



Research Article

Water Distribution during Germination in Sunflower (*Helianthus annus* L.) Seeds Exposed to Magnetic Field

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ABSTRACT

Sunflower (*Helianthus annus* L.) seeds were exposed to static magnetic fields of 50 mT and 200 mT for 2 hours. The characterization of changes in water status were studied during imbibitions, by nuclear magnetic resonance (NMR). Uptake of water during seed germination showed three phases with rapid initial hydration phase I, followed by lag phase II and steady hydration phase III. The water uptake was more in phase II and III in seeds exposed to magnetic field than unexposed seeds. Analysis of transverse relaxation times revealed a three component water proton system, namely hydration, intra-cellular bulk and extra-cellular free water in germinating seeds. The study showed an early appearance of hydration water with least mobility and greater values of relaxation times of cytoplasmic bulk water and hydration water in treated seeds over untreated seeds. It is inferred that early hydration of macromolecules and membranes with greater molecular mobility of bulk and hydration water fractions in magnetically exposed seeds may be responsible for quicker germination and early seedling vigour in sunflower.

Keywords: *Helianthus annus* L., Germination, Imbibition, Nuclear Magnetic Resonance, Transverse relaxation time

Introduction

Information about the distribution and molecular mobility of water during hydration in germinating seeds is necessary to understand the process of germination. Longitudinal (T_1) relaxation, also known as spin-lattice relaxation, is the process by which thermal equilibrium is reached between a spin system and the lattice of atomic nuclei. It is the process by which the longitudinal component of magnetization tries to regain its thermal equilibrium state after a perturbation. The time to regain equilibrium state is characterized by longitudinal (T_1) relaxation or spin lattice relaxation time. Transverse relaxation (T_2) also known as spin-spin relaxation is the process by which the magnetization in the

transverse plane decays towards the zero value. The time period required to reach this state is characterized by the transverse or spin-spin relaxation time. Behaviour of longitudinal (T_1) and transverse relaxation (T_2) time of water protons can be investigated to describe the compartmentation and transport of water in seeds, plants and tissues (Krishnan *et al.*, 2004). The water molecules with different mobilities and their relative amounts can be distinguished by their differences in relaxation times (Van, 1992). Nuclear Magnetic Resonance techniques provide a novel, sensitive and direct method to characterize water status in seeds (Ridenour *et al.*, 1996 and Ratkovic 1987).

Three-component water proton system in germinating seeds of morning glory was observed (Isobe *et al.*, 1999) and T_1 and T_2 relaxation time

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measurements (Ridenour *et al.*, 1996) provided information about molecular mobility within barley grains during imbibition. In general, these NMR studies on hydrated seeds suggest that protons with a short relaxation time are, in part associated with the bound water; protons of medium relaxation time are associated with intercellular water and protons of long relaxation time are associated with the extra-cellular water (Isobe *et al.*, 1999; Brosio *et al.*, 1992). This technique has been used to characterize the water status of primed carrot and tomato seeds and the enhanced performance of primed seeds was attributed to reorganization of seed water during imbibition and increased macromolecular hydration water needed for metabolic activities (Nagarajan *et al.*, 2005).

Exposure to static magnetic fields is one of the physical pre-sowing seed treatments reported to enhance the performance of crop plants. In our laboratory, sunflower seeds were exposed to different magnetic fields (50, 100, 150, 200 and 250 mT) and duration (1, 2, 3 and 4h) and their germination and vigour characteristics up to 10 days old seedlings were evaluated. Among various combinations of magnetic field strength and duration, best results were obtained with 50 mT magnetic field and 200 mT magnetic field for 2h exposure (Vashisth and Nagarajan, 2010). Average increase in germination, speed of germination, seedling length and seedling dry weight over control was 11, 15, 57 and 13%, respectively.

Understanding the differences in seed water status of seeds exposed and unexposed to magnetic field is essential to gain insights on the hydration mechanism and germination process and to explain the mechanism of improvement in magnetically treated seeds. Therefore, the present study was undertaken to characterize *in vivo* changes in water status of magnetically exposed and unexposed maize seeds during germination by NMR spectroscopy.

