Table 4.11.1a: Parameters calculated from application of D'Arcy –Watt equation to sorption isotherm of magnetically treated seeds of maize at 25°C

Treatment	Sorption sites					
	Strong		Weak		Multimolecular	r fit
	K	K′	C	k	k′	
Control	6.062	0.0687	0.0223	0.9457	0.0175	0.998
100mT (2h)	7.108	0.0616	0.0349	0.9956	0.0097	0.999
200mT (1h)	3.301	0.0674	0.0342	0.9499	0.0140	0.997

Table 4.11.1b: Parameters calculated from application of D'Arcy –Watt equation to sorption isotherm of magnetically treated seeds of maize at 35°C

Treatment	Sorption sites					
	Strong		Weak		Multimolecular	r fit
	K	K′	C	k	k′	
Control	14.566	0.0387	0.0547	0.8787	0.0287	0.999
100mT (2h)	34.122	0.0243	0.1063	0.9798	0.0091	0.998
200mT (1h)	29.404	0.0278	0.0765	0.8725	0.0241	0.996

Table 4.11.1c: Number of water binding sites in magnetically treated seed of maize at 25°C

Treatment	Sorption sites X 10^{21} (Sites / g dry weight)			
	Strong	Weak	Multimolecular	Total
Control	2.30	0.23	0.59	3.12
100mT (2h)	2.06	0.37	0.32	2.75
200mT (1h)	2.25	0.36	0.47	3.08

Table 4.11.1d: Number of water binding sites in magnetically treated seed of maize at 35°C

Treatment	Sorption sites X 10^{21} (Sites / g dry weight)			
	Strong	Weak	Multimolecular	Total
Control	1.29	0.33	0.96	2.58
100mT (2h)	0.81	0.63	0.31	1.75
200mT (1h)	0.93	0.45	0.81	2.19

चुम्बकीय क्षेत्रो से सम्बंधित बीजों का जैव भौतिकीय लक्षण वर्णन

BIOPHYSICAL CHARACTERIZATION OF SEEDS SUBJECTED TO MAGNETIC FIELD

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CERTIFICATE

This is to certify that the thesis entitled, "**Biophysical characterization of seeds subjected to magnetic field**" submitted to the Faculty of Post Graduate School, Indian Agricultural Research Institute, New Delhi by **Ananta Vashisth** in partial fulfillment of the requirements for the award of the degree of **Doctor of Philosophy** in **Agricultural Physics**, is a record of *bona-fide* research work carried out by her under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigation have been duly acknowledged.

Place: New Delhi
Date :

(Shantha Nagarajan)
Chairperson
Advisory Committee

BIOPHYSICAL CHARACTERIZATION OF SEEDS SUBJECTED TO MAGNETIC FIELD

by

ANANTA VASHISTH

A thesis
submitted to the Faculty of Post-Graduate School,
Indian Agricultural Research Institute, New Delhi
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for the award of the degree of

DOCTOR OF PHILOSOPHY
IN
AGRICULTURAL PHYSICS

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(ANANTA VASHISTH)

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Chapter - 3
MATERIALS AND METHODS

In order to achieve the objectives of the present study, experiments were carried out in the Indian Agricultural Research Institute, New Delhi. Keeping in view the basic objectives of effect of magnetic field and duration of exposure for maximum enhancement of germination, vigour and field emergence of different crop seeds and the biophysical and biochemical parameters associated with germination process and storability, seed containing predominantly starch, protein or oil were selected. The details of the experiments, the methods followed and techniques adopted in bringing out the desired results are given under the following headings.

3.1. SELECTION OF SEED

The certified seed of maize (Var. Ganga Safed-2) and sunflower (Var. KBSH-1) were obtained from National Seed Cooperation, New Delhi. Breeder seed of chickpea (Var. Pusa-1053) was obtained from Division of Genetics, Indian Agricultural Research Institute, New Delhi. Seeds without visible defects, insect damage and malformation were selected and stored in the desiccators having anhydrous calcium chloride. The moisture content, carbohydrate, protein, oil and germination percentage in seeds were measured (Table 3.1).

3.2 INSTRUMENT USED

3.2.1 Electromagnetic field generator

An electromagnetic field generator "Testron EM-20" with variable magnetic field strength (50 to 500mT) with a gap of 5 cm between pole pieces was fabricated (Plate 3.1). The pole pieces are 9 cm in diameter, 16 cm in length. The number of turns per coil is 3000 and the resistance of the coil is 16 ohm. A DC power supply (80V/ 10A) with continuously variable output current was used for the electromagnet. A digital gauss meter model DGM-30 operating on the principle of Hall effect monitored the field strength produced in the pole gap. The probe is made of Indium Arsenide crystal and is encapsulated to a non- magnetic sheet of 5mmX4mmX1mm and could measure 0-2 Tesla with full-scale range in increments of 5mT. The field strength produced in the

electromagnet at different currents when the pole gap was 3cm, 5cm and 8cm is shown in Fig.3.1. The corresponding values were shown in (Table 3.2).

3.2.2 Root Scanner

Scanning and image analysis for root characteristics was carried out using Root Scanner (LA 1600) and the root morphology and Architecture measurements (total root length, root surface area, root thickness and root volume) were done by win RHIZO program from REGENT INSTRUMENTS Inc. Canada (Plate 3.2). The scanner of RHIZO system had two light sources, one below the scanner glass called flat bed or reflective and one above it (in the scanner cover) called transparency unit or TPU. For root morphological measurements TPU lighting system was used for scanning. The light rays from TPU passed through the sample and then to the camera sensor below the scanner glass. The resulting image was sharp and clear with well-contrasted silhouette. The image analysis system RHIZO acquires a direct digital (grayscale) image from the scanner and then created two other types of images for its analysis. One type of the image created was termed as 'Pixels classification image' in which all parts of the original image that fell below a user defined threshold of grayscale value were removed. The image had only two intensities, black for pixels belonging to roots and white for pixels belonging to the background. Another created image was termed as skeleton image, which consisted of a line, one pixel in diameter, which is superimposed over the earlier root image. RHIZO system measured the root length by scanning the length of the root skeleton. The colour of the skeleton indicated the diameter classification. Measurements of root morphological characteristics were based on Regent's non-statistical method (Arsenault et al., 1995; Guay and Arseneault, 1996) with overlap compensation. The advantage of Regent's non-statistical method over Tennant's (1975) statistical method was that in addition to total root length density, root surface area and volume measurements, it further gave information of their distribution in various size classes based on their diameter.

Length

Total length can be measured with the following formula:

Length = (number of pixels in the skeleton)*(pixel size)

Projected area

Projected area is measured by counting the number of pixels belonging to the root in the pixel classification image. The count is then multiplied by the pixel area.

Pixel area=pixel width * pixel height

Projected area= number of pixels * pixel area

Average diameter, surface area and volume

Average diameter was calculated with the following formula:

Average diameter(D) =Projected area / Total length

This formula is based on the assumption that roots are round.

Surface area=$4\pi(D/2)^2$

Volume=Surface area * Total length

3.2.3 Low Resolution Pulsed NMR Spectrometer

The nuclear magnetic resonance spectrometer (Plate 3.3) comprises the following basic parts:

i. Magnet, capable of producing a very strong and homogenous field

ii. Radio frequency transmitter required for producing r. f. field.

iii. Radio–frequency detector, which consists of nuclear signal processing unit.

iv. Sample circuitry containing the probe to hold the sample and to couple it to both the transmitter and detector.

Characteristics of NMR Spectrometer

Instrument:	Bruker NMS 120 minispec NMR Analyser
Magnet:	Permanent magnet
Field strength:	0.47 Tesla.
Operating frequency:	20MHz
Sample tube diameter:	10mm
Homogenous region of r.f. Coil:	3cc
Detector:	Diode and phase sensitive

90°- pulse width: 5 μsec.

180°- pulse width: 10 μsec.

Dead time of receiver: 10 μsec.

3.2.4 Chemicals

All the chemicals used in the study were of analytical grade.

3.3 EXPERIMENTS

3.3.1 Standardization of magnetic field strength for maximum enhancement of germination characteristics and subsequent field emergence studies

The seeds of maize, chickpea and sunflower were exposed to the magnetic field of 50 to 250mT in a sample holder, cylindrical in shape and made of non-magnetic thin transparent plastic sheet. Visibly sound, mature, healthy 100 seeds were held inside plastic container in the space between the poles of the electromagnet having uniform magnetic field for various durations ranging from 1 to 4h. The required strength of the magnetic field was obtained by regulating the current in the coils of the electromagnet. Gauss meter was used to measure the strength of the magnetic field between the poles. The field strength and duration was standardized for maximum enhancement of germination and vigour in laboratorary conditions. Among the various combinations of field strength and duration best results were obtained with 100mT(2h) and 200mT(1h) exposure in maize, 50mT(2h), 100mT(1h) and 150mT(2h) exposure in chickpea and 50mT, 200mT and 250mT for 2h exposure in sunflower. Therefore these combinations were selected and used for field emergence studies. Based on the field emergence and rooting characteristics of one month old seedling, magnetic treatment of 100mT(2h) and 200mT(1h) exposure in maize, 100mT(1h) and 150mT(2h) exposure in chickpea and 50mT and 200mT for 2h exposure in sunflower were selected for further biophysical and biochemical studies.

3.3.2. Effect of magnetic field on seed viability and vigour loss under accelerated ageing condition

Magnetically treated and untreated seeds of maize, chickpea and sunflower were subjected to accelerated ageing condition as described by Deolouche and Baskin (1973).

The effect of magnetic field on the viability of seeds was investigated. Seeds in muslin cloth bags were placed at high temperature (40°C) and high humidity (95-100%) by placing over sieve in desiccators having water at the bottom. Seeds were sampled on alternate days and analyzed till they lost their viability completely. Following parameters were measured:

i. Germination (%)
ii. Seedling dry weight (g)
iii. Seedling shoot/root length (cm)
iv. Seed moisture content
v. Seed leachate conductivity.

3.3.3. Antioxidant enzymes in aged magnetically treated and untreated seeds

Magnetically treated and untreated seed of maize, chickpea and sunflower were subjected to accelerated ageing condition. The effect of magnetic field on the activity of antioxidant enzymes of seeds when they lost their viability by about 30% was investigated. Seeds (20 each) in muslin cloth bags were placed at high temperature 40°C and high humidity (95-100%) by placing over sieve in a desicator having water at the bottom. Seeds were sampled after five days for maize, four days for chickpea and two days for sunflower. Following antioxidant enzymes were measured in accelerating aged seed and non aged seed of maize, chickpea and sunflower.

a. Superoxidide dismutase (SOD)
b. Peroxidase
c. Catalase

3.3.4. Effect of magnetic treatment of mid storage (partially aged) seeds on germination characters and seed leachate conductivity

Seeds of maize, chickpea and sunflower (300 each) were subjected to accelerated ageing at high temperature (40°C) and high humidity (95-100%) till they lost about 30% of their initial viability. After six days of accelerated ageing for maize and chickpea and two days for sunflower, 100 seeds each were treated by same magnetic fields as mention in 3.3.1. The germination characteristics and seed leachate conductivity in partially aged

magnetically treated and untreated seeds, along with magnetically treated and untreated fresh seeds were measured.

3.3.5. Imbibition kinetics of magnetically exposed and unexposed (control) seeds during germination

The difference in kinetics of imbibition in magnetically treated seeds of maize, chickpea and sunflower along with their untreated control were studied. Magnetically treated and untreated dry seeds of all the crops were placed over moist filter paper pads (distilled water added just sufficient to moisten the filter paper) in covered petri dishes of 4-inch diameter. The experimental seed materials in petri dishes were incubated at 25°C for maize and sunflower and at 20°C for chickpea. At different time intervals of incubation, seeds samples were drawn for the analysis. The seed samples were wiped gently with tissue paper to remove the excess external water before measuring the following parameters.

i. Seed moisture content
ii. Spin- lattice relaxation time (T_1)
iii. Spin- spin relaxation time (T_2)
iv. Component analysis of the spin- spin relaxation time

3.3.6. Effect of magnetic field on enzymes related to germination (α-Amylases, dehydrogenase and protease)

Magnetically treated and untreated seeds of maize, chickpea and sunflower were placed over the moist filter paper in petri dishes for germination and put in the incubator at 25° C for maize and sunflower and at 20°C for chickpea. Enzymes related to germination such as α-amylases, dehydrogenase and protease were studied at different stage of germination in both magnetically treated and untreated seeds of these crops.

3.3.7. Characterisation of water status and energy status in seeds equilibrated at different relative humidities at 25°C and 35°C.

This study was undertaken to characterize the water status and changes in viability of seeds, equilibrated at different relative humidities at 25°C and 35°C. Magnetically exposed and unexposed seeds (25 each) in muslin bags were equilibrated over saturated salt solutions at two different temperatures (25°C and 35°C). The RH of

the salt solutions and sulphuric acid at the given temperatures were as given by Rockland (1960); Winston and Bates (1960); Vertucci and Roos (1993) and covered a range from 1-91% (Table 3.3).

Following measurements and calculations were done:

i. Spin- lattice relaxation time (T_1)
ii. Spin- spin relaxation time (T_2)
iii. Seed leachate conductivity
iv. Germination characteristics
v. Equilibrium moisture content at different relative humidity
vi. Strong, weak and multimolecular water binding sites using D'Arcy–Watt equation.
vii. Differential Enthalpy using Clausius-Clapeyron equation
viii. Differential Entropy
ix. Free energy

3.4. METHODOLOGY

3.4.1. Determination of seed moisture content

Seed moisture was determined in three replicates by oven drying seeds at 95^0C for constant weight (Walters 1998).

Moisture content (%) is calculated as $[(W_1-W_2)/(W_2)]$ x100

where W_1 is initial weight of the seed (g) and W_2 is the final weight of the seed after drying (g). Seed moisture content was expressed as g water/ g dry weight

3.4.2. Germination percentage

The germination of the seeds was determined by using between paper method (ISTA, 1985). One hundred seeds in four replications of 25 seeds each were placed between two layers of moist germination papers. Then the germination papers were rolled carefully ensuring that no excess pressure is placed on the seeds. These were wrapped in a sheet of wax paper to reduce surface evaporation and placed in the germination incubator at 25^0C temperature for maize and sunflower and at 20^0C for chickpea in an upright position. After 7 days (maize), 8 days (chickpea) and 10 days (sunflower) the germinated seeds were evaluated for normal, abnormal seedling, fresh

un-germinated and dead seeds. Germination percentage is given on the basis of normal seedling only.

3.4.3. Seedling growth and seedling vigour

Ten normal seedlings from each replicate were taken at random and the shoot length and root length were recorded by linear scale. Seedlings were dried overnight in an oven set at 90^0C temperature and the weight of 10 seedlings together per replicate was measured. Seedling vigour was calculated following Abdul Baki and Anderson (1973) as

Vigour index I = Germination % x Seedling length (Root +Shoot)

Vigour index II = Germination % x Seedling dry weight (Root +Shoot)

3.4.4. Speed of Germination

Eighty magnetically treated and untreated seeds each of maize, chickpea and sunflower in four replications with 20 each were placed in moistened filter paper in petri dish and kept in an incubator at 25^0C for maize and sunflower and at 20^0C for chickpea. Daily germination count was taken till no more seed germinated. The speed of germination (X) was calculated as:

$$X = \frac{\text{Number of seeds germinated}}{\text{Day of the first count}} + \cdots + \frac{\text{Number of seeds germinated}}{\text{Day of the final count}}$$

3.4.5. Seed leachate conductivity

Based on experimental data for germination character, 200mT (1h), 100mT (2h) for maize, 50mT (2h), 100mT (1h) and 150mT (2h) for chickpea and 50mT, 200mT and 250mT for 2h for sunflower were adjudged to be the best for improving germination and vigour. Therefore the same were used for measurement of seed leachate conductivity and evaluation of field emergence and root characteristics.

Twenty-five seeds in four replicates were soaked in 25 ml of distilled water at 25^0C for 24h for maize and sunflower and 4h for chickpea. A control with distilled water (but without seeds) was also taken. The seed leachate was decanted off in clean 50 ml beaker. The electrical conductance of the leachate was measured at room temperature (ISTA, 1985) using digital conductivity meter (Systronics India).

3.4.6. Field emergence index

Line sowing of 100 seeds of magnetically treated and untreated seeds in four replications (25 seeds each) were carried out in (4mX1m) well prepared soil bed mixed with peat in the net house. Irrigation was provided as and when required. Number of emerged plants was recorded every day and the field emergence index was calculated using the formula (Mock and Skrdla, 1978).

$$\text{Emergence index} = \Sigma \frac{(\text{plants emerged on a day})(\text{days after planting})}{\text{Total plants emerged}}$$

Shoot height, shoot and root dry weight were recorded on one month old seedling, taking five plants from each of the four replications.

3.4.7. Rooting Characteristics

The root samples were washed carefully by gentle stream of water for complete separation of root from soils. Then the roots were air-dried so as to make the root samples ready for scanning. Scanning and image analysis for root characteristics was carried out using Root Scanner (LA 1600) and the root morphology and architecture measurements (total root length, root surface area, root thickness and root volume) were done by win RHIZO program from REGENT INSTRUMENTS Inc. Canada. There were four replications per treatment.

3.4.8. Spin-lattice relaxation time (T_1)

Seed water spin–lattice relaxation time (T_1) was measured using Bruker NMS 120 minispec NMR analyzer operating at 20 MHz and ambient temperature (25°C±1°C). The spin–lattice relaxation measurement is based on rotating the equilibrium magnetization M_0 away from the direction of magnetic field H_0 using either one or more 90° or 180° pulses. The re-establishment of M_0 in the direction of the field is observed at various pulse intervals. In such sequences, the first pulse prepares the spins whereas the second pulse measures the magnetization after the pulse delay. In these experiments the spin-lattice relaxation time is measured by the saturation recovery method using the pulse sequence of 90°-τ-90°. In the saturation recovery method, the two 90° pulses are separated by the variable time duration as denoted as τ. The advantages of the saturation

recovery method are that it is not very sensitive to flip angle missetting and it is suitable for measuring small T_1 values. The sample was inserted into the NMR probe and the resonance of the instrument was adjusted. Using the 90°-pulse sequence programme, the optimum pulse separation and the number of data points were set. Seed materials were tested with the following settings; data points: 18, Duration: 5ms, Number of scans: 4. The parameters such as recycle delay, receiver gain etc. were optimized based on the tissue, and the expected amplitude of the signal. The recovery of NMR signal till the saturation was observed and the in- built programme of the instrument was used to calculate the relaxation time by the equation

$$M_z(\tau) = M_0 (1\text{-}\exp \tau/T_1)$$

Where $M_z(\tau)$ is the magnetization at time τ in z -axis, M_0 is the saturation magnetization in z-axis, T_1 is the spin-lattice relaxation time.

Preparation of sample

Seed samples were randomly collected and quickly taken into the NMR tubes of 10mm diameter, corked immediately to avoid dehydration and placed in the probe of NMS 120. The height of the sample was kept at around 2cm.

3.4.9. Spin- spin relaxation time (T_2)

Spin–spin relaxation or transverse relaxation time is measured by studying the decay of the transverse component of magnetization. The most common and accurate method of T_2 measurement is the Carr-Purcell Meiboom Gill (CPMG) method (Snarr and Van As, 1992). An easy method aimed at avoiding the cumulative errors due to the mis-adjustment of 180° pulses, as proposed by Meiboom and Gill, is to apply 180° pulses along y′ direction, instead of x′ direction (i. e., a phase difference of 90° relative to the initial 90° pulse). This modification of CP sequence gives exactly the correct amplitude for all even-numbered echoes whereas the odd–numbered are only slightly attenuated. For CPMG programme, the optimum number of data points, the 90°-180° pulse separation time and number of dummy echoes were set and the parameters such as the number of scan, the recycle delay, the receiver gain etc. were optimized. Seed materials tested had the following settings:

Number of data points-150; Pulse seperation-0.5ms; Dummy echo-3; Number of scans-10. T_2 values were determined by measuring the exponential decay of the signal.

$M_{xy}(\tau) = M_0 \exp(-\tau/T_1)$

Where $M_{xy}(\tau)$ is the magnetization at the echo time τ along xy. M_0 is the total magnetization along xy just after 90° pulse is applied. T_2 is the spin-spin relaxation time.

3.4.10. Components of NMR relaxation time

Usually, in biological systems multi-exponential relaxation decay curves are measured, and numerous attempts have been made to discriminate between the different water fractions in the various compartments based on the relaxation times (Van AS, 1992). In this study the multi-exponential decay curve analysis has been carried out for T_2 relaxation measurements for the following reasons:

i. A larger number of data points of the decay curves are possible with T_2 than T_1.

ii. Experimentally T_2 relaxation decay is far easier to accomplish than for T_1.

iii. In general the measurement time for T_2 is much shorter than for T_1.

iv. The relative differences in the relaxation times generally are smaller for T_1 than for T_2 yielding a better multi-exponential fit for the T_2.

A single exponential echo decay of the Carr-Purcell measurement using Bruker NMS 120 minispec NMR analyzer, for spin-spin relaxation time is shown in (Fig. 3.2). The signal decay plotted on a logarithmic scale of y-axis using data points for the relaxation time measurements of a seed sample, clearly indicates that there are multiple fractions of tissue water, with distinct spin-spin relaxation times.

One may assume that the measured signal is a summation of the signals from several non- (or slowly) exchanging fractions, i. e.,

$$S(t) = \Sigma_i S_i(t)$$

and $$S_i(t) = P_{oi} \exp\{-t/T_{2,i}\}$$

where S(t) is the normalized echo amplitude at time ($t = 2\tau$), P_{oi} and $T_{2,i}$ are relative population and transverse relaxation time of the i^{th} fraction. This model does not

exclude the possibility that within each $S_i(t)$ there may be several fast- exchanging sub–fractions. But those fractions, which exchange fast with each other, will show a single T_2 and will appear as one fraction. What is important is to know the minimum number of (non- or slowly exchanging) fractions needed to fit the observed data.

