A method for rapid quantification of root hair density in situ

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Introduction

Root hairs enhance plant water and nutrient uptake by greatly increasing root surface area, thereby enabling the root system to explore a large volume of soil relative to root area. Despite the vital contribution of root hairs to plant function, they are often overlooked in plant studies because of the technical difficulties associated with their measurement. Digital imaging of whole root systems in rhizotrons or mini-rhizotrons allows for repeated in situ observation and quantification of root system architecture, including root hairs. Although root hair development and growth can be recorded on digital images, quantifying these very thin and numerous structures is challenging. Current standard methods for quantifying root size and density involve hand tracing and measuring individual root segments and summing the sizes of these individual segments. This is not practical for root hairs due to the enormous time and effort needed to trace and measure these very thin and numerous structures. We have explored a novel technique using open-source image analysis software to quantify root hair density utilizing images from minirhizotrons.

Materials and Methods

Minirhizotron images with root hairs present were converted to gray scale and standardized for brightness and contrast. Selecting an area with root hairs but not other roots was performed in ImageJ image analysis software. Using the R language a threshold value was determined in order to distinguish substrate from roots based on tone (brightness or darkness). Threshold values were determined by placing image selections in visually distinguishable density categories, as well as a fourth category of selections with no root hairs present. The threshold that separated the density categories and estimated the sections with no roots near zero was selected as the best performing threshold. The threshold was then applied to all image selections to estimate root hair density for each image in each category. After selecting a threshold, all image selections with root hairs can be projected as a two-dimensional root hair area, comparable to length and diameter estimates of roots estimated by standard root imaging software.

Results and Discussion

The optimum threshold values vary by plant species and substrate (soil) color and therefore must be adjusted for each plant species-substrate combination. The technique was validated with three different plant-soil combinations: papaya (*Carica papaya* L.) in peat-perlite and sesame (*Sesamum indicum* L.) and castor bean (*Ricinus comunus* L.) in sandy soil. Root hair density in each species-substrate combination was compared with hand tracings of root hair images. The new method compared well with the hand tracing method, but required approximately 5% of the time required for manual tracing.

Conclusion

Utilizing this method for measuring root hair density from minirhizotron images over time allows for the quantification of episodic changes in root hair density during plant development on a scale that was not practical using manual methods.

Rasband WS (2014) ImageJ. U. S. National Institutes of Health, Bethesda, Maryland, USA, (http://imagej.nih.gov/ij/)
R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (http://www.R-project.org/)