

# Growth Response of *Arabidopsis thaliana* to Varied Water Stress

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## Abstract

In the natural environment, plants are subjected to many different levels of water stress, and they adapt and respond to these stresses in different ways. In this paper, we sought to explain the growth responses of *Arabidopsis thaliana* (*A. thaliana*) to different soil water levels. Average leaf length of plants at three different water levels (dry, control and wet) was measured over a period of 20 days. Height of the primary inflorescence was also measured at day 20. Five replicates, each consisting of one pot containing three plants were used in each treatment. The growth of height showed a significant difference between control and dry treatments. However, the results showed no significant difference when comparing overall mean growth and mean outer leaf growth between treatments. Although there were trends observed indicating differences in growth between treatments, we could not reject our null hypothesis that soil water levels higher or lower than standard cause an increase or no change in growth of *A. thaliana*. These results could have differed from those expected because of adaptive physiological responses of *A. thaliana* to non-optimal water levels, or experimental error in measurement. As a result, our data suggested that there were no significant differences found, and thus the null hypothesis could not be rejected.

## Introduction

The flowering plant *Arabidopsis thaliana* (*A. thaliana*), of the Brassicaceae family, is commonly known as thale cress or mouse-eared cress, and grows natively in Europe, Asia and parts of Northwestern Africa (Meinke *et al.* 1998). The entire life cycle of *A. thaliana* is approximately six weeks in which a mature plant reaches a height of 15 to 20 centimeters (Meinke *et al.* 1998). Naturally *A. thaliana* is capable of self-pollinating, however, cross-pollination between other individuals is also possible under laboratory conditions. As *A. thaliana* is a widely used model organism in various fields of study, it is important to determine what conditions are optimal for its maximum growth.

The purpose of the present experiment was to determine the effect of water availability on optimal growth for *A. thaliana*. To investigate this, treatments with less and more water availability than the typical system were designed. If current conditions

are optimal, negative effects of drought or flooding may be observed in the alternate treatments, as has been reported in previous studies where *A. thaliana* responded negatively to both water deficit and flood conditions (Kolodynska and Pigliucci 2003, Aguirrezabal *et al.* 2006). During drought conditions different ecotypes of *Arabidopsis* engage in varied response strategies, with some employing an escape strategy, while other use a tolerance strategy (Meyre *et al.* 2001). Since these response strategies differ in the way they affect both stem and leaf growth, the ecotype of the plant must be taken into consideration when analyzing results (Meyre *et al.* 2001).

Various constraints on *A. thaliana*'s growth are also imposed by flood conditions. As roots are very sensitive to drops in oxygen concentration, flooded soil can negatively impact plants due to resistance to diffusion being much greater in water than air, which can lead to dramatic decrease in gas transport (Kolodynska and Pigliucci 2003). Additionally, microbial growth within flooded soils may occur, the microbial oxygen use further decreases the oxygen concentration available to root, and consequently increases the stress placed on the plants (Kolodynska and Pigliucci 2003). Observable characteristics occur when a plant experiences prolonged flooding, these include leaf chlorosis, necrosis and defoliation (Zhang *et al.* 2000).

Response to water stress at the beginning of an experiment may be different from that observed at the conclusion of the experiment involving water stress, as it was observed by Engelmann and Schlichting (2005). If an environmental component remains stable over a sufficient period of time, or if a plant experiences only that state (such as continuously being wet or dry), the factor is defined as "coarse grained" (Engelmann and Schlichting 2005). Adaptive responses to such factors are known to occur, and may

change the observable behaviour of the plant over time, and therefore these possibilities must be taken into consideration during the course of our experiment (Engelmann and Schlichting 2005).

Our null hypothesis states that standard watering conditions are not optimal for the growth of *Arabidopsis thaliana*, and increasing or decreasing soil water content will increase or have no effect on the growth of *Arabidopsis thaliana*. Our alternate hypothesis states that standard watering conditions are optimal for growth of *Arabidopsis thaliana*, and increasing or decreasing soil water content will decrease the growth.

