

Root Hair Growth in *Arabidopsis thaliana*

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Abstract

We study the factors that affect the root hair growth in the *Arabidopsis thaliana* plant. From experimental data, we measure how the average velocities of growth and tip shapes of the root hairs are affected by pressure and nutrients. Then using a biomechanical model, we can compute the physical parameters of the root hair and aim to correlate our model with experiments by using image processing techniques and the biomechanical approach. Our goal is to determine what percentage of the tip growth is affected by these factors based on time-lapsed images of root hairs. We found that tip shapes stayed fairly consistent as the root hairs grew and average velocity is significantly affected by varying growth media.

1 Introduction

A root hair can be described as a long, thin extension that is a protusion of the root cell wall. Healthy root hairs tend to grow straight and have large surface areas, so plants are able to absorb the majority of their water and nutrients through these root hairs [1]. *Arabidopsis thaliana* is a small weed with white flowers often studied in biology and genetics. This plant is commonly used as a biological model to study the characteristics of plants because it is easy to grow in an experimental lab, has a rapid life cycle, and germinates quickly. The complete genome sequence of *Arabidopsis* helps to determine how much of its growth is influenced by genetics. Its root hairs have a simple growing pattern: the hair-forming cells, trichoblasts, and non-hair-forming cells, atrichoblasts, are arranged in alternating order along the root surface [2]. In addition, the growth of roots and root hairs in *Arabidopsis thaliana* is essentially one-dimensional [3], making them easy to measure as they grow.

Their growth is similar to that of other tip-growing cells, such as plant pollen tube growth [2]. While plants do not need root hairs to grow, root hairs make plants stronger and healthier by absorbing more water and nutrients at a greater distance. Furthermore, it is important to study plant root hairs because if we can determine how to make the root hairs more robust, we can make the whole plant more robust. Overall, the best condition is when the root hairs grow as straight as possible since it gets the most area of water. We obtain a biomechanical model that models the tip growth of actinomyces [4] and use it to analyze the factors affecting root hair growth in *Arabidopsis thaliana*. This will help us determine how much of the growth is caused by nutrients and pressure. Once we have a working model, we can test different parameters to determine which conditions are best for growth. This

will also help us determine how we can improve the growth of root hairs just by changing the soil they are in and the amount of water they receive versus changing their genetics.

In this paper, we analyze various root hairs and root hair tips in their early and later stages (Figure 1) to obtain initial conditions for our biomechanical model of growth. We also explain the procedure used to grow *Arabidopsis thaliana* in the lab. Additionally, we study and modify ordinary differential equations that model the tip growth of filamentary actinomycetes [4] to get similar equations to model the tip growth of plant root hairs.

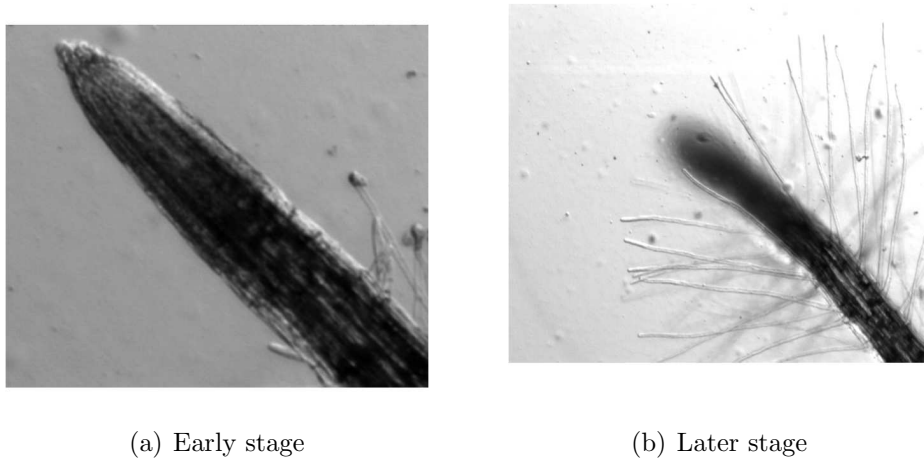


Figure 1: *Arabidopsis* root hairs growing over a period of 32 hours.