Materials and methods

Seed material

Sunflower seeds (Var. KBSH-1) were obtained from National Seeds Corporation, New

Delhi. The moisture content of the seed was 5.2%. Seeds without visible defects, insect damage and malformation were selected and stored in the desiccators over anhydrous calcium chloride. Seed moisture was determined in a set of triplicate seed samples by oven drying seeds at 95°C to constant mass (Walters, 1998). Moisture content (g DW⁻¹) was calculated as $[(W_1 - W_2) / (W_2)]$, where W_1 was initial mass of the seed (g) and W_2 was the final mass after drying (g).

Magnetic treatment

Seeds of sunflower were exposed to varying strength of magnetic field from 50 and 200 mT for 2h respectively with in a cylindrical shaped sample holder of 42cm³ capacity, made from a non-magnetic thin transparent plastic sheet. Visibly sound, mature and healthy 100 seeds were held in the plastic container between the poles of the electromagnet having uniform magnetic field for required duration. The required strength of the magnetic field was obtained by regulating the electric current in the coils of the electromagnet. Gauss meter was used to measure the strength of the magnetic field between the poles. The distance between two pole were 5cm. At low field (50 mT), from centre to end of the poles, the variation was 0.6% in the horizontal direction and 1.6% in the vertical direction of the applied field. At high field (250 mT) the variation was 0.4% and 1.2% of the applied field in horizontal and vertical directions, respectively.

Imbibition kinetics of seeds during germination

The difference in imbibition kinetics of magnetically exposed sunflower seeds along with their unexposed counterpart were studied. Seeds were allowed to imbibe water in covered petri dishes of 10 cm diameter layered with moist filter paper pads at 25°C. After blotting off excess moisture, the wet weight of the seeds and NMR relaxation time (T_2) were measured periodically until all seeds germinated. There were three replications. Seeds were later dried in an oven at 95°C to constant mass and seed moisture was calculated as mentioned above.

Components of transverse relaxation time (T_2)

Transverse relaxation or spin-spin relaxation time was measured by the Carr-Purcell-Meiboom-Gill (CPMG) method (Snaar and van As, 1992) at 20 MHz. Each measurement had the following setting: data points 150, pulse separation 0.5ms, dummy echo 3 and scans 10. Gain was adjusted to maximize the signal to noise ratio. The T_2 value was calculated using the built-in Expspel program with the single exponential decay observed in the CPMG sequence.

In biological systems, multi-exponential relaxation decay curves have been measured, and numerous attempts made to discriminate different components of water fractions based on the relaxation times (Van As 1992). Three water components of the plant and seed systems can be identified with the transverse relaxation times 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} for the extracellular free water (Brosio *et al.*, 1992; Snarr and Van, 1992; Di Nola *et al.*, 1991). The components of transverse relaxation were analyzed by using least square fit analysis in the region of specified limits, based on the t value (x -axis), until the plotted curve showed a visible curvature change with significant r^2 , using a program written in BASIC.

Results

Water uptake during seed germination showed three distinct phases; rapid hydration (imbibition, phase I), lag phase (phase II) and steady hydration phase (germination growth, phase III). The rapid hydration phase I and lag phase II were observed until 13h and between 13-44 h, respectively (Fig. 1). The third phase coinciding with the radicle and plumule emergence showed a steady hydration phase III from 44 h. During the rapid hydration phase I the water uptake was same for both magnetically exposed and unexposed seeds. In magnetically exposed seeds water uptake was slightly more during phase II and significantly greater during phase III of imbibition.