A computer programme was written in BASIC. This programme gives the graphical display of relaxation decay with the signal amplitude on the logarithmic scale along the y-axis and time (t) on the x-axis. Components of spin–spin relaxation was analysed by using the least square fit analysis in the region of limits specified by the user on the basis of visual inspection of the semi log plot. The user can specify the limit based on the t value (x-axis) until which the plotted curve shows a visible curvature change. The usual procedure of curve decomposition was followed, by locating the slowest relaxing fraction from the curve and subtracting this fraction from the observed data (Snarr and Van As, 1992).

Preparation of saturated salt solutions

The saturated salt solutions were prepared by mixing distilled water with the salt to form slurry. It was maintained up to an optimum height. The solutions were observed regularly to ensure that the approximate level of slurry was maintained. Muslin cloth packets were kept on a aluminium net placed above the slurry. Total system was kept in a petri dish, which was made airtight to ensure that actual RH was maintained (Plate 3.4). Muslin cloth packets were placed carefully to avoid contact between seeds and salt slurry. Seeds incubated for equilibrium were weight periodically until a constant weight was attained (at which point the seeds were seemed to be equilibrated). There were three replications for each RH and temperature.

3.4.11. Water binding characteristics:

The sorption data (Equilibrium relative humidity vs seed moisture content) was fitted to D'Arcy-Watt equation by a linear least square fit of each of the three regions of the isotherm (Vertucci and Leopold, 1984) using the computer software 'Curve Fit'. The D'Arcy-Watt equation is given as

Seed water = $\{(KK'a_w/[(1+K)a_w]\} + [ca_w] + \{kk'a_w) / [(1-k)\ a_w]\}$ where

a_w (p/p_0) = water activity (equilibrium relative humidity)

K and K' are the affinity and number of strong binding sites

c is a measure of both affinity and number of weak water-binding sites

k & k' relates to the affinity and number of multi – molecular binding site.

The number of water–binding sites in seed tissues can be calculated from the derived D'Arcy-Watt coefficients. The numbers of strong, weak and multi-molecular binding sites are given as K′N/M, cN/Mp_0 and k′N/M respectively, where N is the Avogadro's number ($6.023X10^{23}$), M is the molecular weight of water and p_0 is the saturated vapour pressure of water at the specific temperature. The amount of water associated with different water binding site in seed tissues were calculated and compared for the seeds of magnetically treated and untreated seeds.

3.4.12. Water activity (a_w)

The seed moisture contents at different relative humidities (RH), for a particular temperature, was fit using the best curve fit method. From the equation of curve fit, for any given moisture content, the corresponding RH was calculated. Water activity is calculated using the equation as described by (Vertucci and Roos, 1993).

$a_w = (p_o\gamma_o / p\gamma) \cong RH/100$ as $RH = p_o/p \times 100$ and $\gamma_0 \approx 1$

where p_o is the water vapour pressure, p is the total pressure of the system and γ is the fugacity coefficient. Thus, RH/100 is the good approximation of water activity of the solution. Water activity of seeds is an intrinsic property; related to the composition, water content and temperature (Walters, 1998).

3.4.13. Chemical potential of water (μ_w)

Chemical potential of water (KJ/mol water) in seeds is calculated from the water activity values as

$$\mu_w \text{ (seeds)} = \mu_w^o + RT \ln (a_w)$$

$$\cong \mu_w^o + RT \ln (RH/100)$$

μ_w^o is the chemical potential of water in its standard state the standard value of (μ_w^o=0), R is the ideal gas constant (8.3143 J mol^{-1} K^{-1}), T is the temperature in Kelvin (Vertucci and Roos, 1993)

3.4.14. Water potential (Ψ_w)

Water potential of seed water (Mpa) is calculated from the water activity values by the following relationship

$$\psi_w = RT \ln (a_w) / \nu$$

Where R=8.314 ml Mpa $mol^{-1}K^{-1}$, T is the temperature in Kelvin (K), ν is the molar volume of water, which is 18.048ml mol^{-1} (Walters et al., 1997).

3.4.15. Differential Enthalpy

The differential enthalpy (J/mol water) is calculated (Vertucci and Leopold, 1987) using the Clausius-Clapeyron equation as

$$\Delta H = R \times [(T_1 \times T_2)/(T_2 - T_1)] \times \ln(a_{w1}/ a_{w2})$$

Where a_{w1} and a_{w2} are the relative vapour pressure or water activities at the lower and higher temperatures T_1 and T_2, R is the ideal gas constant (8.3143 Joule/degree/mol). From the curve fit of relative humidity vs. moisture content relationship at temperatures 25°C and 35°C, the relative vapour pressure for the given moisture content at that particular temperature was calculated. These were used as the corresponding a_w values for the given moisture content for calculating ΔH.

3.4.16. Free energy

The free energy change (joule/mol water) with hydration is calculated (Vertucci and Leopold, 1987) as

$$\Delta G = RT \ln(a_w)$$

R is the ideal gas constant (8.3143 J mol^{-1} K^{-1}), T is the temperature in Kelvin, a_w is the relative vapour pressure at a particular temperature T. a_w was as described in 3.4.12.

3.4.17. Differential Entropy

The differential entropy changes (joules / mol /K) with hydration is calculated (Vertucci and Leopold, 1987) as

$$\Delta S = (\Delta H - \Delta G) / T$$

Where ΔH is the differential enthalpy calculated as 3.4.15.and ΔG is the free energy change at the temperature T in Kelvin.

3.4.18. α-Amylase activity

All enzymes in magnetically treated and untreated germinating seeds of maize, chickpea and sunflower were assayed at different hours of imbibition in distilled water. Seeds were placed in moistened filter paper in petri dish and kept in an incubator at 25^0C for maize and sunflower and at 20^0C for chickpea. 1 g germinating seeds were taken for enzyme extraction at different duration. α-amylases activity was estimated following the method described by Berbfeld (1995).

Reagent required

1. 0.1M Acetate buffer (P^H=4.7) was made using following reagent:
    i. 0.2M Sodium acetate solution (solution A): 3.28 g sodium acetate in 200 ml double distilled water.
    ii. 0.2M Acetic acid solution (solution B): 2.3 ml of acetic acid in 200 ml double distilled water.

    90 ml of solution A and 60 ml of solution B were mixed in 150 ml double distilled water for 300 ml acetate buffer (P^H=4.7). P^H was adjusted by P^H meter.
2. 1% Starch solution: 1 g starch was dissolved in 100 ml acetate buffer.
3. Potassium sodium tartrate solution (Rochelle salt solution): 40 g of potassium sodium tartrate was dissolved in 100 ml double distilled water.
4. 10mM ice cold $CaCl_2$ solution: 73.5 mg of $CaCl_2$ was dissolved in 100 ml double distilled water and kept in the refrigerator.
5. 1% NaOH solution: 5 g NaOH was dissolved overnight in 500 ml double distilled water.
6. Dinitrosalicylic acid reagent (DNS): DNS reagent was made using 1 g DNS acid, 200 mg crystalline phenol, 50 mg of sodium sulphite in 100 ml of 1% NaOH solution.

Preparation of enzyme extraction

1 g of germinating seed were ground in 6 ml ice-cold $CaCl_2$ solution by mortar and pestle placed in ice bucket and kept in centrifuge tube overnight at 4°C. The sample was centrifuged at 12,000 rpm for 25 min at 4°C. Supernatant was decanted in 20 ml centrifuge tube.

Preparation of standard curve for estimating α-amylase activity

25 mg of maltose was dissolved in 100 ml distilled water for working standard and stored in the refrigerator before use. Standard was prepared by taking 0, 0.25, 0.50, 1.00, 1.50 and 2.00 ml of the working standard. '0' served as blank. A standard graph was prepared by plotting concentration of the standard on the x-axis and absorbance at 560 nm on the y-axis.

Procedure for estimating the α-amylase activity

0.1 ml of enzyme extract was taken and volume was made 1 ml by double distilled water. 1 ml starch solution was added and incubated for 15 min at 27° C. 2 ml DNS reagent was added in all test tubes and kept in boiling water bath for 5 min. 1 ml of potassium sodium tartrate solution was added in warm tubes and kept in water for cooling. Total volume was made 10 ml by adding 5 ml double distilled water. Reading was taken at 560 nm in the Spectronic- 20 colorimeter. For control 2 ml double distilled water was taken in place of starch and enzyme extract. From the graph the amount of amylase present in the sample was calculated using formula:

Calculation

$$\text{Amount of amylase (mg maltose/g fresh wt.)} = \frac{\text{value from graph (mg) X Total vol.of extract (ml)}}{\text{Aliquot sample used (ml) X wt. of the sample (g)}}$$

3.4.19. Protease activity

Protease activity was estimated following the method described by Kunitz (1947).

Reagent required

1. 0.2M Phosphate buffer (P^H=7.6) was made using following reagent:
    i. Solution A: 11.6 g of KH_2PO_4 in 500 ml double distilled water

ii. Solution B: 17.4 g of K_2HPO_4 in 500 ml double distilled water

13 ml of solution A and 67 ml of solution B was mixed carefully for 100 ml phosphate buffer and P^H was adjusted by P^H meter .

2. 1% Casein solution: 1 g casein was dissolved in 100 ml phosphate buffer.
3. 5% TCA: 5 g TCA was dissolved in 100 ml double distilled water.

Preparation of enzyme extraction

1g of germinating seed were ground in 5ml 0.2M-phosphate buffer (P^H=7.6) by mortar and pestle placed in ice bucket and centrifuged at 12,000 rpm for 30 min at 4°C. Supernatant was decanted in 20 ml centrifuge tube.

Procedure for estimating the protease activity

0.1 ml of enzyme extract was taken and the volume was made 1 ml by adding phosphate buffer. Sample tube was pre-incubated at 50°C for 10 min. 1 ml of casein (1%) solution was added in all tube and incubated at 50°C for 2 hour. The reaction was stopped by adding 3 ml of 5% TCA solution and centrifuged at 15,000 rpm for 10 min at 4°C. Supernatant was estimated by Lowry's method with BSA as reference standard. The activity was expressed as mg/ g fresh wt.

3.4.20. Dehydrogenase activity

Dehydrogenase activity was estimated following the method described by Kittock and Law (1968).

Reagent required

1. 0.1M Phosphate buffer (P^H=7.2) was made using following reagent:

i. 0.2M monobasic sodium phosphate (solution A): 2.2996 g of NaH_2PO_4 in 100 ml double distilled water

ii. 0.2M dibasic sodium phosphate (solution B: 2.84 g of Na_2HPO_4 in 100 ml double distilled water

28 ml of solution A and 72 ml of solution B were mixed and diluted to 200 ml by double distilled water and P^H was adjusted to 7.2 with P^H meter.

2. 1% tetrazolium (TZ) solution: 1 g 2, 3, 5 triphenyl tetrazolium chloride was dissolved in 100 ml phosphate buffer.

Procedure for estimating dehydrogenase activity

Magnetically treated and untreated germinating seeds were taken. In maize 3 seed were taken in quadruplicate and bisected longitudinally almost full depth through the midsection and the cut surfaces were spread slightly apart. In sunflower 3 seeds were taken in quadruplicate and bisected entirely through the midsection of the distal half. In case of chickpea 5 embryonic axis were taken in quadruplicate. 1ml of tetrazolium solution was added. The sample tube was incubated in dark at 30°C for 2 hour. The excess solution was drained out and washed thoroughly in distilled water. The seed / axis was soaked in 9ml of methyl cellosolve overnight with shacking till the extraction of red coloured formazan was completed. Extract was decanted and intensity of colour was read as absorption at 480 nm in double beam UV-VIS spectrophotometer (Perkin-Elmer). Methyl cellosolve was used to set the absorption at zero.

3.4.21. Estimation of protein content

The supernatant extracted for enzyme activity was used for the estimation of protein. Protein content was estimated as described by Lowry et al. (1951) using the following reagents.

i. Solution A (2% Na_2CO_3 in 0.1N NaOH): 20 g sodium carbonate and 4 g sodium hydroxide were dissolved in distilled water and volume was made to 1litre.

ii. 1% copper sulphate ($CuSO_4.5H_2O$) solution: 1 g of copper sulphate was dissolved in 100 ml double distilled water.

iii. 2% sodium potassium tartrate solution: 2 g of sodium potassium tartrate were dissolved in 100 ml double distilled water.

iv. Working solution of B: Prepared fresh before use by mixing equal volume of (ii) and (iii).

v. Solution C (Alkaline copper solution): 50 ml of solution A and 1 ml of working solution of B was mixed.

vi. Solution D (1N Folin-Ciocalteu reagent): Equal volume of double distilled water and 2N Folin-Ciocalteu reagent was mixed before use and kept in cold dark-amber coloured bottle.

Preparation of standard curve for protein estimation

50 mg of bovine serum albumin (BSA) was dissolved in 50 ml distilled water. Working standard was prepared by taking 10 ml of stock diluted to 100 ml with distilled water and stored in the refrigerator before use. Standard was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard. '0' served as blank. A standard graph was prepared by plotting concentration of the standard on the x-axis and absorbance at 660 nm on the y-axis.

Procedure for estimating protein

Aliquot (0.1 ml) was taken and volume was made 1 ml by double distilled water in a test tube. Blank was taken without aliquot. 5 ml of solution C was added in all test tubes and allowed to stand for at least 10 min at room temperature. This was followed by the addition of 0.5 ml of solution D. Colour was allowed to develop for 30 min and the absorbance was recorded at 660 nm. Protein content was calculated using the standard curve prepared with bovine serum albumin (BSA). Results were expressed as mg protein/g fresh weight. From the graph the amount of protein present in the sample was calculated.

Calculation

$$\text{Amount of protein (mg /g fresh wt.)} = \frac{\text{value from graph (mg) X Total vol. of extract (ml)}}{\text{Aliquot sample used (ml) X wt. of the sample (g)}}$$

3.4.22. Antioxidant enzyme

Enzyme extraction

1 g each of aged and unaged seed of magnetically treated and untreated seeds were ground in 5 ml ice-cold extraction buffer containing 0.25 ml ice cold phosphate buffer (50m mol/L P^H=7.0), 0.2m mol/L Ascorbate and 1% PVPP by mortar and pestle

placed in ice bucket and kept in centrifuge tube at 4°C. The sample was centrifuged at 15,000 rpm for 30 min at 4°C. Supernatant was decanted in 20 ml centrifuge tube and used for estimation of antioxidant enzymes.

Extraction buffer

5 ml of extraction buffer contained following amount of regent:

Phosphate buffer (P^H=7.0): 2.5 ml

Ascorbate: 0.1 ml

PVPP: 0.05 g

Double distilled water: 2.4 ml

Reagent required

1. 0.1M Phosphate buffer (P^H=7.0) was made using following reagent:
    a. Solution A: 6.8 g of KH_2PO_4 in 500 ml double distilled water
    b. Solution B: 8.7 g of K_2HPO_4 in 500 ml double distilled water

    39 ml of solution A and 61 ml of solution B was mixed carefully for 100 ml phosphate buffer and P^H was adjusted by P^H -meter
2. 10mM Ascorbate solution: 80 mg of Ascorbate was dissolved in 50 ml double distilled water.
3. PVPP: 0.05 g of PVPP was added at the time of grinding.

3.4.23. Procedure for estimating activity of superoxide dismutase (SOD)

SOD activity was estimated following the method described by Yu and Rengel (1999). 3 ml reaction mixture contained 13 mM methionine, 25 µM NBT, 0.1 mM EDTA, 50 mmol /L phosphate buffer (P^H=7.8), 50 mmol /L sodium bicarbonate and 0.1 ml enzyme extract. Reaction was started by adding 2 µM riboflavin and placing the tubes below 15 W fluorescent lamps for 15 min. Reaction stopped by switching off the light and covering the tubes with black cloth. Tube without enzyme developed maximum colour, which served as control. A non-irradiated (kept in dark) complete reaction mixture served as a blank. Absorbance was recorded at 560 nm. One unit of enzyme

activity was taken at that quantity of enzyme, which reduced the absorbance reading to 50 % in comparison with tubes lacking enzymes.

Reagent required

1. 0.1M Phosphate buffer (P^H=7.8) was made using following reagent:
    a. Solution A: 6.8 g of KH_2PO_4 in 500 ml double distilled water
    b. Solution B: 8.7 g of K_2HPO_4 in 500 ml double distilled water
    c. 8.5 ml of solution A and 91.5 ml of solution B was mixed carefully for 100 ml phosphate buffer and P^H was measured by P^H -meter properly.
2. Methionine solution: 0.298 g of methionine was dissolved in 10 ml double distilled water.
3. Nitro blue tetrazolium (NBT) solution: 0.0184 g of NBT was dissolved in 10 ml double distilled water.
4. EDTA solution: 0.055 g of EDTA was dissolved in 50 ml double distilled water.
5. Sodium carbonate: 7.942 g of sodium carbonate were dissolved in 50 ml double distilled water.
6. Riboflavin: 0.0023 g of riboflavin was dissolved in 100 ml double distilled water.

3 ml of reaction mixture contained following amount of regent:

Phosphate buffer:	1.5 ml
Methionine:	0.2 ml
NBT:	0.1 ml
EDTA:	0.1 ml
Sodium carbonate	0.1 ml
Enzyme extract:	0.1 ml
Double distilled water:	0.80 ml
Riboflavin:	0.1 ml

Calculation

Amount of SOD = (OD control-OD sample)X Total volume of extract (ml)

(Units/g fresh wt./min) (Control/2) X Aliquot sample used (ml) X wt. of the sample (g)

3.4.24. Procedure for estimating activity of catalase

Catalase activity was estimated following the method described by Aebi (1984). Reaction mixture contained 50 mmol /L phosphate buffer (P^H=7.0), 15 mmol /L H_2O_2 and 50 µl enzyme extract. Reaction was monitored after each 30 min at 240 nm in double beam UV-spectrophotometer and blanked against pure buffer.

3 ml of reaction mixture contained following amount of regent:

Phosphate buffer (P^H=7.0): 1.5 ml

Enzyme extract: 0.1 ml

Double distilled water: 0.94 ml

H_2O_2: 0.464 ml

H_2O_2 was added at last just before taking the observation.

Reagent required

H_2O_2 solution: 62 µl H_2O_2 was dissolved in 100 ml double distilled water

Calculation

Amount of catalase in sample = 38.07 X ΔA (change in OD value per min)

(µmol/min/g fresh wt.) wt. of the sample (g)

3.4.25. Procedure for estimating activity of peroxidase

Peroxidase activity was estimated following the method described by Castillo et al. (1984). Reaction mixture contained 50 mmol /L phosphate buffer (P^H=7.0), 16 mmol /L guaiacol, 10 µl of H_2O_2 and 50 µl enzyme extract. Reaction was monitored after each 30 min by measuring the activity of guiacol at 470 nm and blank against pure buffer.

3ml of reaction mixture contained following amount of regent:

Phosphate buffer (P^H=7.0): 1.5 ml

Enzyme extract: 0.02 ml

Guiacol: 0.96 ml

Double distilled water: 0.46 ml

H_2O_2: 0.06 ml

H_2O_2 was added at last just before taking the observation.

Reagent required

Guiacol solution: 0.55 ml of guiacol was dissolved in 100 ml double distilled water.

H_2O_2 solution: 1 ml H_2O_2 was dissolved in 100 ml double distilled water.

Calculation

Amount of peroxidase in sample (μmol/min/g fresh wt.) = $\frac{281.9 \times \Delta A \text{ (change in OD value per min)}}{\text{wt. of the sample (g)}}$

3.4.26. Estimation of oil content in seeds

Seed materials were ground to a powder. Powder samples (5 g each) were taken in waterman filter paper closed properly, and placed in the Soxhlet funnel. About 200 ml of petroleum ether was taken in the flat bottom flask. The funnel was fixed over the flask and water condenser was attached. Heater was switched on at 80° C temperature with water tap running. The funnel was refluxed for 6h. The heater was switch off and the apparatus was cooled. The condenser and funnel were detached. The petroleum ether in the flat bottom flask was evaporated in an oven at 75°C. When small quantity (about 10 ml) was left in the flask, it was transferred to weighed beaker (w_1) of 50 ml. The flat bottom flask was rinsed five times with small quantity of ether and transferred to the beaker. Beaker was put in the oven at 75°C overnight till all the ether was evaporated (presence of ether can be detected by its smell). After cooling the beaker the weight was taken (w_2). Difference of (w_1- w_2) gave the oil content. The oil percentage was calculated on the basis of the weight of seed material (Agrawal and Dadlani, 1995).

Calculation

Amount of oil in sample (%) = $\frac{\text{oil content (w1- w2)g x 100}}{\text{wt. of the sample (g)}}$

3.4.27. Estimation of total carbohydrate

Carbohydrate was estimated by anthrone method using the following reagent

i. 2.5N-HCl: 21.8 ml of HCl in 100 ml distilled water.

ii. Anthrone reagent: 200 mg anthrone was dissolved in 100 ml of ice cold 95% H_2SO_4. It was prepared fresh before use

Preparation of standard curve for estimating carbohydrate

100 mg of glucose was dissolved in 100 ml distilled water. Working standard was prepared by taking 10 ml of stock diluted to 100 ml with distilled water and stored in the refrigerator before use. Standard was prepared by taking 0, 20, 40, 60, 80 and 100 µl of the working standard. '0' served as blank. A standard graph was prepared by plotting concentration of the standard on the x-axis and absorbance at 630 nm on the y-axis.

