Chapter 5

Live Imaging of Root Hairs

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Abstract

Root hairs are single cells specialized in the absorption of water and nutrients. Growing root hairs requires intensive cell wall changes to accommodate cell expansion at the apical end by a process known as tip growth. The cell wall of plants is a very rigid structure comprised largely of polysaccharides and hydroxyproline-rich *O*-glycoproteins. The importance of root hairs stems from their capacity to expand the surface of interaction between the root and the environment, in search for the necessary nutrients and water to allow plant growth. Therefore, it becomes crucial to deepen our knowledge of them, particularly in the light of the applicability in agriculture by allowing the expansion of croplands. Root hair growth is an extremely fast process, reaching growth rates of up to 1 μm/min and it also is a dynamic process; there can be situations in which the final length might not be affected but the growth rate is. Consequently, in this chapter we focus on a method for studying growth dynamics and rates during a time course. This method is versatile allowing for it to be used in other plant organs such as lateral root, hypocotyl, etc., and also in various conditions.

Key words Roots hairs, Tip growth, Growth rate, Arabidopsis

1 Introduction

Plant root hairs, just like pollen tubes, are single tubular cells and their cell walls are typically comprised of low-crystalline cellulose, xyloglucans, and pectins, together with hydroxyproline-rich *O*-glycoproteins such as extensins and proline-rich proteins [1]. Both the root hairs and the pollen tubes develop by polar cell expansion (tip or polarized growth). This type of growth involves turgor pressure, cell wall loosening at the tip, new cell wall materials synthesis, and their subsequent deposition at the root hair tip by vesicular transport from the endomembrane system [2, 3]. After the fusion of these vesicles with the plasma membrane comes the next step which is the stiffening of the cell wall. Afterwards, the turgor pressure will build again and a new growth cycle will start.

The signaling accompanying these growth pulses involves Reactive Oxygen Species (ROS) [4] which in turn activates Ca^{2+} channels that result in a local apical Ca^{2+} gradient. The Ca^{2+}gradient

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is necessary for the exocytosis of the vesicles [5, 6]. Both ROS and Ca2+, as well as pH, present cycling patterns just like the root hair growth [7–9]. Because vesicular transport is a fundamental part of polarized growth, consequently actin microfilaments are fundamental too [10]. Inhibition of their polymerization impairs elongation of both root hairs and pollen tubes [10, 11]. Root hair growth is an extremely fast process, reaching growth rates of up to 1 μm/min [12, 13].

The root is involved in water, nutrient uptake, nitrogen fixation [8, 14], and plant anchorage to the soil. Given that the plant is a sessile organism, depending only on the root for the uptake and absorption would result in a very limited area available for interaction with the environment. Therefore, the root hairs can expand the surface of interaction, in search for the necessary nutrients and water that allow plant growth. For example, in rye (*Secale cereale* L.) root hairs can represent an interaction surface of 400 m^2 in 50 L of soil [15].

There are various factors, exogenous and endogenous, that can affect the length of the root hair and therefore have a potential impact on agricultural applications by allowing the extension of croplands. Phosphate starvation triggers a set of plant adaptive responses that involve a combination of growth, developmental, and metabolic changes [16–19]. A similar effect is observed upon starvation of nitrate [20]. Among the endogenous factors, auxin is known to have an effect on root hair elongation. Addition of exogenous auxin increases root hair length, whereas inhibition of auxin signaling or disruption of auxin transport results in a decrease in root hair length [21, 22].

Knowing the importance of root hairs in water and nutrient absorption, it becomes highly relevant to be able to fully understand the process by which they grow and the different factors that can affect this process. It is also relevant to see these effects in a live cell context because using measurements of growth at a specific end point can have the negative consequence of missing changes in growth dynamics [23] such as changes in growth rate that perhaps do not have an effect in the final growth length.

In the present chapter, we propose a method for analyzing the root hair growth through a live imaging approach. The current method allows you to determine growth dynamics and rates during a time course, in standard conditions and modified conditions, such as the exogenous addition of auxin.

