Characterization of water distribution and activities of enzymes during germination in magnetically-exposed maize (Zea mays L) seeds

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Magnetic seed treatment is one of the physical pre-sowing seed treatments to enhance the performance of crop plants. In our earlier experiment, we found significant increase in germination and vigour characteristics of maize (Zea mays L.) seeds subjected to magnetic fields. Among various combinations of magnetic field (MF) strength and duration, best results were obtained with MF of 100 mT for 2 h and 200 mT for 1 h exposure. The quicker germination in magnetically-exposed seeds 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. Thus, in the present study, changes in water uptake during imbibition and its distribution and activities of germinating enzymes during germination were investigated in maize seeds exposed to static magnetic fields of 100 and 200 mT for 2 and 1 h respectively by nuclear magnetic resonance (NMR) spectroscopy. The magnetically-exposed seed showed higher water uptake in phase II and III than unexposed seed. The longitudinal relaxation time $T_1$ of seed water showed significantly higher values and hence greater molecular mobility of cellular water in magnetically-exposed seeds as compared to unexposed. Component analysis of $T_2$ relaxation times revealed the early appearance of hydration water with least mobility and higher values of relaxation times of cytoplasmic bulk water and hydration water in magnetically-exposed over unexposed seeds. Activities of $\alpha$-amylase, dehydorgenase and protease during germination were higher in magnetically-exposed over unexposed seeds. The quicker germination in magnetically-exposed seeds 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.

Keywords: Zea mays L, Germination, Imbibition, Nuclear Magnetic Resonance, Longitudinal relaxation time, Transverse relaxation time, Germination enzymes

The process of germination can be divided into three phases. During phase I, dry seeds imbibe water, take up oxygen and increase their mass. In phase II, there is metabolic plateau with very little water absorption. In phase III, the germination process is completed, radicle protrusion takes place through the seed coat and absorption of water and oxygen rapidly increases. Information about the distribution and molecular mobility of seed water during hydration in germinating seeds is necessary to understand the process of seed germination. The water status and changes during imbibition of seeds influence subsequent development and growth. These changes in biological system have been studied non-invasively using low resolution nuclear magnetic resonance (NMR). Longitudinal ($T_1$) and transverse relaxation ($T_2$) behaviour of water protons can be investigated to describe the compartmentation and transport of water in seeds, plants and tissues. The water molecules with different mobilities can be distinguished by their different relaxation times and their relative amounts can be calculated. Thus, NMR techniques provides a novel, sensitive and direct method to characterize water status in seeds.

The NMR studies have been conducted using germinating seeds of oat, cowpeas, and maize seeds. In these studies, NMR relaxation time measurement has identified two to three components of the water proton system and it is suggested that water protons with short, medium and long relaxation times are associated with the bound, cytoplasmic and extra-cellular water, respectively. NMR has been used to characterize the water status of primed seeds of carrot and tomato and the enhanced performance has been attributed to reorganization of seed water during imbibition and increased macromolecular hydration water needed for metabolic activities.

Magnetic seed treatment is reported to be one of the physical pre-sowing seed treatments to enhance...
the performance of crop plants. In our earlier experiment, we evaluated germination and vigour characteristics of 7 days old seedlings of maize (Zea mays L.) seeds, subjected to different magnetic fields (50, 100, 150, 200 and 250 mT) and duration (1, 2, 3 and 4 h). We found average increase in germination, speed of germination, seedling length and seedling dry weight over control was 9, 20, 57 and 35%, respectively. Among various combinations of magnetic field (MF) strength and duration, best results have been obtained with MF of 100 mT for 2 h and 200 mT for 1 h exposure.

The quicker germination in magnetically-exposed seeds 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. Thus, in the present study, changes in water uptake and its distribution and activities of germinating enzymes during germination have been investigated in magnetically-exposed seeds using NMR spectroscopy.

Materials and Methods

Seed material

Maize (Zea mays L.) seeds (Var. Ganga Safed-2) were obtained from National Seed Cooperation, New Delhi. The moisture content of the seed was 7.55 ± 0.018%. Seeds without visible defects, insect damage and malformation were selected and stored in the desiccators having anhydrous calcium chloride. Seed moisture was determined in a set of triplicate samples by oven drying at 95°C to constant weight. Moisture content (g DW\(^{-1}\)) was calculated as \(\frac{(W_1-W_2)/(W_2)}{W_1}\), where \(W_1\) was initial weight of seed (g) and \(W_2\) the final weight of seed after drying (g).