Procedure for estimating total carbohydrate

Seed materials were ground to a powder. Powder samples of maize, chickpea and sunflower (100 mg each) were taken into a boiling tube. It was hydrolysed by keeping in a boiling water bath for 3 hours with 5 ml of 2.5 N HCl. After 3 hours, sample was taken out from the water bath and cooled to room temperature. Sample was neutralized with solid sodium carbonate until the effervescence ceases. The volume was made to 100 ml and centrifuged at 5000 rpm for 25 min, the supernatant was collected. 10 µl (maize and chickpea) and 50 µl (sunflower) aliquots were taken for analysis. The volume was made to 1 ml in all the sample tube by adding distilled water. Blank was also prepared without sample (1 ml distilled water). 4 ml of anthrone reagent was added in all sample tubes and kept in boiling water bath for eight min. The sample tube was cooled rapidly. Green to dark green colour was developed. The reading was taken at 630 nm in the Spectronic-20 colorimeter (Sadasivam and Manickam, 1996). From the graph the amount of carbohydrate present in the sample was calculated using formula:

Calculation

$$\text{Amount of carbohydrate present in sample (\%mg)} = \frac{\text{value from graph (mg) X Total vol. of extract(ml) X100}}{\text{Aliquot sample used (ml) X wt. of the sample (mg)}}$$

3.4.28. Estimation of protein content by micro-Kjeldahl method

The micro-Kjeldahl method is most reliable method for nitrogen estimation. The nitrogen in the protein is determined by digesting the samples in concentrated sulphuric acid in high temperature with catalyst to convert organic and inorganic forms of nitrogen into ammonium sulphate. This salt on steam distillation with excess of NaOH liberates ammonia, which is collected in boric acid solution. The ammonium borate formed is then titrated with standard hydrochloric acid.

Reagents

1. Sulphuric acid, Sp. gr. 1.84, nitrogen free
2. Catalyst mixture: 99.0 g of K_2SO_4, 4.1 g of HgO and 0.8 g $CuSO_4$ were grinded together.
3. Sodium hydroxide and sodium thiosulphate solution: 50 g NaOH and 5 g $Na_2S_2O_3.5H_2O$ were dissolved in 50 ml distilled water and diluted to 100 ml.
4. Boric acid solution: 4 g of boric acid was dissolved in warm distilled water and diluted to 100 ml.
5. 0.02N Standard hydrocholoric acid solution.
6. Mixed indicator: One part of 0.2% methyl red in ethanol was mixed with 5 parts of 0.2% bromocresol green in ethanol.

Procedure for estimating protein content by micro-Kjeldahl method

0.5 g of powdered seed sample was placed in a digestion flask. 1g of catalyst mixture and 5 ml of concentrated sulphuric acid were added. The sample was digested using the digestion unit till the solution became colourless (approximately 40 to 60 min at 370°C). The sample was cooled and minimum quantity of water was added to dissolve the solid. 100 ml conical flask containing 10 ml of boric acid was taken. 2 to 4 drops of mixed indicator dye was added and the flask was placed beneath the condensor with the delivery tip immersed in the solution. The digest was transferred to distillation apparatus and 8 to 10 ml sodium hydroxide-sodium thiosulphate solution was added to the digest and steam distilled until about 20 ml of distillate was collected in the conical flash. The tip was rinsed with water and the distillate was titrated against the standard acid solution

until the first appearance of violet colour, as the end point. A blank was also run containing the same quantities of all the reagents but without sample for every set of nitrogen determination (Anonymous, 1971).

Calculation

Percentage of nitrogen (N) was calculated as follows:

$$\% N = \frac{(\text{ml HCl used in determination} - \text{ml HCl used in blank}) \times \text{Normality of HCl} \times 14 \times 100}{\text{mg sample}}$$

% Protein = %N X Factor for a given cereal (6.25 for maize, chickpea and sunflower).

3.4.29. Statistical analysis of data

The data was analysed using the software SPSS 10.0. For the laboratory experiment, two factor analysis of variance was performed on a split plot randomized complete block design, keeping magnetic field as main plot and duration as sub-plot. The significant level of difference of all measured traits among magnetic fields, duration of exposure and their interaction were calculated. For green house study, one way analysis of variance was carried out on a randomized complete block design and the least significance difference (LSD) among treatments for each trait was calculated. The variance ratio was studied for significance at 1% and 5% level of probability in all cases

Chapter - 4

RESULTS

Results obtained will be discussed under following headings:

4.1 Standardization of magnetic field strength and duration for maximum enhancement in germination characteristics in maize, chickpea and sunflower.

4.2 The field emergence and rooting characteristics of one-month seedling of magnetically exposed seeds of maize, chickpea and sunflower.

4.3 Effect of magnetic field on viability and vigour loss under accelerated ageing condition in maize, chickpea and sunflower seeds.

4.4 Antioxidant enzymes in aged magnetically treated and untreated seeds.

4.5 Effect of magnetic treatment on germination characters and seed leachate conductivity of mid storage (partially aged) seeds of maize, chickpea and sunflower.

4.6 Imbibition kinetics of magnetically exposed seeds during germination using NMR relaxation times.

4.7 Enzymes related to germination (α-amylase, dehydrogenase and protease) in magnetically exposed seeds during germination.

4.8 Seed leachate conductivity of magnetically exposed seeds at different equilibrium RH at 25°C and 35°C.

4.9 Germination characteristics of magnetically exposed seeds at different equilibrium RH at 25°C and 35°C.

4.10 Spin–lattice relaxation time (T_1) and spin–spin relaxation time (T_2) for magnetically exposed seeds of maize, chickpea and sunflower at different equilibrium RH at 25°C and 35°C.

4.11 Equilibrium moisture content at different equilibrium RH and at 25°C and 35°C and characterization of seed water binding in magnetically exposed seeds

4.12 Thermodynamic parameters at different equilibrium moisture content corresponding to different RH and at 25°C and 35°C in magnetically exposed seed

4.1.1. Standardization of magnetic field strength and duration for maximum enhancement in germination characteristics in maize

Exposure of maize seeds to different magnetic field intensities prior to germination increased significantly all the germination related characters, namely, germination percent, speed of germination, shoot and root length, seedling dry weight and calculated vigour indices (Table 4.1.1). The improvement over untreated controlled seeds was 5-9.5% for germination, 9-20 % for speed of germination, 19-87 % for shoot length, 3-34 % for root length and 23-35 % for seedling dry weight. The calculated vigour indices I and II also increased by 25-67 % and 37-44 % respectively. The percent enhancement of different parameters was not linearly related to the field strength of the magnetic field. Among the five magnetic treatments, 100 and 200mT were more effective than others in increasing most of the seedling parameters. Magnetic field exposure time of 1-4 h increased significantly germination characteristics irrespective of the field strength. Even though there was no consistent superiority of a particular duration over others, more than 3h duration was found to reduce some of the germination characters marginally.

The interaction of magnetic field and duration of exposure are given in (Fig. 4.1.1a-h.) In case of germination percent, in most of the field strengths, the enhancement effect was noticed in 1h duration itself. Increasing the duration of exposure seems to reduce the effect in some cases and again there was an increase in germination at 4h duration. Speed of germination was significantly higher than the control in most of the treatments except for 150mT for 1, 2, 3 h exposure, 200mT for 3h exposure and 250mT for 2h exposure. Shoot length of seedling showed significantly greater response for 100 and 200mT fields for all exposure times compared to 50, 150 and 250mT fields. A similar response was seen for seedling root length also with 100 and 200mT fields giving greater response than others for all durations. But seedling dry weight showed a different picture than that was found for seedling length. The greatest values were exhibited by 150mT (2h), 200mT (1h) and 250mT (3h). This was followed by 50mT (3h), 100mT (1h), 250mT (1h). With the exception of 150mT (1,4h) and 200mT (4h), the remaining fields with different exposure times gave similar increment in seedling dry weight compared to control. Seedling vigour based on seedling length and germination percent

showed similar trend as seedling shoot and root length. Both 100 and 200mT fields gave best results for all exposure times compared to other magnetic fields. Seedling vigour based on seedling dry weight and germination percent exhibited highest values for 150mT for 2h exposure and 200mT for 1h exposure. Other fields and exposure times gave similar increase over control with the exception of 150mT (1,4h).

The cumulative germination percentage was found to be higher in magnetically exposed seeds of maize as compared to unexposed control (Fig 4.1.1.2a-e).

4.1.2. Standardization of magnetic field strength and duration for maximum enhancement in germination characteristics in chickpea

Exposure of chickpea seeds to different magnetic field intensities prior to germination increased significantly all the germination related characters, namely, germination percent, speed of germination, shoot and root length, seedling dry weight and calculated vigour indices (Table 4.1.2). The improvement over untreated control seeds was 5-11% for germination, 8-26 % for speed of germination, 12-34 % for shoot length, 5-90 % for root length, 38-57% for total seedling length and 25-47 % for seedling dry weight. The calculated vigour indices I and II also increased by 46-71 % and 32-58 % respectively. The percent enhancement of different parameters was not linearly related to the field strength of the magnetic field. Among the various magnetic treatments, 50mT(2h), 100mT(1h) and 150mT(2h) were more effective than others in increasing most of the seedling parameters. Magnetic field exposure time of 1-4h increased significantly germination characteristics irrespective of the field strength. There was no consistent superiority of a particular duration over others. However exposure for 1h and 2h duration was more effective as compared to 3h and 4h in increasing the germination characters. Also exposure for 250mT was less effective as compare to others. Effect of magnetic fields on speed of germination and seedling growth are shown in (Plate 4.1.1. & Plate 4.1.2.)

The interaction of magnetic field and duration of exposure are given in Fig. 4.1.2a-h. In case of germination percent, in most of the field strengths, the enhancement effect was noticed in 2h duration itself. Exposure for 3h duration seems to reduce the effect in some cases and again there was an increase in germination at 4h duration. Speed of germination was significantly higher than the control in most of the treatments except

for 200mT for 1 and 2h exposure and 250mT for all exposures. Shoot length of seedling showed significantly grater response for 50mT (2h), 100mT(1h) and 150mT(2h) fields compared to other fields. Shoot length was significantly higher than the control in most of the treatments except for 100mT for 3 and 4h exposure, 150mT for 4h and 200mT for 3h exposure. A similar response was seen for seedling root length also with 50mT (2h), 100mT(1h) and 150mT(2h) fields giving greater response than others. The root length and total seedling length was significantly higher than control in all treatments. For seedling dry weight greatest values were exhibited by 50mT(2h), 100mT(1h) and 150mT(2h). Seedling dry weight was significantly higher than the control in all of the treatments except for 150mT for 3 and 4h exposure.

Seedling vigour based on seedling length and germination percent showed similar trend as seedling shoot and root length. 50mT(2h), 100mT(1h) and 150mT(2h) fields gave best results compared to other magnetic fields. Seedling vigour I was significantly higher than the control in all of the treatments. Seedling vigour based on seedling dry weight and germination percent was also significantly higher than the control in all of the treatments except for 150mT for 3 and 4h exposure. Seedling vigour II exhibited highest values for 50mT for 2h, 100mT for 1h and 150mT for 2h exposure.

The cumulative germination percentage was found to be higher in magnetically exposed seeds of chickpea as compared to unexposed control (Fig 4.1.2.2a-e).

4.1.3. Standardization of magnetic field strength and duration for maximum enhancement in germination characteristics in sunflower

Exposure of sunflower seeds to different magnetic field intensities prior to germination increased significantly all the germination related characters, namely, germination percent, speed of germination, shoot and root length, seedling dry weight and calculated vigour indices (Table 4.1.3). The improvement over untreated controlled seeds was 5-11% for germination, 9-15 % for speed of germination, 6-41% for shoot length, 16-80% for root length, 12-57% for total seedling length and 5-13% for seedling dry weight. The calculated vigour indices I and II also increased by 18-74 % and 10-25 % respectively. The percent enhancement of different parameters was not linearly related to the field strength of the magnetic field. Among the five magnetic treatments, 50mT, 200mT and 250mT were more effective than others in increasing most of the seedling

parameters. Magnetic field exposure time of 1-4h increased significantly germination characteristics irrespective of the field strength. Even though there was no consistent superiority of a particular duration over others, 2h duration was found more effective than other in increasing germination characters. Effect of two magnetic fields on speed of germination and seedling growth are given in Plate 4.1.3 & Plate 4.1.4.

The interaction of magnetic field and duration of exposure are given in (Fig.4.1.3a-h). In case of germination percent, in most of the field strengths, the enhancement effect was noticed in 2h duration itself. Speed of germination was significantly higher than the control only in 50mT (2 and 4h) 100mT (1h), 150mT (3h), 200mT (1 and 2h) and 250mT (2 and 3h). Shoot length of seedling showed significantly greater response for most fields and exposure times. However, there was reduction in shoot length compared to unexposed control for some exposures like 100mT (1, 3 and 4h) and 150mT (4h) and 200mT (1h). The highest value was seen in 2h exposure of 200mT followed by 50mT and 250mT fields. A similar response was seen for seedling root length also with 50mT, 200mT and 250mT fields giving greater response than others when exposure time was 2h. But root length was reduced as compared to control for 100mT (1, 3 and 4h), 150mT (4h) and 200mT (1h) exposure fields. Seedling dry weight showed a different picture than that was found for seedling length. The greatest values were exhibited by 50mT, 200mT and 250mT for 2h exposure fields. Also, it was significantly higher than the control in 50mT (1, 2 and 4h) 100mT (2h), 150mT (1h), 200mT (2h) and 250mT (1h and 2h) treatments. Seedling vigour based on seedling length and germination percent showed similar trend as seedling shoot and root length. Seedling vigour based on seedling dry weight and germination percent exhibited highest values for 50mT, 200mT and 250mT magnetic fields for 2h exposure. In case of 100mT exposure, vigour II showed better response due to its greater values of germination percent and seedling dry weight.

The cumulative germination percentage was found to be higher in magnetically exposed seeds of sunflower as compared to unexposed control (Fig 4.1.3.2a-e).

4.2.1 Field emergence and rooting characteristics of one month seedling of magnetically exposed seeds of maize

From the analysis of the data on interaction of magnetic field and the time of exposure, it was found that when all germination characteristics were taken into consideration, best results were obtained with 100mT and 200mT fields for 2h and 1h exposures respectively. Hence further experiments were conducted with these magnetic treatments and the effect on field emergence characteristics, roots and shoot parameters of 1month old plants was studied. The results are given in Tables 4.2.1.1. and Table 4.2.1.2. In magnetically treated seeds, there was significant reduction in the electrical conductivity of the seed leachate (Table 4.2.1.1). The percent field emergence has improved marginally by 5% in 100mT treatment and by 11% in 200mT magnetic treatment compared to control. However, there was no significant increase in field emergence index, which is an index of the speed of seedling emergence in the field. All seedling parameters such as shoot height, shoot and root dry weight and root to shoot ratio of the one month old seedling of treated plants exhibited highly significant increase over the control (Plate 4.2.1). Shoot dry weight increased by about 50% and that of root dry weight by about 14 times in these two treatments. Because of dramatic increase in root weight, the root to shoot ratio was also very high in the treated plants. The root characters of the one month old treated plants also showed interesting results (Plate 4.2.2). Total root length, root surface area, projected area and root volume doubled in plants raised from magnetically exposed seeds as compared untreated control (Table 4.2.1.2.). In case of average diameter, the increase over control was significant for both 100mT(2h) and 200mT(1h) treatments.

4.2.2 Field emergence and rooting characteristics of one month seedling of magnetically exposed seeds of chickpea

From the analysis of the data on interaction of magnetic field and the time of exposure, it was found that when all germination characteristics were taken into consideration, best results were obtained with 50mT (2h), 100mT (1h) and 150mT (2h) magnetic fields. Hence further experiments were conducted with these magnetic treatments and the effect on field emergence characteristics, root and shoot parameters of 1month old plants was studied. The results are given in Tables 4.2.2.1 and Table 4.2.2.2.

In magnetically treated seeds, there was significant reduction (5-13%) in the electrical conductivity of the seed leachate (Table 4.2.2.1). The percent field emergence has increased non significantly marginally by 4% in 150mT (2h) treatment and by 5% in 100mT (1h) magnetic treatment compared to control. However, there was no significant increase in field emergence index (1% for 50(2h) and 100mT(1h) and 6% for 150mT (2h)), which is an index of the speed of seedling emergence in the field. All seedling parameters such as shoot height, shoot and root dry weight and root to shoot ratio of the one month old seedling of treated plants exhibited highly significant increase over the control (Plate 4.2.3). Shoot height increased by 17% in 50mT (2h) and 30% in 100mT(1h). Root length increased by 23% in 50mT (2h) and 38% in 100mT(1h). Total seedling length increased by 12% in 50mT (2h) and 28% in 100mT(1h). Shoot dry weight increased by about 49% in 100mT(1h) to 53% in 50mT(2h) and that of root dry weight by about three times in these treatments. Because of dramatic increase in root weight, the root to shoot ratio was also doubled as compared to control in the treated plants. The number of branches was also significantly increased by 25% in the treated plants. The root characters of one month old treated plants also showed interesting results (Plate 4.2.4). Total root length, root surface area, projected area and root volume doubled in plants raised from magnetically exposed seeds as compared unexposed control (Table 4.2.2.2). However, in case of average diameter, the increase over control was significant only for 100mT(1h) treatment.

4.2.3 Field emergence and rooting characteristics of one month seedling of magnetically exposed seed of sunflower

From the analysis of the data on interaction of magnetic field and the time of exposure, it was found that when all germination characteristics were taken into consideration, best results were obtained with 50mT, 200mT, and 250mT fields for 2h exposures respectively. Hence further experiments were conducted with these magnetic treatments and the effect on field emergence characteristics and roots and shoot parameters of 1month old plants was studied. The results are given in Tables (4.2.3.1 and 4.2.3.2). In magnetically treated seeds, there was significant reduction in the electrical conductivity of the seed leachate (Table 4.2.3.1). The percent field emergence has improved marginally by 5% in 50mT treatment and by 6% in 200mT and 250mT

magnetic treatment compared to control. Field emergence Index was increased by 6% in 200mT and by 10% for 50mT. However, there was no significant increase in field emergence index for 250mT, which is an index of the speed of seedling emergence in the field. All seedling parameters such as shoot height, root length, shoot and root dry weight of the one month old seedling of treated plants exhibited highly significant increase over the control (Plate 4.2.5). Shoot height increased by 7 to 10% and root length increased by 20 to 42%. Shoot dry weight increased by about 83 to 94% and that of root dry weight by 69 to 107% in these treatments. The root characters of the one month old treated plants also showed interesting results (Plate 4.2.6). Total root length increased by 57 to 73 %, root surface area increased by 55 to 81%, projected area increased by 55 to 82%, average root diameter increased by 17 to 35% and root volume doubled in plants raised from magnetically exposed seeds as compared untreated control (Table 4.2.3.2). However, in case of average diameter, the increase over control was non significant for 50mT treatment.

4.3. Effect of magnetic field on viability and vigour loss under accelerated ageing condition

4.3.1. Maize

Seed water content: Changes in the seed water content status with loss in viability of magnetically exposed seeds of maize was studied by keeping the seeds at 40°C and 100% RH. Fig. 4.3.1a gives the change in the water content of the seeds when kept under accelerating ageing condition. Though there was an increase in the seed water content with ageing period, there was no significant difference in seed water content in the magnetically exposed seeds and unexposed seed of maize at same days of ageing.

Seed leachate conductivity: Variation in the electrical conductivity of the seed leachate is given in (Fig. 4.3.1b). Leachate conductivity increased with days of ageing. In general the unexposed seed had higher leachate conductivity than magnetically exposed seeds under accelerated ageing condition throughout the period of ageing. The magnetic treatment of 200mT (1h) had the lowest value for seed leachate conductivity as compared to 100mT(2h).

Germination percentage (%): Change in germination percentage of magnetically exposed and unexposed seeds of maize kept at accelerating ageing condition are shown

in (Fig. 4.3.1c). Both magnetically exposed and unexposed seed lost their germinabiity by 16 days of ageing. Although the initial germination percentage was higher for magnetically exposed seed as compared to unexposed control and it remained higher only up to 12 days of ageing after which the germination percentage reduced sharply in magnetically exposed seed similar to control. Therefore the longevity of the seed is not changed by the magnetic treatment. The reduction in germinability was 15% for 100mT(2h), 22% for 200mT(1h) and 20% for control of initial germinability on 8^{th} day of ageing.

Seedlings dry weight: The change in seedling dry weight with ageing is shown in (Fig. 4.3.1d).There was continuous reduction in the seedling dry weight in both magnetically exposed and unexposed controls. The seedling dry weight for magnetically exposed seed was always higher than unexposed control until 14 days of observation.

Shoot / Root length: The change in shoot and root length of the magnetically exposed and unexposed seed of maize subjected to accelerating ageing are shown in the (Fig.4.3.1e&f). Similar to seedling dry weight shoot and root length had higher value for magnetically exposed seed. After 10 days of observation the shoot length of both magnetically exposed and unexposed seed was drastically reduced, whereas the root length of the magnetically exposed seed declined only after 12 days of ageing which was 2 days later than control

Vigour index I & II: The calculated seedling vigour based on seedling length and germination percent (vigour I) and seedling vigour based on seedling dry weight and germination percent (vigour II) showed similar trend as seedling length and seedling dry weight (Fig. 4.3.1g & h). Both 100(2h) and 200mT(1h) fields exhibited greater values compared to control till 12 days of observation. The decline in vigour II was more than that obtained for vigour I

4.3.2 Chickpea

Seed water content: Changes in the seed water content with accelerated ageing (40°C and 100% RH) is given in Fig.4.3.2a. There was rapid increase in the seed water content during the period of observation. The moisture content of unexposed control was slightly more than the magnetically exposed seed from 2 days of ageing.

Seed leachate conductivity: Variation in the electrical conductivity of the seed leachate of magnetically exposed and unexposed chickpea seeds are given in the (Fig. 4.3.2b). Leachate conductivity increased over the period of observation. In general the unexposed seed had slightly more leachate conductivity than magnetically treated seeds. The leachate conductivity of both treated and untreated seeds increased significantly only after 8 days of ageing.

Germination percentage (%): Change in germination percentage of magnetically exposed and unexposed control seeds of chickpea kept at accelerating ageing condition are shown in (Fig.4.3.2c). Both magnetically exposed and unexposed chickpea seeds lost their germinabiity by 10^{th} days of observation. Therefore the longevity of the seed is not changed by the magnetic treatment. The rapid reduction in germinability was after 6^{th} day of observation in both cases.

Seedlings dry weight: The change in seedling dry weight with ageing is shown in (Fig. 4.3.2d). There was rapid reduction in the seedling dry weight only after 8 days of ageing in both magnetically treated and untreated seeds. The seedling dry weight for magnetically exposed seeds was significantly higher than unexposed control seeds until 8 days of observation.