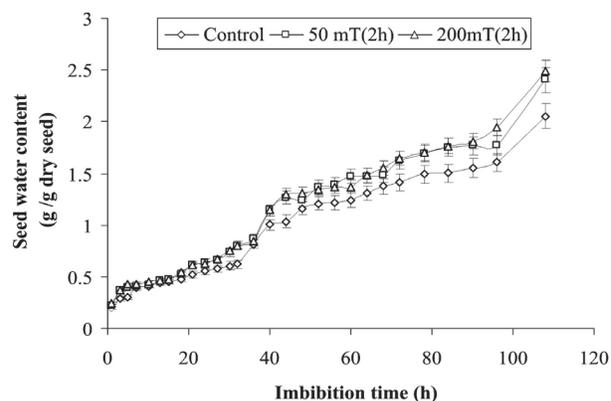


Fig. 1. Changes in seed water content during imbibition of water at 25°C for sunflower seeds exposed and unexposed to magnetic fields

Components of transverse relaxation time (T_2)

The transverse relaxation (T_2) of magnetically exposed and unexposed seeds of sunflower showed an initial decrease when dry seeds were subjected to hydration during germination. This decrease in T_2 was observed both in exposed and unexposed seeds to magnetic field (Fig. 2) over a period of imbibition (7h). During the subsequent period of hydration, marginal increase in T_2 value for magnetically exposed and unexposed seeds, until 60 h, was observed. There was substantial increase in T_2 during the subsequent stage of germination and magnetically exposed seeds had significantly higher T_2 values than the unexposed seeds.

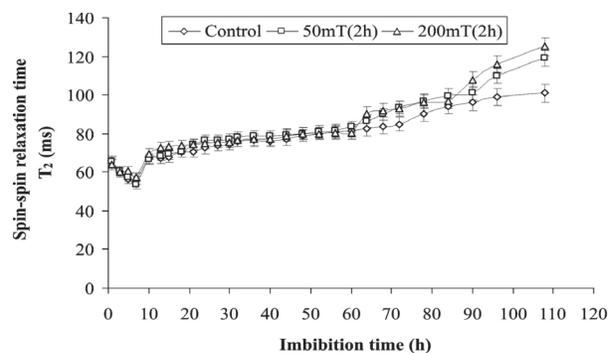


Fig. 2. Changes in weighted average transverse relaxation time, T_2 of seed water during imbibition of water at 25°C for exposed and unexposed sunflower seeds to magnetic fields

The actual relaxation curve showed a marked non-exponentiality that could be accounted by the presence of three clearly recognizable components with different relaxation times. According to the individual values, these three components have been identified with transverse relaxation time T_{2a} , T_{2b} and T_{2c} , respectively. T_{2a} , that corresponds to the extra-cellular free water decreased with increase in hydration time, in exposed and unexposed seeds to magnetic field. Both exposed and unexposed seeds thus showed a decrease until 36 h, thereafter an increase in T_{2a} values. Increase in T_{2a} coincided with sprouting in seeds (Fig 3a).

T_{2b} , which corresponds to cytoplasmic bulk water increased until 36 h in both exposed and

