NMR IN HORTICULTURE: *in situ* plant water balance studies with NMR

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Summary

The information content of NMR is briefly presented. The use of low field low cost proton NMR and NMR imaging in horticulture is emphasized, by presenting selected illustrations of relaxation time studies on plant material in addition to a brief review. Non-invasive water balance studies in intact plants in laboratory as well greenhouse situations are summarized and illustrated, and the possibilities and potential use of a transportable NMR system for *in situ* use in greenhouses and auctions are discussed.

1 Introduction

Nuclear Magnetic Resonance (NMR) is a spectroscopic method based on the interaction between the magnetic moments of atomic nuclei of samples placed in a magnetic field and a radio frequency (rf) magnetic field. The interaction results in absorption of rf energy at a particular frequency, specific for the nucleus under observation (e.g. ^{1}H, ^{13}C, ^{31}P, ^{14}N, ^{23}Na and ^{39}K), the applied magnetic field strength and the local chemical environment of the nucleus. NMR allows the study of structure and kinetics of molecules in solution and is used for compound identification and quantification (Roberts, 1987; Lundberg et al, 1990). In addition, it has long been recognized that NMR can be used for (water and blood) flow measurements (Battocletti, 1986) and for the characterization of the physical state of water in biological systems (Belton & Ratcliffe, 1985). The development of NMR imaging (Lauterbur, 1973) has added a new aspect on NMR, namely image formation, allowing the spatially resolved measurement of all information available with NMR in any selected part (volume, slice, etc) of the material under observation (Moonen et al, 1990)

In the last years, NMR is increasingly applied to the study of plant material, plant products and living plants. The great advantage of NMR is that the method is non-invasive and transparent. Its great disadvantage is its inherent insensitivity. Of the NMR measurable nuclei available in plant material in natural abundance ^{1}H is the most sensitive (relatively set to 100 %) and has far the highest concentration (about 70 M). Other nuclei that have been used for studies of plant material are: ^{13}C (rel. sensitivity with respect to ^{1}H: 1.59 %), ^{13}P (25.8 %), ^{23}Na (16.9 %), ^{39}K (3.0 %) and ^{14}N (4.6 %). In addition, the concentration of these nuclei in plant material ranges from some nM up to 10 - 20 mM, resulting in absolute sensitivities several orders lower than (water) protons. This underlies the reason why the nuclei other than protons needs NMR spectrometers with high magnetic field strengths (>1.5 Tesla). These spectrometers are rather heavy, voluminous and expensive, and operates in well-defined and constant climatological environments only, preventing the *in situ*

application of NMR to plants, e.g. in greenhouses and auctions, or at different positions on plants. Therefore we conclude that in the next future low field proton NMR is the most promising approach for horticulture. Clearly this will not hold for basic plant physiological and horticultural research in general (Pfeffer and Gerasimowicz, 1989)!

In this paper examples of applications of low field proton NMR and NMR imaging to plant material are briefly reviewed. In addition, non-invasive plant water balance studies in intact plants in laboratory and greenhouse situations are illustrated by examples of the work carried out at the Department of Molecular Physics, Wageningen Agricultural University. Attention will be paid to the concept of instrumental design with a small permanent magnet system, resulting in a "portable" NMR spectrometer for application in e.g. greenhouses and auctions. Its potential use is discussed.

2. Applications of low field proton NMR to plant material

2.1. Non spatially resolved measurements

NMR signals are characterized by a number of different parameters. Excitation disturbs the equilibrium in the nuclear spin system. A time dependent rf signal is induced in the NMR measuring probe by that nuclear spin system as it returns to equilibrium. The amplitude of the signal directly after an excitation is a direct measure for the amount of nuclei under observation in the NMR coil, and is primarily a function of nuclei density. Two relaxation time constants describe the rate and manner in which the nuclear spin system returns to equilibrium. One time constant, the spin-lattice relaxation time T1, describes the return in the direction of the magnetic field. The second time constant, the spin-spin relaxation time T2, characterizes the return in the plane perpendicular to the applied magnetic field. These relaxation time constants can be measured independently. T1 and T2 give access to the physical state of the nuclei under observation .

Some general observations of water relaxation in (vacuolated) plant material are that the observed T2 is much shorter than T1, that T2 in plants is shorter or even much shorter than in pure water, and that in general multiexponential decay curves are observed, in general (Belton & Ratcliffe, 1985). In biological systems multiexponential relaxation has been used to obtain information on the relative proportions of water in different environments or in different physical states, e.g. "bound" and "free". In the same way, water and oil fractions have been discriminated in e.g. oil containing seeds (Srinivasan, 1979). In addition, the solid protons of the biomass have very short T2 values (10 - 20 μs) and can be discriminated from the different water protons. This allows the measurement of the total absolute water and oil content (Tiwari & Burk, 1980).

