Nuclear Magnetic Resonance (H^1- Nmr) Spectroscopy and Imaging of Water Uptake and Distribution in Sunflower (*Helianthus Annus.L.*) Seeds Exposed to Magnetic Field

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Abstract

Irradiation of sunflower (Helianthus annus.L.) seeds to magnetic fields (MF) have influence on physiological processes. Seeds exposed to 500 µT for 48 hrs showed an enhancement of 24% in germination on day 3. The experiments carried out show that seeds previously treated with magnetic fields have increased water uptake, which may be the reason for the enhanced percentage germination and growth rate. The physical state of water in the seeds of both control and exposed groups were examined using nuclear magnetic resonance (H^1 NMR) spectroscopy & imaging during imbibitions. NMR images of water distribution and proton transverse relaxation time (T_2) were recorded during the first 15 hrs of imbibitions. NMR images reveal that hydration during this period is a multistage process. Higher concentration of water in the radical of treated seeds revealed that water penetration into the seed is more in treated seeds which must have contributed to faster germination and growth.

Key words: magnetic field(MF), Imbibition, NMR images, Sunflower seeds, proton transverse relaxation time.

Introduction

Visible light consists of electromagnetic radiation of different wavelengths. Light signals regulate change in seed structure and form, such as seed germination, leaf expansion, stem elongation etc. It is therefore of great interest to determine the effects of radiation of different wavelengths and intensities of seed germination.

Presowing exposure of agricultural seeds to electromagnetic fields increase the percent germination, growth rates and causes different physiological changes. Promotive effects of electric field on the germination rate have been reported for hard seeds and dormant seeds, such as corn and soybean by F.W.Wheaton et.al[1] and carrot by M.Matsuo [2]. It has been found that an increase in chemical reactions in plants when exposed to magnetic fields has positive effect on photochemical activity, respiration ratio, and enzyme activity[3]. Carbonell et.al[4] found that magnetic treatment produced a biostimulation of the germination in rice seeds. Such enhancement in germination, seedling vigor in seeds exposed to electric /magnetic/electromagnetic fields were reported in various crop seeds by Aladjadjiyan[5,6], Fischer et.al[7], Rajendra et.al[8] , Florez et.al[9], Vashisth and Nagarajan[10,11,12]. Enhanced percent germination, growth rates, wet and dry weights in sunflower seeds exposed for magnetic fields was reported in our earlier work[13].

The reasons attributed for the enhanced percent germination, growth rates, for other changes in physical and biochemical compositions in seeds exposed for electromagnetic field (EMF) were explained by

different authors. Adey.W.R[14] reported that presowing exposure of seeds to electromagnetic fields leads to physiological signaling across cell membranes and co-operative influence. Garcia and Arza[15] carried out an experimental study on water absorption by lettuce seeds previously treated in a stationary magnetic field of 1-10 mT and reported an increase in water uptake rate due to applied field and noted increase in the germination speed. Aladjadjiyan[5,6] attributed the enhancement in seed germination of Zea mais and tobacco to the increased permeability of the seed coat after exposing the seeds to magnetic field. Leelapriya et.al.[16] quoted that presowing exposure of the seeds to EMF changes in the electrostatic balance of the plant system at the cell membrane level as it is the primary site of action of plant growth. Rajendra et.al[8] stated that energy input by EMF induces changes in growth parameters, causes force on ions and charged cell organelles which in turn stimulates the growth and biochemical changes in broad bean.

H^1 NMR spectroscopy provides a sensitive and nondestructive tool to characterize water status in seeds[17, 18]. Sciichiro Isobe et.al[19] using H^1 NMR technique, observed that the seeds of morning glory exposed to electric and magnetic fields held more water in a condition in restricted motion than the untreated seeds. It is thought that membrane systems were affected by the electrical polarization which leads to an unusual accumulation of water and the hydration of stored macro molecules during the imbibitions process. High and low resolution NMR spectroscopic method have been employed successfully to study the state of water in many biological systems such as nape seed by Miedziejku[20] and plant metabolism by Ratcliffe and Sachan Hill[21]. Krishnan et.al[22,23,24,25] reported that the compartmentalization and transport of water behaviors in wheat and soya bean seeds by the longitudinal and transverse relaxation times of water protons.

