Eur Biophys J (2004) 33: 76–82
DOI 10.1007/s00249-003-0340-9

BIOPHYSICS LETTER

P. Krishnan · D. K. Joshi · Shantha Nagarajan
A. V. Moharir

# Characterisation of germinating and non-germinating wheat seeds by nuclear magnetic resonance (NMR) spectroscopy

Received: 11 November 2002 / Accepted: 26 June 2003 / Published online: 2 August 2003


**Abstract** Experiments were conducted to characterise the changes, especially of water status in germinating and non-germinating wheat seeds by nuclear magnetic resonance (NMR) spectroscopy. NMR relaxation time ($T_2$) measurements showed tri-phasic or bi-phasic characteristics during different stages of hydration, depending on the seed's ability to germinate. Component analysis of $T_2$ data revealed the existence of only two components, bound and bulk water, in dry seeds. In contrast, both the germinating and non-germinating wheat seeds had a three-component water proton system (bound, bulk and free water) in phase I of hydration. During the lag phase (phase II) of hydration, bulk water component of non-germinating seeds disappeared completely, resulting in a two component water proton system. Nevertheless, the three component water proton system was observed in the germinating seeds in phase II. Following phase II, rapid hydration (phase III) was observed in germinating seeds only. Water protons were reorganised and there were increases in bulk and free water but decreases in bound water concomitantly. Comparison of the physical state of water in these seeds by NMR spectroscopy with that of tissue leachate conductivity measurement suggests that the seed membrane system was affected more evidently in non-germinating seeds, leading to the disorganised cell structure. The present study provides evidence that the reorganisation of physical state of water in germinating wheat seeds during hydration is essential for its subsequent event of germination.

**Keywords** Germination · NMR · Relaxation times · Seed germination · $T_2$ · *Triticum aestivum* · Water status

P. Krishnan · A. V. Moharir
Division of Agricultural Physics, Indian Agricultural Research Institute, 10012, New Delhi , India

D. K. Joshi · Shantha. Nagarajan
Nuclear Research Laboratory, Indian Agricultural Research Institute, 110012 New Delhi, India

P. Krishnan (✉)
Division of Plant Physiology, Central Rice Research Institute, 753 006, Cuttack, Orissa, India
E-mail: prameela.krishnan@plant-sciences.oxford.ac.uk, prameela.krishnan@yahoo.com

*Present address:* P. Krishnan
Department of Plant Sciences, University of Oxford, Oxford, OX1 3RB, UK

## Introduction

Germination of seeds is a complex process and the seed water distribution and its molecular mobility during hydration in seeds are not clearly understood (Bewley and Black 1985; Bernal-Lugo and Leopold 1998; Miedziejko 1997; Walters 1998). Glassy state, motional and thermal properties of intra-cellular seed water are considered to change as a function of the total water content and temperature of seed tissues (Bernal-Lugo and Leopold 1998; Sun and Leopold 1994). These changes in seed water status during imbibition and germination of seeds can influence the subsequent development and growth (McDonald 1999).

High and low resolution nuclear magnetic resonance (NMR) have been successfully employed to study the state of water in many biological systems (Miedziejko 1997; Ratcliffe and Shachar Hill 2001). Longitudinal and transverse relaxation behaviours of water protons can be investigated, in particular, to describe the compartmentation and transport of water in tissues, plants and seeds (Ratcliffe 1994; Krishnan et al. 2003a, 2003b, 2003c). The mobile and less mobile water molecules are distinguished by their different relaxation rates and their relative amounts can be calculated (Van As 1992; Krishnan et al. 2003b). Thus, NMR technique provides a novel, sensitive and direct method to characterise water status in seeds (Ridenour et al. 1996; Ratkovic 1987).

Reports are available on the use of NMR relaxation time measurements to characterise the water status of seeds during germination. Ridenour et al. (1996) showed

