Development of a Robot-Based Platform Applied to Simultaneous Root Growth Profiling of Seedlings Growing in a Petri Dish

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Abstract: - A newly developed platform is described which allows automatic detection of root growth characteristics for several seedlings simultaneously with high spatial and temporal resolution. A Petri dish containing young seedlings of Arabidopsis thaliana planted in a row is mounted on a vertical sample stage. This computer controlled two dimensional positioning unit enables screening of the entire Petri dish under the binocular microscope with a resolution of 5.6 x 5.8 µm per pixel. Based on the obtained video images the growth pattern of individual roots is automatically calculated and graphed by our newly developed software package. Proof of the developed system is provided by application to Arabidopsis seedlings (wild type (*col0)* and starch mutants (*sex1* and *pgm*)). The results show decrease of growth rate during dark period in case of *sex*1 and *pgm* mutants, ending to no growth, while the wild type *col0* seedlings continue growing almost the same as during light period.

Key-Words: - Root growth, Image processing, Starch mutants, Time lapse record, Root tip detection

1 Introduction

The growth of plants has traditionally been studied by marking the position of root tips on a transparent surface and measuring the changes over time. Ink, graphite or resin beads were used for marking on the glass window of the Rhizotron, the surface of Petri dishes or acetate papers. Later, photographs were taken and the position of root tip was measured with a ruler, but as technology improved, the photographs were replaced with digital images and the ruler with a video cursor. Nevertheless, the basis of the approach stayed the same: The position of a particle is measured directly in a series of images and the trajectory of this particle defines its velocity. The requirement to make marks limits this approach. A relatively large time interval must elapse to give a measurable displacement, thus only a low density of marks can be applied. This approach is also limited by the tedious, error-prone, subjective nature of the manual measurement process.

The marking approach has been improved by developing software to recognize marks automatically [1]. Using video imaging technology, Ishikawa and Evens [2] developed Multi-ADAPT as an automated system for studying root gravitropism. An alternative means to measure the spatial profile of growth is available, in principle, from image processing techniques for "image sequence" analysis. In these techniques, a sequence of images is captured with a relatively short time interval between images. The sequence is then treated as a three-dimensional image volume, and one or more filters are applied to define spatiotemporal structures in the volume. Modern digital image sequence processing methods make it feasible to study the growth of leaves [3] and roots [4-6] with higher spatial and temporal resolution. These methods are applicable to processes of very different geometries and scales. They are not only more precise than traditional ones, but also less invasive, highly automated and applicable on both above- and below-ground plant organs.

Here we present ROGO1, a newly developed platform for multiple root growth analysis which makes it possible to screen a defined area over the surface of the Petri dish after 30, 60 or 90 minute time periods and analyse time lapse records with spatial resolution of 5.6 x 5.8 µm per pixel.

2 Results

2.1 Hardware component of ROGO1

The hardware component of ROGO1 is basically composed of an infrared light source, a computer controlled robot arm which holds the sample stage and is able to move in two dimensions, the microscope/camera unit and the controlling computer (Fig.1).

The Petri dish containing growing seedlings is mounted vertically on the robot arm. The CCD camera is connected to a vertically placed binocular microscope which, with application of an infrared light source together with an infrared filter, enables continuous

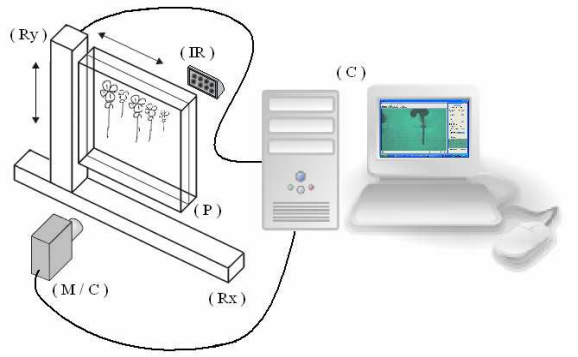

Fig.1. Schematic diagram illustrating structure of the hardware component of the ROGO1 platform. An infra red illuminator supplies the video imaging light. A sequence of three time lapse records with 800ms interval is grabbed before each movement of the robot arm. (C) :Controlling computer, (IR) : Infra red light source, (Ry) : y axis of the robot arm, (M / C) : Microscope/Camera unit, (Rx) : x-axis of the robot arm, (P) : Petri dish containing growing seedlings

monitoring of the surface of the Petri dish regardless of the external light. The photosynthetic light intensity varies in the range of 750-1250 Lux over the movement plane of the Petri dish due to the distance from the light source. To assure constant temperature, the measuring head is enclosed by a temperature controlled Plexiglas shield.