Exposure of sunflower seeds to magnetic fields carried out in our laboratory was found to enhance percent germination, growth rate, wet & dry weights[13]. The objective of our study is to apply ^1H NMR spectroscopy and imaging to determine water uptake and distribution in sunflower seeds (control and exposed for magnetic fields) during the first 15 hrs of imbibitions and to carry out comparative study on proton density with the respective water proton transverse relaxation time (T_2).

Materials and Methods

Selection of sunflower seeds

The Sunflower (*Helianthus annus.L.*) Morden, seeds used in our study were produced from Oil seeds section, National Seed Project, Acharya N.G Ranga Agricultural University, Hyderabad, AP , stored in desiccators having unhydrous calcium chloride. Seed moisture content was determined by Oven Drying seeds at 60 ^{o}C and moisture content (%) was calculated as $\frac{(W1-W2)}{W2}$ X 100, where W_1 and W_2 are the

initial and final weights of the seeds[26]. A moisture content of 5.2% was used for all treatments and experiments.

Magnetic field treatment of seeds

A Custom- Built magnetic enclosure which can produce field intensity ranging from 1-2000 µT was used for the treatment of sunflower seeds [13]. MF strengths of 500 µT was obtained by adjusting electric flow regulator and measured using Tesla meter. Each batch of seeds were exposed for 48 hrs at MF strength of 500 µT.The unexposed seeds were used as control group.

Rate of water absorption:

Water uptake was followed on triplicate, 1gm of healthy sunflower seed samples soaked in 100ml of milliQ at 20^{o}C. Weight increase was recorded after an imbibition of 2, 5, 7, 9, 12 and 15 hrs. Water was drained from the samples for 30 seconds, and the seeds were blotted prior to weighing.

NMR Imaging:

The NMR scans of control and treated Sunflower seeds (exposed for 500µT) were obtained on 600MHz Micro imager, Bruker Avance II spectrometer equipped with the standard micro imager accessories located in The Centre for Cellular and Molecular Biology (CCMB), Tarnaka, Hyderabad, India. The NMR analysis was performed on 10 sets of seeds. The seeds (control and exposed) were fixed at a time on a glass plate and then placed in the NMR tube. Proton density images of the seeds soaked in milliQ water for 2hrs (stage I), 5hrs (stage II), 9hrs (stage III) and 15hrs(stage IV) were taken. During NMR scanning the seeds were removed from the water, blotted with tissue paper before mounting for studies. The Proton density images were compared with the respective water proton transverse (spin-spin) relaxation time (T_2).Proton density and T_2 maps were acquired using spin echo sequence. Transverse relaxation time (T_2) of the water reflects the dynamics which in turn depicts the compactness of the cellular structure. T_2 of the controlled and exposed seeds were measured by recording NMR image with different echo time and fitting the intensity to an exponential function

$M_{xy}(TE)=M_{xy}(0)$ $[1-\exp(-TE/T_2)]$ where $M_{xy}(0)$ and $M_{xy}(TE)$ are the signal intensities:

TE – echotime

T_2 – Transverse relaxation time

T_2 values were calculated using the inbuilt program of the instrument, T_2 determination was done 6 to 10 times for each sample and the mean were calculated.

Results and Discussion:

All the cells of the embryo in dry seeds remain alive and the only requirement for the reinitiation and completion of seed germination is the addition of water.In experiments carried out at our laboratory, it was observed that an enhancement of 24 % in germination and growth rate in sunflower seeds exposed for MF of strength 500µT for 48 hrs compared to the control

samples13. Similar increase in percent germination in different agricultural seeds exposed for electric /magnetic /electromagnetic fields such as musk melon27, rice 4, wheat28, cotton16, tobacco seeds5,6, cucumber seedlings29, maize4,11; chickpea and sunflower seeds10,12were reported.