that $T_1$ and $T_2$ measurements provide information about molecular mobility within barley grains during imbibition. Isobe et al. (1999) observed a three-component water proton system in germinating morning glory seeds. Likewise, reports on NMR studies using germinating seeds of oat (Lenk et al. 1991), rape seeds (Miedziejko 1997), hydrating lettuce (Di Nola et al. 1988), cowpeas (Brosio et al. 1992), soybean (Fukuoka et al. 1994; Noda et al. 1998), wheat (Haranczyk et al. 1996) and maize (Ratkovic 1987) seeds are available. In all these studies, NMR relaxation time measurement has identified two to three components of the water proton system. Haranczyk et al. (1996) described the initial stages of wheat imbibition by proton nuclear magnetic relaxation. Using pulsed $H^1$ NMR spectroscopy, Miedziejko (1997) examined the impact of temperature and chemical stimuli on water transport and mobility in germinating rape-seeds. In general, these NMR studies on hydrated seeds suggest that protons with a short relaxation time are, in part associated with the bound/structural water; protons of medium relaxation time are associated with intracellular/cytoplasmic water; and protons of long relaxation are associated with the extra-cellular water (Brosio et al. 1992; Isobe et al. 1999; Krishnan et al. 2003b). Understanding the differences in seed water status of germinating and non-germinating seeds is essential to gain insights on the hydration mechanism and germination process. The present study was undertaken to monitor the changes in water status of wheat seeds, both germinating and non-germinating during hydration and germination by NMR spectroscopy.

## Materials and methods

### Seed material

Seeds of wheat (*Triticum aestivum* cv. HD 2329) were obtained from the Regional Research Station, Indian Agricultural Research Institute, Karnal, India. These seeds were screened with a magnifying lens and only intact seeds without visible defects due to insect damage or malformations were selected. The contents of moisture, protein, carbohydrate and oil, and the germinability of wheat seeds were tested by the standard procedures (ISTA 1985). Freshly harvested seeds having 100% germination were considered as germinating seeds. Non-germinating seeds were obtained by subjecting them to high temperature (45 °C) and moisture (100%) conditions until they attained 0% germination.

For hydration, the germinating and non-germinating seeds were placed over moist filter paper pads (sterile distilled water added just sufficient to moisten the filter paper) in closed containers and incubated at 25 °C. At different time intervals of incubation, seed samples were withdrawn for analyses. The samples were wiped gently with tissue paper to remove excess external water and immediately taken for NMR analysis.

### Seed moisture content

In a set of triplicate seed samples, moisture content was determined by oven drying seeds at 95 °C to constant weight (Walters 1998). Moisture content (%) was calculated as $[(W_1-W_2)/W_2]\times100$ where $W_1$ was the initial weight of the seed (g) and $W_2$ was the final weight of the seed after drying (g).

### Seed leakage test

Three weighed replicates of seeds (2 g) were soaked in 50 ml of distilled water at 25 °C for 16 h. A control with distilled water (but without seeds) was also maintained. The seed leachate was collected and the electrical conductivity (EC) was measured using a digital conductivity meter (ISTA 1985).

### NMR relaxation measurements

Seed samples (2 g) were immediately placed in NMR tubes of 10 mm diameter, corked to avoid dehydration and placed in the probe of a Bruker NMS 120 pulsed NMR spectrometer as described earlier (Krishnan et al. 2003a, 2003b). The column height of seed sample in the NMR tube was kept at around 2 cm. Each measurement was made with six replicates.

### Spin–spin relaxation time ($T_2$) measurement

Spin–spin relaxation or transverse relaxation time was measured by the Carr–Purcell–Meiboom–Gill method (Snarr and Van As 1992). The settings of data points (150), pulse separation (0.5 ms), dummy echo (3) and scans (10) were maintained for each measurement. The gain was adjusted to maximise the signal:noise ratio. The $T_2$ values were calculated using the in-built program of the instrument. $T_2$ determination was done 6 to 10 times for each sample and the mean values were calculated.

### Components of NMR relaxation time measurement

In biological systems, multi-exponential relaxation decay curves are generally observed and numerous attempts have been made to discriminate between different water fractions in various compartments based on the relaxation times (Van As 1992; Krishnan et al. 2003b). The actual relaxation curve showed a marked non-exponentiality that could be accounted for by the presence of three clearly recognisable components with different relaxation times by the exponential peeling statistical method (Di Nola et al. 1991; Brosio et al. 1992). The assignment of transverse magnetisation to different fractions of water in seeds is made based on the available literature on seed NMR. According to Ratkovic (1987), three water components of the plant and seed systems can be identified with the transverse relaxation time $T_{2c}$, $T_{2b}$ and $T_{2a}$. $T_{2c}$ accounts for the hydration water of macromolecules and is tightly bound, $T_{2b}$ for the cytoplasmic bulk water with lower mobility, and $T_{2a}$ does for the extra-cellular free water (Di Nola et al. 1991; Brosio et al. 1992; Snarr and Van As 1992). Non-exponentiality of spin–spin relaxation is accounted for by the presence of clearly recognisable multi-components with different relaxation times. Three components of spin–spin relaxation with three different relaxation times have been identified as:

$$M_t = C_a \exp(-t/T_{2a}) + C_b \exp(-t/T_{2b}) + C_c \exp(-t/T_{2c})$$

where $C_a$, $C_b$ and $C_c$ are related to the relative population of three components (Di Nola et al. 1991; Brosio et al. 1992). The components of spin–spin relaxation were analysed by using least squares fit analysis in the region of limits specified, based on the $t$ value ($x$-axis) until the plotted curve showed a visible curvature change, using a program written in BASIC. The general procedure of exponential peeling of curve decomposition was followed by locating the slowest relaxing fraction from the curve and subtracting this fraction from the observed data (Fig. 1) (Snarr and Van As 1992; Krishnan et al. 2003b).