2.2 Software component of ROGO1

As illustrated in Fig.2, subunits of the software package are designed for controlling the movement of the robot arm, retrieving the time lapse records and storing them together with light and temperature sensor data, filtering non-informative records and analyzing them.

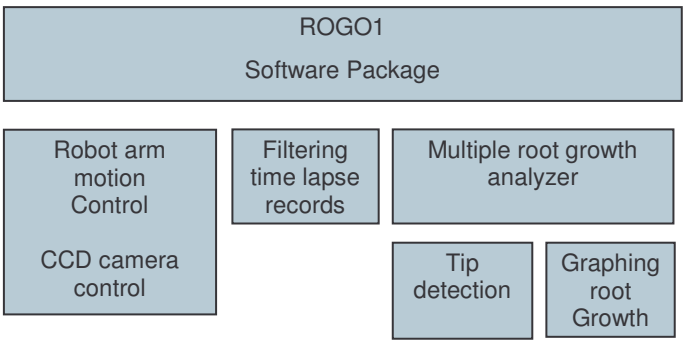

Fig.2. Schematic diagram showing the structure of the ROGO1 software package

2.2.1 Controlling the Robot Arm and CCD Camera

The robot arm changes the position of samples every 6 seconds. To eliminate the effect of vibrations caused by movement of the robot arm, three time lapse records are retrieved, with 800ms intervals, before each movement. Obtained 768x576 pixel colour image files are then stored along with other relevant information including coordinates of the viewing frame and time of retrieval. Each record covers an area of 4.3 x 3.3 mm over the surface of the plate which leads to a resolution of 5.68 x 5.85µm per pixel. Meanwhile, a scaled down copy of one of the records in each position is used to assemble an image of the whole plate which is completed by the end of complete screening of the entire plate (Fig.3). The light intensity and temperature data is also recorded at the end of each screening.

2.2.2 Filtering Time Lapse Records

Screening two third of the plate surface over an hour leads to 1800 files occupying about 105 MB. However since not every single observed frame over this area contains plant root or root tip, this size is reduced by removing records of non-informative positions.

2.2.3 Multiple Root Growth Analyzer

2.2.3.1 Root Tip Detection

Root tip detection starts by applying thresholds on the red, green and blue values of image pixel data. The image is screened from bottom to top; two areas are defined around each candidate pixel, one above and one below. If the number of dark pixels in the upper area exceeds a certain number and the number of dark pixels in the lower area falls below a certain number, then this candidate pixel is considered to be the root tip. The average values of x and y coordinates of the detected pixel from three time lapse records for each plate position is finally returned as the root tip position. However these values are in image coordinates which are then converted to a plate based value using the position of the viewing frame.

An image subtraction method is not applied as the default detection method since roots grow slower under some conditions or even there is no visible growth. However root tip detection based on image subtraction is of crucial importance when a root tip comes close to another root or any permanent dark object in background (mainly air bubbles being formed while pouring the media in Petri dishes), or studying the growth of lateral roots from early emerging states and can be chosen by the user.

