PlantVis: A new software tool for analysis of root growth dynamics.

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ABSTRACT

Root growth is highly dynamic and responds quickly to changes in environmental conditions. Using a combination of confocal laser scanning microscopy and *Arabidopsis thaliana* genotypes expressing a plasma membrane targeted GFP, growth associated motion can be captured in short time-lapse image datasets (e.g. at 1 min intervals). We have developed a new image analysis software tool (*PlantVis*) to estimate displacements at pixel locations in these images to 0.1 pixel resolution, including a confidence measurement for each estimation.

Analysis of the *PlantVis* output using the statistical package R, allows extraction of biologically meaningful information. For example, motion estimates can be calculated relative to the central axis of a root and can be presented graphically as motion at specific distances along the root from the quiescent centre. Furthermore, the length of the division and elongation zones, and the position of maximum expansion can be calculated for individual datasets. Finally, depending on the magnification used during microscopy, elongation can be estimated for individual cells.

Using this methodology we have imaged and analysed root growth under a range of growth conditions, for example with differences in carbon supply and different physical conditions. Currently we are exploring the interaction of roots with their physical environment using glass ballotini beads that impose different physical stresses on the elongating roots.

Keywords: *PlantVis*, cell expansion, elongation, confocal microscopy, motion, root.

1. INTRODUCTION

Root elongation is produced by the cumulative elongation of cells after they divide in the meristem. Whilst the intrinsic rate of root elongation is affected by genetic background, the actual root elongation achieved is influenced greatly by the root environment. In soil these environmental variables include nutrient availability and soil physical properties, such as soil strength. Root responses to these continually changing conditions are highly dynamic and include both changes in longitudinal growth as well as radial motion. To further the study of these processes, tools that can capture and quantify cellular motion and expansion are required. These tools need to be able to measure changes in cell size of $<1\mu m/min$. Tools such as PIV (Bengough, 2009) and *Rootflow* (Van der Weele, 2003) have been used successfully to perform root kinetic analysis. However, in the the case of PIV, estimates of certainty in the tracks reported are not normally available, whilst *Rootflow* involves considerable averaging and interpolation of data. We have implemented a new software tool *PlantVis*, which produces estimates of the motion at pixel locations in an image sequence and, importantly, also provides estimates of the uncertainty of the motion estimation.

2. METHODS

2.1. Germplasm and growth conditions

Arabidopsis thaliana ecotypes Col and C24 expressing the 35S:LTIB:EGFP construct were surface sterilised and sown on to nutrient agar containing x0.5 or x0.1 Murishige and Skoog medium containing vitamins, 1% sucrose, 0.7 or 2% phytoagar, and, in certain experiments, glass beads (ballotini). Plates containing seeds were incubated at 4°C for between 3-5 days, prior to transfer to a growth room, with a 16 h day/8 h night cycle, at 22°C.

2.2. Imaging

Growing *Arabidopsis* roots were imaged using a Leica TCS SP1 confocal scanning laser microsope with a water dipping x10 objective, 488nm excitation argon laser, as transmission images (Figure 1(a)) and fluorescence emission images (Figure 1(b)) at 500-570 nm. Images were captured at 1024 x 1024 pixels at 1-2 min intervals.

2.3. Motion estimation and growth parameter estimation

Motion estimation was performed using *PlantVis* (Roberts, 2009). The software outputs six files: horizontal and vertical motion (pixels/frame), variance of the horizontal and vertical motion, and the covariance and determinant of the motion. The data was imported into the statistical package R for analysis and transformation into biologically meaningful formats (e.g. plots of longitudinal velocity vs distance from the quiescent centre). The step stool function of Peters & Baskin, 2006 was fitted to a transformed version of the longitudinal vs distance graph with the quiescent centre velocity set at zero. The first and second derivatives of this curve were used to extract growth parameters such as the maximum strain rate from the datasets. To test for effects of agar strength, genotype and ballotini size on the growth parameters measured, parameters obtained from roots grown in a range of growth conditions were analysed by unbalanced ANOVA using Genstat 11.