## **Methods**

In order to measure growth of leaves and height, we prepared moderately grown (approximately 3 weeks old) *A. thaliana* individuals of the Columbia ecotype. In the first day (Day 0), we sorted every individual by size to minimize sampling bias, and then transplanted three individuals into each pot using a spatula starting from the largest to the smallest (Figure 1). We labeled three treatments as A, B and C, with each treatment consisting of five replicates that were numbered respectively. Transplantation was performed in a specific order (from A1, B1, C1, C2, B2, A2, A3, and so on) so that each treatment could have equal chance of possessing fairly similar sized individuals at the starting point.



**Figure 1.** Transplanting *A. thaliana* individuals to each replicate using a spatula

We differentiated each treatment by varying the amount of water, and placing them in different trays. Treatment A (hereafter referred to as the dry treatment) only received water once at the beginning of the experiment, with no water provided throughout the remainder of the experiment. In order to do so, the replicates for dry conditions were kept in small separate trays individually (Figure 2).

Treatment B (hereafter referred to as the control treatment) was given an “optimal” amount of water, and we watered them on a regular basis: Day 0 (Oct 26), Day 7 (Nov 2), Day 11 (Nov 6), and Day 14 (Nov 9). Except the period of watering, we kept the control treatment in the absence of water (Figure 2). In order to determine the optimal amount of water for *A. thaliana*’s growth and when to give water to the plants of the control treatment, we measured the average weight of treatment C’s replicates as well as the weight of a dry pot into which the *A. thaliana* plants were to be transplanted. The 75% value of the sum of the average weight of C and the dry pot gave us a minimum weight required for the optimal conditions: 259.95 g. This was a modification of the watering regime used by Engelmann and Schlichting (2005). Therefore, we decided to water the control replicates if their mean weight fell below this minimum weight. When

the control replicates showed a weight below this threshold, we filled water in the tray to make sure that soil was saturated with water, and then discarded the extra water a few hours later to prevent further consistent water saturation. Lastly, we made water available at all times throughout the experiment for treatment C (hereafter referred to as the wet treatment) by maintaining a constant water level within the tray (Figure 2).



**Figure 2.** The different conditions for each treatment, dry (A), control (B) and wet (C)

Throughout the experiment, abiotic factors other than water availability were kept constant. For instance, as all treatments were grown in an incubator, we maintained constant light availability for 24 hours a day, and set temperature for 21 °C during the daytime and 22 °C overnight (Figure 3).



**Figure 3.** The incubator where all treatments were kept, temperature and light availability were kept constant for all treatments

We used a caliper to measure the length of outer and inner leaves of each replicate. The average length of the inner and outer leaves for each replicate was recorded. Means of the average length of the five replicates were calculated for each treatment, and used to examine their 95% confidence interval for a statistical analysis.

The height of the inflorescences was presumably zero millimeters on the transplanting day. The heights of the primary inflorescences were also measured with calipers on day 20. Means and 95% confidence intervals of primary inflorescence height per treatment were calculated for statistical analysis. Only the plants that exhibited inflorescence were included in these calculations.

## **Results**

One major trend observed was that deviations from control conditions produced lower overall growth as measured through average leaf length. This is shown in Figure 4, where it can be seen that the dry and wet conditions exhibit lower growth than the control condition, with mean leaf lengths of 13.6 mm and 12.7 mm. These values are not significantly different from the control treatment, which obtained an average leaf length of 14.6 mm. While the differences were not found to be significant, a general trend can be seen in Figure 4 that the control condition displayed more growth throughout the duration of the experiment.