The water up take measurements of control and magnetically treated seeds were recorded. The weights recorded during imbibition in all stages (after 2, 5, 7, 9 and 15 hrs of imbibitions) showed that exposed seeds absorb significantly more water than the control (Fig.1). Imbibition is the first phase of seed germination and is followed by the mobilization of food reserves to the embryo and protrusion of the radical through surrounding layers during the subsequent phases30. Adey.W.R14 reported that presowing exposure of seeds to ELF EMF leads to physiological signaling across cell membranes and co-operative influence which results in higher accumulation of water in treated seeds. In similar studies Aladjadjiyan 5,6 quoted that the enhancement in Zea mais and tobacco seed germination to the increased permeability of the seed coat after exposing the seeds to magnetic field. Leelapriya et.al16 quoted that presowing exposure of rice seeds to EMF leads to changes in the electrostatic balance of the plant system at the cell membrane level as it is the primary site of action of plant growth resulting in enhancement in percent germination and growth rate in treated seeds. The distribution of water and its molecular mobility in hydrated seeds initiates a sequence of events during germination. These early events, such as the formation of new membranes and transformation of existing membranes, allow changes in permeability of water and gases, among other things[18]. In treated seeds water uptake is higher than that of the control seeds may be due to the increased dynamics, permeability of cell membranes, energy input due to MF .Fig.1, shows water uptake of control and treated Sunflower seeds.

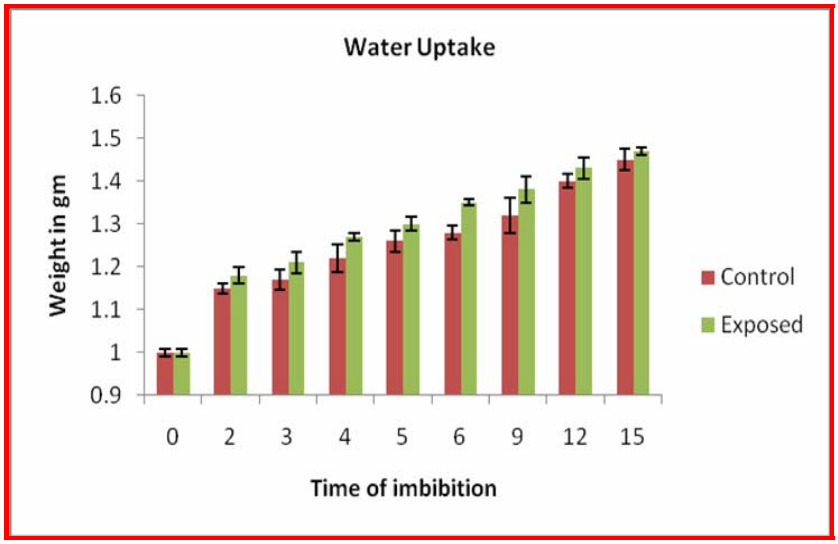

Figure 1 Water uptake of control and treated Sunflower seeds (with standard error bars)

The difference in water uptake and distribution between seeds exposed for MF & control were examined using NMR technique. The proton density images (Fig.2.) showed that in sunflower seeds greater water uptake initially is through radical than through the rest of the seed coat. Observation after 2hrs of imbibitions (Stage I) shows that the imbibitions commenced at the radicle. Radicle, cotyledons and seed coat were distinguishable at this stage. From the radical the water penetrates into the peripheral regions of cotyledons. At this stage water distribution in the outer/peripheral regions of the cotyledons are intense, heterogeneity was observed. Central part of the cotyledons was not wet, vascular system inside the cotyledons can be seen. In treated seeds the water uptake, the penetration through seed coat was found to be higher than in the control.

