Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 87–89, January 1986
Botany

NMR imaging shows water distribution and transport in plant root systems *in situ*

(*Vicia faba* L./stress/germination/pathology/flow)

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Communicated by Paul J. Kramer, August 26, 1985

ABSTRACT **Images of *Vicia faba* L. root systems at 0.6-mm resolution, undisturbed and within the soil medium in which they were grown, have been obtained by using a 1.5-Tesla proton (^{1}H) NMR medical imaging research system. Images of root systems in seven soil media (Cahaba soil, vermiculite, sand, perlite, fritted clay, potting soil, and peatlite) exhibited variable but useful quality owing to a diversity of magnetic properties of the soils. Root structure and pathology in the form of partial decay of hypogeal cotyledons were easily discernible. Water transport in roots with light-stressed foliage was demonstrated by using water doped with a paramagnetic NMR contrast agent, and the process of plant-wilt and recovery was monitored *in situ*. Images of germinated seeds within soil media indicated that dynamic observations of germination and growth are possible. The results suggest that NMR imaging can be an effective noninvasive tool for studying plants *in situ*.**

Existing techniques for the study of plant root structure and function are labor-intensive, often highly destructive, and lack accuracy. Consequently, compared to aboveground plant structures, root systems are seldom a topic of research and investigators have frequently emphasized the difficulty of obtaining root data (1–3). Clearly, there is a need for better approaches.

Proton (^{1}H) NMR imaging is a new technique in medical diagnosis that employs static and radio-frequency (rf) magnetic fields to acquire maps of the mobile water distribution in biological systems (4–7). As soils are largely permeable to these fields and roots are relatively abundant in water, NMR imaging offers a potentially unique means of accessing structural, growth, and hydrodynamic information on root systems *in situ*. Although conventional ^{1}H NMR and NMR imaging have been used to study flow in plant stems (8, 9) and water distribution in vegetables (6, 7), we know of no application of NMR imaging to intact root systems or plant foliage. We present here an investigation of ^{1}H NMR imaging in plant root systems. Spatial resolution, water transport, pathology, seed growth, and the effect of soil type on image quality are examined in the root system of the bean *Vicia faba* L.

MATERIALS AND METHODS

Study plants were produced by pregerminating *V. faba* seeds in germination paper wetted with 0.01 mM $CaCl_2$. At 3 days, the seedlings were potted in 15-cm standard plastic pots of seven different soil media: Cahaba soil at 0.9% moisture by weight with a dry density of 1.23 g/cm^3 (fine-loamy siliceous, thermic Typic Hapludults), sand (medium grain, construction grade) at 1.0% and 1.11 g/cm^3, vermiculite at 143% and 0.19 g/cm^3 and perlite at 266% and 0.08 g/cm^3 (Terra-Lite, W. R. Grace and Co., Cambridge, MA), peatlite at 19% and 0.11 g/cm^3 (Pro-Mix, Premier Brands, New Rochelle, NY), fritted clay at 28% and 0.6 g/cm^3 (Absorb-N-Dry, Balcones Mineral, Flatonia, TX), and potting soil at 105% and 0.19 g/cm^3 (K-Mart Ready-to-Use, Old Fort Industries, Fort Wayne, IN). A 1.5-cm bed of washed gravel in each pot bottom facilitated drainage. All seedlings were grown in a greenhouse. Several pots were seeded directly to permit observation of germination. Plants were grown under relatively dry conditions to avoid waterlogging, which could kill the roots, but were rewatered at the first sign of stress. Root systems were imaged prior to watering, toward the end of drying cycles. Light was supplied to plants outside the NMR machine by three 850-W quartz/iodide lamps (Hedler, Turbo-Lux Super Safe 1250) and within the magnet bore during imaging experiments by two 100-W, 12-VDC quartz/iodide lamps (Sylvania EFP) in nonmagnetic housings. Lights were positioned to provide a suitable spectrum for plant growth with an incident photon flux density of 2000 microeinsteins·m^{-2}·s^{-1}. Air temperature and relative humidity were maintained during imaging experiments at 26.5°C and 38%, respectively. After imaging, root systems were carefully removed from the soil and washed and photographed for comparison with images.