Magnetic treatment

Seeds were exposed to the MF of 100 and 200 mT for 2 and 1 h respectively in a cylindrical shaped sample holder of 42 cm\(^3\) capacity, made of non-magnetic thin transparent plastic sheet. The visibly sound, mature and healthy seeds (100) were held in the plastic container at a volume between the poles of the electromagnet having uniform MF for required duration. The required strength of MF was obtained by regulating the current in the coils of electromagnet. Gauss meter was used to measure the strength of MF between the poles. At low field (50 mT), from centre to end of the poles, the variation was 0.6% in horizontal direction and 1.6% in vertical direction of applied field. At high field (250 mT), they were 0.4% and 1.2% of applied field, respectively.

Seed imbibition during germination

The difference in imbibition kinetics of both magnetically-exposed and unexposed seeds was studied. Seeds were allowed to imbibe water in covered petri dishes of 4-inch diameter layered with moist filter paper pads at 25°C. After blotting off excess moisture, wet weight of the seeds and NMR relaxation time were measured periodically until all seeds were germinated. Seeds were later dried in an oven at 95°C to constant weight and seed moisture was calculated as mentioned above.

Longitudinal relaxation time (\(T_1\)), transverse relaxation time (\(T_2\)) and components of \(T_2\)

Longitudinal relaxation time was measured using Bruker NMS 120 minispec NMR analyser operating at 20 MHz and ambient temperature (25±1°C). Seed water \(T_1\) was measured by saturation recovery method using the pulse sequence of 90°-t-90°. Transverse relaxation was measured by the Carr-Purcell-Meiboom-Gill (CPMG) method. In biological systems, the actual relaxation curve showed a marked non-exponentiality, which could be accounted by the presence of three clearly recognizable components with different relaxation times, using the exponential peeling statistical method. In plant and seed systems, at least three water components can be identified with the transverse relaxation times. \(T_{2a}\) for the extracellular free water with more mobility, \(T_{2b}\) for the cytoplasmic bulk water with less mobility and \(T_{2c}\) for the hydration water of macromolecules with least mobility. Non-exponentiality of transverse relaxation is accounted by the presence of clearly recognizable multi-components with different relaxation times. The total magnetization of the biological sample can be expressed as:

\[
M_t = M_a \exp (-t/T_{2a}) + M_b \exp (-t/T_{2b}) + M_c \exp (-t/T_{2c})
\]

where \(M_a\), \(M_b\) and \(M_c\) are related to the relative population of the three components \(T_{2a}\), \(T_{2b}\) and \(T_{2c}\) respectively.

Enzymes activities during germination

The enzymes related to germination process in magnetically-treated and untreated germinating seeds were assayed at different periods (h) of...
imbibition in distilled water at 25°C. Germinating seeds (1g) were taken for enzyme extraction at different durations. α-Amylase \(^\text{18}\) and protease \(^\text{19}\) activities were measured in triplicate. For dehydrogenase activity \(^\text{20}\) magnetically-exposed and unexposed germinating seeds were taken in quadruplicate and bisected longitudinally almost full depth through the midsection.

**Results**

Water uptake during seed germination showed three phases: rapid hydration (imbibition, phase I), lag phase (phase II) and steady hydration phase (germination growth, phase III). The phases I and II were observed until 22 h and between 22-58 h, respectively (Fig. 1). The phase III, which coincides with the radicle and plumule emergence showed a steady hydration from 58 h. During phase I, the water uptake was similar for both magnetically-exposed and unexposed seeds. In magnetically-exposed seeds water uptake was slightly more during phase II and significantly higher than the unexposed control during phase III. The moisture content increased from the initial level of 0.139 to 1.177 gH_2Og\(^{-1}\) in control seeds, from 0.144 to 1.586 gH_2Og\(^{-1}\) in seeds exposed to 100 mT (2 h) and from 0.145 to 1.322 gH_2Og\(^{-1}\) in seeds exposed to 200 mT (1 h).

Imbibition of seeds initiates a sequence of events that result in the mobilization of food reserves to the embryo, elongation and division of cells and subsequent protrusion of the radicle through surrounding layers \(^\text{21}\). In the present study, germinating seed showed three distinct phases of hydration. Significantly higher water uptake in phase III of hydration after lag phase was observed in magnetically-exposed seeds. Soybean seeds exposed to MF have also shown increased capacity of moisture absorption \(^\text{22}\). Significant increase in the rate of absorption of water is also reported in lettuce seeds exposed to MF \(^\text{23}\).