After imbibitions of 5hrs (stage II) intensity of the water signal has improved in the internal parts of the cotyledons also. From the radical as the period of imbibitions increases the water penetrates into the peripheral regions of cotyledons and then gets distributed throughout the cotyledons. The NMR images readily distinguished the embryo axis, cotyledons and seed coat at this stage. After 5hrs of imbibition the signal was strong enough to reveal such anatomical structures as embrioaxis, cotyledons and seed coat, still the heterogeneity could be observed. The water distribution throughout the cotyledons is higher in treated seeds; the anatomical features in treated seeds are more prominent, indicating the different ranges of penetration of water.

The heterogeneity in water distribution reduced after hrs of imbibitions (stage III) and the changes in water distribution inside the sunflower seed were dynamic. The water content in the cotyledons found to be homogeneous to greater extent, and water content is still found to be more in the radicle. The anatomical features are very clear at this stage. In treated seeds the presence of greater amount water content was observed than in the control sample.

After 15 hrs nearly the entire seed was hydrated, more homogeneous distribution was observed. The changes in water distribution inside the seed were dynamic, intense and anatomical features are very clear. Higher concentration of water content in the treated seeds at the central cotyledons indicates the increased dynamics of the cells.

Therefore, it is observed in sunflower seed radical is the main site of water entry. Water could also penetrate in very small quantity in to seed through seed coat. The water penetrated in to the outer parts of the cotyledons initially from the radical. Seed imbibitions leads to an increase in the water content inside the cells. NMR analysis showed that water distribution in sunflower seeds is inhomogeneous in embryo axes and cotyledons, they hydrate to different extents. It is conceivable that the organs show different metabolic activity during imbibitions and germination. In every stage higher concentration of water content in treated seeds were observed than the in control.

It was quoted that inhomogeneous water distribution may influence the resumption of oxidative metabolism[26]. In pea seed, as well as soybean seed[31] the hilum and the micropyle are the sites of water entry. The micropylar end of seeds was also identified as the major site for water entry in tobacco[32] and in bean seeds [33]. Ganguli and Senmandi[34] reported that the penetration of water into the endosperm in wheat caryopses proceeds from periphery to the interior. Water was said to penetrate into the seed through the seed coat in germinating western white pine seeds[35] . The water uptake and distribution in seeds indicate that it depends on the anatomical structure of the seed, the difference in water uptake between cotyledons and the embryonic axis could be due to the different chemical composition of these two organs. In NMR proton density images it is clear that treated seeds absorbed more water compared to the control seeds.