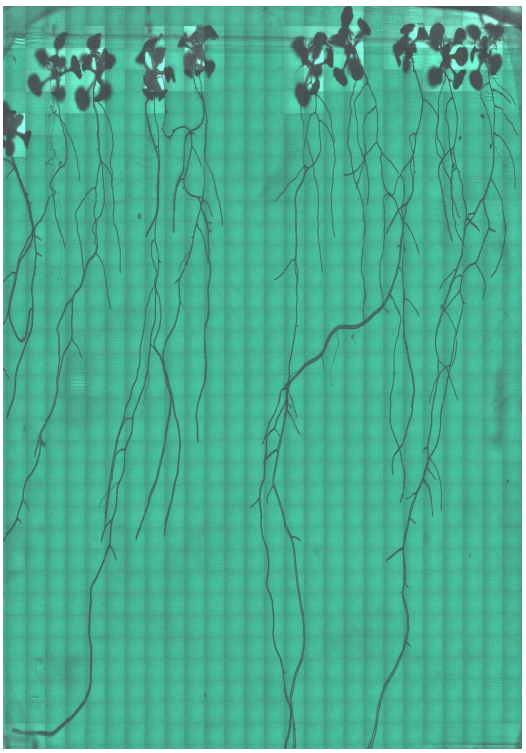

Fig.3. An example of a merged plate image showing the whole surface of a Petri dish containing ten seedlings. Each square in this image originates from the time lapse record grabbed from one view frame.

2.2.3.2 Further Analysis and Visualization of Growth Data

Growth rate values are then calculated from root tip positions and averaged over light/dark periods or different days. The average 24 hour growth behaviour of the sample root is also calculated by overlaying the growth rate values over a 24 hour period and averaging them. Resulting values are depicted as position over time (Fig.4.a) or growth rate over time (Fig.4.b-e) graphs with the possibility of averaging several data sets.

3 Proof of Concept

One of the first experiments done using this platform was studying the growth behaviour of starch mutants, *pgm* and *sex1* in comparison to the *col0* wild type seedlings.

In the light, photosynthetic CO2 fixation drives the synthesis of sucrose in leaves and its export to the remainder of the plant to support growth and storage, whereas at night the plant becomes a net consumer of carbon [7, 8].

Starch normally accumulates in leaves in the light and is remobilized and converted to sucrose at night [9]. The *pgm* mutant lacks plastid phosphoglucomutase activity, which is an essential enzyme for photosynthetic starch synthesis [10]. It accumulates very high levels of sugars in the day, but has very low levels of sugars in the second part of the night [8, 9].

The *sex1* mutant was originally isolated in an iodine-based screen for mutant plants unable to degrade starch in the dark [11].Levels of starch in the leaves of *sex1* plants grown under a 12 h light regime are about five times higher than the maximum levels in wild-type plants, and show less diurnal variation [12]. Initial work indicated that capacity for exchange of glucose across the chloroplast envelope was impaired in the mutant, and it was suggested to lack glucose [12]. Later cloning of the major glucose transporter of the chloroplast envelope showed, however, that the gene encoding this protein is on chromosome 5 whereas the *sex1* mutation maps to chromosome 1 [13].

Map-based cloning approaches have finally shown that the gene at the *sex1* locus encodes the *Arabidopsis* homologue of the R1 protein [14], a glucan, water dikinase (GWD) necessary for normal starch degradation in potato (*Solanum tuberosum)* [15, 16].

3.1 Plant Growth

Surface sterilized seeds of Arabidopsis thaliana (Wild-type and starch mutants *pgm* and *sex1*) were kept overnight in 1ml liquid solution of 15% selective agar containing 1µl Gibberellic acid in room temperature. Seeds were then placed on the surface of AM solid agar media (0.24 % Murashige and Skoog-Medium; 0.055 % MES; 0.7 % agar) prepared in 13cm square Petri dishes in a row 2 cm far from the top border. Plates were then placed vertically in 4°C for 4 days. Seedlings continued their germination in 19°C temperature and under 12 / 12 hour light/dark protocol. For studying root growth, 9 days old Petri dishes were investigated on the platform.

3.2 Growth Results

Fig.4 shows the growth behaviour of the wild type seedlings screened every half an hour. Position of root tips from 9 seedlings over the surface of the plate is shown in (a). Based on these positions, the growth rate for each time period is calculated. The averages of these values over day periods or light/dark periods are shown in (b) and (c) respectively. In addition, the average growth rate value of all the 9 seedlings growing on the plate is presented in (d), and the 24 hour growth behaviour of all seedlings is presented in (e). As shown in Fig.4.a, b and d, these seedlings present a steady growth over this period, with no big change in growth rate during dark and light period (Fig.4.c) and an almost constant growth rate during the day. In case of the starch mutants, a completely different growth pattern is seen; seedlings decrease their growth rate during dark period, and by the beginning of the next light period, they start to grow again (unpublished data).

