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# PROTON SPIN RELAXATION STUDY OF THE GERMINATION OF BARLEY GRAINS

BY

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ABSTRACT

The time-evolution of the spin relaxation of water protons during the germination of barley grains in-situ, supplied continuously by water, was investigated using a "home-made" permanent-magnet NMR relaxometer, working at 25 MHz. It was found that the values of the spin-lattice time, $T_1$, are higher in germinating than in "killed" grains.

RÉSUMÉ

L'évolution dans le temps de la relaxation des spins des protons de l'eau a été examinée in-situ à l'aide d'un relaxomètre en RMN, travaillant à 25 MHz, chez la graine d'orge, alimentée en continu par de l'eau. Chez les graines qui germent normalement, le temps de relaxation spin-réseau, $T_1$, est beaucoup plus élevé que chez les graines tuées.

## INTRODUCTION

Nuclear Magnetic Resonance (NMR) in the pulse regime has proved to be a powerful tool for noninvasive investigation of the living state (GADIAN, 1982), particularly in the domain of molecular dynamics (GRANGE *et al.*, 1980, LENK 1986).

In spite of the vast application of NMR in the field of plant biology, little attention was directed to the NMR Studies of grains *in-vivo*. Consequently, we have introduced the application of the pulse NMR for the study of the fast molecular dynamics in grains of oat (DEGLI AGOSTI *et al.*, 1989, 1991). In the present contribution we report the application of this procedure to the study of the time-evolution of the spin-lattice ($T_1$) and the spin-spin ($T_2$) relaxation times and their ratio for the different physiological states in grains of barley (*Hordeum vulgare*, var. Himalaya).

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## MATERIAL AND METHODS

### *Plant material*

The naked barley cultivar was used. Before the experiments, grains were mixed with a fungicide powder (Thiotox, Sandoz, with 80% Thiram: Bis dimethylthiocarbamoyl disulfide).

In order to obtain killed grains, these were enclosed in a glass tube and dipped in a water-bath at 100° C for 40 min before the experiment.

The barley grains were germinated directly in the NMR 7 mm standard tubes at 19 ± 0.5° C in darkness. In this *in-situ* experimental configuration the investigated grain is supplied by a "water guide" using a strip of paper (Filter paper no. 595 from Schleicher & Schüll) dipped in a reservoir of doubly deionised and distilled water (Fig. 1). Measurements were performed with the aid of a very low-intensity green safelight.

### *NMR spectroscopy*

The $T_1$ relaxation times were measured on a "home-made" pulse NMR spectrometer working at 25 MHz (Fig. 2). The pulse transmitter comprises a crystal oscillator, two double-balance mixers (Anzac MD-108), a broad-band booster amplifier (ZHL-3A, Mini-Circuits) and a final stage RF power amplifier, consisting of two RCA 6417 tubes, hooked in push-pull and gated. The RF-phase incoherence was removed by the 90° phase shifter (quadrature hybrid JH-114, Anzac). The maximum power of the transmitter during the RF pulses was 20 W into a 50 Ohm load. The on/off ratio of the transmitter was better than $10^7$.

The probe used is of the crossed coil type, situated in the 2.4 cm gap of a permanent magnet (Fig. 2). This construction, rather than a single coil, was chosen because, by making the transmitter coil larger than the receiver coil, a homogeneous RF-field over the active part of the cylindrical sample can be obtained. The transmitter coil is made up of two sections (Helmholtz system), one on each side of the sample. Each section is of rectangular shape, the larger dimension being parallel to the sample tube. The receiver coil is cylindrical of diameter 8 mm and of length 4 mm. For the inversion of magnetization, the duration of the RF-field pulse is 85 microsecond.

The NMR signals are amplified by a home-made preamplifier and processed by the Polaron NMR receiver endowed by the phase sensitive detector.

All timing signals are synchronous with the single master clock. Therefore, the oscillator output is changed by the SN7490 counter to 1 MHz to trigger the TTL pulse programmer. The latter makes use of dual retriggerable monostable multivibrators SN74123. The multivibrator "M1" provides the first pulse of the two-pulse sequence, whereas the multivibrator "M3" generates the second pulse and the multivibrator "M2" produces the delay between the two pulses. The output from the pulse programmer is fed to the double balance mixers. The delay between the pulses is measured by the 5302A universal counter (Hewlett Packard). All logic and control signals are TTL compatible.