## Results

In accordance to the kinetics of water uptake (Bewley and Black 1985), hydration of seeds for germination

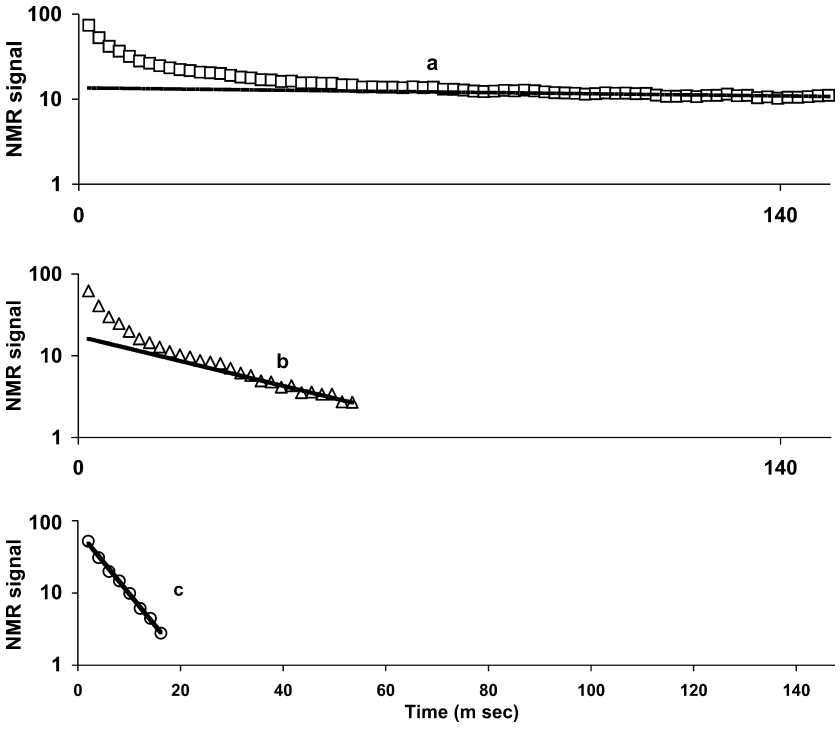


**Fig. 1** Curve a: semilogarithmic plot of the transverse magnetisation decay curve. Curve b: transverse magnetisation decay of $T_{2b} + T_{2c}$ in same sample. Curve b was obtained by subtracting from curve a the extrapolated values of the slow decaying component $T_{2a}$ (dashed line under curve a). Curve c: transverse magnetisation decay of $T_{2c}$. Curve c was obtained by subtracting from curve b the extrapolated value of the slow decaying component of curve b (dashed line)

shows three distinct phases (depending on the rate of water uptake), namely rapid hydration (phase I), lag (phase II) and steady hydration phase (phase III). In the present study, germinating wheat seeds showed all three distinct phases of hydration while there were only the two hydration phases in non-germinating seeds (Fig. 2). Thereafter, non-germinating wheat seeds remained in phase II. Rapid hydration (phase I) was limited to the initial 6 h of hydration with the mean rates of water uptake of 3.64 g $H_2O$ $h^{-1}$ and 2.88 g $H_2O$ $h^{-1}$ in germinating and non-germinating wheat seeds, respectively. The lag period (phase II) with the rates of water uptake of 0.33 g $H_2O$ $h^{-1}$ and 0.24 g $H_2O$ $h^{-1}$ in germinating and non-germinating seeds, respectively, was between 6 and 36 h of hydration. The third phase which coincided with the emergence of radicle and plumule showed a steady hydration with the water uptake of 0.40 g $H_2O$ $h^{-1}$ from 36 h only in germinating seeds. In contrast, the non-germinating seeds continued to be in the lag phase (phase II). The differences in water uptake between germinating and non-germinating wheat seeds became evident during the lag phase (phase II).

