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Title

Advances to Whole Root System Quantification Tools and Techniques

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Introduction

As genetic resources and techniques continue to advance, the identification of genes controlling important aspects of crop performance relies on the accurate and efficient assessment of phenotypic traits. For root system analysis, a number of research groups around the world are beginning to develop projects that work with machine vision and imaging techniques to facilitate more holistic and quantitative studies into root function and development. We are beginning to see the results of these efforts appear in scientific journals (Armengaud, P. et al. 2009; de Dorlodot, S. et al. 2007; Jahnke, S. et al. 2009; Miller, N. D. et al. 2007; Qi, X. et al. 2007). Many of these projects are directly and indirectly affecting how roots systems are viewed and evaluated in relations to environmental and nutritional signals.

Manual methods for quantification of single roots or root systems remain relievable, but often limit the size or even the kinds of data that can be collected in root phenotyping studies. In order to better accommodate large studies, integrated systems are being developed to measure quantifiable root traits with improved efficiency and accuracy. Additionally, these systems are also being designed to investigate mathematical and geometrical characteristics of whole root systems that were not possible before.

Results

To facilitate large scale aluminum (Al) tolerance screening experiments, we have developed and redesigned several aspects of our growth systems, as well as built custom 2D imaging and software tools to capture and analyze whole root systems. Working with hydroponic growth systems we designed a flexible growth setup that allows plants to be grown and photographed with minimal disturbance to their root systems. To capture high quality root images, we constructed a custom imaging system that takes advantage of improved optics and unique lighting techniques to provide high contrast images of both coarse and fine root systems.

Using the Java Web Start Technology, we developed RootReader2D software (Figure 1) to assist in the quantification of individual and whole root system lengths. This software provides an interactive interface with which root lengths can be measured and recorded from root images. While the ability to batch process and measure whole root systems lengths was the main purpose for creating this software, this software also serves as a platform for the incorporation of other phenotypic descriptors such as lateral root counters, width, area and angle measurement. Additionally, these imaging and software tools are being adapted to measure root characteristic from Arabidopsis plants grown on agar plate systems.

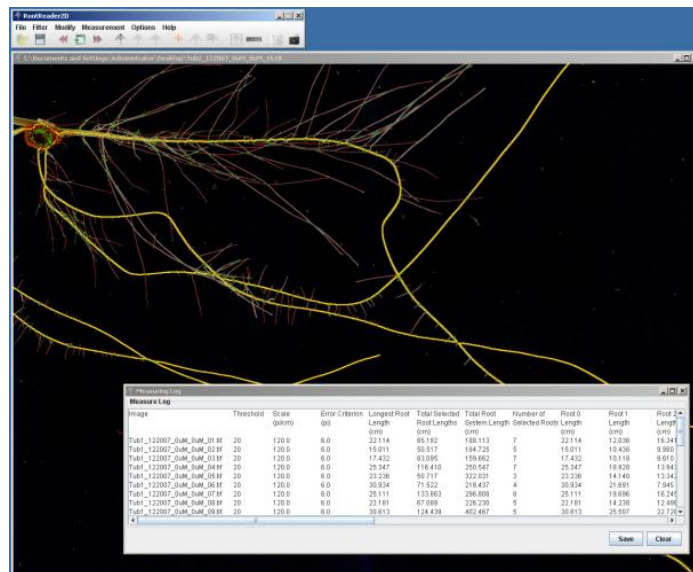


Figure 1: Screenshot of RootReader2D software and sample maize root system image that has been measured for primary, basal and lateral root lengths.

To begin to evaluate 3D root architecture and responses to changes in inorganic phosphorus (P_i) availability, we have developed optical, mechanical and software tools that build on existing silhouette-based 3D root imaging principles (Zhu, T. et al. 2006). Proof of concept work was performed using thin wires as “mock root systems”, and an optical correction tank and axis-of-rotation calibration technique was developed to increase the accuracy and robustness of 3D reconstructions and model generation. Additionally, RootReader3D software (Figure 2) was written to pre-process, reconstruct, and analysis of 3D root system architecture from 2D root image sequences.

Using rice plants grown under semi-sterile conditions in glass cylinders containing purified gellan gum media with a replete nutrient solution, 3D reconstructions of growing root systems were generated. To better control the initial nutrient content of the gellan gum media and to facilitate further P-deficiency studies, mineral contamination found in commercial gellan gum was removed using an adapted purification protocol (Doner, L. W. et al. 1995; Jain, A. et al. 2009).

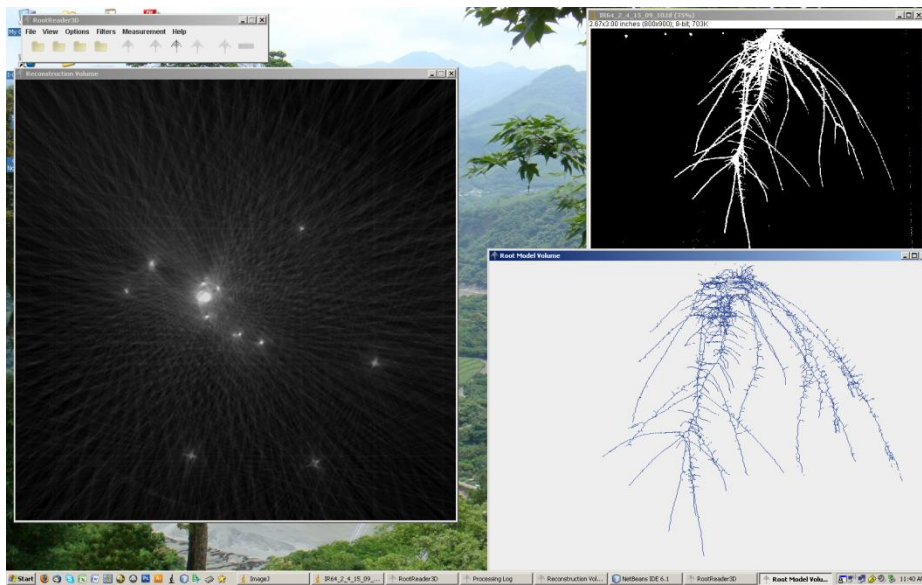


Figure 2: Screenshot of RootReader3D software and a sample reconstructed rice root system cross-section and skeleton.

Improvements to growth systems and techniques combined with advancements to imaging and quantification software will continue provide great opportunities for studying the dynamic growth responses of roots systems under a range of nutrient and stress environments.

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