2 Materials

2.1 Plant Growth

1. *Plant material. Arabidopsis thaliana* Columbia-0 seeds which can be obtained from the Nottingham Arabidopsis Stock Center or Arabidopsis Biological Resource Center.

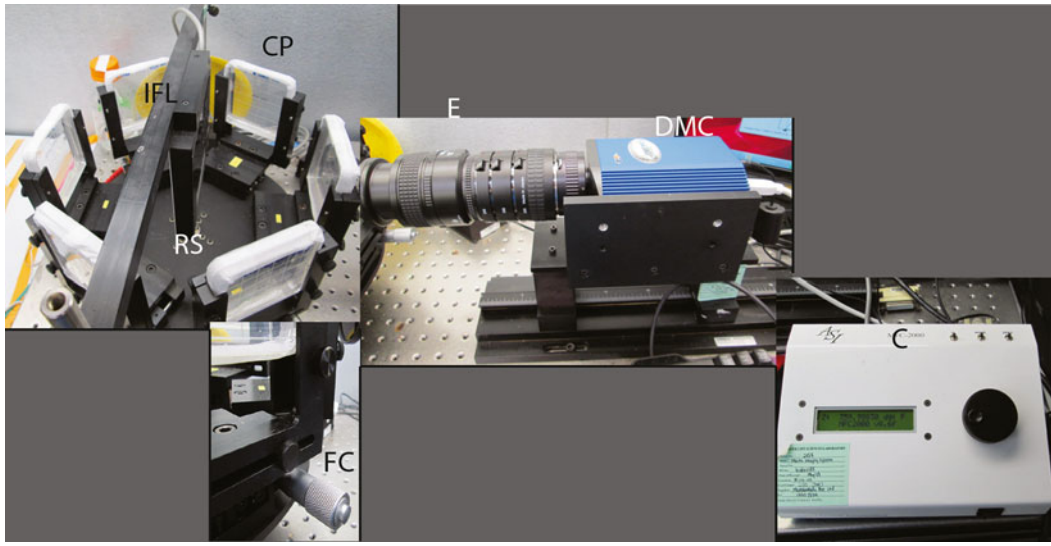

Fig. 1 Custom live-imaging system. *IFL* infrared light, *CP* circular platform, *RS* rotating stage, *E* extension tube, *DMC* digital monochrome camera, *C* controller, *FC* focus controller

2. *Plant growth*. Plants were grown in 0.5× MS medium (Caisson) supplemented with 0.7 % GelRight (Gelzan Sigma) at 21 °C under long day conditions (16 h light/8 h dark) for 7 days in vertical plates of 90 mm × 90 mm.
3. *Auxin treatment*. In the case of the auxin experiments, plants were grown in the same conditions as the standard ones, but supplementing the media with 1 μM Indol Acetic Acid (IAA). The auxin was added after the media had cooled and then poured all together into the plates.

2.2 Equipment

Custom live-imaging system [24] consisting of (Fig. 1):

1. Circular platform with six tissue-culture plate holders.
2. Automated Theta/360° rotary stage.
3. MFC-2000 controller (Applied Scientific Instrumentation).
4. Infrared light-emitting diode panel.
5. Digital monochrome camera (CoolSnap) fitted with a NF Micro-Nikon 60 mm lens (Nikon) and infrared filter.
6. Auto extension tube set DG (Kenko).

Software used to control de equipment:

Micro-Manager Software (Vale Lab, University of California, San Francisco) that allowed to control the stage and automate image acquisition [25].