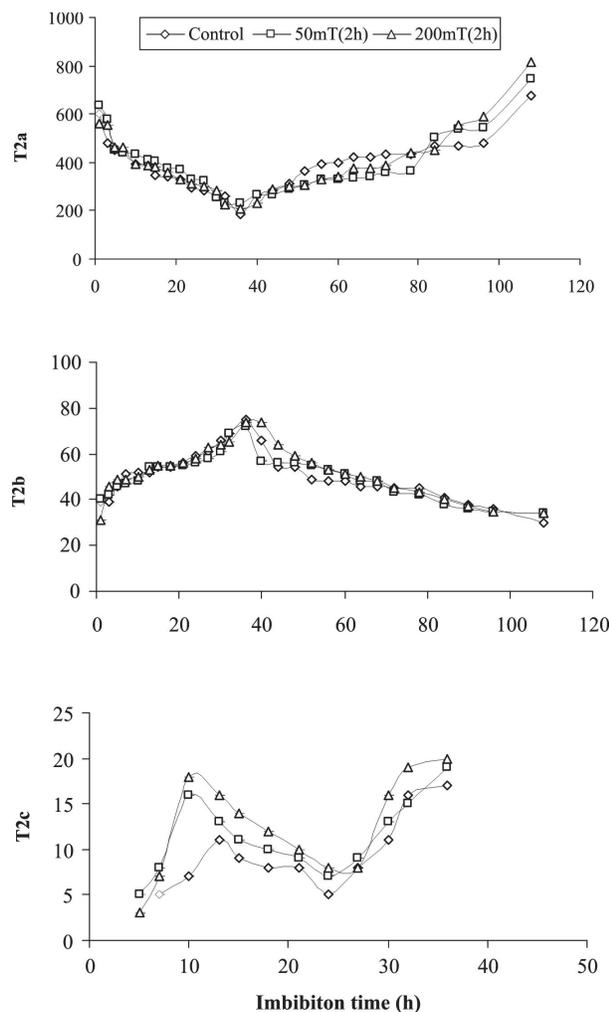


Fig. 3. Changes in components of transverse relaxation time, T_2 of seed water during imbibition of water at 25°C for exposed and unexposed sunflower seeds to magnetic fields

unexposed seeds. However, during subsequent period of germination there was considerable decrease in T_{2b} (Fig 3b). The values of T_{2b} were higher for treated seeds as compared to control during most of the seed hydration duration.

Component T_{2c} was not detectable in dry seeds but resolved at 5 h and 7 h after imbibition in both exposed and unexposed seeds, respectively (Fig. 3c). T_{2c} , which corresponds to the hydration water of macromolecules, initially increased and then decreased to 7 ms in 50mT (2h), 8ms in 200mT (2h) and 5 ms in unexposed seeds, respectively. Thereafter, there was continuous increase in T_{2c} and was greater in the exposed seeds than unexposed. However this third component was undetectable after 36 h of hydration.

The fractional population of different water protons of varying mobilities during germination in sunflower is shown in Fig. 4a-c. After the appearance of the least mobile fraction, the exposed and unexposed seeds to magnetic fields showed an increase in spin population of T_{2b} and T_{2c} together and a decreases in T_{2a} until the radicle emergence took place. However during the subsequent period of imbibition, only two populations of water were observed with T_{2a} fraction being larger than T_{2b} fraction.

Discussion

Imbibition kinetics of seeds during germination

The knowledge of the water status in biological systems is of great importance in understanding their functionality. In particular, a great interest is shown in seeds to understand the water distribution, its molecular mobility in the dry state, as well as during hydration when seeds are re-hydrated for germination. In fact, unless the environmental conditions (e. g. temperature, light, oxygenation etc.) are favourable, the seed germination process can start simply by water imbibition.

In the present study germinating seeds showed three distinct phases of hydration (Fig.

1). Higher uptake of water in phase III of hydration after lag phase was observed in magnetic fields. Soybean seed exposed to magnetic fields have been reported to increase capacity of moisture absorption (Kavi, 1977). Significant increase in the rate of absorption of water was observed in lettuce seeds exposed to magnetic field (Garcia-Reina *et al.*, 2001). The magnetic fields induce changes in the ionic concentration and consequently in the osmotic pressure which regulates the entrance of water in to the seeds. Therefore, there is strong evidence that the magnetic fields alter the water relations in seeds, and thereby the germination rate of seeds.

NMR relaxation times and its components during germination

The distribution of water and its molecular mobility in hydrated seeds are important to initiate a sequence of events during germination. The changes in cellular membrane structure and integrity are reflected in the NMR longitudinal and transverse relaxation times of tissue water (Maheswari *et al.*, 1999; Mathur de-vre 1984) as the relaxation characteristics indicate the molecular mobility and biophysical state of water.