^{1}H NMR images were obtained at 63 MHz on a 1.5-T General Electric medical research system (10) using a 14-cm (diameter) receiver coil. Potted samples were positioned vertically within the coil, and a conventional 256 × 256 point spin-warp spin-echo imaging sequence was applied with 0.56 mm × 0.56 mm resolution in the imaging plane (vertical and horizontal directions) (10). Spatial selection in the third dimension was omitted. To compensate for inhomogeneous NMR line-broadening in soil media, the NMR spin-echo delay time was minimized ($\pi/2$ to π NMR pulse separation, 6 ms), the imaging magnetic field gradient strengths were maximized (52 μT/cm), and an 8-ms data acquisition period was employed. Even so, soil diamagnetism and ferromagnetism caused local spatial distortion and signal loss in some images. The imaging pulse sequence repetition period was either 1.0 or 0.2 s, resulting in image scan times of 8.5 or 3.4 min, respectively.

RESULTS AND DISCUSSION

An 8.5-min root system image of a 30-day-old *V. faba* plant in peatlite is shown in Fig. 1*A*. The image intensity is proportional to the mobile water density: signal from bound water and cellulose with short NMR spin-spin relaxation times ($T_2 \leq 10$ ms) does not appear in the spin-echo or image. The vertical wavy line at the left is a 9-cm long × 1.2-mm inner diameter straight capillary filled with water and inserted into the soil to a depth of 8 cm as an indicator of image distortion. The lower portion of the plant stems, their piths, the hypogeal cotyledons, and the root system are clearly

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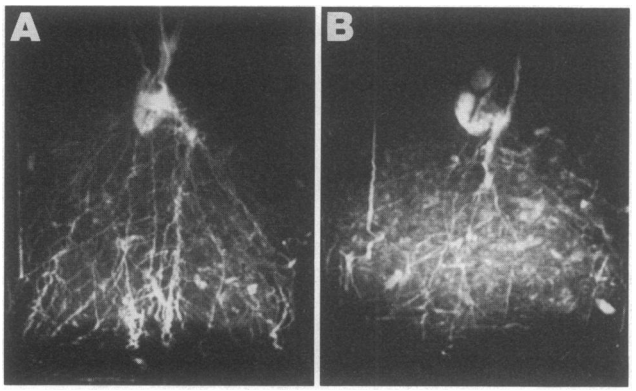

FIG. 1. (*A*) ^1H NMR image of a 30-day-old *V. faba* root system in a 15-cm plastic pot of peatlite (19% moisture by weight; 0.11 g/cm^3, dry density) recorded in 8.5 min at 1.5 T with 1.0-s pulse repetition period. The image consists of 256 × 256, 0.56 mm × 0.56 mm data points unresolved in the third dimension. The plant is viewed from the side with the stem at the top. Lower stems, cotyledons, roots, and a 9 cm × 0.12 cm capillary of $CuSO_4$ solution are seen. The soil–air interface falls just above the cotyledons. (*B*) Image of another *V. faba* root system in peatlite in which the cotyledons show decayed areas at the top (dark grey). (Scale = 1:3.3.)

visible in the image below the soil surface. The cotyledons are filled with water and separated: the bright area at their center is the thick fleshy embryonic axis of *V. faba*. The low intensity to high intensity of roots from the top down is a natural rooting pattern reflecting the higher water content of deeper soil. Fig. 1*B* is an image of a similar root system in peatlite with higher soil moisture giving less root-to-soil contrast and with the cotyledonary attachment seen from a lateral rather than a frontal view. The cotyledons exhibit an irregularly shaped dark grey zone at the top, indicating reduced water content. This zone was later found to have decayed due to a seed rot.

The variation in NMR image quality with soil type is exemplified in Fig. 2, which shows a 12-day-old *V. faba* seedling imaged bare and after potting into fritted clay (scan time, 8.5 min). The smooth nature of the seed coat, the points of secondary root attachment to the taproot, and a bright juncture where the root, cotyledons, and stem attach are visible in the images. Again, the lower secondary roots contain more water than the upper roots: the high water content of the taproot likely derives from these. Although

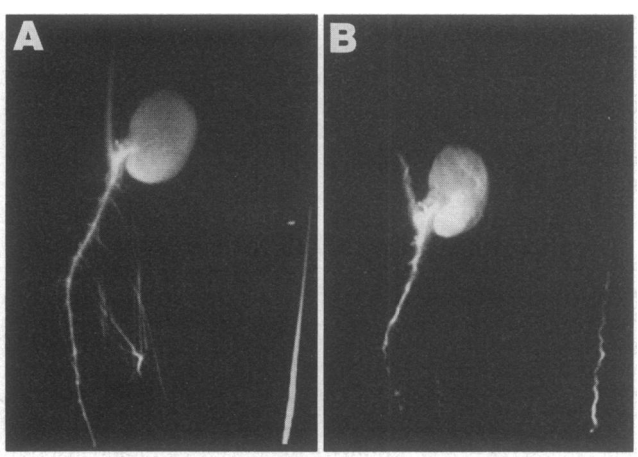

FIG. 2. *V. faba* seedling 12-day-old images bare (no soil) (*A*) and subsequently in fritted clay (*B*). Image parameters are the same as for Fig. 1. (Scale = 1:3.3.)

