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● *Original Contribution*

FUNCTIONAL MAGNETIC RESONANCE IMAGING IN INTACT PLANTS—QUANTITATIVE OBSERVATION OF FLOW IN PLANT VESSELS

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Quantitative magnetic resonance (MR) images of flow velocities in intact corn plants were acquired using magnetization-prepared MR microscopy. A phase contrast flow imaging technique was used to quantitate water flow velocities and total volume flow rates in small xylem vessels. The simultaneous measurement of the transpiration of the whole plant was achieved by using a closed climate chamber within the MR magnet. The total volume flow rate and the transpiration values were in close correlation. Functional magnetic resonance imaging in intact plants was performed by light stimulation of the transpiration inside of the magnet. The change in the flow velocities in the xylem vessels of single vascular bundles was in correlation with the changes in the transpiration. Significant differences were observed between the xylem vessels in different vascular bundles. Furthermore, flow velocity measurements were performed on excised plant stems and visualized by the uptake of the MR contrast agent, gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA). A comparison between the phase contrast flow imaging and the contrast media uptake showed to be in good agreement with each other.
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Keywords: **Magnetic resonance microscopy; Flow velocity; MR contrast agents; MR in plants.**

INTRODUCTION

The sensitivity of magnetic resonance (MR) imaging to flow is well-established.[1] There is an increasing use of imaging and quantification of blood flow in human blood vessels using MR angiography. MR flow-imaging methods are mainly based on in/out-flow effects[2] or MR signal phase changes caused by magnetic field gradient pulses.[3]

Over the last decade, several attempts have been made to measure water flow velocities in plants using MR methods, usually based on phase flow methods. Flow measurements on intact plants are often hampered by the need for strong magnetic field gradients for flow encoding. The expected flow velocities of water in plant vessels are of the order of 1–10 mm/s or less depending on the transpiration condition. Furthermore the xylem and phloem vessels have a diameter of 5 to 200 μm. Therefore, it has been necessary to perform MR microscopy for the study of flow in intact plant systems. These MR magnets give access to high magnetic fields and magnetic gradient strength of the order of 0.5 T/m to improve the signal-to-noise ratio and accuracy of the flow velocity measurements.

Real time measurements of water flow in cucumber plants were performed without spatial resolution[4] and spatially resolved measurements[5–9] were possible, but limited by long total acquisition times of several hours. Time-dependent MR imaging of the regional distribution of flow velocities in intact plant systems under changing biologic stress conditions have not been possible until now.

In this work, we present ^{1}H-MR imaging of young intact corn plants and demonstrate the ability of flow-sensitive MR to yield information both on the total volume flow rates and on flow velocities, with a time resolution of 15 min. Due to the reduced total acquisition

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time, the kinetics of plant responses to environmental changes were monitored. We further analyzed the flow velocities by the observation of the uptake of MR contrast agents in plant systems. The flow velocities measured by the two methods, MR phase contrast imaging and time dependent MR signal changes influenced by contrast media, were compared.

MATERIALS AND METHODS

Plant Materials

All experiments were performed on 10–14-day-old whole corn plants (Zea mays), which were grown in hydroponics. The roots were kept in a nutrition medium throughout the experiments. The total height of the plants was about 20 cm (from the seed to the tip of the uppermost leaf) with two or three fully developed leaves. The shoot had a diameter of 2 to 3 mm and the length of the root system was about 20 cm. The vessel system of a leaf consists of several vascular bundles with typically one or two xylem vessels which form the tube system for the long distance water transport from the roots to the leaves. As visualized by optical microscopy, large and small vessels alternate in each leaf. The plant morphology and MR characteristics are described previously.[10] The plant was positioned in a transmitter and receiver MR coil adjusting an image slice between 3 and 4 cm above the grain in the region of the mesocotyl bundle sheath. The plant shoot was aligned parallel to the main magnetic field.

MR Instrument and Climate Chamber

The MR studies were performed on a Bruker AMX 500 spectrometer. The vertical bore magnet has a bore of a diameter of 89 mm and a field strength of 11.75 T. We used a Bruker actively shielded gradient coil system ($G_{z,max}$ = 762 mT/m, $G_{x,y,max}$ = 381 mT/m). The MR microscopy probe head was equipped with a home-built 5-mm saddle-shaped NMR coil surrounded by a closed climate chamber. The chamber was used to set and control the temperature and relative humidity (25°C and 50% relative humidity) of the environment of the plant. We determined simultanously with the MR experiments the transpiration and assimilation rates[11] of the whole plant. Transpiration was measured by infrared spectroscopy outside of the MR spectrometer using the air circulating in the climate chamber. Illumination of the leaves was performed by an external light source (Leica KL1500, Ismaning, Germany) via a fibre optic inside of the climate chamber. The light intensity was calibrated before the MR measurements. It should be noted that the light source represents a directional entity. Therefore, the light intensity was inversely proportional to the square of the distance of the light source.