The results of transverse relaxation time (T_2) measurements showed distinct changes during various stages of hydration and germination of seeds. This shows that nuclear magnetic resonance (NMR) relaxation time T_2 reflects the *in vivo* dynamic changes and physical states of water in seeds under observations. Seed water transverse relaxation time (T_2) decreased initially during rapid hydration phase and remained relatively constant followed by a steady increase during subsequent hydration in seeds (Fig. 2). Such a trend has been reported earlier in wheat (Krishnan *et al.*, 2004), when the seeds were soaked in water. The reduction in seed water T_2 values, in spite of an increase in seed water content, can be explained on the basis of the reorganization of the different water fractions within the seed tissues. The relaxation times are influenced by a delicate balance between total water content, macroscopic and microscopic

distribution of water at different sites, macromolecular–water interactions and exchange (slow or fast) between different phases with increase in seed moisture content (Mathur De-vre 1984). The decrease in the proportion of mobile water and increase in the proportion of water in less and least mobile forms are apparently responsible for the decline in weighted average T_2 . With initiation of germination, T_2 increased again due to increase in proportion of more mobile water and disappearance of least mobile water fraction. Similar initial decline and subsequent increase in weighted average T_2 along with radicle protrusion has also been reported in tomato seeds (Nagarajan *et al.*, 2005). In seeds exposed to magnetic fields T_2 values were greater than for untreated controls at later stages of imbibition. This may be explained on the basis of higher water uptake by the treated seeds present in more mobile form as indicated by early germination and radicle protrusion in these seeds.

The data on the components of transverse relaxation time T_2 of seed water during imbibition indicate the presence of three different magnetic environments that cause different relaxation rates in seeds (Fig. 3a-c). These three populations correspond to water molecules differing in mobility, such as extra-cellular free water, intercellular bulk water and bound water. NMR studies on hydrated seeds suggest that protons with short relaxation time are associated with bound water, protons of medium relaxation time are associated with intracellular water and protons with long relaxation time are associated with extra-cellular water (Krishnan *et al.*, 2004).

Results of transverse relaxation time analysis indicated the complex water exchange process taking place between components inside the seeds exposed to magnetic fields and untreated seeds. During imbibition the third component with least mobilities appeared after 5 h and 7 h in magnetic field exposed and unexposed seeds respectively (Fig. 3c). Components T_{2b} and T_{2c} , which represent the bulk water and macromolecular hydration water fractions in seeds, increased in all seeds until radicle protrusion. The values of both components are higher in magnetic field

exposed compared to unexposed seeds. They indicate the higher molecular mobility of water protons in these fractions of seed water and availability for metabolic activity. This may be the reason for the faster germination and seedling vigour of the magnetic field treated seeds (Vashishth and Nagarajan 2010; Vashishth and Nagarajan 2007; Vashishth and Nagarajan 2008).

Similar increase in T_2 value of least mobile component within 2.4 h of hydration have reported in lupine seed (Garczarska *et al.*, 2007) indicating that immobilization of water molecules

by macromolecules (carbohydrates, proteins etc.) within the seed matrix becomes less effective in course of hydration time.

Distribution of water proton into three fractions showed clear change with imbibition time indicating rearrangement of cellular water fractions both in magnetic field exposed and unexposed seeds (Fig. 4a-c). Presence of free water in dry seeds at early stages of imbibition is interesting. Magnetic resonance imaging of lupine seed also showed protons corresponding to long relaxation time in dry seeds in vascular bundles