During the rapid hydration (phase I), the moisture content increased from the initial level of 7 to 32% and it was from 40 to 70% during phase III in germinating seeds. The time course of seed leachate conductivity from both germinating and non-germinating wheat seeds is presented in Fig. 3. In general, the non-germinating seeds had higher leachate conductivity than the germinating seeds. During phase I of hydration, both seeds showed an initial decrease, followed by increases in their electrical conductivity. But, during the early lag phase (phase II), the electrical conductivity of germinating seeds decreased and remained almost constant. In

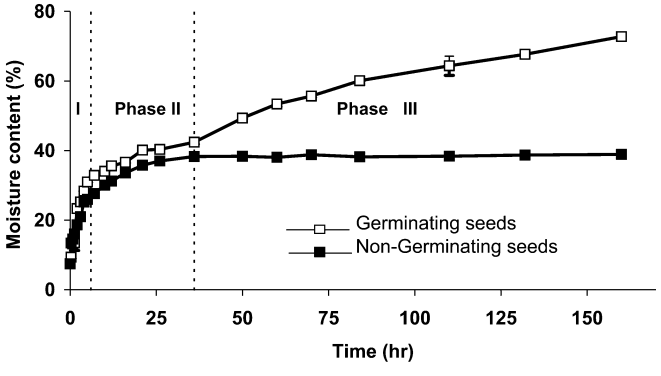


**Fig. 2** The time course of changes in moisture content (%) of wheat seeds during germination

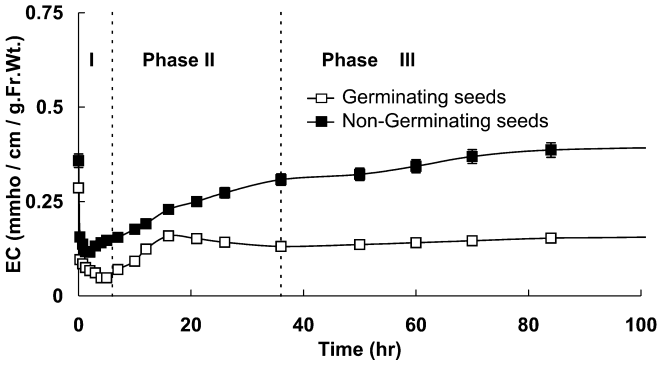


**Fig. 3** Seed leachate conductivity of soybean and wheat seeds during germination

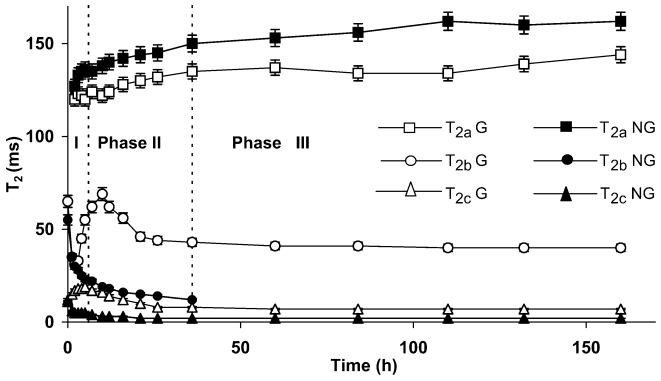


**Fig. 4** Spin–spin relaxation ($T_2$) components of germinating (G) and non-germinating (NG) wheat seeds

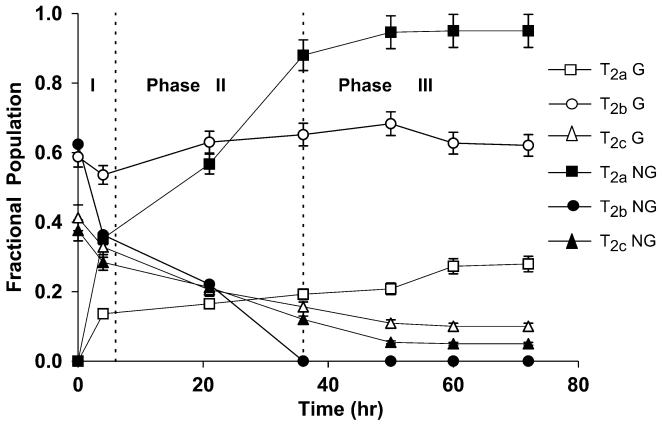


**Fig. 5** Fractional population of various $T_2$ of water in germinating (G) and non-germinating (NG) wheat seeds

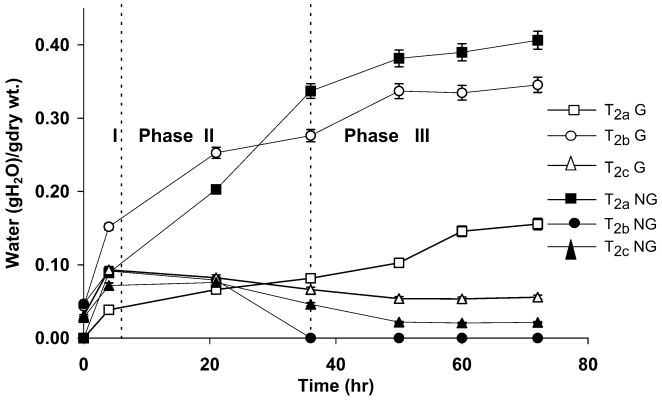


**Fig. 6** Amount of water corresponding to various $T_2$ components in germinating (G) and non-germinating (NG) wheat seeds

contrast, the electrical conductivity of the non-germinating seeds continued to increase from the lag phase (phase II).

## NMR relaxation time measurements

### *$T_2$—transverse relaxation time*

The component of transverse relaxation time ($T_{2a} \approx 142$ ms) which corresponds to the extra-cellular free water in seed tissues was not detectable, particularly in dry seeds. With the hydration of seeds, it was resolved and $T_{2a}$ values increased with increase in hydration time, albeit at different rates in germinating and non-germinating seeds (Fig. 4). During the hydration process, non-germinating seeds had higher $T_{2a}$ values than germinating seeds. $T_{2b}$ ($\approx 20$ ms) which corresponds to the cytoplasmic bulk water, significantly increased in germinating wheat seeds. In contrast, non-germinating seeds showed a declining trend of $T_{2b}$ values and were not resolved, especially after 36 h of hydration. Component $T_{2c}$ ($\approx 9$ ms), which is related to the bound water of macromolecules decreased to 3 ms after 10 h of hydration in non-germinating seeds. Even though the non-germinating seeds showed a least value for $T_{2c}$, this component was detectable even after 60 h of hydration. Conversely, there were significant increases in $T_{2c}$ of the germinating wheat seeds. Though there were increases in $T_{2c}$ components of germinating seeds, there were decreases in their corresponding population and the amount of water in bound state (Figs. 5 and 6). In germinating wheat seeds, the population of water in the bulk state ($T_{2b} \sim 65\%$) was more during the lag phase (phase II) of hydration. But, during the steady hydration phase (phase III) the population of water in the free state increased with a concomitant decrease in the bulk state in germinating seeds. These changes indicate the reorganisation of water in germinating seeds. In the case of non-germinating seeds, the amount of water in the bulk state decreased with a concomitant increase in water in the free state at all stages (or phases)