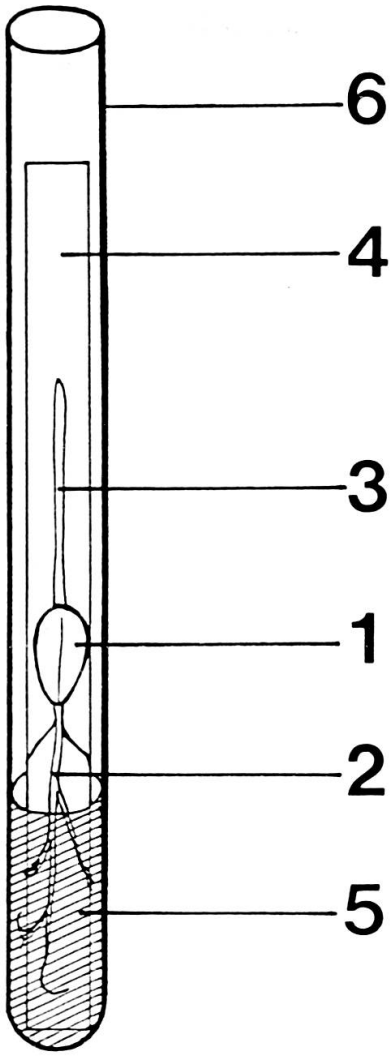


FIG. 1.

The experimental situation of the barley grain: The grain (1) placed on a strip of filter paper (4) dipping in a doubly distilled and deionized water reservoir (5) germinates in the 7 mm NMR tube (6), in darkness. Roots (2) and a shoot (3) develop. The measuring NMR region is located in the grain (1) during the experiment. Germination takes place directly in the NMR tube which is maintained in the NMR spectrometer. Measurements can be done continuously in time.

The spin-lattice relaxation times $T_1$, were measured by the well-known *inversion-recovery* [180° - t - 90°] pulse sequence. The whole equipment is working in the darkness in an underground room with a stable temperature.

The grain, which remains fixed on the band of paper, represents a two-phase system with a slow exchange. Spin relaxation in such a system was studied by ZIMMERMANN AND BRITTIN (1957). Generally, a slow exchange in a multiple phase system yields the weighted average of effects taking place separately in each phase. In this case, the longitudinal magnetization $M_2$, measured by the *inversion-recovery* method, is given by

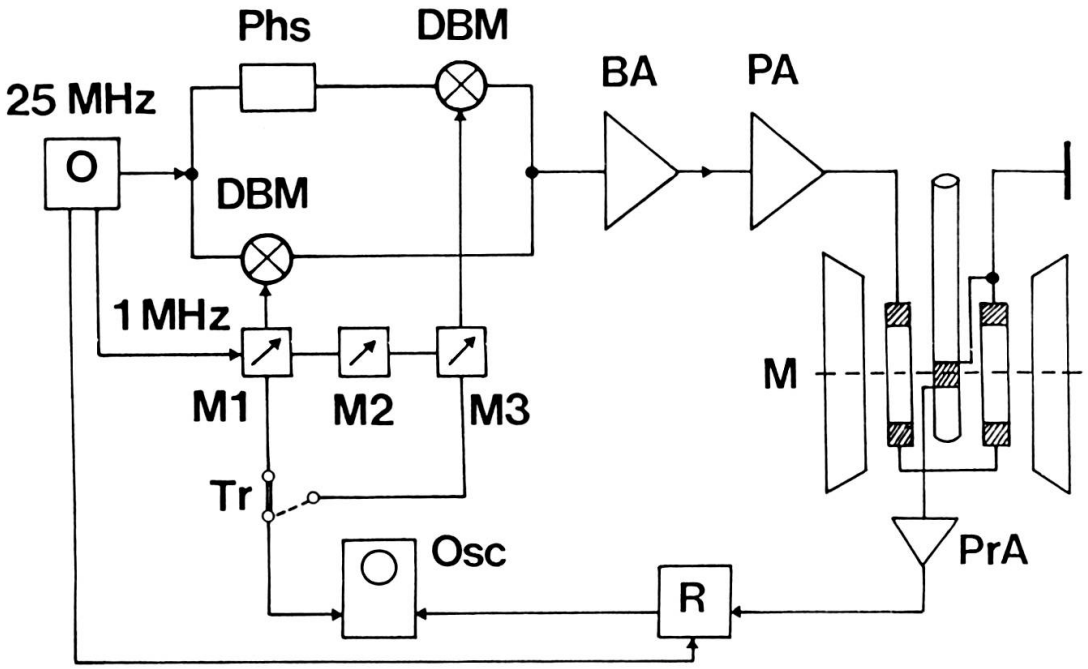


FIG. 2.