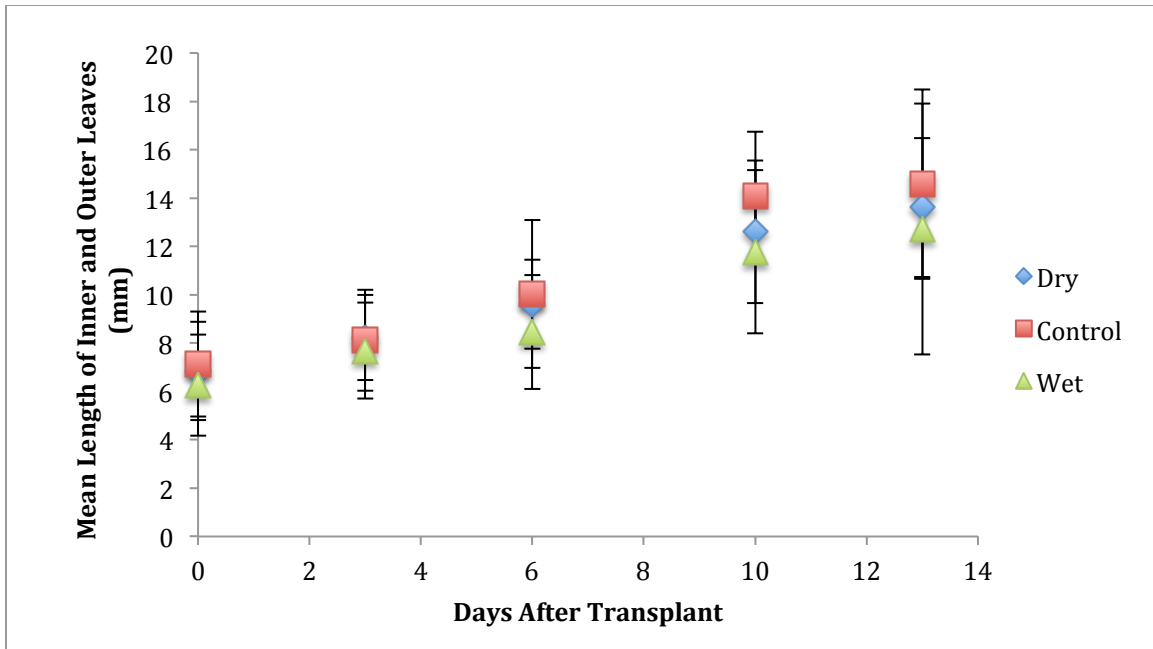
At 6 and 13 days after transplant, there was a significant difference between the dry and wet treatments in inner leaf length. As shown in Figure 5, plants from the dry treatment had significantly larger inner leaves, 7.2 mm at 6 days after transplant and 9.5 mm 13 days after transplant, than the wet treatment which had leaves of 5.3 mm at 6 days

and 5.5 mm at 13 days. This trend is observed throughout the experiment but significant differences are not obtained on any other days.

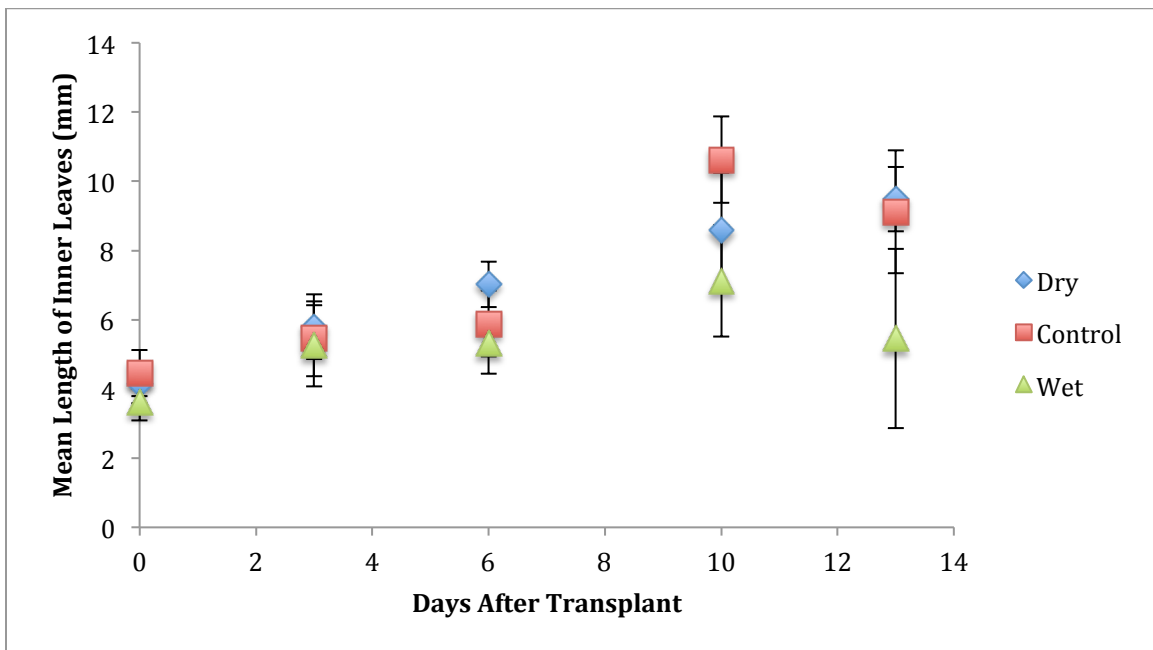
When comparing only outer leaf growth, no significant differences were observed. A trend can be seen in Figure 6 between the control plants and the wet treatment until 13 days after transplant. The control plants continually had larger average outer leaf length than the wet plants, up until 13 days after transplant where the values of the average outer leaf length for the two treatments were equal at 20.0 mm.