2 Experimental Procedure

In this section, we will discuss the equipment and methods used to grow the *Arabidopsis* plants. Our experimental procedure is similarly based on Doerner's protocol [3].

2.1 Materials

1. Six petri dishes that consisted of the following:
 - 3 plates with half of the standard concentration of Murashige-Skoog (MS) salt (2.165 grams per liter)
 - 1 plate with the standard concentration of MS salt (4.33 grams per liter)
 - 1 plate with one-fourth of the standard concentration of MS salt (1.0825 grams per liter)
 - 1 plate with one-eighth of the standard concentration of MS salt (0.54125 grams per liter)

2. One large beaker and three small beakers
3. Double distilled water
4. Parafilm
5. Autoclave
6. *Arabidopsis* seeds

2.2 Preparing the Solution

- First make a medium by mixing 2.165 grams of MS salt with double distilled water to create a liter of the salt-water solution.
- The solution needs a pH of about 5.7.
- We used the three plates with the solution of 2.165 grams of salt per liter as our normal plates for normal growth, and the other three plates were our varied plates.
- Pour 825 milliliters of salt-water solution into a large beaker to use for our normal plates.
- Pour 100 milliliters of solution into a small beaker and add an additional .2165 grams of MS salt to get the solution with the standard MS salt concentration.
- Pour 50 milliliters of solution into a small beaker and add additional 50 milliliters of water to get the solution with one-fourth of MS salt concentration.
- Pour 25 milliliters into a small beaker and add an additional 75 milliliters of water to get the solution with one-eighth of MS salt concentration.
- Add one gram of phytagar to each of the small beakers and 8.25 grams of phytagar to the large beaker to make the solution into a gel.
- Put all the beakers into an autoclave machine for an hour, which sterilizes the equipment and causes the solution to solidify at a certain temperature. It is important not to let the solution cool too fast after it is extracted from the autoclave; otherwise, it will solidify in the beaker.
- After the beakers have cooled for a while, pour the various solutions into six petri dishes.
- Allow the petri dishes to sit for two days before planting the seeds.

2.3 Planting the Seeds

- Sterilize the seeds with a chlorine solution composed of 15 milliliters of chlorine, 35 milliliters of water, and 25 microliters of the surfactant Polysorbate 20 to get the bleach in all the crevasses of the seeds.
- Measure 0.00625 grams of seeds for each plate.

- Add ethanol to the seeds to help them sink in water.
- Let the seeds sit in ethanol for three to five minutes.
- Remove the ethanol and add bleach solution to the seeds to sterilize them.
- Rinse the seeds three to five times with sterile water.
- Add 0.1% of agarose to each set of seeds to evenly disperse them.
- Use a dropper to drop 250 microliters of the seed-agarose solution into each dish and spread out the seeds as much as possible.
- Leave the dishes under direct light at room temperature to grow.

It typically takes between one to two weeks for the root hairs to appear. We were able to image the root hairs in four different solutions on Day 7 (Figure 2). We focused on these roots (Figure 2) on Days 7, 9 and 12 in order to track and measure a specific root hair as they grew. Then we compared the growth rate of each root hair in the varying growth media.

