Magnetic resonance microscopy of changes in water content in stems of transpiring plants

(magnetic resonance imaging/*Pelargonium hortorum*/spin lattice relaxation)

G. ALLAN JOHNSON*[†], JOHN BROWN*[‡], AND PAUL J. KRAMER[‡]

*Duke University Medical Center, Durham, NC 27710; and [‡]Botany Department, Duke University, Durham, NC 27706

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ABSTRACT Differences in water content and degree of binding in the various stem tissues of *Pelargonium hortorum* were observed by magnetic resonance imaging. ¹H images were obtained with a resolution of 100 μm in the transverse plane and a slice thickness of 1250 μm . It was possible to distinguish the principal tissues of the stem by differences in their proton density or apparent water content and spin lattice relaxation time (T_1) or degree of water binding. Measurements were made while the plant was slowly and actively transpiring. In the slowly transpiring plant, T_1 of various tissues ranged from an average of 659 to 865 ms with a proton density variation of from 72 to 100%. In the actively transpiring plant, T_1 ranged from an average of 511 to 736 ms, and the proton density was reduced, ranging between 62 and 88% of the peak value found in the slowly transpiring plant. The fibrous sheath surrounding the vascular tissue and the epidermal region was found to have the highest spin density and T_1 . Both tissues are comprised of relatively small thick-walled cells. Cortical and pith parenchyma are composed of larger, thinner-walled cells with numerous intercellular spaces and lower spin density and T_1 . The differences are attributed to the higher water content by volume in the tissue composed of smaller, more compactly arranged cells. The resolution obtained in this work enables clear definition of tissues in the living plant and quantitative information concerning differences in the distribution and extent of binding of water.

This paper describes an investigation of the use of magnetic resonance imaging (MRI) for nondestructive research on the content and binding of water in plant tissues. Since the initial demonstration of spatially localized magnetic resonance by Lauterbur (1), it has been clear that MRI has potential as a valuable tool in the study of plants. Several early researchers demonstrated images of fruits and nuts (2-4), and MRI has been applied to studies of intact plants (5, 6). Studies have been limited by picture volume elements (voxels) with sampling volumes of $>10\text{ mm}^3$. But the resolution has been extended into the microscopic domain with voxels as small as 0.012 mm^3 with a sufficiently high signal to noise ratio to distinguish subtle differences in signal intensity (7-11). This permits production of images that distinguish the various tissues in a plant stem.

A magnetic resonance image is generated by placing a sample in a strong magnetic field that causes the protons (primarily in water) to precess synchronously about the field at the Larmor frequency. Externally applied radio frequency pulses at the Larmor frequency can be used to manipulate the precessing protons away from their equilibrium position (precessing about the field). In the process the protons absorb energy that is later reemitted. The reemitted signal can be localized in space to create a digital image by application of

magnetic gradients. For the partial saturation or the spin echo sequence (12), the signal S at any point in the image is given by Eq. 1 as

$$S = N_H[1 - 2e^{(-T_R - T_E/2)/T_1} + e^{-T_R/T_1}]e^{-T_E/T_2}, \quad [1]$$

where T_R is the repetition time between pulse sequences, T_E is the time between the 90° radio frequency pulse and the formation of the spin echo signal, N_H is the proton density, and T_1 and T_2 are the spin lattice and spin-spin relaxation times of the sample. For a more complete description of the technology see ref. 13.

One application of MRI in plant science would be to monitor water movement through plant tissue, using change in N_H as a gauge of water content. Eq. 1 demonstrates that signal intensity is dependent on the following three tissue parameters: N_H , T_1 , and T_2 . Thus, signal changes generated when the water content of a plant changes are not necessarily attributable solely to changes in N_H . By using a short T_E , the dependence of the signal strength on T_2 can be minimized. It is, therefore, possible to measure T_1 by acquiring images with several values of T_R and performing a fit to Eq. 1. T_1 is inversely correlated with the fraction of the water that is bound, i.e., water that is hydrogen bonded to larger macromolecules for a period comparable to T_1 (14, 15). It was in fact the observation of prolonged T_1 in tumors that provided much of the early impetus for the development of MRI for clinical uses (16). Thus both T_1 and N_H can be used as markers in physiological studies, N_H as an indicator of relative water content and T_1 as an indicator of the extent to which the water is bound.