## Discussion

Early events in seed germination such as the formation of new membranes and the transformation of existing membranes allow for changes in permeability of water and gases. The distribution of water and its molecular mobility in hydrated seeds are important to initiate a sequence of events during germination. The first phase of germination during hydration of seeds is imbibition, which results in the mobilisation of food reserves to the embryo, and the protrusion of the radicle through surrounding layers (Hou et al. 1997). In seeds which do not exhibit a dormancy mechanism, it is generally considered that water uptake in germinating seeds is more than that of non-germinating seeds. Ganguli and Senmandi (1993) reported that in wheat caryopses, the penetration of water into the large storage tissue is from the periphery to the interior of the tissue. At the cellular level, the areas next to the cell wall and nucleus and the space between storage organelles become hydrated first. Tissue swelling follows and more water uptake occurs until the tissues have 40–60% water content. At the end

of the rapid hydration (phase I), water uptake by seeds often ceases for several hours or days. It then resumes and rises at the time of radicle emergence until the storage tissue and growing seedlings have water contents of 70–90% (Steiner 1998). In the present study, the germinating wheat seeds showed three distinct phases of hydration (Fig. 2) while the non-germinating seeds had only the two phases (phases I and II) of hydration. Higher uptake of water after the lag phase was observed only in germinating seeds.

The cellular membranes are composed primarily of proteins and lipids. During the germination process, disorganisation of proteins and lipid phase transitions influence the membrane structure and integrity, consequently the seed water status. The changes in the conductivity of seed leachate during hydration are often attributed to the membrane reorganisation. The tonoplast and plasmalemma, which normally retain solutes within cells, lose their integrity during drying and do not act as retentive barriers when seeds are first placed in water. The initial leakage of electrolytes, which lasts for about 12 h is from the outermost cell layers (Bewley and Black 1985). Smith and Berjak (1995) suggested that the initial water uptake by seeds is accompanied by the release of a large volume of gas and by rapid leakage of substances, e.g. sugars, organic acids and amino acids. This is immediately minimised in the germinating seeds due to the re-establishment of membranes to prevent further leakage.