Lastly, when comparing the growth of stems over time, the control plants obtained taller stems by the end of the experiment than either the dry or wet treatments. A significant difference was observed between the control and dry treatments. This can be seen in Figure 7 where the control treatment obtained an average stem height of 100.2 mm compared to dry treatment, which only grew to an average height of 26.5 mm.

Additionally, when we examined the different treatments 20 days after transplant, a clear visual difference was observed when comparing the wet and control treatments to the dry treatment. Wet and control plants were larger, had a brighter green color and contained more leaves (Figure 8).

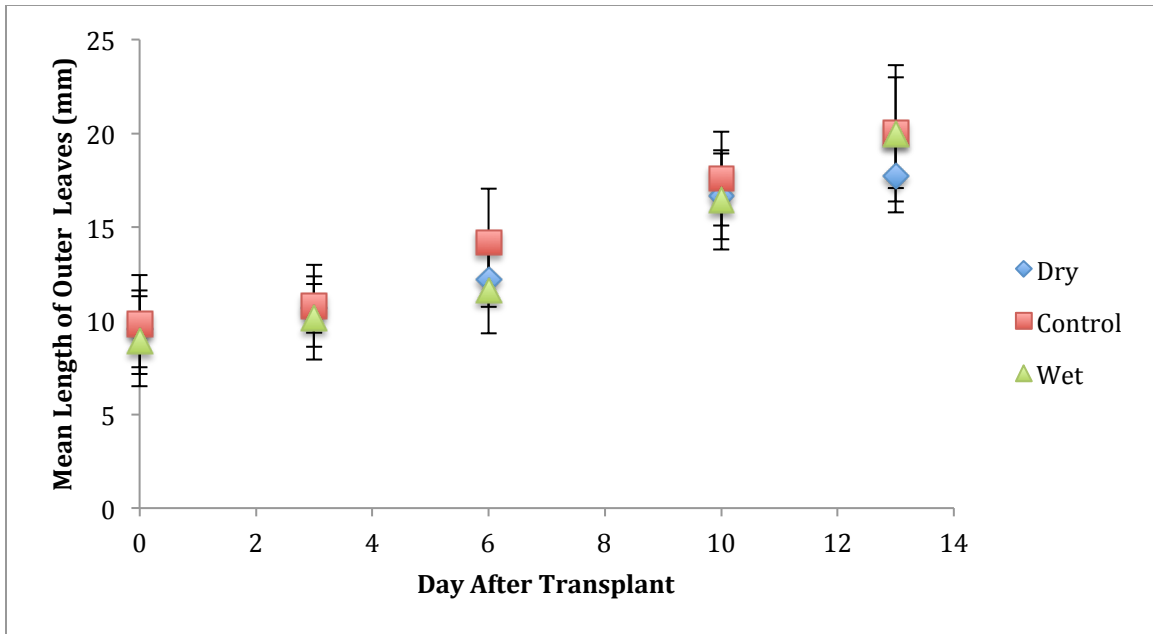


**Figure 4** - Mean length (mm) of *Arabidopsis thaliana* leaves at wet, control and dry treatments versus time (days after transplant). Bars represent 95% confidence intervals. No significant results were observed.