Block diagram of the NMR pulse spectrometer, working at 25 MHz. O: crystal oscillator. DBM: Double balance mixer. Phs: Phase shifter (90°). BA: Booster amplifier. PA: Power amplifier. PrA: Preamplifier. R: Receiver. Osc: Oscilloscope. M1, M2, M3: Monostable multivibrators. Tr: Trigger channel. M: Magnet.

$$M_2(t) = M' [1 - 2\exp(-t/T_1')] + \\ + M'' [1 - 2 \exp(-t/T_1'')] \qquad (1)$$

where M' is the magnetization at t=0 and $T_1'$ the spin-lattice relaxation of the water system in the grain, M'' is the magnetization at t=0 and $T_1''$ the spin-lattice relaxation of the water guide.

The values of M'' and $T_1''$, concerning the water-guide, were measured separately before the experiment.

The values of $T_1'$ were measured by the "null-method". In this case, the right-hand side of eq. (1) equals zero. The approximate solution of this equation was done by the Newton interpolation method.

The values of the spin-spin relaxation times $T_2$ were measured by the standard spin-echo method using the sequence: *90° - t - 180° - echo.*

## RESULTS

The grain, placed inside the receiving coil and supplied by water (Fig. 1), starts the germination process. During the initial stage of imbibition, the $T_1$ relaxation times have

a minimum value between 50-70 ms. After about three days, one can observe the growth of the grainling. This is accompanied by a significant increase of the $T_1$ relaxation time of the water protons in the grain, as presented by curve **A** in Fig. 3.

We studied also the grains, in which the living capacity was "killed" by a pretreatment before the measurement at 100° C for 40 min. The comparison of the time-dependent spin-lattice relaxation values $T_1$ for the germinated grains with those for the un-germinated (killed) grains shows that the former (curve A) are higher than the latter (curve **B**, Fig. 3). This is similar to the result obtained in grains of oat in our preceeding work (DEGLI AGOSTI *et al.* 1991).

The results of the spin-spin relaxation are also presented in Fig. 3. The curve **C** shows also the evolution of the $T_2$ relaxation time for the living grains, while that for the killed grains is given by the curve **D**. It is clear that the spin-spin relaxation times are less different than the spin-lattice relaxation times between living and dead grains.