Shoot / Root length: The change in shoot and root length of the magnetically exposed and unexposed seeds of chickpea subjected to accelerating ageing are shown in the (Fig. 4.3.2e& f). Similar to seedling dry weight, shoot and root length had higher value for magnetically exposed seeds until 8 and 6 days of ageing respectively. Shoot length declined sharply after 8 days in both treated and control seeds whereas root length decreased sharply on 6^{th} day in magnetically treated seeds which was 2 days earlier than controls.

Vigour index I & II: The calculated seedling vigour indices I and II showed similar trend as seedling dry weight and shoot and root length (Fig. 4.3.2g & h). Both 100(1h) and 150mT(2h) fields exhibited higher values compared to control with days of observation.

4.3.3. Sunflower

Seed water content: Changes in the seed water content of sunflower when kept under accelerating ageing condition are given in (Fig. 4.3.3a). There was an increase in the seed

water content during the period of observation and had more values for unexposed than magnetically exposed seeds (50mT(2h) and 200mT(2h)) at different days of ageing.

Seed leachate conductivity: Variation in the electrical conductivity of the sunflower seed leachate are given in the (Fig. 4.3.3b). Leachate conductivity continuously increased over the period of observation in both magnetically exposed and unexposed seeds. In general the unexposed seeds had higher leachate conductivity than magnetically exposed seeds under accelerated ageing condition throughout the period of ageing.

Germination percentage (%): In this study germination of the seeds decreased steadily with artificial ageing in both magnetically exposed and unexposed control seeds. Change in germination percentage of magnetically exposed and unexposed seeds of sunflower kept at accelerating ageing condition are shown in (Fig. 4.3.3c). Both magnetically exposed and unexposed seeds lost their germinabiity by 6^{th} day of observation. Germination percentage was higher for magnetically exposed seed as compared to unexposed control until 4 days of ageing. The reduction in germinability was 32% for 50(2h), 30% for 200mT(2h) and 47% for control from initial germinability on 2^{nd} days of observation. There was rapid decline in germination after 2^{nd} days of observation.

Seedlings dry weight: The change in seedling dry weight is shown in (Fig. 4.3.3d). There was rapid reduction in the seedling dry weight only after 5 days of ageing. The seedling dry weight for magnetically treated seed was always higher than control at all stages of observation.

Shoot / Root length: The change in shoot and root length of the magnetically exposed and unexposed seeds of sunflower subjected to accelerating ageing are shown in the (Fig. 4.3.3e & f). Shoot and root length had higher value for magnetically exposed seeds. There was gradual reduction in the root and shoot length with days of accelerating ageing.

Vigour index I & II: The calculated seedling vigour indices I and II showed similar trend as seedling shoot and root length (Fig. 4.3.3g & h). Both 50mT(2h) and 200mT(2h) fields exhibited higher values compared to control until 3 days of ageing.

4.4. Antioxidant enzymes (SOD, catalase and peroxidase) in aged magnetically treated and untreated seeds

Magnetically exposed and unexposed seeds of maize, chickpea and sunflower (20 each) were subjected to accelerated ageing at high temperature 40°C and high humidity (100%) until they lost their viability by about 30% from the initial value. The activity of antioxidant enzyme was measured in accelerated aged and fresh seeds of maize, chickpea and sunflower.

Soluble protein significantly decreased during artificial ageing in all three crops. The magnetically exposed aged seed had significantly higher protein (18-22%) than unexposed aged seeds of maize (Fig 4.4.1a). In case of chickpea although the magnetically exposed seeds after ageing had more protein (4-5%) than unexposed aged seeds, it was not significant (Fig 4.4.2a). Similar trend was observed in magnetically exposed seeds of sunflower. In sunflower although both treatment 50mT(2h) and 200mT (2h) had higher value of protein (8-10%) in aged seed as compared to unexposed control, only 50mT(2h) was statistically significant as compared to control (Fig 4.4.3a).

The activities of superoxide dismutase (SOD) decreased with ageing in all the crops. In case of maize the activities of SOD decreased significantly (37-46%) with ageing (Fig 4.4.1b). Magnetically exposed aged seed had marginally greater value (3-16%) than that of unexposed aged seed. Exposure of 200mT (1h) had slightly higher activity than 100mT(2h). Similar trend was observed in chickpea. The activity of SOD decreased significantly (5-7%) with ageing (Fig 4.4.2b). Also after ageing the magnetically exposed seed had similar SOD activity for 150mT (2h) and slightly more activity (3%) for 100mT (1h) than that of unexposed aged seed. In case of sunflower the activity of SOD decreased significantly (22-26%) with the ageing (Fig. 4.4.3b). Magnetically exposed aged seed had slightly more (5-7%) activity than that of unexposed aged seed. Exposure of 50mT (1h) had slightly higher activity than 200mT (2h).

The activities of catalase (CAT) also decreased significantly with ageing in all three crops. The decrease was (7-14%) for maize (Fig.4.4.1c), (14-25%) for chickpea (Fig 4.4.2c) and (50-56%) for sunflower (Fig 4.4.3c) seeds. The magnetically exposed seeds of all crops showed slightly more activity than the respective aged unexposed controls.

The activities of peroxidase decreased significantly with ageing in all three crops. The decrease was (8-31%) for maize (Fig.4.4.1d), (17-19%) for chickpea (Fig 4.4.2d) and (31-48%) for sunflower (Fig 4.4.3d) seeds. The magnetically exposed seeds of all crops showed slightly more activity than the respective aged unexposed controls. In sunflower the increased activity of the enzyme in magnetically exposed seeds over unexposed control was significant.

4.5. Effect of magnetic treatment on germination characters and seed leachate conductivity of mid storage (partially aged) seeds of maize, chickpea and sunflower

Seeds of maize, chickpea and sunflower (300 each) were subjected to accelerated ageing at high temperature 40°C and high humidity (100%) until they lost their viability by about 30% from the initial value. This happened in 6 days for maize and chickpea and in two days for sunflower seed. After six days of accelerated ageing for maize and chickpea and two days for sunflower, 100 seeds each were exposed to selected magnetic fields to evaluate the ameliorating effect of magnetic field on age induced adverse changes. They were compared with fresh seeds that were exposed to the same selected magnetic fields. The germination characteristics and seed leachate conductivity of fresh seeds (exposed and unexposed to magnetic field) and partially aged and then exposed to magnetic field along with unexposed aged controls were measured.

4.5.1 Germination characteristics

In maize, magnetic treatment of aged seeds (mid storage) improved the germination by 10-12%, shoot length by 45-52%, root length by 58-68%, total seedling length by 55-57%, dry weight by 24-27% and hence the calculated vigour I and vigour II by 71-74% and 37-42% respectively over aged untreated control (Table 4.5.1). Plate (4.5.1) shows the effect of mid storage magnetic treatment on germination characteristics of accelerated aged seed of maize. In chickpea mid storage magnetic treatment improved the germination by 9-11%, shoot length by 63-69%, root length by 76-80%, total seedling length by 71-74% dry weight by 24-25% and hence the calculated vigour I and vigour II by 90-92% and 37-39% respectively over aged control (Table 4.5.2). In Sunflower mid storage magnetic treatment improved the germination by 10-16%, shoot length by 30-32%, root length by 83-87%, total seedling length by 53-54% dry weight by

6-11% and hence the calculated vigour I and vigour II by 70-79% and 18-29% respectively over aged control (Table 4.5.3).

4.5.2. Seed leachate conductivity

Leachate conductivities of aged seeds were significantly higher irrespective of magnetic treatment in all crops (Fig. 4.5.2 a-c). In fresh seeds, the leachate conductivity decreased significantly in seeds exposed to magnetic field. In aged and then magnetically treated seeds of maize (Fig. 4.5.2a), seed leachate conductivity decreased in 200mT(1h) treatment compared to aged unexposed control. In chickpea, in both magnetic treatments of aged seeds, only marginal decrease in conductivity values were observed compared to aged control seeds (Fig. 4.5.2b). In case of sunflower, exposure to magnetic field of aged seeds has significantly reduced the seed leachate conductivity as compared to unexposed aged controls (Fig. 4.5.2c)

4.6. Imbibition kinetics of magnetic exposed seeds during germination using NMR relaxation times.

The kinetics of seed imbibition was studied to understand the effect of magnetic fields on water uptake in maize, chickpea and sunflower seeds. Water uptake during seed germination showed three phases (i) rapid hydration phase I (ii) lag phase II and (iii) steady hydration phase III. Depending upon the chemical constituents and the seed structure, the duration of these phases was different. The rapid hydration phase I was up to 8 h (Fig. 4.6.1a), 6 h (Fig. 4.6.1b) and 5 h (Fig. 4.6.1c) in maize, chickpea and sunflower respectively. Lag phase II was observed between 8-58 h, 6-36 h and 5-30 h for maize, chickpea and sunflower respectively. The increase in water uptake during the lag phase II was very small in both magnetically exposed and unexposed seed. The third phase, which coincides with the radicle and plumule emergence showed a steady hydration again from 58 h, 36 h and 30 h for maize, chickpea and sunflower respectively. During the rapid hydration phase I the water uptake was same for magnetically exposed and unexposed seed of maize, chickpea and sunflower. The difference in the water uptake in magnetically exposed and unexposed seeds of maize became apparent during the lag phase II and III. The water uptake was more in lag phase II and III in magnetically exposed seed than unexposed seed. Similar trend was observed in magnetically exposed and unexposed seeds of chickpea and sunflower.

4.6.1. Spin lattice relaxation time (T_1) during imbibition

The spin–lattice relaxation (T_1) of magnetically exposed and unexposed seeds of maize, chickpea and sunflower showed an initial decrease when dry seeds were subjected to hydration during germination. This decrease in T_1 was observed both in magnetically exposed and unexposed seeds of maize (Fig. 4.6.2a), chickpea (Fig. 4.6.2b) and sunflower (Fig. 4.6.2c) over a shorter duration of about 6h, 3h and 7h of imbibition. There was increase in T_1 during the subsequent stage of germination. Maize and chickpea seed showed a rapid increase in T_1 up to 12 hours while sunflower showed rapid increase up to 13 hours of imbibition, after which there was no significant increase in T_1 until 72 hours for maize, 48 hours for chickpea and 44 hours for sunflower. After this phase of hydration the seeds of all crops showed a considerable increase in their T_1 values. This corresponds to the steady hydration phase III of the germination. In steady hydration phase III the magnetically exposed seeds had higher value than unexposed control seeds in all three crops.

4.6.2. Spin- Spin relaxation time (T_2) during imbibition

The spin–spin relaxation (T_2) of magnetically exposed and unexposed seeds of maize, chickpea and sunflower showed an initial decrease when dry seeds were subjected to hydration during germination. This decrease in T_2 was observed in magnetically exposed and unexposed seeds of maize (Fig. 4.6.3a), chickpea (Fig. 4.6.3b) and sunflower (Fig. 4.6.3c) over a period of about 18h, 3h and 7h of imbibition. During the subsequent period of observation there was marginal increase in T_2 of both magnetically exposed and unexposed seeds of all crops up to 44 hours for maize, 48 hours for chickpea and 60 hours for sunflower. Then there was substantial increase in T_2 during the subsequent stage of germination. The magnetically exposed seeds had higher value than unexposed control seeds in all three crops in third phase of imbibition.

4.6.3. Spin-spin relaxation time components during imbibition

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. According to the individual values, these 3 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

magnetically exposed and unexposed control seeds of all three crops under study. Both magnetically exposed and unexposed seeds showed a decrease up to 58 h in maize (Fig. 4.6.4a), 44 h for unexposed control seeds of chickpea and 40h for magnetically exposed seeds of chickpea (Fig. 4.6.5a). In case of sunflower the decrease in T_{2a} is up to 36 h in both magnetically exposed and unexposed seeds (Fig. 4.6.6a). Thereafter there was an increase in T_{2a} in both magnetically exposed and unexposed control seeds of all crops. It is interesting to observe that increase in T_{2a} coincides with the sprouting in all the crop seeds.

$T_{2b,}$ which corresponds to cytoplasmic bulk water increased up to 58 h in magnetically exposed and unexposed control seeds of maize ((Fig. 4.6.4b), 44 h in magnetically exposed seeds of chickpea and 48 h of unexposed control seeds of chickpea (Fig. 4.6.5b). In case of sunflower T_{2b} increased up to 36 h in both magnetically exposed and unexposed control seeds (Fig. 4.6.6b). However during subsequent period of germination there was considerable decrease in T_{2b} in all the crop seeds.

Components T_{2c} was not detectable in a dry seed of maize but was resolved at 12 h and 15 h after imbibition in magnetically exposed and unexposed control seeds of maize respectively (Fig. 4.6.4c). In case of chickpea components T_{2c} was resolved after 1h and 2h of imbibition in magnetically exposed and unexposed control seeds (Fig. 4.6.5c). Components T_{2c} was not detectable in a dry seed of sunflower. It was resolved at 5 h and 7 h after imbibition in magnetically exposed and unexposed control seeds of sunflower respectively (Fig.4.6.6c). T_{2c}, which corresponds to the hydration water of macromolecules, initially increased in both magnetically exposed and unexposed control seeds of all three crops. Then it decreased to 2 ms and 5 ms in unexposed control and magnetically exposed seeds of maize respectively; Reduced to 3 ms in unexposed control, to 6 ms in 100mT (1h) and to 4 ms in 150mT (2h) magnetically exposed seeds of chickpea. T_{2c} decreased to 5 ms in unexposed control, to 7 ms in 50mT (2h) and to 8 ms in 200mT (2h) magnetically exposed seeds of sunflower. Thereafter there was increase in T_{2c} in magnetically exposed and unexposed seeds of all the crops. However this third component again was undetectable after 72 h hydration in maize, 48 h in chickpea and 36 h in sunflower.

Fig. 4.6.7a-c, Fig. 4.6.8a-c and Fig. 4.6.9a-c gives the fractional population of different water protons of varying mobilities with hours of imbibition during germination in maize, chickpea and sunflower. In all crops magnetically exposed and unexposed control seeds 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 a reverse trend was observed with only two populations of water and the T_{2a} fraction being larger than T_{2b} fraction.

4.7. Enzymes related to germination (α-amylase, dehydrogenase and protease) in magnetically exposed seeds during germination

α-Amylase activity:

α-Amylase activity was measured in the magnetically exposed seeds of maize, chickpea and sunflower during different stage of germination. Seed exposed to different magnetic field strength such as 100mT (2h), 200mT(1h) for maize, 100mT(1h), 150mT (2h) for chickpea and 50mT(2h), 200mT(2h) for sunflower maintained significantly higher activity of these enzymes as compared to unexposed control in most stages of germination. Changes in activity were similar among maize, chickpea and sunflower that increase in enzyme activity occurred with germination process, reached maximum value and then activities decreased.

In case of maize the α-amylase activity increased significantly until the 64 h of imbibition, reached maximum value, that was 17% higher in 100mT(2h) and 22% higher in case of 200mT(1h) as compared to corresponding value of unexposed control (Fig. 4.7.1a). In case of chickpea the α-amylase activity increased significantly until 48 h of imbibition reached maximum that was 18% higher in 100mT(1h) and 18% higher in case of 150mT(2h) as compared to corresponding values of unexposed control (Fig.4.7.2a). In case of sunflower the maximum activities occurred at 48h after imbibition. The maximum value of α-amylase activities in sunflower was 43% higher in 50mT(2h) and 41% higher in 200mT(2h) as compared to unexposed control (Fig. 4.7.3a).

The soluble protein was significantly decreased during germination in both magnetically exposed and unexposed seeds of all three crops. The magnetically exposed seed had slightly higher protein after imbibition in all stage of germination than unexposed seeds. The decrease in protein was (80-81%) of their initial value in maize

(Fig. 4.7.1b). In chickpea (Fig.4.7.2b) decrease in protein was (43-45%) of the initial value and in sunflower the decrease in protein was (61-63%) to the initial value (Fig. 4.7.3b).

Dehydrogenase activity:

Dehydrogenase activity was measured in the magnetically exposed seeds of maize, chickpea and sunflower during different stages of germination. The dehydrogenase activities were significantly higher in magnetically exposed seeds as compared to unexposed control seeds in most stages of germination in all three crops. Changes in activity were similar among maize, chickpea and sunflower that increase in enzyme activity occurred during germination, reached maximum value and then activities decreased.

In case of maize, the dehydrogenase activity increased significantly until 60h of imbibition, reached maximum value that was 44% higher in 100mT(2h) and 48% higher in case of 200mT(1h) as compared to corresponding values of unexposed control (Fig. 4.7.4a). In case of chickpea the dehydrogenase activity increased significantly until 36h of imbibition, reached maximum value that was 19% higher in 100mT(1h) and 23% higher in case of 150mT(2h) as compared to corresponding value of unexposed control (Fig.4.7.4b). In case of sunflower the maximum activities occurred at 36h after imbibition in magnetically treated seeds while in unexposed control seeds maximum dehydrogenase activity occurred at 42h after imbibition. The maximum value of dehydrogenase activities was 12% higher in 50mT(2h) and 27% higher in 200mT(2h) as compared to corresponding values of unexposed control (Fig. 4.7.4c).

Protease activity:

Protease activity was measured in the magnetically exposed seeds of maize, chickpea and sunflower during different stage of germination. Seed exposed by different magnetic field strengths such as 100mT (2h), 200mT(1h) for maize, 100mT(1h), 150mT (2h) for chickpea and 50mT(2h), 200mT(2h) for sunflower maintained higher activity of these enzymes as compared to unexposed control. Changes in activity were similar among maize, chickpea and sunflower that increase in enzyme activity occurred with germination, reached maximum value and then activities decreased.

In case of maize the protease activity increased significantly until 64h of imbibition reached maximum value, which was 8% higher in 100mT(2h) and 5% higher in 200mT(1h) as compared to corresponding values of unexposed control (Fig. 4.7.5a). In case of chickpea the protease activity increased significantly until 40h of imbibition reached maximum value that was 13% higher in 150mT(2h) and 13% higher in case of 100mT(1h) as compared to corresponding values of unexposed control (Fig. 4.7.6a). In case of sunflower the maximum activities occurred at 24h after imbibition in magnetically exposed seeds as well as in unexposed seeds. The maximum value of protease activities was 22% higher in 50mT(2h) and 15% higher in 200mT(2h) as compared to corresponding value of unexposed control (Fig. 4.7.7a). The protease activities had significantly higher values in magnetically exposed seeds of sunflower as compared to unexposed control seeds in most stages of germination.

4.8. Seed leachate conductivity at different equilibrium RH and temperature of magnetically exposed seeds

The effect of equilibrating seeds to different RH by placing them over salt solution at temperature 25^0C and 35^0C on the seed coat membrane stability of maize seeds exposed to magnetic field of 100mT for 2h duration and 200mT for 1h duration, chickpea seeds exposed to magnetic field of 100mT for 1h duration and 150mT for 2h duration and sunflower seeds exposed to magnetic field of 50mT and 200mT for 2h duration was evaluated by measuring the conductivity of the seed leachate.

The leachate conductivity of the magnetically exposed seeds was lower than unexposed seeds at all relative humidity and at both temperatures in all three crops. At higher temperature (35°C) the conductivity was higher for both unexposed and magnetically exposed seeds due to faster ageing than those equilibrated at low temperature (25°C) in all three crops. The leachate conductivity of all treatments of maize had sharp increase at higher (≥ 75%) as well as at lower relative humidity (≤ 5.5%) (Fig.4.8.1a-b). However, the RH range over which the leachate conductivities were lower was similar for both chickpea and sunflower seeds (Fig.4.8.2a-b, 3a-b). This range was reduced when the equilibrium temperature increased to 35°C. The lowest value of conductivity was found for 29.5% RH at 25°C and for 21-22% RH at 35°C in

maize, chickpea and sunflower. Chickpea had higher value of leachate conductivity as compared to sunflower and maize.

4.9. Germination characteristics of magnetically exposed seeds of maize, chickpea and sunflower at different equilibrium RH at 25°C and 35°C

Under any equilibrium relative humidity the germination for magnetically exposed seeds was slightly higher than unexposed control at both the temperatures in all three crops. The viability was more at 25°C than at 35°C for all humidities. At low equilibrium temperature (25°C) the highest germination percentage in maize seed was about 85% for control, 90% for 100mT(2h) and 200mT(1h) at 29-32% relative humidity (Fig. 4.9.1a). At higher temperature (35°C) maximum germination percentage was 80% for control and 85% for 100mT(2h) and 200mT(1h) at 21-22% relative humidity (Fig. 4.9.1b).

In case of chickpea highest germination percentage at 25°C was 90% for control, 92% for 100mT (1h) and 92.5% for 150mT(2h) at 29-32% relative humidity (Fig. 4.9.2a). Whereas at 35°C maximum germination percentage was 78% for control, 82% for 100mT (1h) and 85% for 150mT (2h) at 21% relative humidity (Fig. 4.9.2b).

The highest germination percentage in sunflower at 25°C was 78% for control, 84% for 50mT(2h) and 85% for 200mT(2h) at 29% relative humidity (Fig. 4.9.3a). Whereas at 35°C maximum germination percentage was 58% for control, 65% for 50mT(2h) and 67.5% for 200mT(2h) at 21% relative humidity (Fig. 4.9.3b).

There was sharp decline in germination at both high and low equilibrium relative humidities. The optimum range of RH for optimal germination in maize was 5.5-78% at 25°C and 11-62% at 35°C. In chickpea optimum range of RH for maximum germination was 8-64% at 25°C and 21-43% at 35°C and in sunflower it was 13-64% at 25°C and 21-43% for 35°C.

The shoot and root length of maize (Fig. 4.9.4a-d), chickpea (Fig. 4.9.5a-d) and sunflower (Fig. 4.9.6a-d) seeds for magnetically exposed seeds was higher than unexposed control at both the temperature under all equilibrium of relative humidity. The shoot and root length had lower value at 35°C than at 25°C. The highest value for shoot / root length was at 21-22% relative humidity for higher temperature (35°C), whereas at

low temperature (25°C) peak value of shoot / root length was at 29-32% relative humidity for both magnetically exposed and unexposed seeds of all three crops. Both high and low equilibrium RH reduced root and shoot length drastically. Similar trends were observed for seedling dry weight and vigour indices I and II.

