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. © 1998 Elsevier Science Inc.

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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 effects² 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 resolution⁴ and spatially resolved measurements⁵–⁹ 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 ¹H-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...
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.\textsuperscript{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 simultaneously with the MR experiments the transpiration and assimilation rates\textsuperscript{13} of the whole plant. Transpiration was measured by infrared spectroscopy 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.

MR Methods and Parameters

MR flow imaging was performed using magnetization-prepared MR microscopy.\textsuperscript{12} It has been recently shown that the corn tissue exhibits short T2 and long T1 values\textsuperscript{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)\textsuperscript{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\degree slice-selective and 180\degree 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\degree 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 90\degree 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
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:

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

(1)

where $\alpha = \gamma G \delta \tau$, $\gamma$ = gyromagnetic ratio, $\tau$ = time interval between the gradient pulses, $\delta$ = 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 $\delta$ 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. This constant was included in the fitting procedure.

The flow encoding was performed with the following parameters: maximum gradient strength $G = 150$ mT/m, $\tau = 99$ ms, and $\delta = 2$ 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 $128 \times 32$. 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 = $128 \times 32$ 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...
prepared flow-encoded cross-sectional images of the plant stem. 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 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.

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.

Figure 3A shows a T1-weighted high resolution spin echo image of the plant stem. 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/m² 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.](image-url)
with a calibration factor $c$. Factor $c$ was determined by an additional MR experiment with eight averages and 16 flow-encoded images to improve accuracy. The factor $c$ was calculated by adjusting $V_t$ of the calibration image with the transpiration rate $E$. In comparison to the flow images measured within 34 min, we reduced also for a few images the measuring time to 14 min. Here, we improved the time resolution by the acquisition of four flow-encoded images as the data base for each calculated flow image and reduced the repetition time to 1 s. Figure 4 demonstrates the close agreement between $V_t$ as determined by the MR method and the transpiration rate. It further shows that a reduction of the measuring time of the flow images is possible without a reduction in the accuracy of the flow quantification.

It was also possible to compare the flow velocities of single vascular bundles at different transpiration rates. As compared to Fig. 4, in Fig. 5 the same transpiration data are shown together with the flow velocities in different vascular bundles indicated by the different symbols. Due to the large range of velocities in the plant, all values are calibrated according to the calibration experiment. The flow velocities in all vascular bundles follow the change in the transpiration rate of the whole plant. However, there are significant differences of the flow velocities in different vascular bundles.

**Gd-DTPA Measurements**

Three cross-sectional images are shown in Fig. 6 after changing the nutrient solution to the Gd-DTPA doped solution. The images were acquired 31.2 s, 140 s and 357 s ($\pm 1$ s) after the administration of the contrast agent. The images have been subtracted from the original image which was measured before the application of the contrast agent. The time-dependent signal increase in the vascular bundles is clearly visible. Again, two systems of vascular bundles were detected: one system showing no or only very low flow velocities and a second system with flow velocities of about 1 mm/s.

In Fig. 7 the time course of signal enhancement for two vascular bundles is shown. The symbols indicate the image signal intensities in a vessel. The solid line is a fit
assuming a parabolic flow profile in the vessels. The fitting function \( f(t) \) is for:

\[
\begin{align*}
\text{for } t < t_0 & \quad f(t) = M_s \\
\text{for } t > t_0 & \quad f(t) = M_s + (M_f - M_s) \left(1 - \frac{t}{t_0}\right) \\
\end{align*}
\]

(3)

\( t_0 \) is the time point where the contrast agent reaches the MR image plane. \( M_s \) is the MR signal intensity without the contrast agent, and \( M_f \) is the MR signal intensity at equilibrium, when the vessel is completely filled with the contrast agent. According to the equation\(^3\) the signal intensity increases from the value \( M_s \) to \( M_f \) when a parabolic flow is present.

We compared the PGSTE method and the contrast media flow measurement using the same plant after cut-off the roots. In Fig. 8 the flow velocities, as determined by the PGSTE method, were plotted vs. the flow values, as determined by the Gd-DTPA uptake. The two methods gave approximately the same results, but the PGSTE sequence yielded systematically higher values. Because the uptake of Gd-DTPA into the plant vessels is almost completely reversible by changing the Gd-DTPA containing solution against a normal nutrition medium, the study can also be performed under changing illumination conditions. Furthermore, the flow velocity determination is also possible by taking into account the time course of signal decrease when the contrast agent is washed out from the image slice.

Figure 9 shows a summary of the experiments using Gd-DTPA. The solid line represents a transpiration curve at different illumination conditions. The symbols indicate relative changes in flow velocities in the same vascular bundles as in Fig. 5. It can be seen that the transpiration and the flow velocity as determined by the Gd-DTPA measurements follow the same kinetics. Furthermore, the significant difference of the relative change of flow velocities in the different vascular bundles is also visible and supports the results of the measurements on the intact plants.