Any increase in leachate conductivity is primarily a function of the loss of cell membrane integrity. In the present study (Fig. 3), non-germinating seeds had higher solute leakage than germinating wheat seeds. The inability of seeds to germinate can be related to the difficulties in the membrane competency. But, in the present study, the seeds were experimentally subjected to get non-germinating seeds. The use of these seeds helps to gain greater insights on the hydration mechanism and seed water status. Higher leachate conductivity in non-germinating seeds could be due to the enhanced seed membrane permeability. Similar observations were reported in several other crop seeds. Damage to seed coats prior to imbibition is reported to be the cause for this phenomenon in soybean (Golovina and Tikhonov 1994).

The changes in cellular membrane structure and integrity are reflected in the NMR longitudinal and transverse relaxation times of tissue water (Millard et al. 1996; Maheswari et al. 1999; Ratcliffe and Shachar Hill2001; Krishnan et al. 2003a) as the relaxation characteristics indicate the molecular mobility and biophysical state of water. The spin–spin relaxation time measurements of seed cellular water are known to be dependent on membrane permeability (Krishnan et al. 2003a). The results of the present study on the changes in $T_2$ components and the hydration-dependent transverse relaxation time of seed water during hydration and germination corroborate the above hypothesis. The data on the components of transverse relaxation time measurements (Fig. 4) indicate the presence of rearrangement mechanism taking place, especially in germinating seeds during hydration and germination. The state and quantity (Figs. 5 and 6) of water present in the localised sites within cells could provide a medium suitable for metabolic activity to proceed, even though the total water content of the whole seed is low. Three types of water-binding sites, namely strong water-binding, weak water-binding and multi-molecular sorption sites in seed tissues were previously reported from the studies on water sorption isotherms (Walters 1998). Sun and Leopold (1994) also made similar observations in the hydrated soybean seeds. The strong and multi-molecular sites exchange for the weak binding sites during imbibition of the partially germinated soybean seeds.

Pulsed $^1$H NMR measurements of germinating and non-germinating wheat seeds in the present study suggest that there were three different populations of protons, each with a different magnetic environment that causes a different relaxation rate (Figs. 4 and 5). These NMR relaxation rates are too low to be accounted for by lipid and protein macromolecules (Miedziejko 1997; Ratkovic 1987) and must reflect different water proton categories. These three populations may correspond to water molecules differing in mobility such as extra cellular free water, intracellular bulk water and solid or bound water (Ishida et al. 1988). Several workers have already exploited this feature in attempts to determine tissue water partitioning (Di Nola et al. 1988, 1991; Brosio et al. 1992; Foucat et al. 1993). A general conclusion is that high relaxation rates relate to extracellular free water and low relaxation rates relate to intracellular bulk water and very low relaxation rates relate to bound structural water.

Intracellular and extracellular water mutually exchange across the plasma membrane and the rate of exchange depends on plasma membrane permeability. For example, high seed deterioration was related to the decreases in molecular mobility of ultra dried seeds of soybean and wheat (Krishnan et al. 2003a, 2003c). The data on leachate conductivity (Fig. 3) of germinating and non-germinating seeds also suggest that the cellular membranes in non-germinating seeds were injured which is paralleled by their inability to reorganise during hydration. The evaluation of the relationship between the electrolyte leakage and $T_2$ components during seed hydration shows that the membrane permeability increases with increase in time during hydration of non-germinating seeds while there was a decline followed by a steady state in germinating seeds. The free water component of seed water as measured by the NMR transverse relaxation time component analysis follows a similar trend; an opposite trend was observed in bulk water populations (Fig. 5). The probable reason for a higher population of water in the bulk state could be attributed to the disorganisation of macromolecules, resulting in release of water held in hydration layers. The consistency of the relationship between the electrolyte leakage (Fig. 3) and $T_2$ components (Fig. 4) and water population (Fig. 5) substantiates the fact that biological

processes such as seed germination alter the physical state of water in seeds.