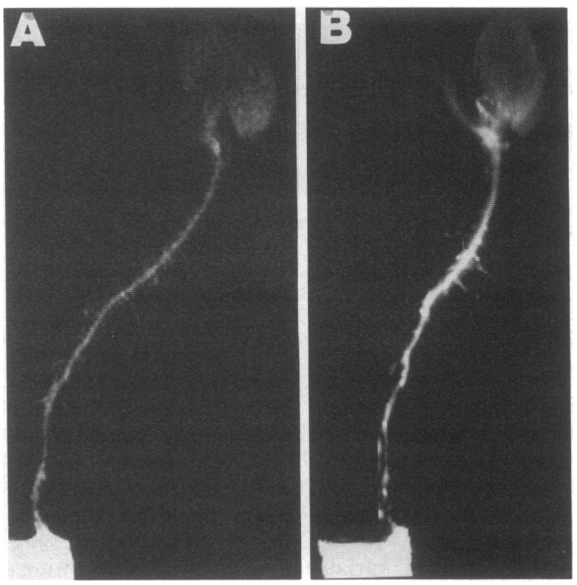

FIG. 3. Image of a *V. faba* seedling immediately after the root tip has been immersed in a 15-ml vial containing 0.04% $CuSO_4$ solution (*A*) and after 60 min of exposure to light (*B*) showing uptake of the contrast agent into the bottom 3/4 of the taproot. Scans were acquired in 3.4 min, with a 0.2-s pulse repetition period. (Scale = 1:2.2.)

some injury occurred when transplanting the seedling into the fritted clay, the rooting pattern and much of the other information is still apparent.

Spatial distortion of the capillary in the fritted clay (Fig. 2*B*) is greater than that in the peatlite (Fig. 1), with displacements of up to about 2 mm. In order of increasing image distortion and signal loss, the ranking of the different soil media examined was peatlite, potting soil, perlite, fritted clay, vermiculite, sand, and Cahaba soil. The first six media produced usable results, but only the cotyledons and upper part of the taproot were visible in the Cahaba soil images due to the presence of ferromagnetic particles. Indeed, differences between soil magnetic properties appeared to be the principal source of variability in image quality among the various soil media examined.

Water flow in *V. faba* was investigated by using a 0.04% aqueous $CuSO_4 \cdot 5H_2O$ solution as a tracer. Cu^{2+} is paramagnetic and decreases the ^1H NMR relaxation times in accessible regions, thereby enhancing the image contrast when the shorter NMR pulse sequence repetition period is used. It was recognized that this level of $CuSO_4$ could be phytotoxic. Fig. 3*A* is an image of a 12-day-old seedling removed from the soil and imaged (3.4 min) with the bottom 1-cm tip of the taproot immersed in 5 ml of the $CuSO_4$ solution. The seedling, with root-tip immersed, was then exposed to the light (2000 microeinsteins$\cdot m^{-2} \cdot s^{-1}$) for 1 hr, whereupon symptoms of wilting were apparent and the seedling was reimaged. The scan (Fig. 3*B*) shows ascension of the tracer into the taproot to about 3/4 of the root length. Brightened points along this zone correspond to secondary root branch attachments.

Water transport was studied *in situ* by injecting a 20-ml bolus of water into the soil base of the plant/soil system of Fig. 1*A*. The soil medium was otherwise fairly dry, but the plant showed no signs of water stress. An NMR scan was immediately acquired and the light level was applied at 2000 microeinsteins$\cdot m^{-2} \cdot s^{-1}$ (Fig. 4*A*). After 87 min of light exposure, initial signs of wilting were apparent (leaves slightly drooping with edges of top young leaves beginning to roll). An image at this time showed water being transported from the cotyledons and their associated embryonic axis (Fig. 4*B*). After 200 min of exposure to the light, the plant was severely