3. RESULTS AND DISCUSSION

3.1. Root growth kinetic analysis

Time-lapse datasets of *Arabidopsis* plants expressing LTI-eGFP were obtained in both transmission (e.g Root growing in media containing ballotini - Figure 1(a) and fluorescent channels - Figure 1(b). Using fluorescent imaging of the *Arabidopsis* roots allowed visualisation of the roots separately from the background of the ballotini beads. Analysis of the fluorescent images using *PlantVis*, allowed the calculation and representation of the motion between images as velocity in µm/min at each pixel coordinate. The data can then be transformed to produce velocity profiles along the root length for both longitudinal - Figure 1(c) and radial velocity - Figure 1(d), such that a step-stool spline function maybe fitted. Biologically relevant growth parameters (e.g. root tip velocity, position of the start and end of the elongation zone, the position of, and the quantity of the maximum strain rate) can be calculated using the first and second derivatives of this curve.

3.2. Effect of phytoagar, genotype, nutrient strength and ballotini on root growth.

Figure 2(a) shows the overall average velocity of the root tips calculated for a range of treatments. Columbia plants were found to have an average tip velocity of 2.0 µm/min compared with C24 tip velocity of 1.2 µm/min (p=0.026). Ballotini slowed root elongation, with root

velocity minimised for ballotini of 0.2μm diameter (p<0.001). *Arabidopsis* root diameter is approximately 100μm. This finding is probably because the ballotini packing allows roots to grow around larger beads, whilst the very smallest beads provoke more of continuum type behaviour with the embedding agar. In this set of data neither phytoagar nor nutrient concentration significantly affected root tip velocity.

More detailed analysis of changes in the kinetics of the roots in response to the growth media and as a result of the genotype are shown in Figure 2(b). This figure shows the position of the transition between the division zone and the elongation zone, the end of the elongation zone and the position of the maximum strain rate. 20g/L phytoagar resulted in both the beginning (p=0.001) and the end of the elongation zone (p<0.001) being closer to the quiescent centre without a change in overall elongation zone length (p=0.32). In Columbia plants the position of both the beginning and end of the elongation zone and the position of the maximum strain rate were further from the quiescent centre (p<0.018, p=0.004, p<0.001)

In Ballotini treatments the beginning and the end of the elongation zone was significantly affected (p<0.001, p=0.023). Radial motion in the ballotini datasets can also reveal interesting interactions between the roots and their physical environment - Figure 1(d) and also Bengough, 2009. Further exploration of these datasets with respect to the radial motion is needed to fully understand the physical interactions between the ballotini and the growing roots.

In conclusion, *PlantVis – R* is a useful and flexible tool that can be used to explore root kinetic responses to a wide range of environmental conditions.