2.3 Software Used for Image Analysis

The following plug-ins for ImageJ [26] software will be used to analyze the stack of images:

1. StackReg.
2. RootTipPrecalculate [27].
3. Manual Tracking.

3 Methods

3.1 Preparation of Seedlings Plates

1. 90 mm × 90 mm, half-strength MS, 1 % GelRight vertical plates were left to dry in a laminar flow hood for 20 min (*see* **Notes 1** and **2**).
2. 7-day-old seedlings were transferred with the help of forceps to the vertical plates. Up to 4 seedlings per plate were transferred (*see* **Note 3**). The seedlings were placed at the center bottom of the plate (2.6 cm from the bottom of the plate and 3.5 cm from each border, respectively) (Fig. 2).
3. A slice of 10 μl pipette tip was placed in between the roots of 2 seedlings as a reference point (*see* **Note 4**) (Fig. 2).
4. The plates need to be sealed at the bottom with parafilm so as to avoid leaking of water onto the equipment and then sealed all around with micropore tape.

3.2 Setting of the Plates on the Platform

1. The plate was set and secured in each plate holder (*see* **Note 5**).
2. Have the Micro-Manager software open on Live Snap.

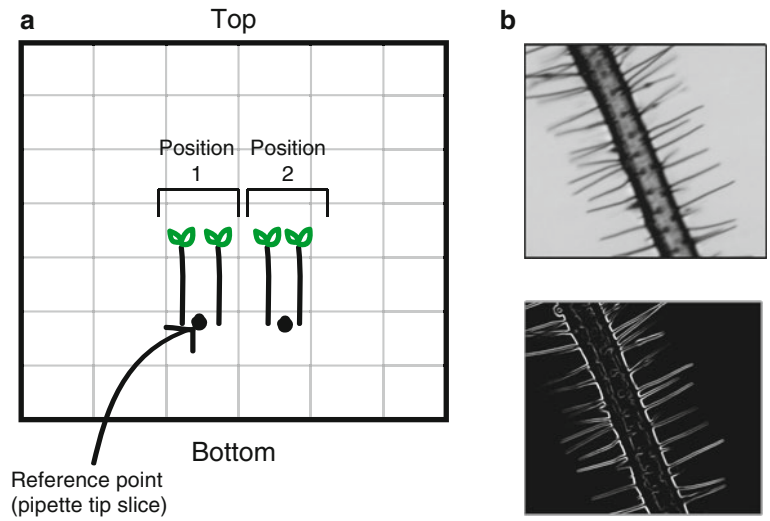

Fig. 2 (**a**) Schematic illustration of vertical plate for the live imaging set up. (**b**) Example of processed imaged ready for measurement

3. The focus was set for each plate individually with the aid of the micro-actuator focus control located at the bottom of each plate holder (Fig. 1). The focus is one for the entire plate so if you have several positions, it might happen that one or more of the positions are not in focus.

3.3 Setting of the Acquisition Parameters

1. For root hair imaging the exposition was set at 100 μs and the binning at 1.
2. When imaging more than one seedling, then the Multi-Dimensional Acquisition must be set up.
3. Up to two positions for imaging can be set up per plate (Fig. 2). To determine the positions one must go the Edit positions submenu, at the Multi-Dimensional Acquisitions panel.
4. Set up the zero position.
5. With the help of the platform controller place yourself on each position you want to acquire and mark it twice (*see* **Note 6**).
6. For root hairs, the images are taken every 5 min and for a time course of 24 h. That adds up to a total of 284 number of time points (*see* **Note 7**).
7. Create a folder to store the acquired images.
8. Press Acquire.

3.4 Processing of Images and Data Acquisition

1. Sequential images have to be imported to ImageJ as a stack.
2. The stack of images is aligned using the StackReg plug-in.
3. The RootTip Precalculate plug-in is used to enhance the contrast between the background and the root (Fig. 3).