**Longitudinal relaxation time (T\(_1\)), Transverse relaxation time (T\(_2\)) and its components**

The longitudinal relaxation (T\(_1\)) and transverse relaxation (T\(_2\)) of magnetically-exposed and unexposed seeds showed an initial decrease over a period of imbibition, when dry seeds were subjected to hydration during germination (Fig. 2a,b). During the subsequent period of hydration, marginal increase in T\(_1\) and T\(_2\) value was observed. There was substantial increase in T\(_1\) and T\(_2\) during the subsequent stage of germination and magnetically-exposed seeds had significantly higher T\(_2\) values than unexposed seeds.

![Fig. 1—Changes in seed water content with imbibition time at 25°C for seeds exposed to MFs.](image1)

![Fig. 2—Changes in (a) longitudinal relaxation time, T\(_1\) and (b) transverse relaxation time, T\(_2\) of seed water with imbibition time at 25°C for seeds exposed to MFs.](image2)
Similar results have been reported in cowpea \(^1\), wheat and soybean \(^3\) during imbibition in water. The decrease in the proportion of water in more mobile form and increase in the proportion of water in less and least mobile forms are 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 been reported in tomato seeds \(^13\). In magnetically treated seeds, \(T_2\) values were greater than untreated controls at later stages of imbibition. This might be explained on the basis of higher water uptake by magnetically-treated seeds, which might be present in more mobile form, as indicated by early germination and radicle protrusion in these seeds.

NMR longitudinal and transverse relaxation times of tissue water are used to study changes in structure and integrity of cellular membranes as the relaxation characteristics indicate the distribution of water and its molecular mobility \(^6\). Initial dip in \(T_1\) values during hydration and then a gradual increase has been observed in maize \(^8\) and wheat seeds \(^26\). Comparison of these results with Bloembergen, Purcell and Pound (BPP) \(^27\) theory of magnetic relaxation suggests that during initial hydration decrease in \(T_1\) may correspond to relaxation of immobilized water molecules within the seed matrix. In present study, \(T_1\) values were greater for magnetically-exposed seeds as compared to unexposed seeds corresponding to similar seed moisture levels. Longitudinal relaxation time of water in leaves has been directly related to water activity \((a_w)\) of the cell water \(^26,28\), which in turn is related to availability for metabolic activities. This might explain the greater speed of germination and increased seedling vigour of magnetically-exposed seeds \(^14,29\).

The actual relaxation curve showed a marked non-exponentially that could be accounted by the presence of three clearly recognizable components with different relaxation times. \(T_{2a}\) decreased with increase in hydration time in both magnetically-exposed and unexposed seeds until 58 h, followed by an increase. Increase in \(T_{2a}\) coincided with sprouting in seeds (Fig. 3a). Similarly, \(T_{2b}\) increased until 58 h in both magnetically-exposed and unexposed seeds, but considerably decreased during subsequent period of germination (Fig 3b). The values of \(T_{2b}\) were higher for magnetically-exposed seeds compared to control during most of the seed hydration. Component \(T_{2c}\) was not detectable in a dry seeds, but resolved at 12 and 15 h after imbibition in magnetically-exposed and unexposed seeds, respectively (Fig. 3c). \(T_{2c}\), initially increased in both magnetically-exposed and unexposed seeds respectively until 18 h, but decreased considerably until 32 h followed by continuous increase and was significantly higher in
magnetically-exposed seeds than unexposed. However, $T_{2c}$ was again undetectable after 72 h of hydration.

Figures 4a-c show the fractional population of different water protons of varying mobilities during germination. After appearance of the least mobile fraction, magnetically-exposed and unexposed seeds showed an increase in spin population of $T_{2b}$ and $T_{2c}$ together and a decrease in $T_{2a}$ until the radicle emergence took place. However, during subsequent period of imbibition, only two populations of water were observed with $T_{2a}$ fraction being larger than $T_{2b}$ fraction. Figures 5a-c give the amount of seed water corresponding to different $T_2$ components in magnetically-exposed and unexposed seeds. Amount of water in free form increased only after radicle emergence and the values were significantly greater for treated seeds. $T_{2a}$ increased after an initial dip in both treated and untreated seeds albeit at different rates.