In the experimentally obtained non-germinating wheat seeds, membranes can be considered to be porous from the fact that the leakage of solute was fast at the first stage of hydration. When these seeds were hydrated, water that is rapidly incorporated through the porous membrane is absorbed by the materials stored in the seeds such as proteins and saccharides which swell suddenly upon hydration, resulting in the breakdown of the dry and inactive membrane organisations. On the other hand, in germinating seeds, hydration of stored materials without breaking down the organisation of membranes restores the biological function of seed germination, which regulates further incorporation of water (Ishida et al. 1988).

The sharp decline in bulk water in non-germinating seeds (Figs. 5 and 6), followed by its disappearance after 36 h as observed in the present experiments could be due to the loss of membrane integrity and the collapse of sub-cellular compartmentation of water. The disappearance of bulk water in non-germinating seeds with increased time of hydration could be due to the breakdown of organisation of cellular membranes and cellular organelles. Loss of cellular compartmentation would result in an exchange of water protons between the mobile intracellular water and more mobile extracellular water, contributing to the decline and finally the collapse of the bulk water component in non-germinating seeds. In germinating wheat seeds, though there are decreases in bulk water, hydration of stored materials without breaking down the organisation of membranes restores the biological function of water in the bulk state. A higher fraction of water in the free state in non-germinating seeds suggests that external water of hydration was not used for cell division and elongation. The trace of bound water that could be detected in non-germinating seeds is probably that associated with the denatured proteins and nucleic acids.

The variations in $T_2$ components (Fig. 4) with increasing tissue hydration were not reflected in the total tissue water content (Fig. 2). Many factors influence proton relaxation including cell size, structure, the chemical composition and viscosity of the cellular contents and magnetic susceptibility. The $T_2$ components, as analysed in the present study also, help to identify the bound water, cytoplasmic/bulk water and extracellular/free water. The relaxation times of tissue water components are influenced by the delicate balance between the total water content, macroscopic and microscopic distribution of water in different sites/phases, macromolecular–water interactions and exchange (slow or fast) between water phases.

The pattern of changes in electrolytic leakage, often used to estimate the damage to cell membranes in many previous studies (Maheswari et al. 1999: Krishnan et al. 2003a, 2003b, 2003c), at increasing tissue hydration supports the inferences based on NMR measurements of transverse time components of seed water in the present study. The structure and/or motions of seed water and other biomolecules are affected by the macromolecular interface during seed germination causing changes in the NMR relaxation time. In conclusion, there is a rearrangement of cellular water during germination, and water with medium relaxation times and with relatively less restricted mobility is associated with the germination process. More research is needed to quantify the water status of individual seed organs and to use this information for predictable control of seed deterioration.

**Acknowledgements** We thank Mr. A.P.S Verma for skilled technical assistance, the Project Director, NRL, IARI, New Delhi for providing the facilities and P G School, IARI, New Delhi, India, for providing the financial support.

## References

Bernal-Lugo I, Leopold AC (1998) The dynamics of seed mortality. J Exp Bot 49:1455–1461

Bewley B, Black M (1985) Storage, imbibition and germination In: Bewley B, Black M (eds) Seed physiology of development and germination. Plenum, New York, pp 89–128

Brosio E, Verzegnassi B, Di Nola A (1992) NMR study of seed hydration: The role of the seed anatomical structures in water uptake of soaked cowpeas. Cell Mol Biol 38:693–697

Di Nola A, Brosio E, Delfini M, Manes F, Quattrocchi S (1988) Hydration mechanism study in lettuce seeds by proton NMR relaxation. Cell Mol Biol 34:636–648

Di Nola A, Daubaldo A, Fracast M, Brosio E (1991) NMR study of seed hydration with deutrated water. Dependence of proton signals on hydration level. Cell Mol Biol 37:9-13

Foucat L, Chavagnat A, Renou JP (1993) Nuclear-magnetic-resonance microimaging and X-radiography as possible techniques to study seed-germination. Sci Hort 55:323–326

Fukuoka M, Watananabe H, Mihori T, Shimda S (1994) Moisture diffusion in a dry soybean seed measured using pulsed-field-gradient NMR. J Food Eng 23:533–541

Gambhir PN, Pramila RK (Krishnan P), Nagarajan S, Joshi DK, Tiwari PN (1997) Relationship between NMR relaxation characteristics and water activity in cereal leaves. Cell Mol Biol 43:1191–1196

Ganguli S, Senmandi S (1993) Effect of ageing on amylase activity and scutellar cell structure during imbibition in wheat seed. Ann Bot 71: 411–416

Golovina EA, Tikhonov AN (1994) The structural differences between the embryos of viable and non-viable wheat seeds as studied with the EPR spectroscopy of lipid-soluble spin labels. Biochim Biophys Acta—Biomembranes 1190:385–392