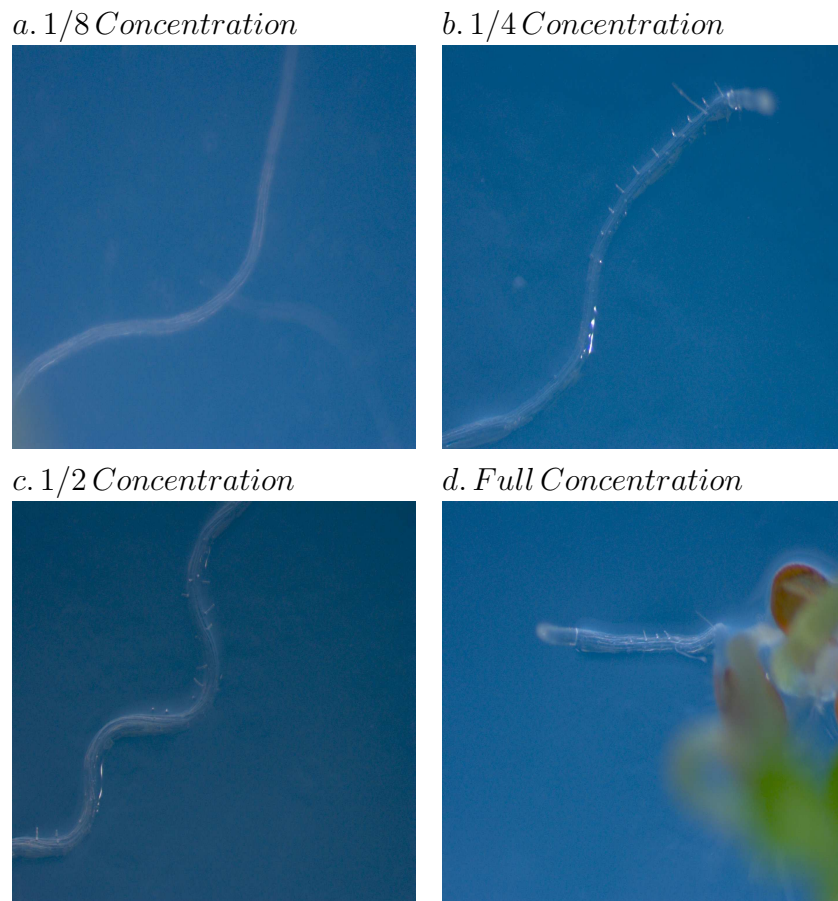


Figure 2: Root Hairs on Day 7.

3 Preprocessing

The images we analyzed were taken by Dr. Claudia Uhde-Stone from CSU East Bay. We analyzed these images of different root hairs at various stages of growth using the program ImageJ. Our goals are to find equations that model the tip shape of the root hair and to use these equations as initial conditions for our biomechanical model.

3.1 Analyzing Tip Shape

We compared tip shapes of five root hairs from the earlier stages and five root hairs from the later stages of growth. We plotted twenty to thirty points around the tips of these root hairs and graphed these points in Microsoft Excel. Since these root hairs are all different shapes and orientation, we needed to put them in a standard orientation to compare them.

1. For each graph, we first found the point closest to the vertex of the graph and subtracted each point on the graph by this point in order to shift the graph so that the vertex was at the origin.
2. We then found the axis of symmetry of the graph, found the angle that this line made with the x-axis, and inputted this angle into the rotation matrix.
3. By multiplying this matrix by every point on the graph, we got a new set of points that created a parabolic shape with the vertex at the origin.