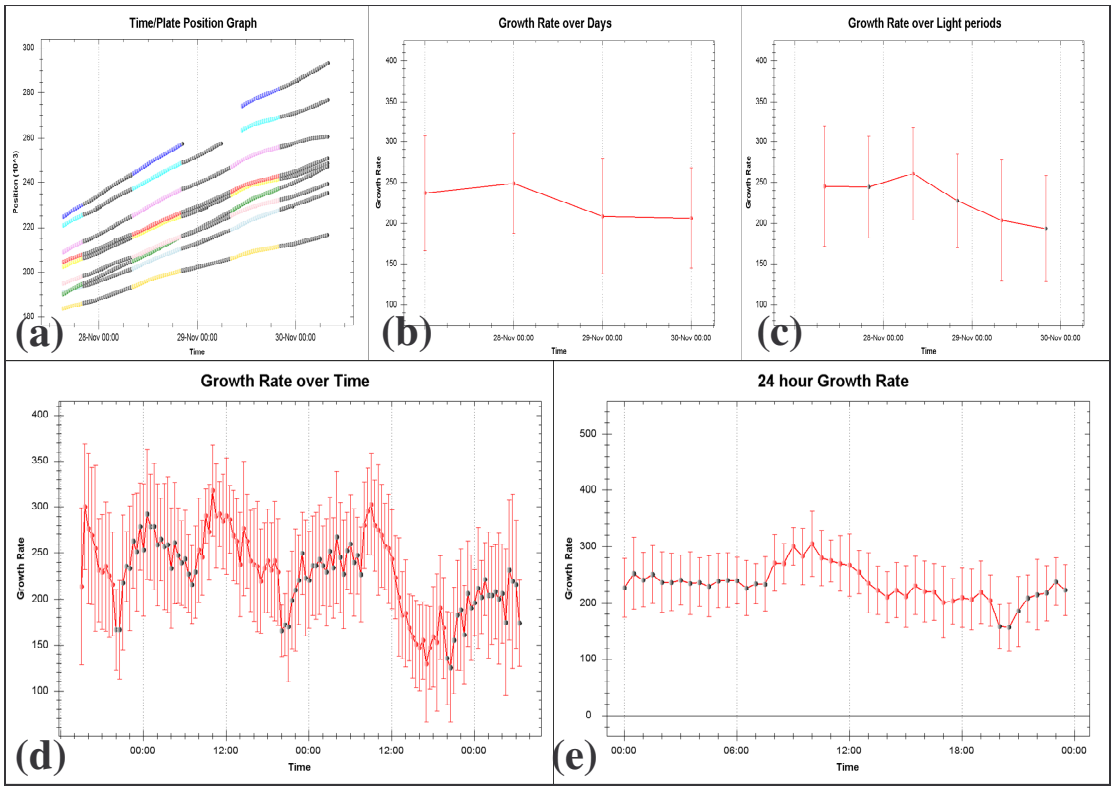

Fig.4. Root Growth behavior of Col0 seedlings depicted as graphs. The light period is shown with colored dots and dark period as black dots.
(a) : Plate Position over time graph, (b) : Average of growth rate during one day period, averaged between all seedlings, (c) : Average of growth rate over light/dark periods, averaged among seedlings, (d) : Growth rate over time averaged among different seedlings, (e) : 24 hour growth behavior averaged among seedlings.

4 Conclusion

A robot based platform and its controlling software package is developed for analysing root growth by screening the surface of a Petri dish with spatial resolution of 5.68 x 5.85 µm per pixel over periods of 30, 60 or 90 minutes. Processing of the time lapse records by the developed software package results to the growth profile of the seedlings and is visualized as position over time or growth rate over time graphs.
Application of this system will enable to unravel fundamental questions in plant physiology in an unprecedented manner. In particular, determinants of root growth and thus plant performance can be investigated with highest spatial resolution down to the single cell level. In combination with various mutated plants and the ability to modify the growth media, the system will provide a high throughput detector for improvement of plant nutritional parameters and due to its capacity to simultaneously observe many roots it will furthermore enable a high throughput screening of root growth mutants.

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