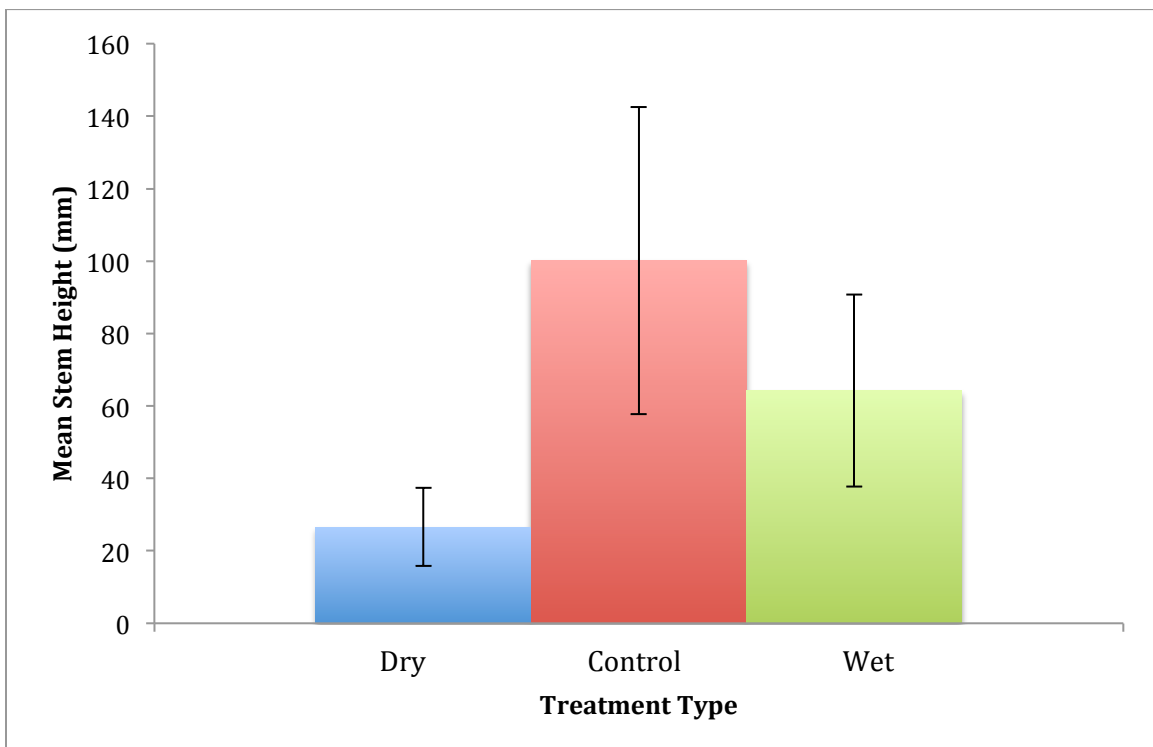


**Figure 5** - Mean inner leaf length (mm) versus time (days after transplant) of *Arabidopsis thaliana* plants at wet, control and dry treatments. Bars represent 95% confidence intervals. Significant differences were observed at 6 and 13 days after transplant between dry and wet treatments.





**Figure 6** - Mean outer leaf length (mm) versus time (days after transplant) of *Arabidopsis thaliana* plants at dry, control and wet treatments. Bars represent 95% confidence intervals. Significant differences were not observed between any treatments.



**Figure 7** - Mean stem height (mm) at twenty days after transplant of *Arabidopsis thaliana* plants at wet, control and dry treatments. A significant difference was observed between the dry and control treatments. Bars represent 95% confidence intervals.