4.10.1. Spin–lattice relaxation time (T_1) for magnetically exposed seeds of maize, chickpea and sunflower at different equilibrium relative humidity at 25°C and 35°C

The spin–lattice relaxation time (T_1) for magnetically exposed as well as unexposed seeds of maize, chickpea and sunflower equilibrated at high temperature (35°C) was higher than those at low temperature (25°C). Initially there was increase in T_1 value with increase in equilibrium RH at both temperatures and in all three crops. Further increase in relative humidity decreased seed water T_1 value. Spin-lattice relaxation time showed a maximum for maize seed at 7.5% RH when equilibrium temperature was 25°C or 35°C for both magnetically exposed and unexposed seeds (Fig. 4.10.1a-b). In magnetically exposed and unexposed control seeds of chickpea the maximum value of spin–lattice relaxation occurred at 8% RH and 7.5% RH when equilibrium temperature was 25°C and 35°C respectively (Fig. 4.10.2a-b). Spin-lattice relaxation time showed a maximum for sunflower seed at 13% RH and at 11.5% RH when equilibrium temperature was 25°C and 35°C respectively for both magnetically exposed and unexposed seeds (Fig. 4.10.3a-b). The unexposed seed showed slightly higher T_1 value than those of the magnetically exposed seeds of maize, chickpea and sunflower.

4.10.2. Spin–spin relaxation time (T_2) for magnetically exposed seeds of maize, chickpea and sunflower at different equilibrium relative humidity at 25°C and 35°C

Spin–spin relaxation time (T_2) was measured for maize seeds exposed to 100mT for 2h exposure and 200mT for 1h exposure, chickpea seeds exposed to 100mT for 1h exposure and 150mT for 2h exposure and sunflower seeds exposed to 50mT and 200mT for 2h exposure along with unexposed seed at different RH and temperature (25°C and 35°C). A reverse trend was observed in the spin-spin relaxation behaviour of seed water compared to the spin–lattice relaxation time (T_1). At a given RH, spin-spin relaxation values were lesser at higher temperature than at low temperature in both magnetically exposed and unexposed control seeds of all three crops. Initially T_2 increased with increase in RH reached maximum and subsequently decreased sharply in all three crops.

The maximum value for maize was observed at 8% RH at 25°C and at 35°C (Fig. 4.10.4a-b). The maximum value for chickpea (Fig. 4.10.5a-b) and sunflower (Fig. 4.10.6a-b) was observed at 13% RH and 11.5% RH at 25°C and 35°C respectively. Similar trend was observed in magnetically exposed seeds of all three crops.

4.11. Equilibrium moisture content at different relative humidity and characterization of seed water binding in magnetically exposed seeds.

Equilibrium moisture content (mc) of seeds under storage conditions is controlled by two important factors, namely temperature and relative humidity (RH). By equilibrating seeds at different relative humidities and temperatures, changes in the physical status of water associated with the changes in physiological activities were studied in magnetically treated and untreated seeds of maize, chickpea and sunflower. The water sorption characteristics of the seeds vary depending on the temperature and chemical constituents of the seed.

Equilibrium water content

Moisture sorption isotherm for magnetically exposed and unexposed control seeds of maize (Fig. 4.11.1a-b), chickpea (Fig. 4.11 2a-b) and sunflower (Fig. 4.11.3a-b) measured at 25°C and 35°C gave the equilibrium relationships between moisture content (mc) and relative humidity (RH). The extent of water sorption varied with the species. In general it was greater for chickpea and smaller for sunflower. The equilibrium moisture content increased with increase in relative humidity. For all the species the equilibrium moisture content at lower temperature was considerably higher than at higher temperature.

Water binding characteristics

Seed water sorption isotherms, which relates to the seed moisture content with equilibrium relative humidity was developed for magnetically exposed and unexposed seeds of maize, chickpea and sunflower at 25°C and 35°C. All of them showed a typical reverse sigmoidal shape with a three distinct regions of water bindings. The three regions observed in the isotherm belong to strong, weak and multimolecular water binding sites. The analysis of the isotherm at 25°C and 35°C using D'Arcy – Watt equation are given in Table 4.11.1a-b and Table 4.11.1c-d for maize, Table 4.11.2a-b and Table 4.11.2c-d for chickpea and Table 4.11.3a-b and Table 4.11.3c-d for sunflower, which gives the

different parameters of the equation and the number of water binding sites in the strong, weak and multimolecular regions of the sorption isotherms.

In maize the value of K, which signifies the strength of attraction for strong sites, had less value for 200mT(1h) at 25°C (Table 4.11.1a) and more value for magnetically exposed seed at 35°C (Table 4.11.1b) compared to respective unexposed control seeds. At 25°C and 35^0C the magnetically exposed seeds of maize had more weak binding sites and less strong and multimolecular binding sites as compared to the unexposed control seeds (Table 4.11.1c and Table 4.11.1d). The total number of binding sites was greater in unexposed control seeds.

In chickpea the strength of the attraction (K) for strong water binding sites was more for exposed seed at 25°C (Table 4.11.2a) and had less value at 35°C (Table 4.11.2b) than respective unexposed control seeds. The total number of binding sites was greater in unexposed control seeds. At 25°C the magnetically exposed seeds of chickpea had less strong binding sites, more weak binding sites as compared to the unexposed control seeds (Table 4.11.2c). However at 35°C the magnetically treated seeds had more strong and weak binding sites and less multimolecular binding sites as compared to unexposed control seeds (Table 4.11.2d).

In sunflower the strength of the attraction (K) for strong water binding sites was less for magnetically exposed seed at 25°C (Table 4.11.3a) and had less value at 35°C for 200mT(2h) and more value for 50mT(2h) (Table 4.11.3b) than respective unexposed control. The total number of binding sites was greater in unexposed control seeds. At 25°C and 35^0C the magnetically exposed seeds of sunflower had more weak binding sites and less strong and multimolecular binding sites as compared to the unexposed control seeds (Table 4.11.3c and Table 4.11.3d).

The seed moisture distribution among strong, weak and multimolecular binding sites were also calculated from the three parts of the D' Arcy –Watt equation and are presented in Fig.4.11.4a-f for maize, Fig.4.11.5a-f for chickpea and Fig.4.11.5a-f for sunflower. At 25°C and 35°C magnetically exposed seeds of maize had more seed water associated with weak binding sites. However the seed moisture associated with strong and multimolecular binding sites was more in the unexposed control seeds (Fig.4.11.4a-f). With increase in RH there was continuous increase in moisture related to weak

binding sites. In chickpea at 25°C unexposed seeds had more water associated with strong and multimolecular binding sites. However, the weak water binding sites was more in the magnetically exposed seeds (Fig.4.11.5a-c). At 35°C the magnetically exposed seeds had more water related to strong water binding sites as well as weak water binding sites and less multimolecular binding sites (Fig.4.11.5d-f. In sunflower at 25°C and 35°C magnetically exposed seeds of sunflower had more water associated with weak binding sites. However the strong and multimolecular water binding sites was more in the unexposed control seeds (Fig.4.11.5a-f). With increase in RH there was large increase in water related to weak binding sites for treated seeds.

4.12. Thermodynamic parameters at different equilibrium moisture content corresponding to different RH in magnetically exposed seeds

Water activity (a_w), Chemical potential (μ_w) and Water potential (Ψ_w)

The water activity, chemical potential and water potential calculated for magnetically exposed and unexposed seeds of maize, chickpea and sunflower at 25°C and 35°C are presented in (Table 4.12.1a-b), (Table 4.12.2a-b) and (Table 4.12.3a-b). Chemical potential and water potential of magnetically exposed and unexposed seeds equilibrated at different RH and temperature conditions showed variation depending on the condition in which seeds were equilibrated. Chemical potential and water potential decreased with decrease in RH (water activity) and hence the seed moisture content.

Differential Enthalpy (ΔH)

The differential enthalpy (ΔH) for magnetically exposed and unexposed seeds of maize, chickpea and sunflower, calculated from the sorption data using the Clausius Claperon equation at different water contents are shown in Fig.4.12.1, Fig.4.12.2 and Fig.4.12.3. The calculated ΔH is highly negative and for different magnetic treatment the peak negative value was at different water content. The maximum negative differential enthalpy was at 32.5% and 22% relative humidity at 25°C and 35°C respectively. Corresponding water content for maximum negative value in maize seeds was 0.057 g/g for control, 0.058g/g for 100mT(2h), 0.053 g/g for 200mT(1h) (Fig 4.12.1). For chickpea seeds the corresponding water content for maximum negative value was 0.063 g/g for control, 0.062 g/g for 100mT(1h), 0.061 g/g for 150mT(2h) (Fig 4.12.2). And for sunflower seeds, these values were 0.035 g/g for control, 0.034 g/g for 50mT(2h), 0.031

g/g for 200mT(2h) (Fig 4.12.3). In general, the seed moisture content corresponding to minimum differential enthalpy was smaller for magnetically treated seeds as compared to untreated controls. The differential enthalpy decreased with increase in relative humidity reached peak value and than increased with further increase in relative humidity. Similar trend was observed in magnetically exposed seeds.

Gibb's free energy

Gibb's free energy of magnetically exposed and unexposed seeds of maize, chickpea and sunflower are presented in (Fig. 4.12.4a-b), (Fig. 4.12.5a-b) and (Fig. 4.12.6a-b). Gibb's free energy increased with increase in relative humidity for all crops seeds at both temperature 25°C and 35°C. The sharp decrease in Gibb's free energy was for RH less than 64% and 75% in maize, for RH less than 50.5% and 75% in chickpea and for RH less than 75% and 84% in sunflower at 25°C and 35°C temperature respectively. The moisture content corresponding to sharp decline in Gibb's free energy was less at 25°C than at 35°C in all three crops.

Differential entropy

The differential entropy ΔS was calculated as TΔS= ΔH-ΔG for magnetically exposed and unexposed seeds of maize (Fig. 4.12.7a-b), chickpea (Fig. 4.12.8a-b) and sunflower (Fig. 4.12.9a-b). Differential entropy showed a large negative entropy change associated with high negative enthalpy change. The variation in differential entropy was from -0.039 KJ/mol/degree at 32.5% RH to -0.0007 KJ/mol/degree at 85% RH and 0.037 KJ/mol/degree at 22% RH to -0.0006 KJ/mol/degree at 84% RH at 25°C and 35°C respectively. The corresponding water content was different for different crops and different treatments

The maximum germination characteristics were obtained at 32.5% and 22% RH at 25°C and 35°C respectively corresponding to maximum negative differential enthalpy and entropy in all three crops.

Chapter - 2

REVIEW OF LITERATURE

2.1. Seed orientation, germination, seedling growth and yield of magnetically treated seeds

Pittman (1962) observed that winter wheat generally mature 4 to 6 days earlier when seeded in rows oriented north and south than when seeded in rows oriented in east and west. Boe and Salunkhe (1963) indicated that tomatoes placed in a magnetic field ripened faster than controls and that the fruits nearest the south magnetic pole ripened faster than those nearest the north magnetic pole. Pittman (1965) reported that speed of germination and seedling growth of corn (*Zea mays L.)* and beans (*Phaseolus vulgaris L.*) were affected by pre-germination exposure of the dry seed to an introduced magnetic field. Duration of pre-germination exposure as well as temperature and seed orientation during germination affected total visible seedling growth. Bhatnagar and Deb (1977) conducted experiments on germination and early growth and reported that pre germination exposure of wheat seeds (Cv, Sonalika) increased the rate of germination, shoot length, maximum root length and total root length significantly. Chauhan and Agrawal (1977) studied the effect of geomagnetic and introduced magnetic fields on the germination of seeds of Sonalika wheat. The seeds oriented towards geomagnetic south and magnetic north sprouted earlier and showed more growth of coleoptile and roots. Pittman (1977) reported that pre-seeding magnetic treatment of barley *(Hordeum vulgare L.)* seed resulted in seed yield increase in 13 out of 19 field tests. Similarly treatment of spring and winter wheat seed *(Triticum aestivum L.)* resulted in yield increase in 14 out of 23 tests. Otas (*Avena sativa L.)* showed no yield response to magnetic treatment of the seed. Freyman (1980) reported the growth of two barley cultivars grown from magnetically treated and untreated seed. The only apparent effect of magnetic seed treatment was a small stimulation of net assimilation rate (NAR) under controlled environment conditions, between 29 and 57 days after planting. No response to magnetic seed treatment was detected under field conditions. Gubbels, et al. (1982) observed that seed lots of flax (*Linum usitatissimum L.*), buckwheat (*Fagopyrum esculentum Moench*.),

sunflower (*Helianthus annuus L.)* and field pea (*Pisum sativum L.)* exposed to magnetic field produced earlier and more vigorous seedlings in some seed lots and increased the yield of sunflower. Dayal and Singh (1986) reported that the treated tomato cultivar 'Pusa Ruby' plants on an average produced 1.76 more branches / plant at early stages and 1.48 more branches / plant at later stages as compared with control. The increase was maximum in treatment of 1,250 gauss for 15 min. The cultivar 'Pusa Early Dwarf' produced more branches than the control only at early stage. The increase was noticed in magnetic field treatments of 500, 900 and 1550 gauss for 30 min. An increase in the number of primary branches may be a good index to increase fruit yield. The untreated plants of both cultivars grew to a height of 18.63 cm on an average while magnetically treated plants attained a height of 27.5 cm. Kato (1988) reported that the orientation of the maize root in terms of the direction of the magnetic field (from the north to the south-seeking pole) affected the rate of growth of the root. When the direction of root growth was in line with the direction of the magnetic field of 5 k gauss or in the direction opposite to that of the field, growth rates increased by 27% and 22%, respectively, of the growth rate of the controls. When the direction of growth was perpendicular to the direction of the field, the growth rate increased by 15% of that of the control. Kato et al. (1989) reported that the hairy roots of *Daucus carota* and *Atropa belladonna*, which were induced by inoculation with Agrobacterium rhizogenes that harboured the Ri plasmid, were cultured on Murashige and Skoog's solid medium in magnetic fields of 5k gauss or 50 μ gauss. The growth rate of roots exposed to 5k gauss was 25% greater than that of the control (0.01 k gauss) in both types. In the case of *A. belladonna*, the growth rate of the roots cultured in a field of 50 μ gauss was 40 to 56% greater than that of the control (0.5 gauss). Saktheeswari and Subrahmanyam (1989) reported that there was an increase in the number of parenchymatous cells in the root and leaf of paddy, the root hairs were more in number and increased cell division in the roots. They also reported increase in the uptake of calcium ions, which may be responsible for proper root development and prevention of chlorosis of leaves and for enhancement of chlorophyll contents. Increase in the amount of chlorophyll a, b and total chlorophyll contents of the primary and secondary leaves, both in 10 and 14 days old seedlings were observed when fresh paddy seeds exposed to pulsed magnetic field. Exposure of the seeds to pulsed

magnetic field with their germination tip towards east and south was more beneficial than other orientations. Kato (1990) observed that the gravitropic curvature of maize roots incubated in a field of 50 μ gauss was 37% larger than the control value, while the control roots grew more rapidly. Phirke et al. (1990) studied the germination of sunflower crop exposed to different magnetic field of low strength (7-78 gauss) and at different speed of rotation found that the higher speed of rotation (800, 900 and 1000 rpm) enhanced seed germination percentage. Higher speed than these showed reduction in seed germination, whereas at lower speed the results were at par with control. Pietruszewski (1993) reported a positive effect of magnetic field on yield of wheat cultivars. Kiranmai (1994) studied the mutagenic effect of magnetic field in two varieties of *Helianthus annus L*. exposed to1000, 2000 and 3000 G for over 90 min. He found that among the three doses studied, 2000G produced positive mutations in both varieties of sunflower. Alexander and Doijode (1995) found that onion and rice seeds exposed to a weak electromagnetic field for 12h showed significantly increased germination, shoot and root length of seedlings. Phirke et al. (1996) reported using response surface analysis, the optimum magnetic field strength (MFS) for enhancing yield of magnetically treated seed in the field. The range for MFS was 0.072 to 0.128 tesla in combination with seed exposure time (SET) varying from 13 to 27 min. The study revealed that SET was found to be more effective factor than MFS for soybean, cotton and wheat seed sown in the field. An MFS of 0.10 tesla was the optimal level for all three crops and SET optimal levels of 25 minutes were obtained for soybean and cotton but only 13 minutes for wheat seed. The respective yields of the three crops were increased by about 46%, 32% and 35%. It was concluded that at constant rotational speed of the disc and MFS level, SET varies from crop to crop. Hirota, et al. (1999) observed that magnetic forces influence the geotaxis of the cucumber. The observations in this study indicated that the material distribution in the living bodies is affected by the magnetic field. Carbonell et al. (2000) evaluated the seeds of rice (*Oryza sativa L*.) exposed to 150 and 250mT magnetic fields both chronically and for 20 min after seedling emergence. Chronic exposure to a 150mT magnetic field increased ($p<0.05$) both the rate and percentage of germination relative to non exposed seeds (18% at 48h). Significant differences were also obtained for seeds exposed to 250mT magnetic field for 20min (12% at 48h). Additionally, seeds were

moistened with water magnetically treated by static and dynamic methods. Dynamic and static treatment of water improved the germination of seeds related to the control, but significant differences ($p<0.05$) were only obtained for the dynamic method (16% at 48h). Celestino et al. (2000) have reported enhanced sprouting rate, main shoot length, axillary shoot formation, fresh and dry weights of the emerged shoot of *Querus suber* seedlings when exposed to chronic EM field. Moon and Chung (2000) found that the percent germination rates of tomato seed treated with AC electric fields ranging from 4 to 12 kV/cm and AC magnetic flux densities ranging from 3 to 1000 G exposed to 15 to 60 s time were accelerated about 1.1–2.8 times compared with that of the untreated seed. However, an inhibitory effect on germination was shown in the case of the electric field more than 12 kV/cm and the exposure time more than 60 s. Martinez et al. (2000) reported the influence of a stationary magnetic field on the initial stages of barley plant development. When germinating barley seeds were subjected to a magnetic field of 125mT for different times (1, 10, 20, and 60 min, 24 h, and chronic exposure), increases in length and weight were observed. Maximum increases in the measured parameters were obtained when the time of exposure to magnetic field was long (24 h and chronic). Harchand, et al. (2002) reported that field trial of 100 gauss with 40h exposure indicated an increase in plant height, seed weight per spike and yield of wheat. Aladjadjiyan (2002) observed that the magnetic field stimulated the shoot development of maize and led to the increase of the germinating energy, germination, fresh weight and shoot length. Fischer et al. (2004) reported that sunflower seedlings exposed to $16^{2/3}$HZ sinusoidal 20μT (rms) vertical magnetic field showed small but significant increases in total fresh weight, shoot fresh weight and root fresh weight, whereas dry weight and germination rates remained unaffected. Experimentally treated wheat exhibited marginally (but significantly) higher root fresh and dry weights, total fresh weights and higher germination rates. Podlesny et al. (2004, 2005) confirmed the positive effect of the magnetic treatment on the germination and emergence of both broad bean and pea cultivars. The magnetic stimulation of seeds favourably influenced the sprouting and emergence of seed. As a result of the application of this treatment plant emergence was more uniform and took place 2-3 days earlier than the emergence of plant in the control. Pea seedling grown from seeds treated with magnetic field were better formed and the

plants grown from them produced a temporarily bigger leaf surface. The gain in seed yield resulting from the pre-sowing treatment of seeds with a magnetic field for both broad bean and pea was due to the higher number of pods per plant and fewer plant losses in the unit area in the growing season. No significant differences were found in the course of most developmental phases of those plants grown from the treated and non-treated seeds. However, a few days acceleration was reported concerning the maturity of plants obtained from those seeds pre-treated magnetically in comparison to the control. Galland and Pazur (2005) reported the unsystematic manner in which the research on magetoresponse in biology has been carried out in the past and explains presently accepted mechanisms of magnetoreception. He reported two mechanisms for magnetoreception, the 'radical-pair mechanism' consisting of the modulation of singlet-triplet interconversion rates of a radical pair by weak magnetic fields and the 'ion cyclotron resonance' mechanism that centres around the fact that ions should circulate in a plane perpendicular to an external magnetic field with their Larmor frequencies, which can interfere with an alternating electromagnetic field. Rajendra et al. (2005) have observed significant increase in mitotic index as well as ^{3}H-thymidine incorporation into DNA in seeds of *Vicia faba* exposed to 100 μT power frequency electromagnetic field. These are clear indications of enhancement of growth of germinated seedlings exposed to magnetic field. De Souza et al. (2006) observed the effects of pre-sowing magnetic treatments on growth and yield of tomato exposed to full-wave rectified sinusoidal non-uniform magnetic fields (MFs) induced by an electromagnet at 100mT (rms) for 10 min and at 170mT (rms) for 3 min. In the vegetative stage, the treatments led to a significant increase in leaf area, leaf dry weight, and specific leaf area (SLA) per plant. Also, the leaf, stem, and root relative growth rates of plants derived from magnetically treated seeds were greater than those shown by the control plants. In the generative stage, leaf area per plant and relative growth rates of fruits from plants from magnetically exposed seeds were greater than those of the control plant fruits. At fruit maturity stage, all magnetic treatments increased significantly ($P < .05$) the mean fruit weight, the fruit yield per plant, the fruit yield per area, and the equatorial diameter of fruits in comparison with the controls. At the end of the experiment, total dry matter was significantly higher for plants from magnetically treated seeds than that of the controls.

Florez et al. (2007) reported that exposure of maize seed to stationary magnetic field enhanced the germination and early growth of seedlings. The greatest increases were obtained for plants continuously exposed to 125 or 250mT.