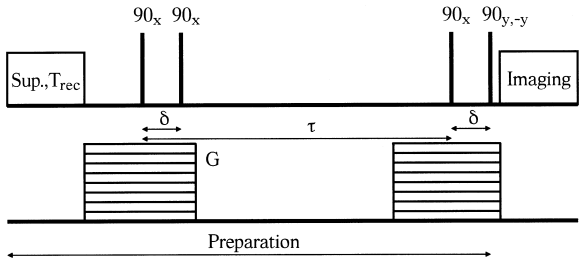

Fig. 1. Pulse program for spatially resolved flow velocity MR imaging. The magnetization preparation consists of a pulsed gradient stimulated echo sequence. The initial suppression sequence consists of a 90° pulse followed by a spoiling gradients and a recovery time T_{rec} to ensure the same initial magnetization at each step of phase cycling and imaging step. The flow encoding gradient pulses are switched on before the first radiofrequency pulse to avoid eddy current effects. The radiofrequency pulse lengths are 10μs. The fourth 90° pulse creates longitudinal magnetization. The subsequent pulse sequence consists of a spin-echo imaging sequence. The complete sequence is repeated using different steps of the phase encoding imaging sequence. The gradient pulses G are stepped from one image to the next to acquire different flow encoded images. The phase cycling of the fourth 90° pulse cancels pulse imperfections.

MR Methods and Parameters

MR flow imaging was performed using magnetization-prepared MR microscopy.[12] It has been recently shown that the corn tissue exhibits short T2 and long T1 values[10] of the ^{1}H-MR signal of the water resonance. Therefore, it was necessary to perform the magnetization preparation with a stimulated echo preparation sequence (PGSTE)[13] for flow encoding. Here, the time interval between the second and third radiofrequency pulse needed to produce a stimulated echo could be used for flow encoding. After the stimulated echo preparation sequence and the final pulse for the return of the prepared magnetization to longitudinal magnetization, one line in k-space was sampled with a spin echo sequence consisting of a 90° slice-selective and 180° non-selective radiofrequency pulse. The total sequence was repeated to cover the whole k-space. The MR imaging pulse program is given in Fig. 1.

The magnetization preparation method suppressed stationary water. The longitudinal magnetization after the fourth 90° pulse is determined by the transverse magnetization of the stimulated echo, and hence by magnetization which accumulates a net phase shift by the two gradient pulses. The stationary water signal and pulse imperfections could be cancelled completely by the phase cycling of the fourth pulse.

Eight images having eight different gradient values were acquired for flow-encoded image contrast. The flow velocities were determined by a two-parameter fit using the signal intensities of all eight images. We assumed a