The correlation between relaxation time data and water content, relative water content, tissue hydration and water potential has been shown in a number of papers (Van As et al, 1986; Colire et al, 1988; Veres et al, 1991). There exists a good, though not always quantitative, correlation between the NMR data and the classical measurements.

Recent results concerning the interpretation of the proton relaxation time measurements on water in plant material (Bacic & Ratkovic, 1984 and 1987; Hills & Duce, 1990; Snaar & Van As, 1990; Van As & Snaar, 1990) are

summarized in the model as presented in Fig. 1. Depending on field strength and experimental parameters, two or three exponentials are observed, which can be assigned more or less uniquely to water in the vacuole, cytoplasm and cell wall/extracellular space, respectively. The observed T2 of the vacuole compartment depends on the vacuole size/geometry, proton tonoplast permeability and T2 of the cytoplasm. The observed T2 of the cytoplasm compartment depends on the water content of the cytoplasm, the tonoplast and plasmalemma proton permeability, and the T2 of the cell wall/extracellular space. In combination with the amplitude information this allows the study and measurement of water distribution on subcellular level. An example of the effect of growth and fungal infection on dehydration patterns on subcellular water distribution is given in Fig. 2.

In general, relaxation time measurements give access to damage and quality detection of fruits and vegetables, allows the determination of growth, stage of maturation and water content, even on subcellular level, and are applied to growth, ripening, cold hardiness, drying and senescence studies of plant tissue, fruits and plant products (e.g. Hills & Duce, 1990; Kaku et al, 1985; see also § 2.2).

The frequency of the time dependent rf signal given off by the nuclear spin system as it returns to equilibrium after being excited depends strongly on the magnetic field strength felt by the spins. When a well defined linear magnetic field gradient is created spins at different positions give off rf signal with different frequencies, each frequency being characteristic for a particular position. This is the basis for NMR imaging (see § 2.2). At the same time, when spins move from one position to the other (diffusion, flow) the frequency of the detected rf signal will change in time. This principle has been used to measure xylem flow in the plant stem (Van As & Schaafsma, 1984) and diffusion of water in plant cells (Cory & Garroway, 1990). The diffusion measurements can be done by varying the observation time, resulting in the distance over which the spins can diffuse freely and the permeability of the surrounding wall (membranes). In this way the size of e.g. the vacuoles in vacuolated plant tissue (Merboldt et al, 1987) or oil droplets in oil containing seeds have been measured (Fleischer et al, 1990).

2.2. NMR imaging

All above mentioned information available with low field NMR can also be measured spatially resolved in any selected part of the material under observation by NMR imaging. In this way anatomical and functional information, and even functional anatomical information, is obtained. Contrast in the NMR images can be manipulated. Depending on the actual rf pulse and magnetic field gradient sequence the pixel intensity represents proton density, T1 or T2 relaxation values, flow, diffusion, exchange or a combination of these parameters (Moonen et al, 1990; Merboldt et al, 1987). NMR imaging has been used to study stem, leaf, root anatomy and soil water distribution around the root (based on proton density and differences in relaxation times) (Bottemley et al, 1986; Cofer et al, 1989; Conneley et al, 1987; Johnson et al, 1987; Veres et al, 1991). Its value for internal quality assessment of fruits and vegetables has been investigated (Chen et al, 1989; Wang et al, 1988), turning out that voids, pits, bruises, worm, dry regions can well be observed. In addition studies on the evaluation of maturation and stage of ripeness have been performed (Ishida et al, 1989).

The potential applications of NMR imaging are fascinating and enormous,

especially for (water) distribution studies (Veres et al, 1991). However, for quality assessment (e.g. damage, maturation, ripeness) NMR imaging gives no new functional information with respect to non spatially resolved NMR measurements. The only gain is a better figure of the location and amount of e.g. damaged tissue. The increase of investigation costs of NMR imaging with respect to non spatially resolved low field NMR is relatively enormous: at least a factor 15. Therefore, the use of NMR imaging will be limited to basic plant physiological and horticultural research in general, unless the development of relatively cheap and small sized NMR imagers as presented recently (WNS, 1990) will be further developed.