Results of transverse relaxation time analysis indicated the complex water exchange process taking place between components inside magnetically-exposed and unexposed seeds. They indicated the higher molecular mobility of water protons in $T_{2b}$ and $T_{2c}$ fractions of seed water and, therefore, better availability for metabolic activity. This may be the reason for the faster germination of the magnetically-exposed seeds.

Distribution of water proton into three fractions showed clear change with imbibition time indicating rearrangement of cellular water fractions both in magnetically exposed and unexposed seeds (Fig. 4a-c). Presence of free water in dry seeds at early stages of imbibition shows that the state and quality of different water components during early stages of imbibition could provide a medium suitable for metabolic activity to proceed\textsuperscript{13}, although the total water content was still low.

Relatively greater amounts of cytoplasmic bulk and bound water in magnetically-exposed seeds might be responsible for the speed of germination. Disappearance of bound fraction and presence of only two components corresponding to bulk and free water fractions with radicle protrusion indicated the exchange of water in different compartment by the rearrangement of membrane permeability during germination and the formation of vacuoles in association with growth of embryo. The early appearance of structure-associated least mobile water in magnetically-treated seeds indicated early hydration of macromolecules which bind water and reduce its mobility and maintain ongoing metabolism and cellular membrane integrity. Similar results of early appearance of bound water fraction have been reported in primed seeds of carrot and tomato seeds\textsuperscript{12, 13}. Increase in water content in less mobile (cytoplasmic bulk water) and least mobile (bound water) fractions during phase II of imbibition indicated that these fractions were involved in germination related metabolic processes until radicle protrusion (Fig. 5 b,c).

Component $T_{2a}$, which represents relaxation time of extracellular more mobile (free water) decreased with imbibition time till radicle protrusion and then increased sharply (Fig. 5a). This might be due to the hydrolysis of storage material like protein and sugar when mixed restrict the mobility of extracellular free water. Once the radicle protrusion takes place, tissue growth begins and the bound fraction disappears, the relaxation time of this free component increased.
T2b, which represent the relaxation time of bulk water fractions in seeds increased in all seeds until radicle protrusion and then decreased (Fig. 5b). T2c, representing the relaxation time of macromolecular hydration water fraction decreased initially until 20 h of imbibition and then increased (Fig. 5c). This might be explained as the initial hydration of water tightly held by macromolecules with least mobility and subsequent layers of water had relatively greater mobility due to protein hydrolysis and exchange with bulk or free water. The values of both components were higher in magnetically-exposed compared to unexposed seeds, indicating the higher molecular mobility of water protons in these fractions of seed water and, therefore, better availability for metabolic activity.

Enzymes related to germination

Activities of α-amylase, dehydrogenase and protease in magnetically-exposed seeds showed significantly higher values, as compared to unexposed control in most stages of germination. α-Amylase activity increased significantly until 64 h of imbibition and was 17% higher in 100 mT (2 h) and 22% higher in case of 200 mT (1 h) as compared to corresponding values of unexposed control (Fig. 6a). Dehydrogenase activity increased significantly until 60 h of imbibition and was 44% higher in 100 mT (2h) and 48% higher in case of 200 mT (1h), as compared to unexposed control (Fig. 6b). Protease activity increased significantly until 64 h of imbibition, which was 8% higher in 100 mT (2 h) and 5% higher in 200 mT (1 h) as compared to unexposed control (Fig. 6c).
α-Amylase is responsible for the degradation of food reserves of the seedling during germination. In present study, α-amylase activity increased up to 64 h (Fig. 6a) beyond which it started declining. Increase in speed of germination in magnetically-treated seeds can be explained as a consequence of increased activity of α-amylase. α-Amylase activity in broad bean is reported to be significantly decreased at 5, 50 and 100 µT on day 2 and 4 of growth at 100 mT which

In addition, the present study revealed that NMR relaxation time of seed water indicated early appearance of hydration water, greater amount of cytoplasmic bulk water as well as hydration water in magnetically-exposed seeds, compared to unexposed controls. Also, in treated seeds, molecular mobility of cytoplasmic bulk water and hydration water of macromolecules were greater, as indicated by their respective relaxation times. Thus, early hydration of macromolecules as well as membranes and greater activities of enzymes during germination were responsible for quicker germination of magnetically-exposed seeds as compared to unexposed seeds.

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