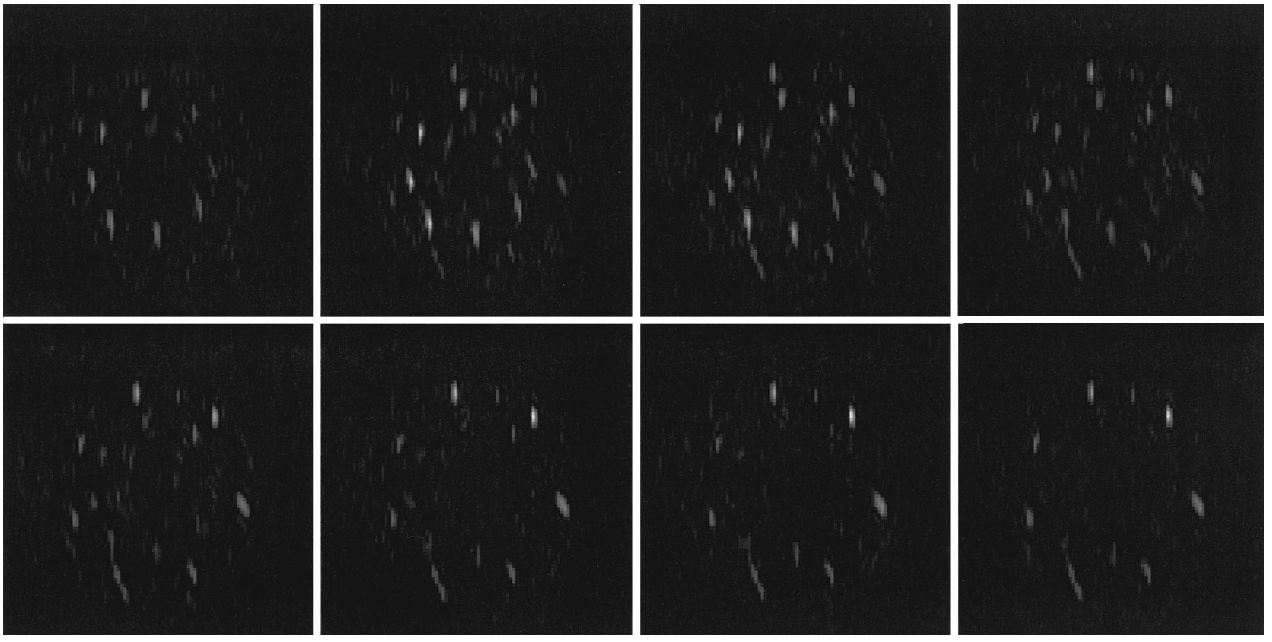

Fig. 2. Eight flow-encoded cross-sectional images of the plant stem using eight different flow-encoding gradient strengths ranging from 18 mT/m to 150 mT/m. The FOV is 3.9 mm.

laminar flow in all vessels and hence a parabolic flow profile. We also assumed a circular vessel geometry. The signal dependence S(G) on the gradient strength G, which is the appropriate function to calculate the flow velocities, is defined accordingly:[13]

$$S(G) = M_0 \sin^2(\alpha v)/(\alpha v) \quad (1)$$

where $\alpha = \gamma G \delta \tau$, γ = gyromagnetic ratio, τ = time interval between the gradient pulses, δ = duration of the gradient pulses, G = gradient strength, v = average flow velocity and M_0 = signal amplitude of flowing spins. The time interval t is the sum of the echo time and the middle interval between the second and third pulse. The time interval δ is the echo time.

The MR sequence produces a signal loss due to the self-diffusion of water molecules. This effect has been taken into account for the calculation of flow velocities. In a previous work, we determined the self-diffusion constant within the vascular bundles of this plant.[10] This constant was included in the fitting procedure.

The flow encoding was performed with the following parameters: maximum gradient strength G = 150 mT/m, τ = 99 ms, and δ = 2 ms. In order to avoid any residual transverse magnetization and to produce a constant value of the longitudinal magnetization, we used a suppression sequence followed by a recovery period (Sup., Trec) during the repetition time immediately following the acquisition of the MR signal. The spin-echo image was acquired with a field of view (FOV) of 5 mm, the repetition time (TR) was 2 s, and the echo time in the imaging sequence 3.58 ms. Slice thickness was 3 mm. This was necessary to significantly improve the signal-to-noise ratio without loss of information about the localization of the various vascular bundles in the image cross-sections. The inplane number of pixels was 128 × 32. The total measuring time amounted to 34 min with four averages.

The results of the flow MR imaging method were compared by the observation of the ascend of an MR contrast agent, Gd-DTPA on the excised plants of the same type. The plants were cut above the grain and placed in a tube containing nutrient solution. The plant was positioned in the probe head adjusting an image slice 57 mm above the cutting plane. The nutrient solution was exchanged by a 5 mM/liter Gd-DTPA doped nutrient solution. The nutrient solution had a T1 value of 50 ms. The ascent of the solution was imaged by the time course of signal enhancement in a rapid series of spin echo images. The experimental parameters for these experiments were: FOV = 4 mm, slice thickness = 1.5 mm, image matrix = 128 × 32 pixel, TR = 131 ms, 2 averages. These parameters gave an image acquisition time of 8.4 s.