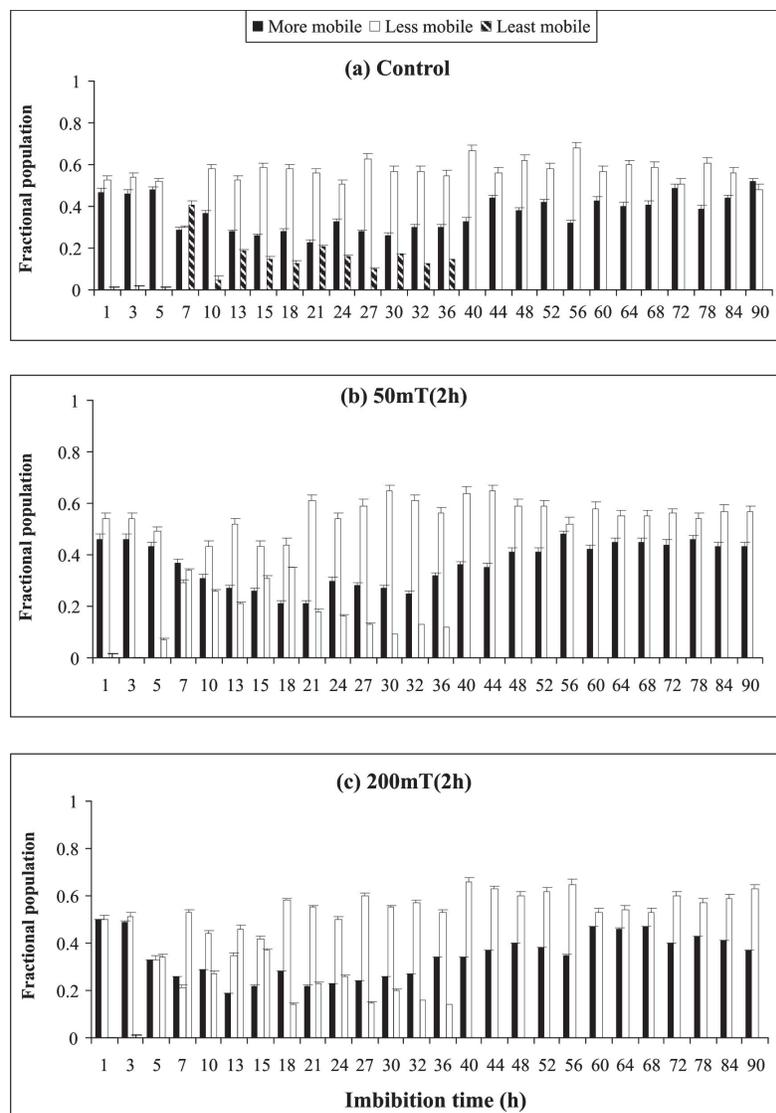


Fig. 4. Fractional population of different water protons of varying mobilities during imbibition in water at 25°C for exposed and unexposed sunflower seeds to magnetic fields. The plain, dotted and crossed bars denote protons associated with more, less and least mobilities, respectively

adjacent to embryonic axis (Garnczarska *et al.*, 2007). The ^1H NMR 2D micro imaging of low-hydrated tobacco seeds showed that there were localized tissue areas with higher proton mobility (Leubner-Metzger, 2005). This indicates that the state and quality of different components of water during early stages of imbibition provides a medium suitable for metabolic activity to proceed despite the total water content being still low. The bulk water fraction in non-viable wheat seeds during imbibition were reported to completely disappear (Krishnan *et al.*, 2004). Disappearance of bound water fraction and presence of only two components corresponding to bulk and free water fractions with radicle protrusion, indicate the exchange of water in different compartments by the rearrangement of membrane permeability during germination and the formation of vacuoles in association with growth of embryo. Magnetic resonance imaging of germinating lupine seeds clearly showed that germination was accompanied by swelling of protein bodies and changes in the organization of stored reserves with gradual disappearance of protein (and the bound water associated with it) from the cells (Garnczarska *et al.*, 2007). The early appearance of structure-associated least mobile water in magnetic field treated seeds indicates early hydration of macromolecules and membranes which bind water, reduce its mobility besides maintaining ongoing metabolism and cellular membrane integrity. Similar results of early appearance of bound water fraction have been reported in primed seeds of tomato (Nagarajan *et al.*, 2005).

Conclusions

The study showed evidence for rearrangement of cellular water in seeds exposed to static magnetic field and in un-exposed controls. NMR relaxation time of seed water and its analysis indicated early appearance of structural/hydration water and greater molecular mobility of cytoplasmic bulk water and hydration water of macromolecules in magnetic field treated seeds compared to untreated controls. This may be responsible for early germination and higher seedling vigour of these seeds over untreated controls.

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