2.2. Respiration, biochemical activity and moisture absorption in magnetically treated seeds

Akoyunoglou (1964) observed that the activity of enzyme carboxydismutase increased considerably as the magnet was turned on, while the activity decreased as soon as the magnet was turned off. Pittman and Ormrod (1970) reported that seed of winter wheat magnetically treated before germination respired more slowly, released less heat energy, and grew faster than untreated seed. Pittman and Ormrod (1970) also reported that wheat seedlings grown from magnetically treated seed absorbed more moisture and contained more reducing sugar than untreated seed. Roots and shoots were longer for magnetically treated than untreated wheat.

Pitman (1972) observed an elongation of the primary cortical cells in potato. It was reported to intensify the glycolytic process, activate the Kreb's cycle reactions, and stimulate the activities of oxidase containing heavy metals. Kavi (1977) observed that soybean seed exposed to magnetic fields were found to have increased capacity of moisture absorption and the higher rate of growth was attributed to increased physiological activity when it was subjected to magnetic field. Lebedev, et al. (1977) reported that exposure to a magnetic field increased photochemical activities in a unit of chlorophyll molecule, resulting in an increase in the green pigment of wheat and bean. Tarakanova (1978) reported the after effects of a permanent magnetic field persisted in the Hill reaction of seedlings and in the mature plants. Bhatnagar and Deb (1978) studied the effect of pre-germination exposure of wheat seeds and showed higher respiratory quotient and α-amylase activity compared to control seeds. The result indicated more effective metabolic activity in the magnetically treated seed samples. Pittman et al. (1979) reported that pre-imbibition exposure of quiescent seeds of barley and of hard red winter wheat to a non-homogeneous magnetic field reduced the amount of amylolytic enzyme activity in subsequently imbibed seed parts, whole seed, or very young seedlings. Kavi (1983) observed that by subjecting the ragi (*Eleusine coracana Gaertn*) seed to a magnetic field, its internal potential energy changed and this could be used for

higher yield by fixing suitable strength of magnetic field and exposure time of seeds. The pre-sowing magnetic treatment on wheat cultivars increased the percentage of albumin, starch and gluten in the wheat seed (Pietruszewski, 1993). Francisco (2001) observed significant increase in the rate with which the lettuce seeds absorbed water in the interval 0-10mT of magnetic treatment. An increment in the total mass of absorbed water in this interval was also observed. These results were consistent with the reports on the increase of germination rate of the seeds, and the theoretical calculation of the variations induced by magnetic fields in the ionic currents across the cellular membrane. The fields originate in changes in the ionic concentration and thus in the osmotic pressure which regulates the entrance of water to the seeds. The good correlation between the theoretical approach and experimental results provides strong evidence that the magnetic field alters the water relations in seeds, and this effect may be the explanation of the reported alterations in germination rate of seeds by the magnetic field. Aladjadjiyan and Ylieva (2003) reported that the influence of a stationary magnetic field with induction of 0, 15 T at expositions of 10 min, 20 min and 30 min on tobacco seeds. It was found that the magnetic field stimulates the development of the germ and leads to increasing germination energy and germination.

2.3. Studies on rooting characteristics

Quantification of root growth is necessary to study water and nutrient dynamics in rhizosphere. It forms an important part of models of soil–plant interaction required for management guidance (Hanson et al., 1999). Root characteristics such as mass, length, average diameter, surface area and volume were used to assess the quality of roots and functional size of the root system. Total root mass being easier to measure than root length and surface area, was used frequently to compare root systems (Murphy and Smucker, 1995). However, later on it was seen that root mass alone could not describe many root functions involved in studying plant-soil relationships such as uptake of water and nutrient by roots from soil. It was finer roots with larger length density and surface area which contributed to more water and nutrient uptake from surface as well subsurface soil than the thicker roots, which mainly contribute to the root mass and remained confined to upper surface layer only. Hence for studying soil-water-plant relationship it

is better to use root length density rather than root weight density (Brewster and Tinker, 1970; Raper et al; 1978; Fiscus, 1981 and Box and Ramseur, 1993).

Computer assisted electronic image analysis have made root analysis less time consuming and allowed more accurate and less subjective measurement of root characteristics than the human eye is capable of making (Merrill et al., 2002). Electronic methods acquire images through video camera or optical scanner. Fine roots were underestimated when roots were measured using image analysis as they could not be successfully detected due to their small diameter and near transparency (Burke and LeBlanc, 1988; Pan and Bolton, 1991 and Murphy and Smucker, 1995). Such roots account for a substantial proportion of total root length in a number of species (Merrill et al., 2002). Improvement of lighting source and technical development in scanning technology and development of image analysis software made root length measurement easy and accurate. One such image analysis software developed by RHIZO (Reagent instruments, Quebec) was tested for its accuracy in measuring root length for samples in which root overlap and also for measuring the distribution of length in different diameter classes (Bauhus and Messier, 1999).

2.4. Loss of seed viability during accelerating ageing

Temperature and relative humidity can combine to cause rapid decline in seed quality during storage. The pattern of seed longevity and storage potential of different species have been studied by several workers both for long and medium term under ambient and controlled storage conditions and for short term under accelerated ageing conditions. The accelerating ageing test, in which seed is incubated for a short period (a few days) under high humidity and high temperature conditions, was first developed by Delouche and Baskin (1973) at Mississippi State University, USA for predicting the storability of seed. The accelerating ageing test has been widely used to know the pattern of deterioration and to assess the seed quality in various species (Tekrony et al., 1997). Likhlatchev et al. (1984) concluded that physiological changes in seeds subjected to accelerated ageing were largely similar to those during natural ageing with the main difference being the rate at which they occur. In general, the pattern of seed ageing has been described in terms of its water content during storage. Bound water, water activity, water potential and aqueous glasses are introduced as factors that determine the

mechanisms and kinetics of longevity (Ellis et al., 1989; Roberts and Ellis, 1989; Ellis et al., 1990 and Vertucci and Roos 1990).

The irreversible changes taking place within the seed, leading to its ageing and non viability, are collectively known as deterioration. The possible sequence of events, occurring during the seed ageing has been described by (Roberts, 1972 and McDonald, 1999). Generally it is believed that the damage to cellular membranes is one of the key changes, followed by impairment of cellular repair and biosynthetic processes, reduction in the speed of germination and the seedling growth rate, increased susceptibility to abiotic and biotic stresses, poor field emergence and finally the inability to germinate. The physiological changes, which appear earliest, would be seen most likely to have a causative relation to ageing. The intrinsic factors that are believed to be closely associated with the seed deterioration are: loss of membrane integrity (Priestley and Leopold, 1979; Basavarajappa et al., 1991), alteration of chemical composition (Wilson and McDonald, 1986; Wett laufer and Leopold, 1991), changes in enzyme activities (Agrawal and Kharlukhi, 1987; Bailly et al., 1998 and Ravikumar et al., 1998), depletion of food reserves (Ravikumar et al., 1998) and genetic aberrations (Roberts 1972, Elder and Osborne, 1993). Survival of seeds in dry storage depends more on its moisture content than on any other factor (Justice and Bass, 1978). This dependence is attributed, intuitively to the notion that the physiological reactions may quantitatively increase as the water content increases. Deteriorative reactions also proceed in seeds more readily if the moisture content is higher, and subsequently, the high moisture conditions would constitute threat to the longevity of seed survival (Vertucci 1990). With decreasing water content the molecular mobility reaches a minimum and it was observed that molecular mobility is inversely correlated with storage stability. (Walters 1998). Thus with decreasing water content storage stability increases. The viability equation (Eillis and Roberts, 1980) describes the relationship between the seed longevity and storage environment as,

$$V= K_i\text{-}p/10^{K_E - (C_w \log_{10} m) - (C_H t) - (C_Q t^2)}$$

Where V is the probit or normal equivalent deviate of germination (%), p is the period of storage in days at moisture content m (% fresh weight) and temperature t (°C), K_E, C_w, C_H and C_Q are constants and K_i is the initial viability. This equation incorporates a

negative logarithmic relationship between seed moisture content and longevity. Application of this equation has been shown in several crop species (Ellis et al., 1982). At one temperature, this component relation can be written as

$\log_{10} \sigma = K - Cw \log_{10} m$

Where σ is the standard deviation of the frequency distribution of seed deaths in time (days) and

$K = K_E - C_H t - C_Q t^2$

These models are extremely useful in predicting how long seeds will survive under ambient conditions (RH between 30 and 90%, and temperature between 10 and 40° C).

2.5. Effect of R.H. on seed moisture content

Seed moisture content is directly influenced by the relative humidity and temperature of the surrounding. Both temperature and seed moisture content influences seed metabolism. High relative humidity increases seed moisture content, which results in biochemical event such as increased hydrolytic enzyme activity, enhanced respiration and increase in free fatty acid. High temperature enhanced the rate at which many enzymatic and metabolic reactions occur, causing higher rate of deterioration.

Electrical conductivity

Increased membrane permeability has been reported to result in an increase in electrical conductivity of seed leachate in different crop species with ageing (Dadlani and Agrawal, 1983; Ferguson et al., 1990 and Kalpana and Rao, 1996). Electrical conductance of seed leachate is low in fresh seeds which increases as the ageing period increases, due to loss in membrane integrity during ageing process leading to more loss of electrolytes into the imbibing medium.

The conductivity of the seed steep water is influenced both by seed moisture and soak temperature. Low seed moisture (<10%) increased conductivity readings for mungbean, soybean (Dadlani and Agrawal, 1983) and lotus species (Hampton et al., 1994), which was ascribed to imbibitional damage. Dadlani and Agrawal (1983) and later Hampton et al. (1992) studied the effect of soak temperature and found that at 25^0C soak produced greater conductivity readings than 20^oC soak even though the seed lot ranking was unchanged.

Losses of seed viability are highly dependent on temperature and seed moisture content (Roberts 1972) and may be associated with various biochemical and metabolic alterations that could result in loss of membrane integrity, and impaired RNA and protein synthesis and ATP production. Free radicals and lipid peroxidation are widely considered to be major contributors to seed deterioration (Wilson and McDonald 1986). They generate change in unsaturated fatty acids that affect the structure and functional properties of cell membranes, such as the inactivation of membrane–bound protein and an increase in membrane permeability (Simon 1974).

2.6. Activity of antioxidant enzymes during ageing

Seed deteriorate and loose their germinability during periods of prolonged storage. Factors such as accumulation of toxic compounds, loss of vitamins or hormones, degradation of nucleic acids, proteins or membranes take place (Roberts, 1972). Many studies have been shown that membrane integrity play a key role in seed viability (Simon 1974). The main cause for membrane deterioration would be lipid peroxidation (Parrish, and Leopold 1978). High temperature and high seed moisture further accelerate the seed deterioration (Abba and Lovato, 1999). A large number of reactive oxygen species are generated in the seed during ageing which causes lipid peroxidation (McDonald, 1999). This free radical induced non-enzymatic peroxidation, which has the potential to damage membrane, is the major cause of stored seed deterioration. Some protective mechanisms involving free radical and peroxide scavenging enzymes, such as catalase (CAT), peroxidase (POD), glutathione reductase (GR) and superoxide dismutase (SOD) have evolved within the seed (Bowler et al., 1992, McDonald, 1999). Bailly et al. (1996) reported that the sunflower seeds progressively lost their ability to germinate at 25°C, the optimal temperature for germination, after accelerated ageing was carried out at 45°C and 100% RH. The deleterious effects on the high temperature treatment increased with increasing seed moisture content. Loss of seed viability was also associated with decreases in superoxide dismutase, catalase and glutathione reductase activities. The sunflower seed deterioration during accelerated ageing is closely related to a decrease in the activities of detoxifying enzymes and to increase in lipid peroxidation.

Cellular damage caused by lipid peroxidation might be reduced or prevented by protective mechanisms involving free radical and peroxide scavenging enzymes such as

superoxide dismutase (SOD), catalase, and glutathione reductase (GR). SOD is generally considered as a key enzyme in the regulation of intercellular concentration of superoxide radical and peroxides, which can react to form hydroxyl radicals (Bowler et al., 1992) leading to lipid peroxidation (Gutteridge and Halliwell, 1990). Catalase is implicated in removal of hydrogen peroxide (Fridovich, 1986). The effect of seed ageing has been demonstrated on the activities of different enzymes. Ageing inhibits the activities of peroxide-scavenging enzymes like peroxidase, catalase, superoxide dismutase, ascrobate peroxidase (Jeng and Sung, 1994; Sung and Chiu, 1995 and Bailly et al., 1996). The peroxide scavenging enzymes play a vital role in reducing the damaging effects of peroxide radicals. Hence, a reduction in the activity of these enzymes results in greater–damage caused by radical mediated reaction leading to deterioration of seeds.

2.7. Enzymes during germination

Germination and seedling emergence have a high demand for energy via respiration of seed food reserve. The energy in the chemical bond of the carbohydrate, fat and protein is released by digestion and oxidative phosphorylation. Starches are hydrolyses by α and β amylases to maltose and glucose sugar, fats are hydrolyses by lipases into glycerol and fatty acid. Protease breaks peptide bond of protein molecules yielding amino acid. Bhatnagar and Deb (1978) reported that effect of pregermination exposure of wheat seeds to magnetic fields on α-amylase activity. α-Amylase activity increased with increase in imbibition time reached maximum and than decreased. The activity was higher in magnetically treated seeds. Rajendra et al. (2005) reported that α-amylase activity significantly decreased at 5, 50 and 100μT on day 2 and 4 of growth. Protease showed a significant decreses in activity on day 2 and 4 of growth at 100μT. All living cells of the seed which, respire can reduce a colourless solution of 2, 3, 5–triphenyl tetrazolium chloride into red coloured compound formazan. The reduction of the chemical is achieved by the action of group of enzymes called dehydrogenase. These enzymes are involved in the H-transfer during respiratory activity of the biological systems. Nagarajan et al (2003) reported that the dehydrogenase activity increased in the osmo and halo primed seeds of Asiatic carrot seeds. Pandita et al (2003) also reported that priming increased significantly activities of dehydrogenase in tomato seeds. Saha et

al (1990) reported that priming caused increased amylase and dehydrogenase activity in soybean seeds.

The mobilization of seed storage proteins represents one of the most important post germinative events in the growth and development of seedling. Proteolytic enzymes play central role in the biochemical mechanism of germination (Bewley and Black, 1994; Shewry et al., 1995 and Muntz, 1996). Numerous reports in which increase in activity of proteases are correlated with the breakdown of storage proteins support that these proteases are responsible for protein degradation (Storey and Beevers, 1977; Nandi et al., 1995 and Senyuk et al., 1998). The positive correlation between the developments of proteases with protein depletion suggested the involvement of these proteases in the degradation of proteins in germinating Indian bean. These proteases increased in the early stages of germination and decreased later (Ramakrishna and Ramakrishna Rao, 2005).

2.8. Water in seeds

Water is the major constituent of cells in all-living organisms. Since most biochemical reactions proceed in aqueous solutions, the dynamic state of water affects many physiological phenomena. Water is also an important substrate in many reactions (Buitnik et al., 1998). Its removal can lead to reduced activities due to low substrate concentrations. The most important function is the role of water as the solvent for many biochemical reactions. Loss of water as solvent will reduce the diffusion rate of solute substrates to an active site (Leopold and Vertucci, 1989). Water also affects the intermolecular motions of proteins that are essential for catalytic activities. In plants, water is present in all tissues including the seed propagules. In order to germinate, seeds imbibe water from the surrounding environment (Chai et al., 1998).

For the purpose of cultivation, seeds are stored till they are required for propagation. Seed storage is also essential for the preservation of biological diversity (Walters, 1998). When seeds deteriorate, they lose vigour and become more sensitive to stress before germination. The factors, which determine the rate of this ageing, are temperature and moisture content at which seeds are stored (Fang et al., 1998). The deteriorative reactions proceed at the higher moisture levels in seeds while they may be restrained at low levels. Recent advances in the understanding of the thermodynamic

status of water in seeds and its relationship to reactions, have led to a considerable departure from the intuitive notions of seed deterioration (Desmarchelier, 1999).

2.9. Biophysical characterization of water in seed

Seed water status refers to the measurement of state of water in relation to seed, and it is used in a relative sense (Vertucci and Roos, 1990, 1993 and Vertucci et al., 1994). Water status can be described either by measuring the moisture levels of tissue water content or by measuring the energy status of cell water. Moisture levels of seeds are the important determinant of seed longevity (reviewed by Cromarty et al., 1985). Moisture levels in seeds can be described in several ways: moisture content (mc), water activity (a_w) and chemical potential (μ_w). While related, these parameters have very different thermodynamic implications.

Water / Moisture content

This simply is a measure of the concentration of water in the seed. Tissue water content is generally expressed as a percentage of fresh weight or dry weight of the tissue. Often this quantity is expressed as the amount of water per unit fresh weight of the tissue (Vertucci, 1994).

Water activity

This specifies the relative purity of water, measures how many times as effective as water is at promoting aqueous reaction at a given mc compared with a standard reference state (usually, pure liquid water). Water activity describes the tendency for a chemical reaction involving water at a given temperature; it is always ≤ 1 (Ellis et al., 1989; Roberts and Ellis, 1989; Ellis et al., 1990 and Vertucci and Ross, 1990).

$a_w = (p_o\gamma_o / p\gamma) \cong RH/100$ as $RH = (p_o/p) \times 100$ and $\gamma_0 \approx 1$

where p_o is the water vapour pressure, p is the total pressure of the system and γ is the fugacity coefficient. Thus, RH/100 is the good approximation of water activity of the solution. Water activity of seeds is an intrinsic property, related to the composition, water content and temperature (Walters, 1998).

Chemical potential of water (μ_w)

Chemical potential is viewed as the potential for chemical or physical change (Vertucci and Roos, 1993). The chemical concerned is water and so its chemical potential is referred as the chemical potential of water. Chemical potential of water is the component of free energy (G) of a system.

$$\mu_w \text{ (seeds)} = \mu_w^o + RT \ln (a_w)$$

$$\cong \mu_w^o + RT \ln (RH/100)$$

μ_w^o is the chemical potential of the standard i.e. pure liquid water

Water potential

The parameters "water potential" (Ψ_w) is basic to the study of water relations in plants. Water potential is actually a measure of pressure, which can be derived from the chemical potential (energy) (Walters et al., 1997). By convention, Ψ_w is calculated from μ_w by dividing by molar volume of water (ν) at 20°C and 1 atm (18.048 ml/mole) and by setting the value of $\mu_w^o / \nu = 0$. Thus, the water potential can be calculated as

$$\psi_w = RT \ln (p\gamma/p\gamma_o) / \nu$$

In general, water activity and the chemical potential of water are believed to be the relevant parameters to evaluate the role of water in deteriorative reactions (Ellis et al., 1989, Roberts and Ellis, 1989; Ellis et al., 1990; Vertucci and Roos, 1990; 1993; Vertucci et al., 1994 and Walters, 1998).

2.10. Physical status of water in seed

Discrete changes in metabolic activities with moisture content are to be associated with discrete changes in the physical status of water in seeds. At least, five types of water in seeds are distinguished from the calorimetric and the motional properties (Myers et al., 1992).

(i) At moisture level below 8% (-150 MPa) water association is in ionic sites

(ii) When moisture content is between 8-25% (-150 to -11 MPa), water is in association with hydrophilic sites and catabolic activity starts.

(iii) At moisture levels between 24-45%, the catabolic activities continue unabated and processes utilizing the high-energy intermediates are impaired.

(iv) At about 45% (-3 MPa) protein synthesis ceases and repair processes become inoperative.

(v) When the moisture level are below -1.5 MPa (>70%), tissues no longer grow or expand.

With the moisture contents at which seeds are ordinarily stored, water exists as bound water. The term bound water refers to water associated with a macromolecular surface and is sufficiently structured so that its thermodynamic properties differ from free or bulk water; mostly it is not readily freezable. Vertucci and Leopold (1984) showed that there were at least three types of water from their studies on water mobility and thermodynamics in both protein samples and seeds. The types of bound water are determined by the strength with which it is sorbed to a macromolecular surface.

Type 1 water is believed to "chemi -sorb" to macromolecules through bonding.

Type2 water condenses over the hydrophilic sites of macromolecules

Type 3 water forms bridges over hydrophobic sites

Consequently, type 1 water is extremely tightly bound; type 2 is bound weakly and type 3 water is bound with negligible energy. The affinity of water for seed components can be calculated from the enthalpy of binding (Vertucci and Roos, 1993). Two different methods are used. Using Clausius-Clapyron equation (Vertucci and Leopold, 1987).

$$\Delta H = (R\ T_1\ T_2) / (T_2 - T_1)\ [\ln (a_{w1}/a_{w2})]$$

Where ΔH is the differential enthalpy of hydration, R is the gas constant, T_1 and T_2 are the lower and higher temperature; and a_{w1} and a_{w2} are the relative vapour pressure at T_1 and T_2, respectively. This compares the RH values at which similar wetting are observed at two temperatures.

D'Acry-Watt equation is used to characterize three regions of moisture isotherms to define the thermodynamic properties of binding (D'Arcy and Watt, 1970).

$$W = \{(KK'a_w/[(1+K)a_w]\} + [c\ a_w] + \{kk'\ a_w) / [(1-k)\ a_w]\}$$

Where W is the grams of water sorbed per gram sorbent; a_w is the relative vapour pressure, K and K' are the affinity and number of sites in water binding region 1, respectively; c is the number x affinity of sites in water-binding region 2; and k & k' are the affinity and number of sites in water-binding region 3 (Vertucci and Leopold, 1987). This equation is based on the algebraic separation of a nearly linear region in the middle of the moisture isotherms from the non-linear regions above and below it (Vertucci and Roos, 1990).

These studies emphasize that the water in dry seed is structured and non freezable, which can be separated into 3 regions with distinct affinities. The region with lowest water content has the highest affinity for water. A clear relationship between water binding characteristics and desiccation tolerance has been shown through water sorption isotherms constructed for germinated and un-germinated soybean axes and several species of *Polypodium* with varying tolerance to dehydration (Vertucci and Leopold, 1987). The loss of strong water binding sites and gain of weak binding sites were observed in desiccation intolerant, partially germinated soybean axes compared to un-germinated axes. Sun et al. (1997) have correlated the storage stability of osmo-primed mung bean seeds with the modifications of seed water sorption properties. Similarly, the enhanced germination characters of primed seeds of carrot and tomato have been related to changes in seed water binding properties (Nagarajan et al., 2003, 2005). Chatterjee and Nagarajan (2006) have shown that the intrinsic drought tolerance behaviour of wheat varieties can be attributed to their seed water binding properties and seed coat membrane stability. The tolerant variety showed greater amount of seed water in strong binding sites and higher membrane integrity compared to susceptible variety.