RESULTS

PGSTE Measurement

Figure 2 shows a series of PGSTE magnetization

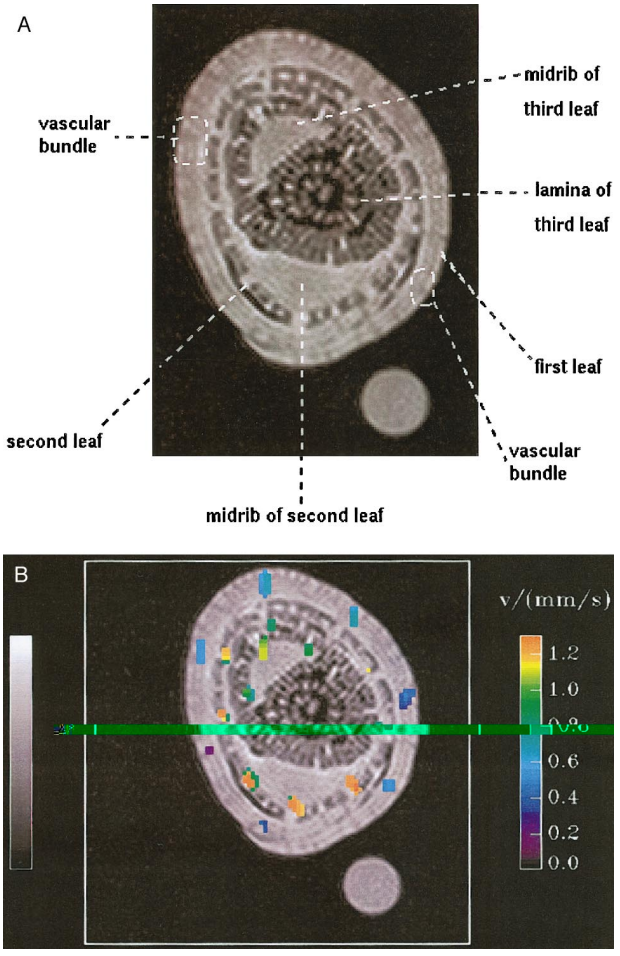

Fig. 3. A) High-resolution spin-echo MR image of the plant stem of a corn plant showing the anatomical details. B) Colour-coded flow velocity cross-sectional image of the plant stem of a corn plant superimposed on a high-resolution spin echo NMR image (in-plane resolution = 39 μm, slice thickness = 500 μm). The FOV is 3.6 mm. The color encoding shows the flow velocities on a linear scale. The image on the bottom right is a water-filled reference tube showing no flow.

prepared flow-encoded cross-sectional images of the plant shoot. The eight images were acquired with eight different flow-encoding gradients. Net flow is limited to specific regions of the plant which are identified as the vascular bundles. Flow velocities showed large differences between the vascular bundles. The efficiency of stationary water suppression is further proven.

Figure 3A shows a T1-weighted high resolution spin echo image of the plant shoot. Detailed anatomical information can be seen in the plant morphology, although single cells were not resolved. The mid-rib of the third and youngest leaf and the mid-rib and lamina of two other leaves are visible. The vascular bundles are seen as lower intensity regions within the leaves. Figure 3B shows a colour-coded calculated flow velocity map superimposed on the high resolution MR image (in-plane resolution = 39 μm, slice thickness = 500 μm). In this study, the corn plant was illuminated with a light intensity of 190 W/m and the transpiration rate was 1.3 μmol/s. Detailed information can be seen on the plant morphology, even if single cells were not resolved in the spin echo image. The sheath of one leaf as well as the mid-rib and the lamina of two further leaves can be distinguished, for example. Regions of different flow velocities which are related to different vascular bundles can be resolved. Two groups of vascular bundles independent of the leaf were identified. One bundle system was either very weakly or even non-conducting, whereas the second system displayed flow velocities of the order of 1 mm/s. By comparison with light microscopy, it was easy to associate the very weakly conducting regions with the smaller vascular bundles in the different leaves.

Figure 4 shows the result of a time dependent series of MR flow measurements under changing light conditions. The solid line in Fig. 4 indicates the curve of the transpiration rate E as measured by the gas exchange device. The arrows mark changes of the illumination induced by switching the light on and off. The transpiration clearly varies with the changes of illumination. The data points give the total water flow V_t calculated from the two parameters M_0 and v using the equation:

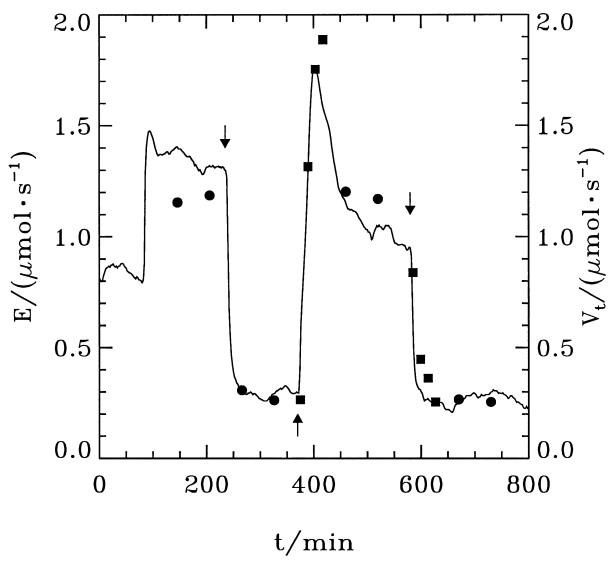

Fig. 4. Comparison of total volume flow rate V_t (symbols) calculated from the MR flow data and the transpiration rate E (solid line) at different stages of illumination (190 W/m^2 and darkness). The upward and downward arrows indicate time points when light was switched on and off, respectively. The total acquisition time for one MR flow data set was 34 min and 14 min for the experiments indicated by the circles and squares, respectively.