leaf, which was thought to reflect cells of a similar age within the individual leaf.

It was found that cer10 mutant type leaf cell lengths were shorter than those of the wild type. According to previous work by Zheng et al. (2005), this is due to the lack of ECR synthesis in the mutant.

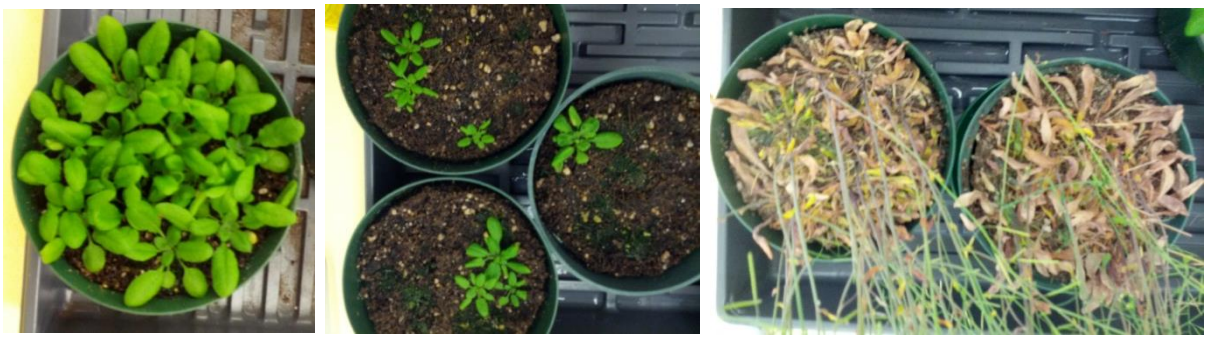

Figure 5: These images show *Arabidopsis thaliana* planted on October 2nd : Wild type (Left), October 4th : Mutant type (Middle), and September 4th : Wild type (Right). The first two images show the phenotypic differences between the wild type and the mutant type. The right image shows the September 4th wild type plant that reached maturity and leaf death.

CONCLUSION

Statistical analysis was conducted on data from days 28, 49, and 56, where a two-sided t-test analysis indicates that if we assume that the cer10 mutation results in no change or an elongation in cell length in mutant *Arabidopsis thaliana*, there is a less than 0.1% chance that our observed results is due to random chance or variation. Based on the 95% confidence intervals along with the statistical analysis of the t-test, we reject the null hypothesis and provide support for the alternate hypothesis that the length of mutant type leaf cells will be less than the cell lengths observed in wild type *Arabidopsis thaliana* leaf cells of the same age.

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