3. Water balance studies with NMR

3.1. Laboratory studies

^1H NMR for the non-invasive and non-destructive studies of plant water relations in intact plants has been demonstrated in cucumber plants by combined NMR measurement of water flow in the xylem and water T2 measurements of the surrounding tissue (Reinders et al, 1988a and b). Water flow has been measured as presented before (Van As & Schaafsma, 1984), resulting in the linear and the volume flow rate. The T2 data have been either approximated by a single exponential (Reinders et al, 1988) or presented by the weighted average value (Van As et al, 1986), and are used to monitor the total water content or the water potential of the tissue (cf. § 2.1) in the NMR measuring probe. As discussed in § 2.1 water relations up to subcellular level can now be traced by more accurate T2 measurements.

The NMR method appears to be a valuable tool for quantitative studies of the plant water balance. If the method is applied to the part of the stem just above the root, the combination of flow and water content measurements allows the determination of changes in resistance for water transport and where this change is located. This has been demonstrated by the discrimination between changes in water uptake and transpiration rate in response to changes in environmental parameters as root temperature, light intensity, rh and air CO2 concentration variations (Reinders et al, 1988a and b; Reinders, 1987).

The absolute accuracy of the NMR flow measurements in these studies has been estimated to be better than +10 %. The relative accuracy in one plant under observation is even better, within a few percent (Reinders, 1987). This high accuracy has also been demonstrated in the high correlation between NMR flow results and dendrometer results (see Fig. 3) and between water uptake (weight balance measurements), transport (NMR) and transpiration rates (based on relative air humidity measurements) as measured in one and the same plant under steady state transport conditions (Van As & Duyvis, unpublished results).

The NMR method allows for a high time resolution (one measurement takes typically in the order of one minute) and for a long observation period (observation periods of longer than two months have been realized).

3.2. *In situ* studies

All above mentioned NMR studies on plant material have been done using NMR spectrometers which are very heavy, voluminous, and expensive and which operates in a well-defined and constant climatological environment, preventing the *in situ* application of NMR to plants. Recently, small sized (trans)portable NMR equipment has been developed

overcoming this problem (de Jager et al, 1988). A high degree of automation of data acquisition and spectrometer control has been realized to allow for unattended operation over long periods of time under realistic greenhouse conditions. The permanent magnet system is a U-shaped 30 kg magnet with a 2 cm air gap. Magnetic field drifts as a result of the varying climatological conditions as met in greenhouses are corrected by an automatic field locking procedure. The rf probe, with a single hinged Helmholtz type transmitter/receiver coil (inner diameter 1 cm) that can be opened and folded around (part of) the plant stem, is connected to the electronics by a single coaxial cable. Cable lengths up to 50 m have been used, allowing the electronics to be placed outside the greenhouse climate.

This (trans)portable NMR will become commercially available (Bruker Spectrospin N.V., Wormer, The Netherlands). It allows the measurement of water flow, water content and relaxation times as described above (§ 3.1), and has been successfully applied to plants in a phytotron and in greenhouses (Van As et al, in prep). The system can easily be applied to different positions in a plant, allowing e.g. the study of the flux in the stem at different heights in a canopy in relation to the transpiration rate and the study of plant water hydraulics. Its applications include the use as a monitoring device for screening in plant breeding experiments to select plants with sufficient transport in roots and shoots for water and nutrients, and the use as a sensor for the 'speaking plant' approach has been suggested. This latter application, a quantitative sensor of internal plant water relations for greenhouse irrigation and climate control, is presented and discussed in this Acta in more detail (van de Sanden et al, this Acta).

4. Prospects

The applications of NMR for basic horticultural research are numerous in general. The possibility to study water balance by flow and water content measurements simultaneously is unique. The *in situ* applications in horticulture are limited to the information available from small sized low field NMR systems.

Due to the costs and possible information content we have to discriminate between conventional and imaging systems. NMR imaging will become a unique method for the non-invasive study of water content distributions and spatially resolved water balance studies, especially in the soil/root system and developing fruits. The costs of the available systems, however, will be to high for routine use in horticulture in general, unless the development of relatively cheap and small sized NMR imagers will be further developed. The transportable NMR system as discussed above, based on relatively low cost conventional low field proton NMR systems (magnetic field strength < 1.5 Tesla), can be extended for imaging. Transportable systems are the most promising systems for use in horticulture, allowing the *in situ* application in greenhouses and auctions. Up to now applications have been limited to objects with a diameter of < 1 cm. Magnet systems with a larger air gap are available, at the cost of a increased weight or a lower field strength, however. For the use as water balance sensor in greenhouses NMR has to compete with much cheaper, though less informative, devices. Its penetration will strongly depend on the price of the total system. The combination of one NMR console with a number of magnet systems, used in time sharing, can lower the costs per sensor. Anyhow, NMR can serve as a calibration method

for other flow meters. The most promising application in the next future will become the use of NMR as an objective tool for quality assessment of fruits and other horticultural products.