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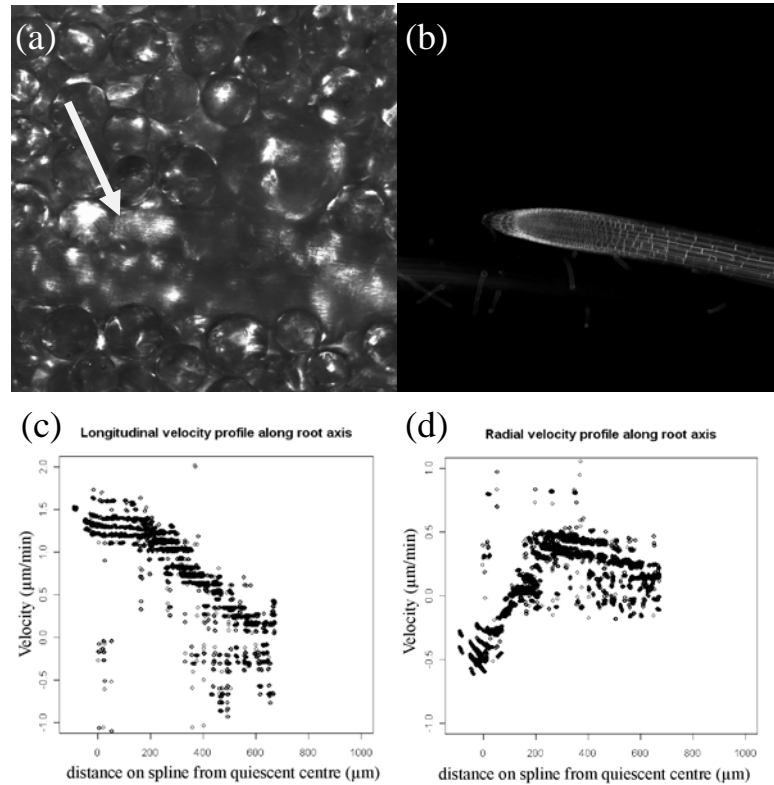
Figure 1. Image analysis of *Arabidopsis* root growth using *PlantVis-R* – Confocal laser scanning microscopy image of *Arabidopsis* LTIb-eGFP growing in nutrient medium containing ballotini beads, (a) transmission image (arrow - position of quiescent centre) (b) fluorescence image. (c) Longitudinal velocity and (d) radial velocity at pixels relative to their distance along the root from the quiescent centre.
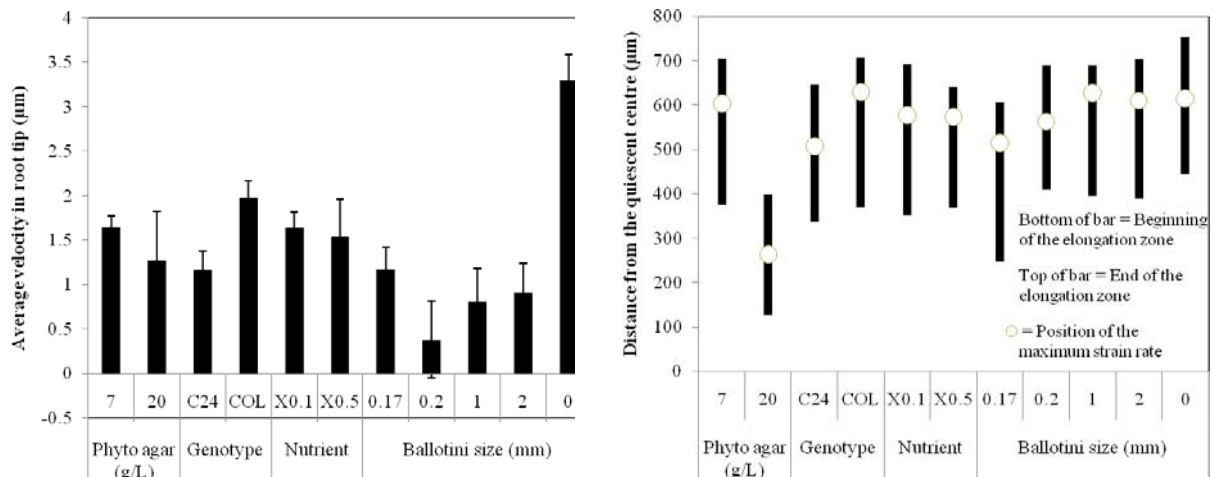

Figure 2. (a) Average velocity of root tip (Agar strength p=0.530, Genotype p=0.026, Nutrient p=0.204, Ballotini p<0.001). (b) Position of the beginning and end of the elongation zone, maximum strain rate position (Agar strength p=0.001, p<0.001, p<0.001, Genotype, p=0.018, p=0.004, p<0.001, Nutrient p=0.924, p=0.159, p=0.832, Ballotini p=<0.001, p=0.023, p=0.194)