**Figure 8** - Photos of dry plants (A), control plants (B) and wet plants (C) at 20 days after transplant.

### Sample Calculations

Mean leaf length of dry treatment plants at 3 days after transplant:

$$\bar{x} = \frac{12.51+11.25+11.23+9.35+8.92}{5} = 10.65 \text{ mm}$$

Standard deviation of outer leaf lengths of 'A' plants 3 days after transplant:

$$\sqrt{\frac{(12.51 - 10.65)^2 + (11.25 - 10.65)^2 + (11.23 - 10.65)^2 + (9.35 - 10.65)^2 + (8.92 - 10.65)^2}{4}} = 1.49 \text{ mm}$$

95% Confidence interval, outer leaf lengths of 'A' plants, 3 days after transplant:

$$10.65 \pm 1.96 \times \frac{1.49}{\sqrt{5}} = 10.65 \pm 1.30$$

### Discussion

The aim of this experiment was to examine how high or low water levels would affect the growth of *A. thaliana*, compared to a control treatment grown at an optimal water level. Although there was no significant evidence of a difference in average leaf length for any of the three treatments, except for inner wet and dry leaves (Figures 4, 5 and 6), there were some other indications that there might have been a difference in growth among the treatments. The difference in inner leaf size between the dry and wet

treatments could indicate greater growth, but it is unconvincing when compared to the outer and overall leaf lengths. Primary inflorescence height can be used as a measure of growth in *A. thaliana*. However, since this was not our primary mode of measurement and a significant difference between the stem heights was only observed on the final day of the experiment (Figure 7), we cannot reject our null hypothesis. The trend observed in Figure 4 agrees with the findings of Kolodynska and Pigliucci (2003) and, Aguirrezabal *et al.* (2006) that a high or low soil water level decreases growth in *A. thaliana*; however, our results were not statistically significant.

Despite these differences in stem height, our primary method of measurement showed no statistical difference in growth, therefore we could not reject our null hypothesis, that soil water levels higher or lower than an optimal level increase or have no effect on the growth of *A. thaliana*.

There are several reasons as to why the result was not as hypothesized and these can be divided into two categories; physiological responses in the plants, and experimental error or mistakes in procedure.

#### Physiological Responses: Wet treatment

In heavily watered or saturated soil, non-photosynthetic tissues, such as roots, have a highly decreased level of available oxygen ( $O_2$ ), as  $O_2$  has a lower diffusion rate in water than in air (Voeselek *et al.* 2006). This causes a reduction in ATP energy availability and consequently growth, since there is less  $O_2$  to be used in cellular respiration (Vartapetian and Jackson 1997). During the daytime, however, this drop in  $O_2$  levels can be mitigated to some extent by photosynthesis (which depletes carbon dioxide,

and produces O<sub>2</sub> as a by-product), and it has been found that the more light available to a plant, the better it deals with flooding stress (Voosenek *et al.* 2006, Mommer and Visser 2005). Since the incubators used in this experiment were lit constantly, this could have caused the wet treatment to have better growth than it would have had in natural conditions of day and night, compensating for the decrease in energy availability caused by the high water levels. This is a potential explanation as to why there was no significant difference in leaf length between the wet treatment and the control.

#### Physiological responses: Dry treatment

Decreased soil water level has been shown to reduce leaf growth in *A. thaliana* (Baerenfaller *et al.* 2012, Aguirrezabal *et al.* 2006). This reduction in leaf size reduces the transpiration rate of the leaves, which in turn helps to conserve water in the plant (Aguirrezabal *et al.* 2006). However, different ecotypes of *Arabidopsis* are known to exhibit differential adaptive strategies in dealing with drought conditions (Meyre *et al.* 2001). In particular, Columbia, the ecotype used in this experiment, employs a drought tolerance strategy that involves focusing growth on leaves and roots as opposed to shoots and reproductive structures (Meyre *et al.* 2001). Therefore it is possible that the ecotype used in this experiment may have compensated for the dry growing conditions by increasing leaf growth to a similar level as the control plants.

Although there was no significant difference in leaf length observed, there was a significant difference in height of inflorescence between the dry and control treatments. It has been shown that *A. thaliana* grows a shorter inflorescence in low water conditions than in a high water environment (Engelmann and Schlichting 2005). In fact, the

Columbia ecotype has been shown to have virtually no floral stem elongation in drought conditions (Meyre *et al.* 2001). This result could lend credence to the theory that a strong ecotype specific response to dry conditions was occurring in the dry treatment.