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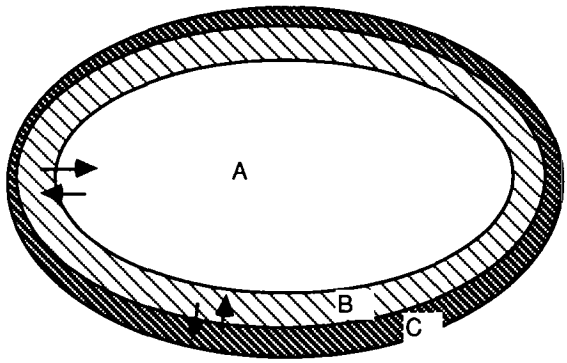

A: vacuole
B: cytoplasm
C: cell wall/extracellular space

Figure 1.
information content of NMR relaxation time measurements. At low magnetic field strength (< 0.5 Tesla) the intrinsical relaxation times are: T1 = T2 for water in the vacuole (about 2 s); T1 > T2 for water in the cytoplasm and the cell wall. T2 of water in the cytoplasm is about 0.2 s. T2 for the cell wall ranges from 5 ms to 1 s, strongly depending on the water content. Within these compartments diffusive exchange results in single exponential relaxation behaviour. Due to exchange of water protons with protons of the biomass the T2 values depends on the magnetic field strength and the pulse spacing in a CPMG sequence. Proton exchange over the tonoplast and the plasmalemma effects the observed relaxation times. Since the effect of exchange is with respect to the relaxation times, T1 and T2 are in general different and the results strongly depend on pulse spacing and magnetic field strength, even for the number of exponentials observed.