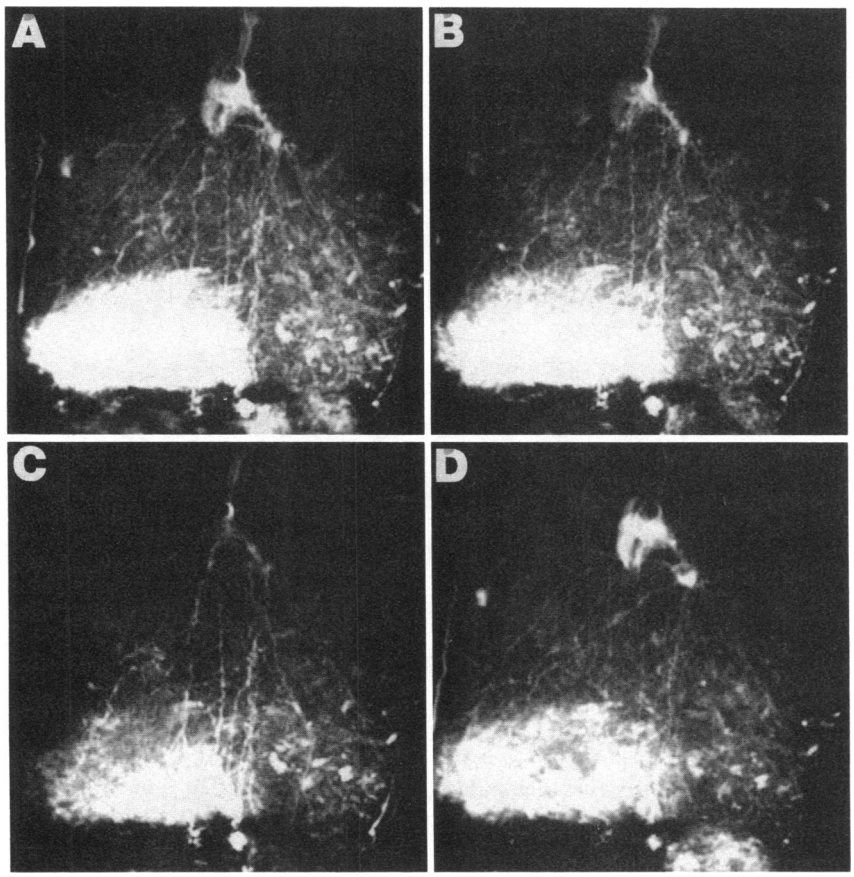

FIG. 4. (*A*) Image of the *V. faba* root/soil system from Fig. 1, with a 20-ml bolus of water injected into the soil at the base. (*B*) The root system image after 87 min of exposure to light stress shows water leaving the cotyledonary area. (*C*) After 200 min of light exposure, water has almost cleared from this area. (*D*) The image after 275 min, light having been fully removed at 200 min, shows recovery (return of water) in the cotyledonary area. All images were obtained in 3.4 min, with a 0.2-s pulse repetition period. (Scale = 1:2.7.)

wilted (all leaves rolled and mainstem somewhat bent) and an NMR scan revealed that the cotyledonary area and an attached root section had lost most of their water (Fig. 4*C*). The plant was then placed in a dark cool area to recover. Seventy-five minutes later the plant was fully turgid without signs of stress, even in the youngest top leaves. The corresponding image (Fig. 4*D*) shows that water had been restored to the root tissues from the injected bolus, the only available source.

Seeds that had germinated in potting soil were also imaged. Most water was concentrated in the cotyledons, and the emerging shoot and radicle were visible. Except possibly for neutron shadowgraphs (11), there are no other noninvasive means of assessing the germination process *in situ*. Such information is very important for optimizing growth conditions in seedbeds.

In conclusion, the NMR imaging procedure is entirely nondestructive, employs nonionizing radiation that can permeate soil media, and has no known effect on plants. Thus, imaging experiments could be repeated indefinitely over the lifetime of the plant to monitor growth and development and the response of the plant to light, CO_2 level, and nutrients. The sensitivity of the image intensity to water content ideally suits the technique to the characterization of the water distribution in plants and the investigation of its movement through the soil–plant–air continuum. The observation of image intensity changes associated with seed rot suggests applications of the technique to plant injury studies involving disease, chemicals including herbicides and pollutants, and physical stress. Clearly, NMR imaging can be an effective tool for studying plant problems *in situ*.

We thank A. R. Mitchell, F. A. Johnson, H. Davis, M. G. Huck, C. B. Elkins, and J. H. Shinn for fruitful discussion, J. N. McCrimmon and J. A. Batchelor for technical assistance, and R. W. Redington for scientific support in Schenectady. Partial support by the Carbon Dioxide Research Division (Office of Basic Energy Sciences, U.S. Department of Energy) through the Plant Ecology Group, Environmental Sciences Division, Lawrence Livermore National Laboratory, is acknowledged.

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