METHODS

¹H images were obtained at 64 MHz on a prototype magnetic resonance system designed for clinical imaging (General Electric Medical Systems, Milwaukee, WI). The system, configured about a 1.0-m bore 1.5-T superconducting magnet, has been modified for microscopy by the addition of a smaller (28-cm diameter) set of gradient coils to produce the high gradients (0.47 mT/cm) necessary for microscopy. A 5-cm diameter radio frequency coil was constructed using the low-pass "birdcage" design suggested by Hayes *et al.* (17). Utilization of three-dimensional spin-warp imaging (18) allowed efficient acquisition of 16 contiguous 1.25-mm slices, each reconstructed on a 256×256 matrix. With a 25-mm field

Abbreviations: N_H , relative proton density; MRI, magnetic resonance imaging; T_1 , spin lattice relaxation time; T_2 , spin-spin relaxation time; T_E , time between the 90° radio frequency pulse and the formation of the spin echo signal; T_R , repetition time between pulse sequences.

[†]To whom reprint requests should be addressed at: Department of Radiology, Box 3302, Duke University Medical Center, Durham, NC 27710.

FIG. 1. Levels 5–13 of a 16-slice three-dimensional acquisition showing a series of 1250- μm transaxial slices through the branching point on a geranium stem. T_R , 1600 ms; T_E , 20 ms.

of view, this resulted in images with individual elements of $100 \times 100 \times 1250 \mu\text{m}$.

Pelargonium hortorum var. Bailey plants were produced from stem cuttings rooted in a synthetic foam medium (Rootcube[®]; Smithers Oasis, Kent, OH) under intermittent mist and grown under greenhouse conditions. Individual unrooted cuttings were placed in foam cylinders 5.0-cm diameter $\times$ 8-cm long that were wrapped in polyethylene film and aluminum foil to prevent evaporation and exposure of roots to sunlight. Synthetic foam medium was used to avoid soil diamagnetism and ferromagnetism, which may cause spatial distortion and signal loss in MRI data acquisition (5). In ≈ 5 weeks, after the cuttings were well rooted, the plant stems were imaged.

FIG. 3. Relaxation recovery for pith parenchyma (curve a) and fibrous sheath (curve b).

The sample plant was placed in the radio frequency coil with the stem in the central imaging section of the coil and the foliage protruding from one end. A catheter was inserted in the foam medium to supply water to the roots without moving the plant. The coil and sample were positioned in the gradients so foliage could be fully illuminated at one end. Illumination was provided by two 650 W, 120 V quartz–iodide lamps mounted in flood light housings on adjustable stands placed 4.5 m from the end of the magnet. The high magnetic field near the magnet precludes operation of the lamps closer to the plants. The incident photon flux density was $\approx 450 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the leaf surface.

The initial studies were performed on a plant that had been kept in darkness at 22°C and 37% relative humidity. Three-dimensional image sets were acquired at T_R s of 200, 400, 800, 1600, and 3200 ms with a T_E of 20 ms. The lights were then turned on and 30 ml of distilled water was introduced to the foam medium. The plant was allowed to stabilize in the light

FIG. 2. (A) Magnified view of slice 9 showing the following tissues: a, pith parenchyma; b, vascular tissue; c, fibrous sheath; d, cortical parenchyma; e, epidermal tissue. (B) Light microscopy section of a similar stem.

Table 1. T_1 and relative spin density of plant tissues at different levels of transpiration

Tissue	Transpiration			
	Low		High	
	T_1 , ms	N_H^*	T_1 , ms	N_H^*
Pith	769 ± 56	0.72 ± 0.01	511 ± 107	0.62 ± 0.05
Vascular	865 ± 101	0.95 ± 0.03	649 ± 80	0.76 ± 0.05
Fiber	659 ± 34	1.00 ± 0.02	612 ± 37	0.88 ± 0.03
Cortical	689 ± 56	0.98 ± 0.06	736 ± 25	0.87 ± 0.04
Epidermal	716 ± 75	0.97 ± 0.06	653 ± 72	0.86 ± 0.05

The data represent the averages of six randomly selected 3×3 pixel areas acquired from within each of the individual tissue regions. They not only are averages of free and bound water, but are spatial averages across cells and intercellular spaces.

*Spin density relative to fiber.

for 4 hr at 30°C before a second set of data was acquired as described above. The acquisition at a T_R of 3200 ms was eliminated in this second set for reasons given in the next paragraph.

T_1 and N_H for a given tissue were calculated by performing a two-parameter fit of signal intensity in a pixel vs. T_R to Eq. 1 (19). Since we did not know how long the T_1 of the tissues might be, we initially used the following five values of T_R : 200, 400, 800, 1600, and 3200 ms. Since there was <2% variation on T_1 calculated with and without inclusion of the data from 3200 ms, acquisition of data at 3200 ms was omitted in later measurements.