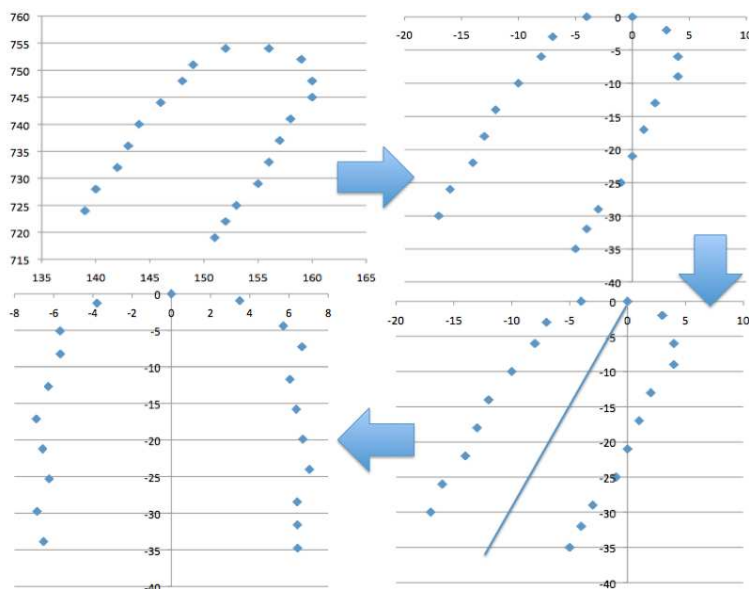


Figure 3: Procedure for rotating the curve

After repeating this procedure for each graph, we plotted the new sets of points for the tips in the earlier stages of growth on one graph and all of the new sets of points for the tips in the later stages of growth on another graph, which resulted in two new graphs. From our tip shape analysis, we found that tip shapes stay fairly consistent as they grow. This suggests that as pressure and nutrients are pushed into the root hairs, they grow longer but

do not expand outwards much. Then we used Microsoft Excel to find the best curve fit for each of these two new graphs.

We use the same coordinate system from Goriely's and Tabor's biomechanical model of the growth of filamentary actinomycetes to analyze the growth of root hairs [4]. Figure 4 uses an $r - z$ coordinate system, so we replaced x with r and y with z in our previous curve fit equations to obtain an equation for z in terms of r . The initial condition that we use for our model is $r_0 = -0.411549 + 1.93125\sqrt{16.4462 - 1.0356\sigma}$.

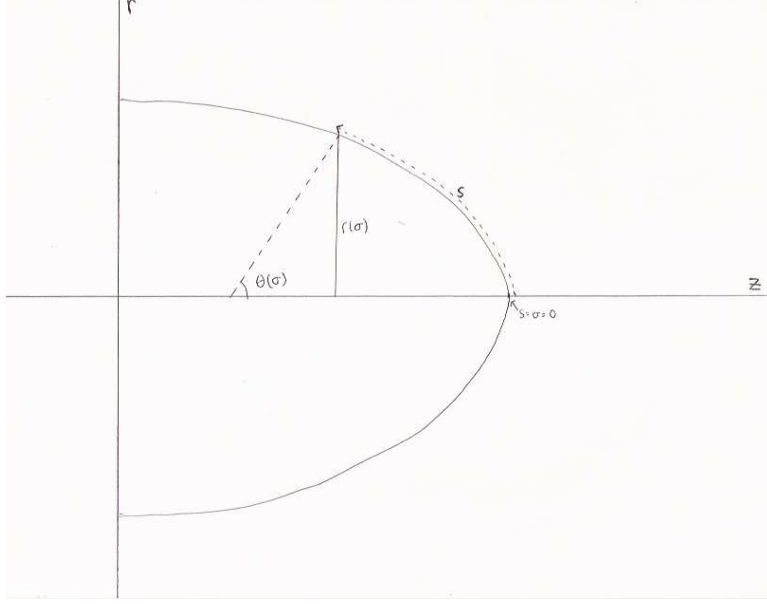


Figure 4: Initial shape of curve

According to Goriely and Tabor [4], the parameters are as follows:

- r and θ characterize the shell geometry
- s is the arclength of the curve
- σ is the material parameter identified with arclength before growth of the curve
- $r_0(\sigma)$ is the function that defines the initial shape
- P is the turgor pressure
- μ describes the elastic properties of the root hairs
- σ_1 and α both represent the length of the apical extension zone which is where the tip grows
- β is the effective pressure in distal regions of the root hair

We define p as

$$p = \frac{P}{2}(1 - \tanh(\frac{\sigma - \sigma_1}{\alpha})) + \beta$$

and λ as

$$\lambda = \frac{1}{2} \sqrt{4 + 4\mu(1 - \frac{r(\sigma)^2}{r_0(\sigma)^2}) + \frac{2r(\sigma)p}{\sin(\theta(\sigma))}}$$

where p is denoted as the effective pressure and λ is the extensional stretch ratio [4].