2.11. Water status characterization by Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) is the most convenient spectroscopic technique for non-destructive study of wide varieties of samples having nuclei of non-zero spin. NMR techniques are unique in their ability to monitor several essential material properties related to both structure and dynamics non-invasively. The potential

importance of these techniques to biologists meant that their interests and requirement began to be reflected in the design of NMR equipment and this accelerated the application of in vivo NMR methods and problem solving techniques in plant biology. The wide range of application of NMR in agriculture and food can be classified based on the different types of instrumentation available such as,

1. Low Resolution / Low Field NMR,
2. High Resolution NMR Spectroscopy

   (a) Solution state (b) Solid state (c) In vivo
3. NMR imaging

Amongst these, the applications of low- field NMR have been relatively more exploited because of less expensive and simple instrumentation.

One of the main requirements in agricultural research is to analyze large number of samples for there one or more chemical constituents and physical properties. The agricultural samples are complex and heterogeneous structures composed of many different components like water, fat, protein, carbohydrates etc. Also, in plant –breeding programmes and germplasm evaluation, it is desirable that the samples are not destroyed in the process of analysis so that the identified genetic materials remain viaible for further propagation. Pulsed Nuclear Magnetic Resonance (NMR) is a potential means for developing methods of such analysis and its application in the plant water status are discussed in the following sections.

2.11.1 Basic Principles of pulsed NMR

NMR is a spectroscopic technique that exploits the magnetic properties of the atomic nucleus. Like electrons, nuclei of certain atoms spin and the spinning of these electrically charged particles generate a magnetic field along the axis of spin, so that these nuclei act as tiny bar magnets. The ability of an element/isotope to be detected depends on the magnetic properties of its nucleus and has a characteristic resonance frequency at a given magnetic field strength and can be detected in a NMR spectrometer that in tuned to operate in the correct frequency range.

If such nuclei with magnetic moment (for eg. ^{1}H, ^{31}P, ^{13}C) are placed in an external magnetic field H_0, the majority of the nuclei get aligned to it. The frequency (ω_0) of the precession is given by well-known Larmor equation:

$$\omega_0 = \gamma H_0$$

Where γ is a constant known as gyromagnetic ratio, which is characteristic for each type of nuclei. Therefore, the different types of nuclei in a sample generate NMR signals at characteristic frequencies. The alignment of nuclei along H_0 is exponential with time. Consequently, the magnetization M_t in the sample builds up exponentially to its maximum value M_0 with a time constant T_1 called longitudinal or spin-lattice relaxation time, which is different for different kinds of samples. The magnetization M_t is flipped away form H_0 (Z-axis) when a radio frequency magnetic field H_1 is applied perpendicular to the Z-axis by passing an r.f. current in a coil with its axis in the XY plane at the resonance frequency of a particular nuclei which equals larmor frequency ω_0. The flip angle (α) is given by:

$$\alpha = \gamma H_1 t_w$$

where t_w is the duration of the r.f. pulse. When the duration of the r.f. pulse is such that $\alpha = 90^0$, it is called 90° or $\pi/2$ pulse and when t_w is such that α = 180° it is called 180°or π pulse.

The application of 90° pulse flips the magnetization in a plane perpendicular to z-axis resulting in induction of voltage in the radio frequency coil. This induced voltage, called NMR signal, is proportional to the number of the particular nuclei under study in the sample. After the r.f. pulse, the NMR signal decays with time as the nuclei precess freely without the influence of any field. This process is called Free Induction Decay (FID). The signal in a homogeneous magnetic field decays exponentially with the characteristic time constant T_2 called transverse relaxation time or spin-spin relaxation time.

The availability of water for participation in metabolic activities is closely related to randomness of water molecules (Ratcliffe, 1994). The proton spin–lattice relaxation time (T_1) of water is directly related to translational and rotational motion (randomness)

of water molecules (Bloembergen et al., 1948 and Samuilov et al., 1976; 1979). Therefore T_1 of water in biological tissues may indicate the availability of water for metabolic activities.

A large amount of evidence supporting the structural water hypothesis comes from NMR study of living tissues of animals and plants. Hazelwood (1979) had listed the data on T_1 and T_2 measured at various frequencies ranging from 2.7 MHz to 100MHz using mainly H^1 NMR. Several studies have shown more than one component of T_1 and T_2 (Aksenov et al., 1969; Ratkovic, 1987; Bacic et al., 1992 and Noda et al., 1998).

Changes in the membrane permeability are manifested in the molecular mobility and the biophysical state of water (Millard et al., 1996). These changes are in turn reflected in longitudinal and transverse relaxations of tissue water. Leaf water relaxation properties have been successfully used to develop a screening method for assessing heat and drought tolerance in wheat cultivars (Tiwari et al., 1993 and Maheshwari et at., 1999).

In flag leaf of wheat, the changes in leaf water content with maturity showed similar pattern of changes in T_1 (Gambhir et al., 1997a). However, in seeds, the T_1 values of developing seeds of wheat cultivars decreased in spite of a sharp increase in seed water content during 5 h of imbibition. They assumed that this might be due to active accumulation of storage materials, which in turn affect the relaxation time of cellular water.

The relationship between the imposed water activity and a_w (equilibrium relative humidity), and the conventional water status parameters and the proton spin-lattice relaxation time T_1 of leaf water of pearl-millet were investigated by Gambhir et al. (1997b). With the decreasing a_w, the relative water content and T_1 decreased linearly but other variables including the leaf water potential and leaf water content decreased exponentially. Similar results with leaf water content, relative water content and T_1 in wheat were reported by Nagarajan et al. (1993).

Hydrophilic compounds such as sugar and protein could affect the physical state of water (Nagarajan et al., 1993). T_1 was negatively correlated with soluble protein and sugar in cereal leaves. The spin lattice relaxation time of water protons in wheat and

barley leaves showed at least two fractions, which were linearly related with dry matter and protein content when expressed on per gram water basis (Gambhir et al., 1991).

2.11.2 Characterization of water status in seeds by NMR

Askochenskaya (1982) used the proton NMR method to study the state of water in seeds. Most of the NMR studies of water in seed use either spin-lattice (T_1) or spin-spin relaxation time (T_2). It was found that changes of T_1 during maturation of bean (*phaseoulus vulgare L.*) grains reflected the morphological transformations and water status of the kernel.

Ratkovic et al. (1982) made T_1 measurements to follow the changes of water status in kernels of *Zea mays L*. during maturation. Samuilov et al. (1976) studied in seeds of *Vicia fava L.* at rest and during imbibition. Aksenov et al. (1977) investigated the fractions of water in different seeds from the proton T_2 measurements.

Proton NMR signals in seeds is dependent on hydration level (Di Nola et al., 1991). At low water amount, as it occurs in many native seeds, protons have a restricted mobility, hence the lower signal height as measured by NMR. The hydration process of soaked cowpea was monitored by analysis of the transverse magnetization decay curves of water protons, measured at different times after the addition of water (Brosio et al., 1992). The measured curves do not show a single exponential behaviour. That indicated the presence of more than one type of protons with different transverse relaxation times. The amounts of different components measured at different times after the addition of water showed that the amount of "external water" exponentially decreases, with time to a constant value.

In maize embryos, Bacic et al., (1992) observed the proton NMR spin–spin relaxation time (T_2) of water fractions. Seeds were kept at six different relative humidities (0-65%) i. e., at relatively dry conditions. These T_2 components were $T_{2a} \approx 7$ ms (macromolecules and closely bound water); $T_{2b} \approx$ 15-120 ms (water protons at different humidities) and $T_{2c} \approx$ 100-200 ms (triglycerides). Germination test indicated that those water fractions with the shortest values were essential for maintaining seed viability.

Water uptake in seed of *vigna spp*. by NMR spectroscopy was investigated by Marconi et al. (1993). Water absorbed by the intact seeds was measured at different

intervals using analysis of transverse magnetization decay of water protons. Widely different kinetics of first and higher orders was found amongst the samples. The time required to reach saturation hydration capacity ranged from 3 to 16 h: the total amount of absorbed water ranged from 1.06 to 1.39 g H_2Og^{-1} sample.

Golovina et al. (1993) investigated the state of water in wheat seeds and changes during imbibition by the pulsed NMR. The distribution of bulk and sorbed water changed with the time after the end of the imbibition phase. The trigger role of liquid water in reactivating the metabolism of seed was related to its intercellular localization.

The physical state of water in dehydrating soybean seeds was determined by the NMR relaxation time (T_1) and analysed using AIC (Akaike's information criterion, a procedure for statistical model identification) by Noda et al. (1998). A three- phase regression model was identified as the most appropriate. The first transition occurred at 44% water content and the second at 18%. In the first phase, the water content was 60% at the "physiological maturity" stage, at which time the developmental processes in the majority of seeds either, cease or slow down. In the second phase, the seeds quickly lost the loosely bound water from the cytoplasm during a very short period. In the third phase, a glassy state could represent a useful mechanism to trap residual water molecules and to prevent damaging interactions between the cell components. The three phases demonstrating cytoplasmic water might thus correspond to the desiccation tolerance with different dehydration processes.

The relationship between seed viability and water status in hybrid rice seeds using chemical shift studies of NMR was reported by Jiang et al. (1997). The relative contents of free water and bound water in seeds in terms of chemical shift (ppm) were measured before and after storage. They also observed that the water status especially the free water content was closely associated with the shortage quality of seeds. When rice seeds were stored at 5-6% water content (WC), without free water components, they could display the storage tolerance. However, the seed viability would rapidly fall down if its water components increased. Similar results were observed in corn, sorghum and peanut seeds (Jiang et al., 1997).

From these studies it can be observed that molecular mobility is inversely correlated with storage stability (Walters, 1988). With decreasing water content, the

molecular mobility reaches a minimum, and increases again at very low water content, Minimum mobility and maximum storage stability occur at similar water content. This correlation also suggests that storage stability might be controlled by molecular mobility (Bernal and Leopold, 1998). Long ago, Bloemergen et al. (1948) had shown the use of NMR for the phase transition studies based on the motional properties of water molecules.

Longitudinal and transverse relaxation (T_1 & T_2) behaviour of water protons can be investigated to describe the compartmentation and transport of water in tissues. The mobile and less mobile water molecules are distinguished by their different relaxation times and their relative amounts can be calculated. The relationship between seed viability and water status in rice seeds, using chemical shift studies of NMR was reported by Jiang et al. (1997). The water status, especially the free water content was closely associated with the storage quality of seeds, having an inverse relationship between free water content and storability in case of rice seeds. Nagarajan et al. (2003, 2005) attributed that the better performance of primed carrot and tomato seeds to the modification of seed water binding properties studied through sorption isotherm and redistribution of cellular water during germination studies using NMR relaxation time. Krishnan et al. (2004a, 2004b) characterized the distinct changes in water status between germinating and non-germinating wheat and soybean seeds by nuclear magnetic resonance. They concluded that there was a rearrangement of cellular water during germination and water with medium relaxation times and with relatively less restricted mobility was associated with the germination process. Garnczarska et al. (2007) reported that the components of transverse relaxation time indicate the complex exchange processes taking place between water components inside lupine seed over first 2.5h of hydration, with a distinguished increase in structural water and decrease in other components. This is in favor of the high water absorbing capacity of lupine seeds as related to high protein content.

Table 4.11.3a: Parameters calculated from application of D'Arcy –Watt equation to sorption isotherm of magnetically treated seeds of sunflower at 25°C

Treatment	Sorption sites					
	Strong		Weak		Multimolecular	r fit
	K	K′	C	k	k′	
Control	89.228	0.0155	0.0015	0.6259	0.0954	0.990
50mT (2h)	80.076	0.0122	0.0477	0.7681	0.0371	0.990
200mT (2h)	80.913	0.0108	0.0525	0.8236	0.0271	0.989

Table 4.11.3b: Parameters calculated from application of D'Arcy –Watt equation to sorption isotherm of magnetically treated seeds of sunflower at 35°C

Treatment	Sorption sites					
	Strong		Weak		Multimolecular	r fit
	K	K′	C	k	k′	
Control	63.106	0.0087	0.0312	0.5639	0.0949	0.995
50mT (2h)	64.651	0.0062	0.0669	0.7026	0.0386	0.995
200mT (2h)	59.929	0.0066	0.0750	0.7261	0.0316	0.996

Table 4.11.3c: Number of water binding sites in magnetically treated seed of sunflower at 25°C

Treatment	Sorption sites X 10^{21} (Sites / g dry weight)			
	Strong	Weak	Multimolecular	Total
Control	0.518	0.016	3.194	3.728
50mT (2h)	0.408	0.500	1.241	2.149
200mT (2h)	0.361	0.550	0.906	1.821

Table 4.11.3d: Number of water binding sites in magnetically treated seed of sunflower at 35°C

Treatment	Sorption sites X 10^{21} (Sites / g dry weight)			
	Strong	Weak	Multimolecular	Total
Control	0.291	0.186	3.174	3.651
50mT (2h)	0.201	0.399	1.292	1.891
200mT (2h)	0.221	0.447	1.057	1.725

ABSTRACT

A study was carried out for standardizing the static magnetic field and duration for maximum enhancement in germination characteristics of seeds of different chemical constituents. The effects of the standardized magnetic field exposure of seeds on field emergence, shoot and root characteristics, quality changes during accelerating ageing were studied. Biophysical and biochemical characterization of magnetically treated seeds were also undertaken to elucidate the mechanism of magnetic field enhancement during germination and storage. Maize (Var. Ganga Safed-2), chickpea (Var. Pusa 1053) and sunflower (Var. KBSH-1) were used as the experimental material and magnetic field exposure was done using the electromagnetic field generator "Testron EM-20" with variable magnetic field strength (50 to 500mT). The seeds were exposed to different magnetic fields of strength from 0 to 250mT in steps of 50mT for different duration from 1 hour to 4 hour in steps of 1 hour for all fields. Initial experiments were conducted to standardize magnetic field and duration for maximum enhancement in germination of maize, chickpea and sunflower. Results indicate that magnetic field application enhanced the seed performance in terms of laboratory germination, speed of germination, seedling length and seedling dry weight significantly compared to unexposed control. But the response varied with field strength and duration of exposure without any particular trend. Among the various combinations of field strength and duration best results were obtained with 100mT(2h) and 200mT(1h) in maize with 50mT(2h), 100mT(1h) and 150mT(2h) in chickpea and with 50mT, 200mT and 250mT for 2h exposure in sunflower. Exposure of seeds to these magnetic fields improved seed coat membrane integrity as it reduced the cellular leakage electrical conductivity. In the field, seeds exposed to these treatments showed increased field emergence and significantly increased seedling dry weights of one-month-old plants. The root characteristics of the plants showed dramatic increase in root length, root surface area and root volume. In chickpea there was increase in the number of branches from the magnetically treated seeds.

During artificial ageing magnetically treated seeds had lower leachate conductivity than unexposed control. Age induced reductions in seedling dry weight, shoot /root length, vigour I, vigour II of magnetically exposed seeds were lower than

unexposed control seeds. Magnetic treatment on aged seeds of maize, chickpea and sunflower improved the germination characteristics over aged control. Seeds exposed to different magnetic field strength maintained higher activity of antioxidant enzymes in aged seed as compared to unexposed control. The magnetically exposed seed had higher protein after ageing than unexposed aged seeds. In steady hydration phase III, the magnetically exposed seeds had higher value of spin–lattice relaxation time (T_1) and spin–spin relaxation time (T_2) than unexposed control seeds in all three crops. Proton NMR spin-spin relaxation time (T_2) of magnetically exposed and unexposed control seeds showed two different fractions of slowly exchanging water in seed in the initial periods of imbibition. During the subsequent process of imbibition seed water proton could be classified into a three-component system and after sprouting water protons re-organised into two different fractions. In magnetically exposed seeds, the third component corresponding to hydration water appeared earlier than unexposed control seeds. α-Amylase, dehydrogenase and protease activities were significantly higher in magnetically exposed seeds as compared to unexposed control seeds during germination.

Equilibrating over different relative humidity, the germination characteristics of magnetically exposed seeds were higher than unexposed control. The leachate conductivity of the magnetically exposed seeds was lower than unexposed seeds at all relative humidities. Analyzing water sorption isotherms showed that magnetically exposed seeds had less strong and multimolecular binding sites and more weak binding sites as compared to the unexposed seeds. The germination characteristics were maximum corresponding to maximum negative differential enthalpy and entropy in all three crops.

Hence the exposure of seeds of maize, chickpea and sunflower to specific magnetic fields was found to enhance seedling growth and also reduce the seed deterioration during ageing. The improved functional root parameters suggested that the magnetically treated seeds could be used profitably under rainfed conditions. An increase in the number of branches from the magnetically treated chickpea seeds may be a good index to increase yield. The enhancement effect could be partly explained on the basis of cellular water distribution and activity of germination related and antioxidant enzymes.

Chapter - 6
SUMMARY & CONCLUSION

Experiments were carried out to standardize the intensity and duration of exposure to static magnetic field for improving germination and field emergence characteristics in seeds of different chemical constituent (maize, chickpea and sunflower). The positive input of magnetic field on seeds was characterized for their effect during accelerated ageing and during germination process based on biophysical and biochemical parameters. Seed water binding properties were calculated from water sorption isotherms generated for magnetically exposed seeds to further support the observed enhancement. From the study, the following conclusions were drawn:

1. Exposure of dry seeds of maize, chickpea and sunflower to static magnetic field ranging from 0 to 250mT for a period of 1-4h increased laboratory germination, speed of germination, seedling length and dry weights significantly compared to unexposed control. But the response varied with field strength and duration of exposure with no particular trend. The observation that certain combinations of magnetic field and duration were highly effective in enhancing most of the germination characters suggests that there may be a resonance like phenomena which increases the internal energy of the seed.
2. Among the various combinations of field strength and duration, 100mT(2h), 200mT(1h) for maize, 100mT(1h), 150mT(2h) for chickpea, 50mT(2h), 200mT(2h) for sunflower gave best results.
3. The seeds exposed to these specific treatments showed increased field emergence percent and significantly increased shoot and root length, shoot and root dry weights of one month old plants. The root characteristics of the plants showed dramatic two fold increase in root length, root surface area and root volume.
4. In all three crops the decreased viability and vigour with days of artificial ageing was partially ameliorated in magnetically exposed seeds with significantly less reduction compared to untreated controls. Similarly, magnetically treated seeds had lower leachate conductivity than unexposed control during ageing process which indicated maintenance of greater membrane integrity.

5. Activities of antioxidant enzymes, viz., superoxide dismutase, catalase and peroxidase were reduced and level of soluble protein was decreased in aged seeds. Seed exposed to different magnetic fields strength maintained greater activity of these enzymes and higher levels of soluble proteins compared to unexposed aged and fresh controls in all three crops.
6. During mid storage (partially aged), seeds exposed to magnetic field had greater germination percent, seedling length and seedling dry weight than aged unexposed control in all three crops.
7. The imbibition kinetics during germination showed that the water uptake was significantly greater in lag phase II and rapid hydration phase III in magnetically exposed seed than unexposed seed in all three crops. In phase III hydration, the magnetically exposed seeds had higher value of spin–spin relaxation (T_2) and spin–lattice relaxation (T_1) than unexposed control seeds implying higher molecular mobility of seed water and better availability for metabolic activities.
8. The component analysis of seed water spin-spin relaxation time T_2 showed three populations of water protons with varying relaxation times. With imbibition, the fraction representing free water with long T_2 decreased and that representing cytoplasmic bulk water and hydration / bound water together increased. When radicle protrusion took place, the bound water fraction disappeared and free water increased. Interesting observation in this study was the early appearance of hydration water in magnetically treated seeds than untreated seeds in all crops. Hence, this early hydration of embryo may be responsible for better and faster germination of the treated seeds.
9. α-Amylase, dehydrogenase and protease enzyme activities and total soluble protein content of magnetically exposed seeds were significantly higher than unexposed control seeds at most stages of germination process in all three crops. The higher activities of hydrolyzing enzymes were responsible for higher germination and vigour of the treated seeds.
10. Irrespective of magnetic treatment, seeds equilibrated at 35^0C had less germination and vigour related traits and more leachate conductivity than those equilibrated at 25 ^{0}C.

11. At all equilibrium RH of 25 and 35 ^{0}C, germination characteristics of seeds exposed to magnetic field were higher than unexposed seeds. This may be explained as due to better membrane integrity maintained by the treated seeds as indicated by relatively low leachate conductivity of these seeds.
12. Irrespective of magnetic treatment, the spin-lattice relaxation time (T_1) of seeds of maize, chickpea and sunflower equilibrated at high temperature (35°C) was higher than those at low temperature (25°C), which may be attributed to increased randomness of the water molecules. A reverse trend was observed in the spin-spin relaxation (T_2) behaviour of seeds compared to the spin–lattice relaxation time (T_1). As the temperature increases exchange increases and contribute more to T_2 relaxation and hence T_2 decreases with increase in temperature
13. Sorption isotherm of seeds is function of their major chemical constituents. Maize and chickpea seeds with higher amount of starch and protein which are hydrophilic absorbed more moisture than lipid containing sunflower seed at the same equilibrium relative humidity.
14. Analysis of water sorption isotherms revealed that magnetic exposure decreased the number of strong and multimolecular binding sites and increased weak binding sites. This might have increased the molecular mobility of seed water and its availability for various biochemical reactions involved in the germination process.
15. The differential enthalpy decreased with increase in relative humidity reached a minimum value and than increased with increase in relative humidity. Differential entropy showed large negative change associated with high negative enthalpy change. Similar trend was observed in magnetically exposed seeds.

FUTURE STUDY

1. The study should be extended to the field and the effect of these magnetic field exposures on yield and yield components in these three crops should be evaluated.
2. These studies can be extended to other crops and the positive effects of static magnetic field if present can be evaluated.