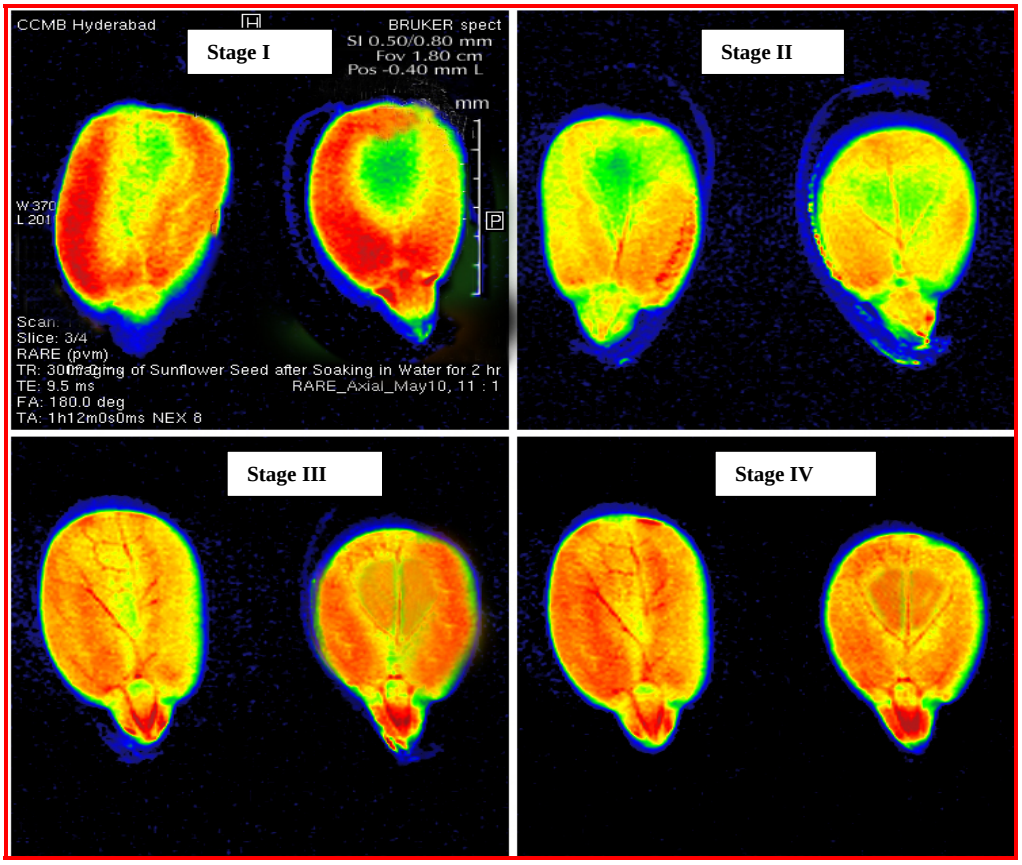

Figure 2 Hydration of seed at different stages. Control (left), Treated (right)

Hydration of seed –NMR Transverse relaxation time measurements

NMR longitudinal and transverse relaxation times of tissue water reflect the changes in cellular membrane structure and integrity[21,24] as the relaxation characteristics indicate the molecular mobility and biophysical state of water. The integrity of cellular membranes, which are composed primarily of protein and lipids, is influenced during the germination process. Disorganization of proteins and lipid phase transitions alter membrane structure and integrity and consequently, seed water status. The spin- spin

relaxation time measurements of seed cellular water are dependent on membrane permeability[24]. Our data suggest that there is rearrangement of cellular water during germination. Seed water, with medium relaxation times and with relatively less restricted mobility, is associated with the germination process since seeds are non-homogeneous, different cell types and tissue compartments can have different T_2 values.

The results of present study on the changes in T_2 components and hydration dependent transverse relaxation time of seed water corroborate the above hypothesis. Even though the total water content of the whole seed is low the state and quantity of water present in the localized sites within cells could provide a medium suitable for metabolic activity to proceed. The general conclusion is that extracellular free water is related by high relaxation times, intracellular bulk water by low relaxation times and bound – structural water by very low relaxation times.