The ordinary differential equations (ODE) we use are:

$$\frac{\partial r}{\partial \sigma} = \lambda \cos \theta$$

$$\frac{\partial \theta}{\partial \sigma} = \frac{-\lambda \sin \theta [r^2 - r_0^2(1 + \mu(1 - \lambda^2))] - p r r_0^2}{r^2 \mu - r_0^2(1 + \mu - \lambda^2)}$$

where $\frac{\partial r}{\partial \sigma}$ stretches perpendicular to the growth axis z and $\frac{\partial \theta}{\partial \sigma}$ is how the angle changes with respect to the reference point [4].

By using these equations, our goal is to model the growth process of the root hairs through re-parametrization of the extended membrane [4]. We define the best curve fitting equation for the early stage as $r_0(\sigma)$, which is the initial shape for the curve. Then we solve the system of differential equations that results in a new shape $r(\sigma)$ with certain boundary conditions. This process is repeated many times. Once we apply this process to the curve fit equation for the later stage, we can compare the growth of the root hair during the early and later stages.

4 Results and Discussion

In this study, we used time lapsed images of the experimental *Arabidopsis* root hairs to compare the tip shape and average velocities of the root hairs grown in different solutions. We selected a specific root hair from each solution and measured their lengths at various points in time. Then we calculated their velocities of growth by determining the change in length over a finite period of time. This gave us an idea of how fast the root hairs grew on average. Each time-lapsed image would be taken every twenty minutes. Below is a table of the average velocities of root hairs in different solutions. The Hoagland solution is a solution that provides all the necessary nutrients for plant growth and Polyethylene glycol (PEG) reduces the amount of water that the plants absorb. Our analysis show that the root hairs grew most quickly in the 1x Hoagland solution, and they grew very slowly in the 1x Hoagland solution with 60% PEG. We eventually want to compare these velocities to the velocities computed by our biomechanical model.

<i>Solution</i>	<i>Velocity(micrometers/minute)</i>
1x Hoagland	0.0277621577
1x Hoagland	0.016479409
1/8x Hoagland	0.0054395501
1x Hoagland + 60% PEG	0.0006380818
1x Hoagland + 60% PEG	0.0012741331

Since we also grew our own plants in four different solutions, we were able to image them and compare their average velocities. The results are shown in the following table. On day seven, day nine, and day twelve of growth, we imaged a specific root from each solution and focused on one root hair to track. The root hairs in the solution with the full concentration of MS salt grew the fastest. We hypothesized that the root hairs in solutions with one-eighth and one-fourth of the standard concentration of MS salt would grow the slowest out of all the plants. However, the root hairs in the solution with one-half the standard concentration of MS salt grew the slowest out of all the plants. This is during the early stages of growth so there was not a significant change in root hair growth.

<i>Solution</i>	<i>Velocity(micrometers/day)</i>
Full Concentration MS Salt	13.876
One-Half Concentration MS Salt	1.186
One-Fourth Concentration MS Salt	9.964
One-Eighth Concentration MS Salt	1.373

In the future, we would like to use our model to test different parameters in order to determine which parameters give us the best growth and tip shape for root hairs. We also want to isolate the differences in root hair growth between the genetic factors such as elasticity ratio of the root hair and environmental factors such as water, pressure, and nutrients. Our goal is to determine how much is influenced by genetic factors and physical parameters. Furthermore, it would be useful to study the differences between wild-type and mutations in *Arabidopsis thaliana*. Finally, we would work to develop an algorithm that can automatically detect root hairs using image processing techniques.

5 Acknowledgements

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