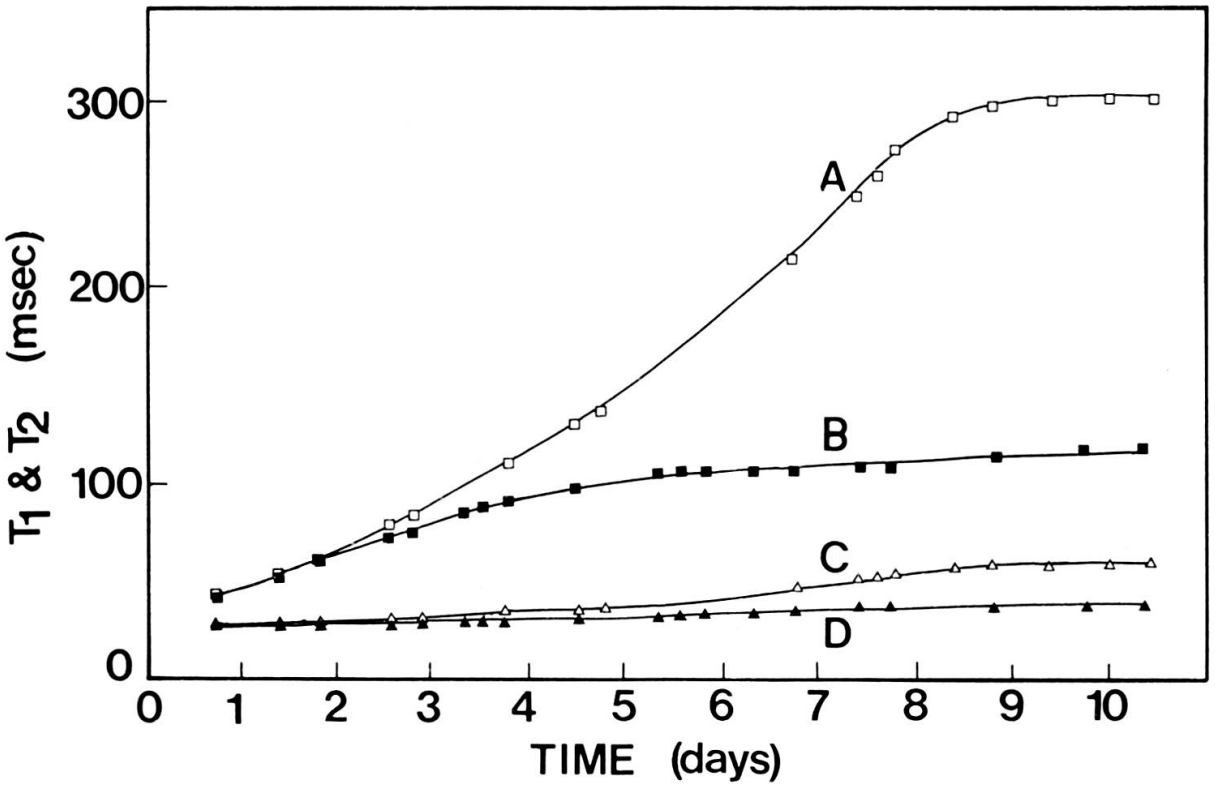


FIG. 3.

The time-evolution of the spin-lattice relaxation time $T_1$ and the spin-spin relaxation time $T_2$ in a grain of barley at 25 MHz. A) $T_1$ in the germinating grain. B) $T_1$ in the "killed" grain. C) $T_2$ in the germinating grain. D) $T_2$ in the "killed" grain.

## DISCUSSION

The spin-lattice relaxation time $T_1$ measures the fast brownian dynamics of the water-proton system and indicates the thermodynamic state of the system (LENK, 1986). It the fast brownian dynamics of the trapped water molecules has the rotational character, then following BLOEMBERGEN *et al.* (1948), the spin-lattice relaxation rate is proportional to the sum of the Lorentzian spectral densities

$$1/T_1 = K.\ [J(2\pi f) + 4J(4\pi f)]/5 \qquad (2)$$

where the related spectral densities are:
$J(2\pi f) = t_c/[1+(2\pi f t_c)^2]$, $J(4\pi f) = t_c/[1+(4\pi f t_c)^2]$, f is the frequency, $K = I(I+1)\ \gamma^4 h^2/R^6$, R is the interprotonic distance, $\gamma$ is the gyromagnetic ratio and I is the spin value.

Using eq. (2), one obtains at the room temperature $[(2\pi f t_c)^2 << 1]$

$$1/T_1 = K.\ t_c \qquad (3)$$

This means that the rotational correlation time $t_c$ is proportional to the spin-lattice relaxation rate $1/T_1$, which can be particularly applied in the study of the phase transitions from the state "A" to the state "B", using the following ratio

$$T_{1A}/T_{1B} = t_{cB}/t_{cA} \qquad (4)$$

The spin-spin relaxation rate is given by

$$1/T_2 = K.\ [3t_c + 5J(2\pi f) + 2J(4\pi f)]/10 \qquad (5)$$

At the room temperature $[(2\pi f t_c << 1]$, eq. (5) yields

$$1/T_2 = K.\ t_c \qquad (6)$$

Relations (2) and (6) show that for $2\pi f t_c)^2 << 1$, the spin-spin relaxation rate equals to the spin-lattice relaxation rate ($1/T_2 = 1/T_1$).

Furthermore we define the parameter *r*

$$r = T_1/T_2 \qquad (7)$$

which measures the deviation of the brownian dynamics from the isotropic rotation.

Figure 4 illustrates the changes of the parameter r as a function of time. The parameter r increases monotonically with the final value of r = 5.1 for the living grains and of r = 3.4 for the killed grains. The brownian dynamics in the investigated samples is probably a mixture of the rotation and translation (see also LENK, 1972). Note also

that the values of $r$ in Fig. 4 are smaller than that obtained by us for the agar-water samples (LENK *et al.*, 1982).

The approach described in this paper allows one to follow *continuously* and *in-situ*, by pulse-NMR, the evolution of germination and the further development of the barley grain in darkness. In this situation, the $T_1$ relaxation time gives information on the dynamics of water protons. Initially, the molecules of water diffuse into the grains (imbibition <2-3 days) and its dynamics is very restricted, as shown by a very low $T_1$ (the $T_1$ relaxation time of pure water is 3.5 s). After the begining of germination (2-3 days), the radicle protrudes and the embryo develops. At the same time, the $T_1$ relaxation time also increases to reach its maximum value after about 9 days. Thus the water dynamics has significantly increased (eq. 4).

