Visualization of Soybean Root Morphology Using Magnetic Resonance Imaging

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Introduction

Magnetic Resonance Imaging (MRI) is one of several techniques within the broader field of Nuclear Magnetic Resonance (NMR) technology. NMR concerns the differentiation of certain isotopes based on the magnetic properties of their atomic nuclei. These nuclei possess a physical quality called non-zero spin, which resonates at a particular frequency proportional to a local magnetic field. ^1H (hydronium) has a spin quantum number of 1/2 making it ideal for NMR detection. MRI is accomplished through recording and arranging frequency signal units (voxels) within a specimen volume exposed to a magnetic field to form a visual model.

Water, of which 2/3 is hydronium, is the most abundant chemical substance in living systems and an ideal pool of nuclei for imaging. Differences in nuclear relaxation properties as the magnetic field is manipulated varies the signal intensity from ^1H within each voxel to provide contrast in the image.

Here, the experimental goal was to image juvenile soybean root systems growing *in situ* to visualize root morphology and estimate root volume by counting root voxels.

Materials and Methods

Soybean seeds were germinated in paper rolls for 2d and transferred to 7 x 10 cm cylindrical cups containing a fine sand. The seedlings were grown for 6d in a phytotron (SEPEL, North Carolina State University) at optimum light, temperature, and fertility. The seedlings were transferred to the Center for In Vivo Microscopy, Duke University for MR imaging. Root systems were imaged in a General Electric Signa MRI unit customized for high resolution research studies. Specimens were placed inside a radio frequency coil tuned to 85 MHz, then placed into the bore of a 2T superconducting magnet. Three dimensional image sets arranged in a 256 x 256 x 128 voxel array were acquired with 390 um in plane resolution. Images were viewed using Voxelview 5 on a Unix platform workstation.

Root volumes were calculated by counting the number of voxels (390 x 390 x 780 um) viewed to be part of the root system. The measurements were compared to water displacement and water weight volume (derived from subtracting dry weight from fresh weight, then dividing by the density of water) estimates of fresh root tissues following imaging.

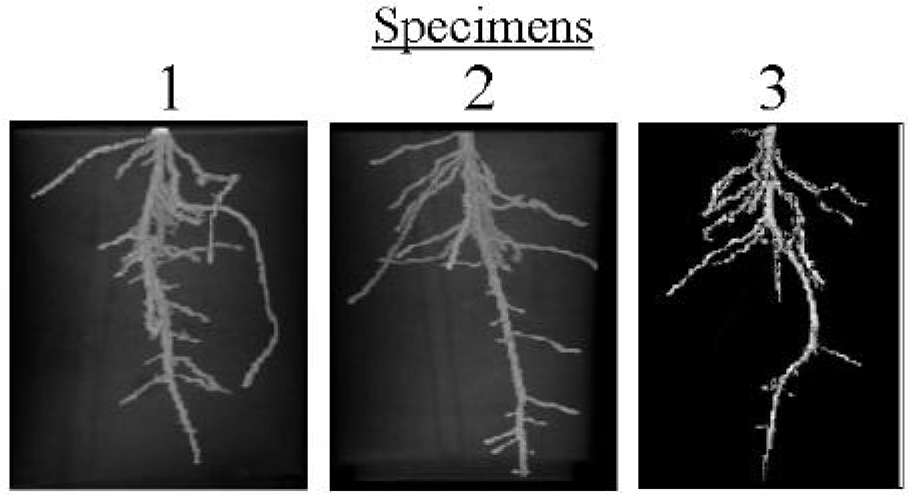

Table 1

Specimen	Voxelview Volume Estimate (mm^3)	Water Displacement Volume (mm^3)	Water Weight Volume Estimate (mm^3)
1	609	500	590
2	683	600	600
3	739	600	530

Results

Three dimensional image stacks were rendered for each specimen using Voxelview. The renderings can be rotated and viewed from any angle as well as captured into a movie format as demonstrated on the computer below. Visual inspection of the images revealed that each plant had a prominent tap root structure with longer upper basal and shorter lower lateral roots radiating out towards the container sides. In addition to qualitative visualization, each specimen was analyzed for total root volume using the “Seedfill” function in Voxelview. This function assesses the intensity of each voxel and all adjacent voxels and collects all connected voxels determined to be above a certain threshold intensity. Once the number of root voxels was estimated and knowing the volume per voxel, total root volume was estimated. Table 1 shows the total volume estimates for each specimen along with two corroborative physical measurements: water displacement and water content by weight. The values for the three volume estimates for each method were similar.

Conclusions

* NMR images of soybean root systems provided qualitative visualization of root morphology of 3 specimens.

* Voxelview 5 provided quantitative data for total root volume for each specimen and was corroborated by two physical measurement methods.

* MRI is an effective tool in studying soybean root development *in situ*.

Appendix

We use a complementary method to estimate total root length and surface area (Wright et al. 1998). The method involves removing the roots from sand, placing them in a shallow film of water and capturing 2 dimensional images. The images were subsequently analyzed using computer software which estimates total root length and surface area (Table2).

This method combined with the MRI technique gives a comprehensive qualitative and quantitative picture of root morphology for a given soybean specimen.

Table 2

Specimen	Total Estimated Root Length (mm)	Total Estimated Root Surface Area (mm^2)
1	1457	2289
2	1116	1752
3	1007	1582

References

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