Haranczyk H, Strzalka K, Jaisinski G, MosnaBojarska K (1996) The initial stages of wheat (*Triticum aestivum, L*) seed imbibition as observed by proton nuclear magnetic relaxation. Colloids Surf A—Physicochem Eng Aspects 115:47-54

Hou JQ, Kendall EJ, Simpson GM (1997) Water uptake and distribution in non-dormant wild oat (*Avena fatua* L.) caryopses. J Exp Bot 48:683–692

Ishida N, Hiromi K, Toshio K, Takashi Y (1988) Analysis of physical states of water in soybean seeds by NMR. Agric Biol Chem 52:2777–2781

Isobe S, Ishida N, Koizumi M, Kano H, Hazlwood CF (1999) Effect of electric field on physical states of cell associated water in germinating morning glory seeds observed by $^1$H NMR. Biochim Biophys Acta—Gen Subj 1426: 17–31

ISTA (1985) International rules of seed testing. (Rules + annexes) Seed Sci Technol 13:299–513

Krishnan P, Nagarajan S, Moharir AV (2003a) Changes in NMR relaxation times as a measure of membrane stability in soybean

and wheat seeds equilibrated under different temperature and relative humidity conditions. Ind J Biochem Biophys 40:46–50

Krishnan P, Nagarajan S, Dadlani M, Moharir AV (2003b) Characterisation of wheat (*Triticum aestivum*) and soybean (*Glycine max*) seeds under accelerated ageing conditions by proton nuclear magnetic spectroscopy. Seed Sci Technol 31:541–550

Krishnan P, Joshi DK, Maheswari M, Nagarajan S, Moharir AV (2003c) Characterisation of soybean and wheat seeds during equilibration at different temperatures and relative humidities by nuclear magnetic resonance spectroscopy. Biol Plant (in press)

Lenk R, Degliagosti R, Greppin H (1991) Studies of oat seeds germination by NMR. Biol Plant 33:501–505

Maheswari M, Joshi DK, Saha R, Nagarajan S, Gambhir PN (1999) Transverse relaxation time of leaf protons and membrane injury in wheat (*Triticum aestivum* L.) in response to high temperature. Ann Bot 84:741–745

McDonald MB (1999) Seed deterioration: physiology, repair and assessment. Seed Sci Technol 27:177–237

Miedziejko EM (1997) Impact of chemical stimuli and temperature on water transport and mobility in germinating rapeseeds by pulsed H-1 NMR spectroscopy. Mag Res Chem 35:S61–S68

Millard MM, Veisz OB, Krizek D, Line M (1996) Thermodynamic analysis of physical state of water during freezing in plant tissue, based on the temperature dependence of proton spin-relaxation. Plant Cell Environ 19:33–42

Noda N, Sakaguchi K, Kumamoto Y, Iwaya Inoue M (1998) Determination of the phase change in the $H^1$-NMR relaxation behaviour of dehydrating soybean seed using AIC method. J Fac Agric 43:67–74

Ratcliffe RG (1994) In vivo NMR studies of higher plants and algae. Adv Bot Res 20:44–123

Ratcliffe RG, Shachar Hill Y (2001) Probing plant metabolism with NMR. Annu Rev Plant Physiol Plant Mol Biol 52:499–526

Ratkovic S (1987) Proton NMR of maize seed water: the relationship between spin-lattice relaxation time and water content. Seed Sci Technol 15:147–154

Ridenour CF, Xiong J, Maciel GE (1996) Investigation of germination and aging in Moravvian III barley grain by nuclear magnetic resonance. Biophys J 70:511–531

Smith MT, Berjak P (1995) Deteriorative changes associated with the loss of viability of stored desiccation-tolerant and desiccation-sensitive seeds. In: Kigel J, Galilli G (eds) Seed development and germination. Marcel Dekker, New York, pp 701–746

Snarr JEM, Van As H (1992) Probing water compartments and membrane permeability in plant cells by $H^1$ NMR relaxation measurements. Biophys J 63:1654–1658

Steiner M, Stahl (1998) Germination and vigour loss of non-sprouted and sprouted wheat seeds during storage—testing the viability constant. Am Seed Sci Res 8:123–128

Sun WQ, Leopold AC (1994) Glassy state and accelerated ageing of soybeans. Physiol Plant 89:767–774

Van As H (1992) NMR in horticulture: in situ plant water balance studies with NMR. Acta Hort 304:103–112

Walters C (1998) Understanding the mechanisms and kinetics of seed ageing. Seed Sci Res 8:223–244