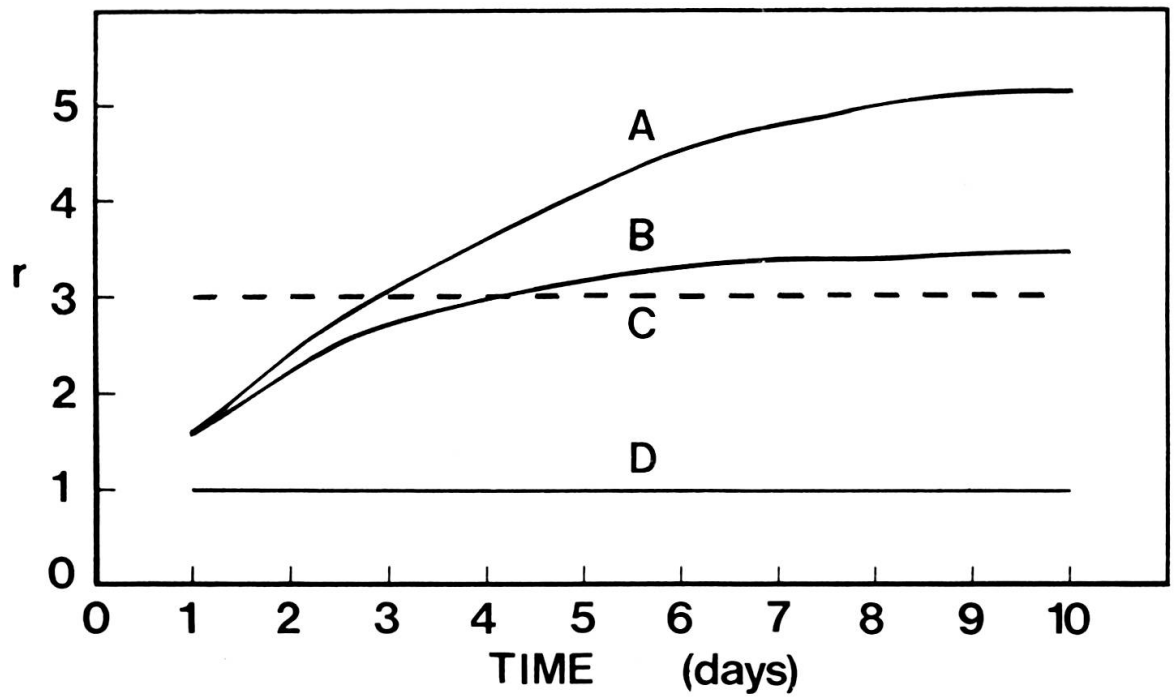


FIG. 4.

The time evolution of the parameter $r = T_1/T_2$. A) Germinating grain. B) "Dead" grain. C) $r = 3.0$ for distilled water. D) $r = 1$ for pure isotropic rotation.

This can be explained by the known transformation of stored substances in the grains and their transfer to growing tissues. The increase of $T_1$ is clearly related to the physiological development of a normal living grain, since the *non-germinating grains* do not exhibit this phenomenon (inspite of a small initial increase of the $T_1$ relaxation time due to pure physico-chemical properties of the grain).

In the absence of light, the physiology of the seedling is purely heterotrophic. The system is thus characterized by a strong irreversibility towards the degradation of the grain storage.

According to eq. (4), our results can be interpreted from the thermodynamical point of view: the grain can be considered as a *heat reservoir* of the plant. At the begining of germination, the state of this system is characterized by a very restricted molecular dynamics and low internal energy.

During the germination and further development of the plantlet, the molecular dynamics and consequently, the internal energy of the grain, increases dramatically. A high molecular dynamics in this organ is necessary in order to assume an adequate distribution of organic matter to the growing part of the grainling (shoot and roots) and the cell nutrition. In the higher dynamic state, the molecular components can easily find their final configuration and the nutrients can easily diffuse toward their destination. This is experimentally illustrated by the evolution of the parameter r (see Fig. 4), in which the translational brownian component (diffusion) of the motion of water molecules is probably increased at the expense of its rotational component.

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