We compared the calculated flow velocities of the Gd-DTPA flow-in and flow-out experiments. Figure 10 shows a plot of flow velocities measured by the Gd-DTPA flow-in experiment vs. the Gd-DTPA flow-out experiment. There is good agreement between these two types of experiments but with systematically higher values for the flow-in experiment.

**DISCUSSION AND CONCLUSION**

The results reported here have shown that the water flow velocity in xylem vessels of intact plants can be monitored by two different nuclear MR (NMR)-imaging methods. It was further possible to detect functional information from the plants by the observation of dynamic changes of flow velocities under variable light conditions. The flow velocity changes were simultaneously observed with an NMR-independent measurement of the transpiration rate of the plant. The temporal resolution of the NMR-imaging experiment is sufficient to follow the changes in the flow velocities in the vas-
cular bundles. We were able to quantify the water flow velocity in small xylem vessels, even though it was impossible to resolve the individual xylem vessels due to the low spatial resolution of the NMR-imaging method. However, both flow-imaging methods suppress the stationary magnetization. Therefore, the signal of the flow images only reflects the moving magnetization. Therefore, no contamination of the flow information due to stationary magnetization is observed.

The results show an excellent agreement of transpiration rates measured by a gas exchange system in the climate chamber and calculated volume flow rates of NMR studies. Comparison of transpiration rates and water flow velocities in individual vascular bundles showed the expected behaviour, but with significant differences in individual vascular bundles. Furthermore, the studies on plant dynamics showed pronounced differences in the onset of xylem water flow to changes in transpiration in different vascular bundles.

It should be noted, that in vivo flow imaging on plants\(^5\)\(^-\)\(^9\) can also be measured by using q-space imaging technique\(^5\)\(^,\)\(^8\)\(^,\)\(^9\)\(^,\)\(^14\). The disadvantage is that this method needs more gradient steps for a good velocity resolution, which results in a long measurement times. On the other hand, the advantage of the q-space technique is that it offers the possibility to evaluate the flow profile within an image. The method reported here needs information about the velocity profile, which we assumed is parabolic. The time course of signal enhancement in the contrast agent measurements show that this assumption is valid indeed.

It should be further emphasized that both methods, the phase contrast flow imaging and the application of the contrast agent, give almost identical flow velocities. In principle, phase contrast-flow images could be sensitive to the flow velocities in both directions from the root to the leaves and vice versa. It would be impossible to distinguish both directions without the use of q-space imaging sequences. On the other hand, the flow-imaging technique using the contrast agent is sensitive to net flow from the roof to the leaves. We observed that both techniques give almost identical results. This proves that the flow velocity measured by the phase contrast is indeed directed from the roof to the leaves of the plant. There is no contribution from velocities in the other direction. Otherwise, both techniques would result in different flow velocities.

The somewhat higher flow velocities measured by the phase contrast approach (Fig. 8) are not surprising, because the uptake of Gd-DTPA or any other contrast agent in the plant could be hindered by diffusion barriers. The discrepancy between flow velocities determined by Gd-DTPA wash-in compared to Gd-DTPA wash-out experiments suggests that some interactions of the contrast

![Fig. 9. Comparison of the transpiration rate E and the flow velocity measured by the application of the contrast agent at different stages of illumination. The illumination was changed at the time points represented by the arrows (first arrow: 75 W/m\(^2\)→300 W/m\(^2\) second arrow: 300 W/m\(^2\)→5 W/m\(^2\) third arrow: 5 W/m\(^2\)→190 W/m\(^2\)). The different symbols indicate the flow velocities in different vascular bundles. All velocity values were calibrated according to the first two experiments and thus display relative changes. The first and each uneven number of measuring points represents flow measurements taken from the contrast agent in-flow data, the second and all even number of points show the out-flow data.](image1)

![Fig. 10. Comparison of the flow velocities determined by Gd-DTPA in-flow and out-flow signal intensity variations. The solid line represents the ideal case of equal values.](image2)
agent with the structure of the vessel walls exist. Further studies to observe this interaction in more detail are necessary, for example by using other ionic or non-ionic contrast agents. Despite this unsolved problem, the application of contrast agents for the detection of flow and flow changes in the intact plant shows that it offers a straightforward approach to the fast determination of xylem flow velocities under variable environmental conditions.

It seems to be surprising that both methods, the phase contrast flow imaging and the application of the contrast agent yield almost identical flow velocities, although the roots were excised in the contrast agent experiments. Roots provide a source of resistance to water movement and might result in a lower flow velocity. However, the resistance will provide a higher pressure gradient. Due to the Hagen-Poiseuille-law, a higher pressure gradient will give a higher flow velocity. Therefore, the flow velocity in plant vessels will not be changed by cutting the roots.

Both NMR methods show that NMR flow imaging is a non-invasive method to detect quantitative flow velocities and time dependent changes. Despite the fact that NMR imaging has a lower spatial and temporal resolution compared to many other imaging methods in plant physiology, it has the clear advantage of giving access to functional information about the water distribution and transport of intact plants under normal physiological conditions.

REFERENCES