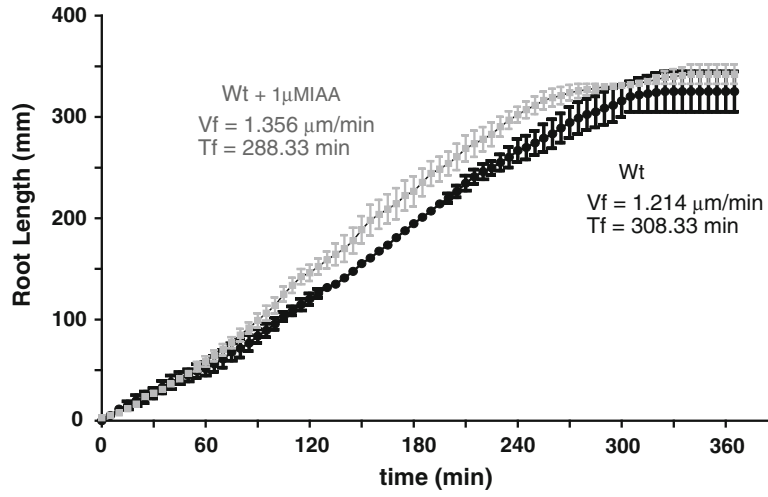

Fig. 3 Example of root hair length vs. time graph for Wt Col-0, in standard conditions and when 1 μM IAA had been added to the media. Total time of growth and growth rate are also included in this graph

4. A 200 % zoom was done on the areas where the root hairs were longer (which would be an indicative of a mature root hair) and where the entire development could be observed.
5. Quantification of the root hair growth is done with the Manual Tracking plug-in.
6. You must set the following parameters: time interval between the frames, *x/y* calibration (the inverse of the number of pixel per unit distance) (*see* **Note 8**).
7. The growth of the root hair is tracked manually from frame to frame, from its initiation to its completion (*see* **Note 9**). To start a new track, click on "Add Track." This will turn the tracking mode on.
8. To record *x/y* coordinates of a structure of interest, simply click on it on the image window. The results table will appear, showing recorded *x/y* coordinates, as well as the distance travelled by the root hair tip during the time interval between two successive images, and velocity of movement. The intensity of the corresponding pixel will also be recorded.

3.5 Data Analysis

1. In the table of measurements generated by ImageJ, each track is marked by a –1.
2. With the values of distance travelled by the root hair tip between each frame, you can calculate the total distance for each time point by adding the increments of travelled distance (e.g., for the time point 10 min, you must add the distance travelled between 0 and 5 min plus the distance between 5 and 10 min); the last value calculated being that of the total length of the root hair.
3. With the distances calculated above, you can generate a graph of root hair length vs. time. This graph will show you the growth dynamics of the root hair (Fig. 3) (*see* **Note 10**).
4. By adding all the time points, you can calculate the total time of growth and afterwards generate the corresponding graph.
5. By diving the total root hair length with the total time of growth, you can calculate the growth rate (*see* **Note 11**).

4 Notes

1. The preparation of the seedling plates to be imaged was all done in sterile conditions. 50 ml of media was added for every plate used.
2. The media can be supplemented with other compounds such as NaCl, hormones, chemical compounds, etc., so as to try other growth conditions other than the standard.

3. The seedlings selected for transfer had all the same root length.
4. Two reference points (=two slices of pipette tips) will be needed if there are 4 seedlings in the plate.
5. Up to 6 plates can be set up simultaneously (one for each plate holder available). Make sure the plates are properly secured as they may slide during the movement of the platform and you can lose the focus on the root.
6. Each position must be entered twice because due to the movement of the platform, the first set of images will probably be shaky. For each plate, if you have placed four seedlings you will end up with 4 sets of images.
7. You must set up the number of time positions manually based on the time course and the frequency of the acquisition of the images.
8. Remember to set the scale for each stack of images if you want the distances travelled to be expressed in units of length instead of pixels.
9. The initiation of the root hair was determined as the frame prior to the emergence of the root hair. Only root hairs that were growing in contact with the agar surface and at a 90° angle from the primary root were measured.
10. When representing the data for various mutant lines, that do not have the same final growth time, more time points can be added for the lines that end sooner but maintaining their final root hair length. In this way, the graphs will be more consistent.
11. Since the root hairs presented an initial lag phase in their growth, the growth rate was calculated for the constant growth phase.

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