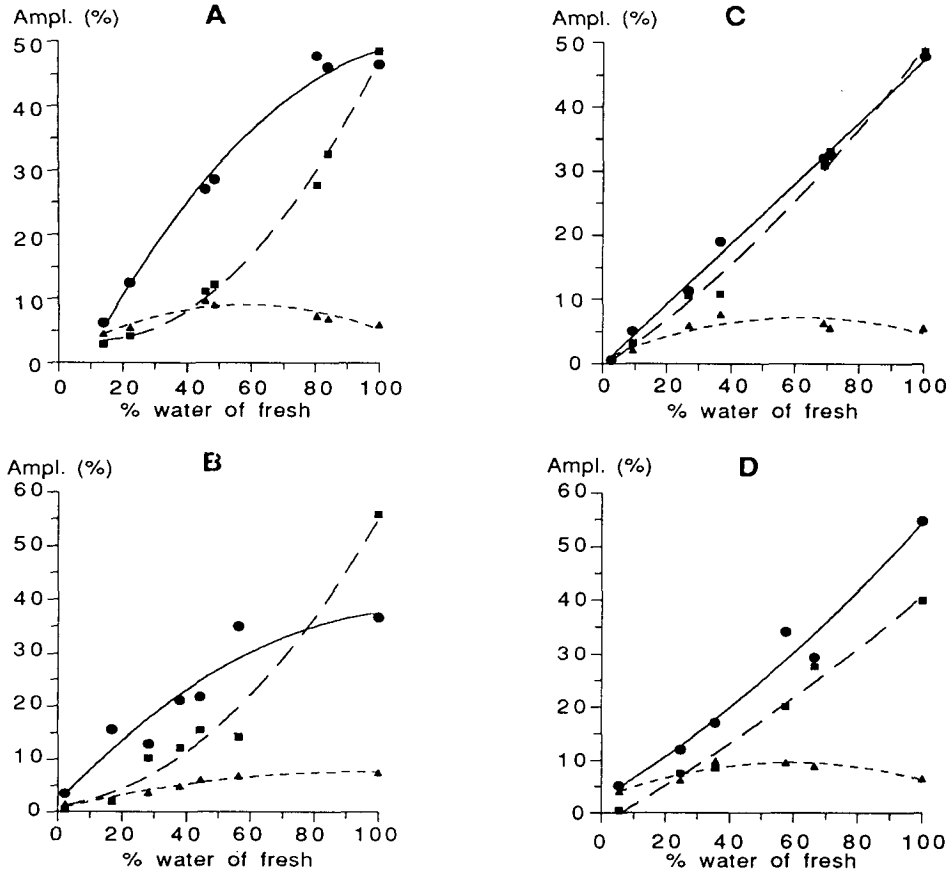

Figure 2.
Relative amplitudes corresponding to the longest (▪), intermediate (•) and shortest (▵) NMR T2 relaxation times of water in the first leaf of wheat plants as a function of water content during dehydration. A and B: healthy, 2 and 4 weeks old, resp.; C and D infected by the fungus *Puccinia Recondita*, 2 and 4 weeks old, resp..
During maturation of the healthy leaf, the relative amplitude belonging to the longest T2 increases (see 100 % water content data). The (dehydration) pattern of the relative amplitudes as a function of decreasing water content during dehydration differs for younger and mature leaves (Fig. 2A and B). Simultaneously, all observed T2 values decrease during dehydration. These typical dehydration patterns have been observed in several other plant tissues, e.g. cucumber stem, mushroom, apple fruit. Fungal infection, resulting in a increased water permeability of the cell membranes, results in a total different dehydration behaviour of the relative amplitudes: these amplitudes no longer behave independent of each other (Fig. 2C and D). In addition, the relative amplitudes as a function of leaf age behave differently in infected leaves compared to healthy leaves.

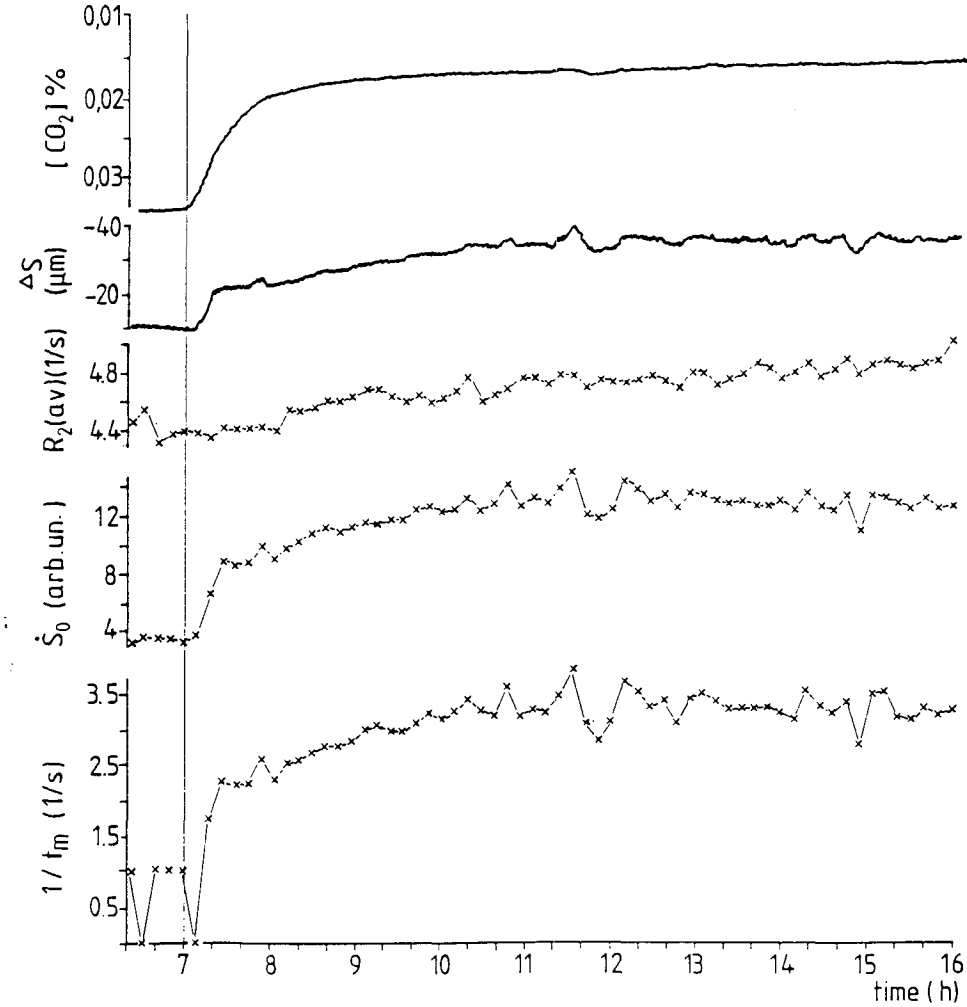

Figure 3.
Illustration of the correlation between various measured parameters in stem of a cucumber plant: linear flow rate (NMR, presented by t_{max}^{-1}), volume flow rate (NMR, presented by SO), water content or potential (NMR, presented by R2 = $T2^{-1}$) and dendrometer reading (Δ5, sign inverted to emphasis the correlation between the various parameters), and air CO2 concentration. At 7.00 h illumination was switched on (Courtesy of J.E.A. Reinders, H. Van As, and T.J. Schaafsma).