RESULTS AND DISCUSSION

A representative set of transaxial data acquired from the slowly transpiring plant at a T_R of 1600 ms is shown in Fig. 1. Slices 5–13 of the 16 contiguous 1250- μ m slices are shown, demonstrating the progression of images from the lower stem through a branching point. Fig. 2A is a magnified image of slice 9 permitting more careful inspection of the detail and identification of the tissues. Fig. 2B is a stem cross-section for reference, photographed under a microscope. The long T_R used for the magnetic resonance image in Fig. 2A decreases the influence of T_1 on signal intensity. This implies that the differences in image intensity are due primarily to differences in water content. The central pith parenchyma is surrounded by much brighter vascular tissue and a bright ring of fibrous tissue. At this point one is not sure how much of the difference in signal intensity is due to differences in N_H and how much is due to T_1 . If, for example, the T_1 of water in the

pith parenchyma was 2.0 s, which could be possible for free water, a large part of the tissue contrast could be attributed to incomplete relaxation of spins in the water in the pith parenchyma compared with water in the tissue with smaller, thicker-walled cells. Thus, one must actually measure T_1 and N_H .

Fig. 3 is a graph of signal intensity as a function of T_R from a 3×3 pixel region in (curve a) the pith parenchyma and (curve b) the fibrous sheath surrounding the vascular tissue. These regions are identified on Fig. 2A. The data are fitted to Eq. 1. The shorter relaxation time of the pith parenchyma shows a more rapid attainment of equilibrium. The asymptote indicates the equilibrium signal that in this instance is the water concentration. By contrast, curve b for the fibrous sheath rises more slowly as a consequence of the longer T_1 and reaches an equilibrium value at 3200 ms, which is 30% higher.

Six separate regions were fitted for each of the different tissues of slowly and rapidly transpiring plants, and the data are summarized in Table 1. The decrease in T_1 in most tissues after a 4-hr period of transpiration presumably occurred because there was a decrease in water content, characteristic of transpiring plants (20). The variations in relative proton density are the most dramatic, with the lowest density in the pith parenchyma and the highest in the vascular regions. There is a clear gradient in N_H as one moves outward from the central pith parenchyma. This is seen more clearly in Fig. 4A, which shows the measured N_H for both the slowly and actively transpiring plants as one moves across the cross sectional image. The N_H is reduced in all tissues during active

FIG. 4. (A) Plot of relative N_H in 3×3 pixel regions across the stem. □, Low T_s ; +, high T_s . (B) Plot of calculated T_1 for a 3×3 pixel strip across the stem. □, Low T_s ; +, high T_s . Areas labeled are as follows: a, pith parenchyma; b, vascular tissue; c, fibrous sheath; d, cortical parenchyma; and e, epidermal tissue.

transpiration. Similarly, as is seen in Fig. 4B and Table 1, the T_1 is reduced in most tissues during active transpiration.

There is an obvious correlation between increased N_H and longer T_1 . This is not surprising in light of earlier work (15) that developed the fast-exchange two-state model, which assumes water in two distinct states. Water bound to larger molecules will have a short T_1 since the energy of the excited spins can be more readily coupled to the surrounding lattice. Free water, on the other hand, has a much longer T_1 . Rapid exchange of protons between the two states yields an average of the two. As the percentage of free water increases with an accompanying increase in relative N_H , the weighted T_1 also increases. Examination of the light microscopy section (Fig. 2B) shows smaller cells in the epidermal region and outer cortex and in the fibrous sheath. The increased N_H and T_1 in the tissues with small cellular structure is consistent with more water per volume in these tissues. The larger pith and cortical parenchymal cells possess large vacuoles containing starch grains, and parenchyma tissue contains more intercellular spaces, resulting in lower water content per volume.

The T_1 and N_H values listed in Table 1 for several tissues were obtained over reasonably large tissue samples ($300 \times 300 \times 1250 \mu\text{m}$) and represent averages over 9 pixels. One can see, however, in Fig. 4 evidence of differences in T_1 and N_H within a single tissue, probably related to structural differences. Note, for example, the four peaks in the graph of N_H in the pith parenchyma between $350 \mu\text{m}$ and $550 \mu\text{m}$. The variations in a single tissue cannot be due to random signal variations in the imaging procedure since the difference between several points is evident in both slowly and actively transpiring plants. Thus the values for T_1 and N_H for pith and cortical parenchyma reported in Table 1 represent not only an average of free and bound water in the cell, but also a spatial average across cell walls, protoplasts, and intercellular spaces. Further improvements in resolution will permit even more detailed examination of water distribution at the cellular level.

In summary, we have shown that magnetic resonance microscopy of the plant stem can distinguish several different tissue types. The signal differences from these tissues are due to differences in N_H and T_1 , indicating differences in water content and degree of binding. Tissues with smaller, thicker-walled cells and few or no intercellular spaces had both higher T_1 and higher N_H consistent with more water per unit volume

of tissue. All tissues demonstrated changes in N_H and T_1 after a period of relatively rapid transpiration. The ability to noninvasively measure relative water concentration and T_1 in the live plant at the cellular level promises an entirely new approach to plant studies using magnetic resonance microscopy.

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