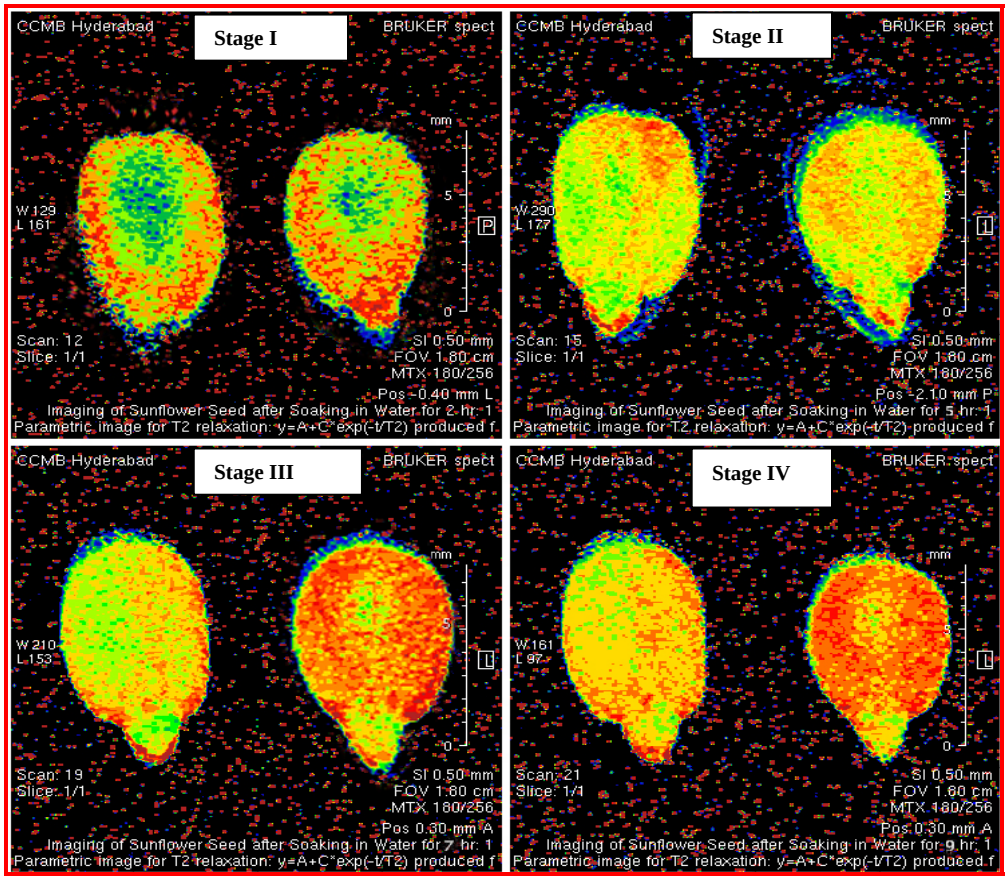

Fig.3. Transverse relaxation time (T_2) of water measured at different hydration stages

In dry seeds here has no detectable component of transverse relaxation time, which corresponds to extracellular free water in seed tissues. Hydration of seeds lead to better resolution and T_2 values varied over the time course of the entire experiment in control and treated seeds (Table.1) Fig.3. Exposed seeds

have higher T_2 values. T_2 varied in radical, outer cotyledons and mid cotyledons with increasing hydration time. At stages III & IV, T_2values are significantly higher in treated seeds. It suggests that seeds exposed for MF increase the mobility of water in the cells which may be due to increased dynamics of cell. The permeability of the plasma membrane determines the rate and mutual exchange of intracellular and intercellular water across the membrane.

Table 1 T_2 of water measured at different hydration stages:

Location	Proton relaxation time of Water (T_2) / ms							
	Stage - I		Stage - II		Stage - III		Stage - IV	
	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
Radicle	13.7- 16	16 – 18.5	16 - 21	18.5- 23	16.8–21.2	19–23.8	17.2 - 22	20.5-24.5
Outer cotyledons	14.2 – 16	15–16.8	15.5 -20	17 – 21.8	17–21.2	17.5–23.6	18–22.5	20–24.3
Mid cotyledons	13-14	13.6 –14.2	14.3 - 17	15.2 – 19.8	16–18	17.5–20	18-21	20-23.5

The treatment of seeds to MF might have increased the permeability of the membrane which in turn increased the water permeability in the seeds. The increase in the T_2 values and water population provides in treated seeds show strong evidence for the fact that the physical state of water is altered during germination in seeds.

Conclusions

The effect of 48 hrs application of 500 μT MF at 50Hz on sunflower seeds carried out showed the enhanced water absorption by sunflower seeds, which may be the explanation for the increase in the germination and growth rate of treated seeds. The changes in water status of control and exposed seeds characterized by proton density images and proton relaxation time measurements during imbibitions reveal that the pathway and pattern is strongly related with the anatomical structure of the seed and water hydration during this period is a multistage process. The quicker germination of seeds in MF exposed might be due to greater activities of germination related enzymes, early hydration of membranes as well as greater molecular mobility of bulk and hydration water fractions.

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