### Experimental error

The leaf measurement method used in this experiment was based on a loose adaptation of the methods of Baerenfaller *et al.* (2012), in which they measured a single leaf's growth. In this experiment, a mean length was calculated from all leaves in a treatment. It was expected that differing growth in leaves would show up as a difference in the average leaf length (either inner leaves, outer leaves, or both together). However, the growth of the different treatments varied not just in leaf length, but also in number of leaves. For example, the control treatment plants grew many small leaves as well as larger leaves, while the dry treatment plants grew fewer, but evenly sized leaves. This growth of many smaller leaves likely reduced the observed measurement of average leaf length, even as total leaf area and above ground biomass increased. As shown in Figure 8, the dry treatment clearly had less leaf area and above ground biomass than the other two treatments. If total leaf area or biomass had been measured, there would have been a significant difference between the dry and non-dry treatments.

Another possible source of error was that the watering procedures were not carried out as strictly as possible. If the dry and wet treatments were closer in actual water level to the control than was expected from the following methods, this could help to account for the lack of significant difference between treatments. However, it is unlikely that this was a major factor because the treatments were weighed every three or

four days to check their water level and the results were in close accordance with the procedure in Engelmann *et al.* (2005).

## **Conclusion**

Not enough convincing evidence was found to allow us to reject our null hypothesis, that *A. thaliana* growth increases or remains the same in non-optimal soil water conditions. This may have been due to adaptive physiological responses, or to experimental or measurement error. Some results of this experiment suggest that the alternative hypothesis may be correct, but more research needs to be done before this can be shown conclusively.

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## Literature Cited

- Aguirrezabal, L., Bouchier-Combaud, S., Radziejwoski, A., Dauzat, M., Cookson, S. J., and Granier, C. 2006. Plasticity to soil water deficit in *Arabidopsis thaliana*: dissection of leaf development into underlying growth dynamic and cellular variables reveals invisible phenotypes [online]. *Plant Cell and Environment*, **29**(12): 2216-2227.
- Baerenfaller, K., Massonnet, C., Walsh, S., Baginsky, S., Buehlmann, P., Hennig, L., Hirsch-Hoffmann, M., Howell, K. A., Kahlau, S., Radziejwoski, A., Russenberger, D., Rutishauser, D., Small, I., Stekhoven, D., Sulpice, R., Svozil, J., Nathalie, W., Stitt, M., Hilson, P., Granier, C., and Gruissem, W. 2012. Systems-based analysis of *Arabidopsis* leaf growth reveals adaptation to water deficit [online]. *Molecular Systems Biology*, **8**: 606.
- Engelmann, K., and Schlichting, C. 2005. Coarse-versus fine-grained water stress in *Arabidopsis thaliana* (brassicaceae) [online]. *American Journal of Botany*, **92**(1): 101-106.
- Kolodynska, A. and Pigliucci, M. 2003. Multivariate responses to flooding in *Arabidopsis*: an experimental evolutionary investigation. *Functional Ecology*, **17**(1): 131-140.
- Meinke, D., Cherry, M. J., Dean, C., Rounsley, S. D., and Koornneef, M. 1998. *Arabidopsis thaliana*: A model plant for genome analysis [online]. *Science*, **282**(5389): 662-682.
- Meyre, D., Leonardi, A., Brisson, G., and Vartanian, N. 2001. Drought-adaptive mechanisms involved in the escape/tolerance strategies of *Arabidopsis* landsberg erecta and columbia ecotypes and their F1 reciprocal progeny [online]. *Journal of Plant Physiology*, **158**(9): 1145-1152.
- Mommer, L., and Visser, E. 2005. Underwater photosynthesis in flooded terrestrial plants: A matter of leaf plasticity [online]. *Annals of Botany*, **96**(4): 581-589.
- Vartapetian, B., and Jackson, M. (1997). Plant adaptations to anaerobic stress. *Annals of Botany*, **79**: 3-20.
- Voesenek, L., Colmer, T., Pierik, R., Millenaar, F., and Peeters, A. 2006. How plants cope with complete submergence [online]. *New Phytologist*, **170**(2): 213-226.
- Zhang, J., Van Toai, T., Huynh, L., and Preiszner, J. 2000. Development of flooding-tolerant *Arabidopsis thaliana* by autoregulated cytokinin production [online]. *Molecular Breeding*, **6**(2): 135-144.