3. The usefulness of the large improvement in root characteristics of plants from magnetic field exposed seeds can be tested for their tolerance to drought by subjecting them to soil moisture stresses of different levels.
4. Water use efficiency and Nutrient use efficiency can be studied in magnetically exposed seeds of different crops to exploit the increased root development of the treated seeds.
5. Protein profile and rate of DNA/RNA synthesis can be studied during germination in the magnetically exposed seeds to understand the molecular mechanism of the enhancement effect.

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Table 4.11.2a: Parameters calculated from application of D'Arcy –Watt equation to sorption isotherm of magnetically treated seeds of chickpea at 25°C

Treatment	Sorption sites					
	Strong		Weak		Multimolecular	r fit
	K	K′	C	k	k′	
Control	7.172	0.0562	0.0226	0.6132	0.0976	0.993
100mT (1h)	10.654	0.0385	0.0496	0.5640	0.1065	0.995
150mT (2h)	14.607	0.0365	0.0617	0.5838	0.0907	0.995

Table 4.11.2b: Parameters calculated from application of D'Arcy –Watt equation to sorption isotherm of magnetically treated seeds of chickpea at 35°C

Treatment	Sorption sites					
	Strong		Weak		Multimolecular	r fit
	K	K′	C	k	k′	
Control	63.750	0.0247	0.0252	0.6572	0.0684	0.989
100mT (1h)	16.154	0.0356	0.0263	0.6849	0.0572	0.996
150mT (2h)	14.421	0.0341	0.0350	0.6035	0.0638	0.995

Table 4.11.2c: Number of water binding sites in magnetically treated seed of chickpea at 25°C

Treatment	Sorption sites X 10^{21} (Sites / g dry weight)			
	Strong	Weak	Multimolecular	Total
Control	1.87	0.24	3.27	5.38
100mT (1h)	1.27	0.52	3.56	5.35
150mT (2h)	1.22	0.65	3.04	4.91

Table 4.11.2d: Number of water binding sites in magnetically treated seed of chickpea at 35°C

Treatment	Sorption sites X 10^{21} (Sites / g dry weight)			
	Strong	Weak	Multimolecular	Total
Control	0.83	0.15	2.89	3.87
100mT (1h)	1.19	0.16	1.91	3.26
150mT (2h)	1.14	0.21	2.14	3.49

Chapter - 5

DISCUSSIONS

5.1. Influence of pre-germination static magnetic field exposure of maize, chickpea and sunflower seeds on germination and early growth characteristics

Results of exposure of maize, chickpea and sunflower seeds to different magnetic fields showed that there was overall stimulating effect of magnetic field with respect to all germination characters (Table 4.1.1, Table 4.1.2 and Table 4.1.3). Such enhanced performance of seeds in their germination characteristics have been reported (Pittman, 1965; Gubbels, 1982; Kato, 1988; Phirke, 1990**;** Aladjadjiyan, 2002; Fischer, 2004 and Florez, 2007). But the mechanism for such an increase has not been completely understood. In wheat, Pittman and Ormrod (1970) reported that the seedlings grown from magnetically treated seed absorbed more moisture, respired more slowly, released less heat energy and grew faster than the untreated controls. In soybean, Kavi (1977) observed that the seeds exposed to magnetic field had increased capacity to absorb moisture. The increased physiological activity due to greater absorption of moisture by treated seeds may be responsible for the increase in seedling length, seedling dry weight and vigour indices in our study. But some fields were more effective than others and there was no linear increase with increase in field strength. In the same way, the response to exposure time also varied and no direct relation between improvement in seedling parameters and time of exposure was observed. However, Florez et al. (2007) reported that the accumulation of dry weight of 10 day old seedling from seeds exposed to magnetic field increased logarithmically with duration of magnetic field induction. In their study the duration of magnetic field induction was increased from 1 minute to 24h and also continuous exposure was used whereas we have gone until 4h of exposure only. Moreover, the seeds in their study were imbibed in water before the exposure to magnetic field whereas dry seeds were exposed to magnetic field in our study. Fischer et al. (2004) reported that sunflower seedlings exposed to magnetic field showed small but significant increases in total fresh weight, shoot fresh weight and root fresh weight, whereas dry weight and germination rates remained unaffected. Experimentally treated wheat exhibited marginally (but significantly) higher root fresh and dry weights, total

fresh weights and higher germination rates. Kiranmai (1994) found that 2000G magnetic field produce positive mutations in sunflower.

The interaction of magnetic field and exposure time indicated that certain combinations of magnetic field and duration were highly effective in enhancing most of the germination characters compared to other combinations (Fig. 4.1.1a-h, Fig.4.1.2a-h and Fig.4.1.3a-h). This observation suggests that there may be a resonance like phenomena which increases the internal energy of the seed and that occurs when there is appropriate combination of magnetic field and exposure time. In ragi (*Eleusine coracana Gaertn*) seeds, exposure to magnetic field changed its internal potential energy which could be used to get higher yields by suitably selecting the magnetic field and exposure time (Kavi, 1983).

The reduced conductivity of leachate from seeds exposed to magnetic fields compared control (Table 4.2.1.1, Table 4.2.2.1 and Table 4.2.3.1) indicated greater seed coat membrane integrity of these seeds. However, exposure of barley seeds to 37.5 mT field had no effect on leakage of cellular electrolytes (Gusta et al., 1978). In field studies, percent emergence was not affected by magnetic field exposure albeit the field emergence index was increased compared to control. This is in accordance with our laboratory observation where the speed of germination was increased due to magnetic treatment. Similar enhancement in speed of germination due to magnetic field exposure of seeds has been reported in maize (Florez et al., 2007), in rice (Carbonell et al., 2000), and in wheat (Bhatnagar and Deb, 1977). Most useful observation was the dramatic increase in shoot and root weights of one month old plants and the greatly improved root characteristics in the plants from magnetically treated seeds. Rajendra et al. (2005) have observed significant increase in mitotic index as well as ^{3}H-thymidine incorporation into DNA in seeds of *Vicia faba* exposed to 100 µT power frequency electromagnetic field. These are clear indications of enhancement of growth of germinated seedlings exposed to magnetic field. It is postulated that the ion-cyclotron resonance may interfere with the Ca^{2+} ion sequestering and thereby enabling the raise in free Ca^{2+} concentration in the system. The increased Ca^{2+} concentration may signal the cell to enter into early mitotic cycle. Also, increased uptake of Ca^{2+} ions in rice seedlings grown from seeds exposed to pulsed magnetic field was found responsible for better leaf growth, meristematic tissues

in stems and roots (Saktheeswari and Subrahmanyam, 1989). There was significant increase in number of branches from the magnetically treated seeds of tomato. An increase in the number of primary branches may be a good index to increases fruit yield (Dayal and Singh, 1986). Podlesny et al. (2004, 2005) confirmed the positive effect of the magnetic treatment on the germination and emergence of both broad bean and pea cultivars. The magnetic stimulation of seeds favourably influenced the sprouting and emergence of seed. As a result of the application of this treatment plant emergence was more uniform and took place 2-3 days earlier than the emergence of plant in the control. Pea seedling grown from seeds treated with magnetic field were better formed and the plants grown from them produced a temporarily bigger leaf surface. The gain in seed yield resulting from the pre-sowing treatment of seeds with a magnetic field for both broad bean and pea was due to the higher number of pods per plant and the fewer plant losses in unit area in the growing season.

5.2 Effect of magnetic field on viability and vigour loss under accelerating ageing conditions

The rate of decrease in germination percentage was different for the three crops tested. Among the three species the fall was faster in sunflower followed by chickpea and maize, confirming the pattern of poor storability for sunflower and chickpea and good storability for maize seed (Agrawal, 1980). Maize and chickpea showed same decrease in germination until the 6^{th} day of ageing. A sudden drop was observed in chickpea beyond 6 days of ageing. Though the pattern of deterioration was typically sigmoid for all species (Fig. 4.3.1c, 2c and 3c), the slope was greater for chickpea and sunflower than maize seeds.

Membrane permeability: Earliest symptoms of seed ageing are enhanced leakage of solutes (Jung and Sung, 1994 and McDonald, 1999). In the present study a sharp increase in the solute leakage could be seen in seeds. This confirms that measurement of solute leakage is a good indicator of the physiological status of the seed in terms of its viability and vigour (McDonald, 1999). The decrease in germinability related well with increased electrolyte leakage (Fig. 4.3.1b, 2b and 3b), thus reflecting a loss in membrane integrity. Seed viability loss is often attributed to the loss of integrity of the plasmalema (Bernal and Leopold 1998; Bewley and Black 1994). This results in increase of leachate solutes

from the seed as it undergoes ageing. Golovina and Tikhonov (1994) have shown that the loss of germinability with ageing coincided with considerably increased plasma membrane permeability.

In magnetically treated seeds, all the three crops showed decrease in seed water content and seed leachate conductivity with days of ageing as compared to unexposed controls. The significantly lower values of leachate conductivity indicate the maintenance of membrane integrity in treated seeds as compared to untreated controls during ageing process. This may be the reason for marginally higher germination and significantly greater seedling characteristics in magnetically exposed seed subjected to ageing. Similar improvement in germination and vigour characteristics had been observed with associated reduction in seed leachate conductivity in okra seed conditioned to low moisture levels (Nagarajan et al., 2004).

5.3 Effect of magnetic field on antioxidant enzymes in aged seeds

In the presence of oxygen, ageing of seed can lead to peroxidative change in polyunsaturated fatty acid (PUFA) (Stewart and Bewley, 1980; Wilson and McDonald, 1986). This free radical induced non- enzymatic peroxidation may lead to membrane damages and is likely to cause seed deterioration (Jung and chiu, 1995). Protective mechanism that could scavenge the peroxidatively produced free radicals and peroxides prevail within the cellular system to minimize seed deterioration reactions. Superoxide dismutase, peroxidase and catalase enzyme system provide one such protective mechanism and inhibition of activities of these enzymes is reported to cause faster deterioration of seed (Jung and Chiu, 1995).

Loss of seed viability of maize (Fig. 4.3.1c), chickpea (Fig. 4.3.2c) and sunflower (Fig. 4.3.3c) during accelerated ageing at 40°C and 100% RH was associated with a decrease in the activities of SOD (Fig 4.4.1b, 2b and 3b), catalase (Fig 4.4.1c, 2c and 3c) and peroxidase (Fig 4.4.1d, 2d and 3d) in both magnetically treated and untreated control seeds. The decrease in activities of SOD, catalase and peroxidase were related with an increase in the seed leachate conductivity in all three crops (Fig. 4.3.1b, 2b and 3b).

The protein content in the aged seed decreased significantly in maize (Fig 4.4.1a), in chickpea (Fig 4.4.2a) and in sunflower (Fig 4.4.3a) irrespective of magnetic treatment.

This may be due to the ageing induced deterioration in soluble protein (Wettlaufer and Leopold, 1991, Sun and Leopold, 1995).

SOD activity was detected in both aged and unaged seeds of all three crops and the value was lowest in sunflower seeds. The greatest reduction due to ageing was observed in maize seed. SOD seems to play a minor role in sunflower seed ageing which is consistent with the observation in sunflower (Bailly et al., 1996) and also supported in soybean by (Stewart and Bewley, 1980) and in peanut seeds (Sung and Jeng, 1994). However in our study this enzyme seems to play a major role in chickpea, where its levels are maintained under accelerated ageing condition.

Catalase enzyme activity in unaged seeds of maize and sunflower was significantly higher than in chickpea irrespective of magnetic treatment. The activity was drastically reduced in aged sunflower seeds while the reduction was marginal in maize and chickpea. Similar decrease in catalase activity due to ageing was reported in sunflower (Bailly et al., 1996) and pigeon pea (Kalpana and Madhav Rao, 1994). No significant change was however observed in peanut seeds (Sung and Jeng, 1994).

Peroxidase activity declined significantly in aged seeds of sunflower irrespective of magnetic treatment whereas the activity was marginally reduced in chickpea and maize. The reduction in sunflower seeds was more than maize and chickpea. Similar decrease in peroxidase activity due to ageing was reported in peanut seed by (Sung and Jeng, 1994) and Sung (1996) in soybean seeds.

Many workers reported improved seed vigour when the seeds were pre-treated with antioxidants prior to accelerated and natural ageing in seeds of various crops (McDonald, 1999). Reduction in seed deterioration and improvement in seed vigour by the use of antioxidant like ascorbic acid is primarily due to quenching of free radicals, which prevents the peroxidative damage and enhances the activities of peroxide and radical scavenging enzymes (Hailstone and Smith, 1991). In present study, magnetic exposure of seeds prior to ageing was more effective in improving the percent germination as compared to unexposed control seeds (Fig.4.3.1c, 2c and 3c). The activities of various antioxidant enzymes, which decreased during artificial ageing treatment, maintained marginally higher levels in magnetically exposed seeds as compared to unexposed controls.

5.4 Imbibition kinetics of magnetically treated seeds during germination

The knowledge of the water status in biological systems is of great importance in understanding the functionality of these systems. In particular, a great interest is shown in seeds to understand the water distribution, its molecular mobility in the dry state, as well as the hydration mechanism when seeds are rehydrated during germination. In fact, unless the environmental conditions (e. g. temperature, light, oxygenation etc.) are unfavourable, the seed germination process can start simply by water imbibition (Bewley and Black, 1994).

The cellular membranes are composed primarily of proteins and lipids. During the germination process, disorganization of proteins and lipid phase transitions influence the membranes structure and integrity, consequently the seed water status. The total water potential of the seed is composed of several components, including osmotic, matric and turgor potentials. In dry seeds, matric and osmotic potentials contribute primarily to the total water potential (Hou et al., 1997). The matric potential is a function of the water binding capacity of macromolecules, which varies with the chemical composition of seeds of different species. Affinity of the major macromolecules to water is as follows, protein> carbohydrate>lipids (oil). Seeds containing more carbohydrate and protein (which are hydrophilic and are primarily responsible for the absorption of water) accounts for a higher water uptake by the viable seeds (Walters et al., 1997). Therefore, chickpea, which is rich in protein, show higher moisture content followed by maize and sunflower during the process of hydration and germination.

The first phase of germination of seed is imbibition, which results in the mobilization of food reserve to the embryo, and subsequent protrusion of the radicle through surrounding layers (Hou et al., 1997). 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 occur until the tissues have 40-60% water content. At the end of the rapid hydration phase, 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 seedling have water contents of 70-90% (Steiner, 1998). In the present study germinating seed showed three distinct phases of hydration (Fig. 4.6.1a, 1b and 1c). Higher uptake of water in phase III of hydration after the lag phase was

observed in magnetically exposed seeds. Kavi (1977) observed that soybean seed exposed to magnetic fields have increased capacity of moisture absorption. Francisco (2001) observed significant increase in the rate of absorption of water in lettuce seeds exposed to magnetic field. He correlated the observed increase of germination rate of the seeds with the theoretically calculated variations induced by magnetic fields in the ionic currents across the cellular membrane. The fields induce changes in the ionic concentration and thus in the osmotic pressure which regulates the entrance of water to the seeds. Hence there is strong evidence that the magnetic field alters the water relations in seeds, and thereby alter the germination rate of seeds.

5.5 NMR relaxation times and its components during germination of magnetically exposed and unexposed control seeds.

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 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 Hill, 2001 and Krishanan et al., 2003) 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., 2003).

The results of spin-lattice relaxation time (T_1) and spin-spin 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 times T_1 and T_2 measurement reflects the *in vivo* changes in dynamic and physical states of water in seed system. Few studies (Di Nola et al., 1991 and Brosio et al., 1992) have shown the usefulness of the unique information from NMR on water absorption, mobility and types in seed.

Seed water spin-lattice relaxation time (T_1) (Fig. 4.6.2a-c) and spin-spin relaxation time (T_2) (Fig. 4.6.3a-c) decreased initially during rapid hydration phase and remained relatively constant followed by a steady increase during subsequent hydration

in all three crop seeds. Such a trend has been reported in cowpea (Brosio et al., 1992) and in wheat and soybean (Krishnan et al., 2003) when the seeds were soaked in water. The reduction in seed water T_1 and 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 relaxation times of the different components of water and relatively higher proportion of protons in the less mobile phase may be responsible for the initial decline in weighted average T_2 (Nagarajan et al., 2005). In magnetically treated seeds, both T_1 and T_2 were higher than untreated controls at later stages of imbibition. This may explained on the basis of higher water uptake by magnetically treated seeds, which may be present in more mobile form as indicated by early germination and radicle protrusion in these seeds.

The difference in the values of these components observed in the study clearly showed the presence of rearrangement mechanism taking place in the seed during hydration and germination. This also shows that the state and quantity of water present in the localized sites in cells could provide a medium suitable for metabolic activity to proceed, even though the total water content is low for whole seed. 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. 4.6.4a-c, 5a-c and 6a-c). Ishida et al. (1988) suggested that these three populations correspond to water molecules differing in mobility, such as extracellular free water, intercellular bulk water and solid or bound water. This suggestion has been extensively used by many researchers to explain tissue water partitioning (Di Nola et al., 1988, 1991, Brosio et al, 1993 and Foucat et al., 1993). The general conclusion is that extracellular free water is characterized by high relaxation times, intercellular bulk water by low relaxation time, and bound–structural water by very low relaxation times.

During imbibition the third component in magnetically exposed maize appeared around 12 h (Fig. 4.6.4c), 1h in chickpea (Fig. 4.6.5c) and 5h in sunflower (Fig 4.6.6c) seeds. The third fraction with least mobilities appeared after 15h, 2h and 7h in unexposed

seeds of maize, chickpea and sunflower respectively. In general the components T_{2b} and T_{2c}, which represent the bulk water and hydration water fraction in the seed are higher in magnetically exposed seed compared to unexposed control during imbibition. This indicates the higher molecular mobility of the water proton in these fractions of seed water and therefore the better availability for metabolic activity (Lewin, 1974). This may be the reason for the faster germination and vigour of the magnetically treated seeds in our experiments.

The distribution of water proton into three fractions showed clear change with imbibition time indicating the rearrangement of cellular water both in magnetically exposed and unexposed seeds in all three crops (Fig. 4.6.7a-c, 8a-c &9a-c). With imbibition time, the free water fraction declined and that of cytoplasmic bulk water and bound water together increased till radicle protrusion took place. After this only two fraction belonging to free water and bulk water were detected.

The proportion of hydration water and cytoplasmic water together seems to play an important role in germination process. Krishanan (2004a) reported the complete disappearance of bulk water fraction in non-viable wheat seeds during imbibition. The appearance of only two components of water after radicle protrusion indicate 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 appearance of the bound water fraction earlier in magnetically treated seeds indicates the early synthesis and mobilization of proteins with seed hydration, which binds water and reduces its mobility. Similar results of early appearance of bound water fraction have been reported in primed seeds of carrot and tomato seeds (Nagarajan et al., 2003, 2005).

5.6. Enzyme related to germination in magnetically exposed seeds during germination.

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 hours in maize (Fig. 4.7.1a) and 48 hours in chickpea (Fig. 4.7.2a) and sunflower seeds (Fig. 4.7.3a) 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 similar to our

results. Rajendra et al. (2005) reported that α-amylase activity in broad bean significantly decreased at 5, 50 and 100 μT on day 2 and 4 of growth. In their experiment the seed exposure was at very low magnetic fields. The investigation of Lebedev et al. (1975) indicated profound influence of magnetic field on the basic aspects of metabolism of winter wheat, soybean and sunflower plants, including photosynthesis, respiration and enzymatic activity. Bhatnagar and Deb (1978) observed that the seeds treated at 1500 and 1000 Oe had significantly higher ($P<0.01$) α- amylase activity than the control or the other treatments. Lebedev et al. (1975) had also reported a similar result. Pitman and Ormrod (1970) found more reducing sugars in germinating magnetically treated seeds.

In the present study, dehydrogenase activity in both magnetically exposed and unexposed control seeds increased with imbibition, reached maximum at 60 h in maize (Fig. 4.7.4a) and 36 h in chickpea (Fig. 4.7.4b). In sunflower seeds the maximum activity was observed at 36 h after imbibition in magnetically treated seeds while in unexposed control seeds it occurred at 42h after imbibition (Fig. 4.7.4c) beyond which it started declining. Increase in dehydrogenase activity has been reported in primed soybean (Saha et al., 1990), carrot (Nagarajan et al., 2003) and tomato (Pandita et al., 2003) compared to unprimed seeds. Also greater glucose-6-phosphate dehydrogenase activity has been reported in primed sweet corn seeds (Smith and Cobb, 1991).

In our study protease activity of maize, chickpea and sunflower seeds showed an increase up to 64 h, 40 h and 24 h of imbibition respectively followed by a decrease with onset of germination (Fig. 4.7.5a-7a.)

The activity of this enzyme was significantly higher in magnetically treated seeds of all three crops. Protease are involved in the degradation of proteins in the germinating seeds and the reduction being initiated by endoproteases which convert the water insoluble storage protein into soluble peptides that can be further hydrolyzed to amino acids by exopeptidases (Callis, 1995, Shutov and Vaintraub, 1987). Vidyavathi et al. (1983) observed maximal proteolytic activity on the third day of germination in germinating finger millet seeds. The storage protein degradation and the increase in proteolytic activity observed in germinating chickpea seeds are consistent with various reports (Ahmed et al., 1995, Alvarez et al., 1985 and Nielsen et al., 1984). Ahmed et al. (1995) observed that storage proteins were degraded and enzymes synthesized at

different times of germination as a characteristic of the seed. Rajendra et al. (2005) however, reported that protease showed significant decrease in activity on day 2 and 4 of growth at 100 μT which may be due to extremely low magnetic fields that were used for exposure as compared to our fields.

5.7 Seed viability for magnetically exposed seeds at different equilibrium RH and temperatures

Seeds are hygroscopic absorb and desorbs the moisture depending on the relative humidity of the surroundings. The moisture absorbed by the seed is held with different types of binding at varying strengths. In this experiment, the effect of equilibrating the magnetically exposed and unexposed control seeds at two different temperatures over ranges of RH (1-91%) has been studied to understand the changes in seed water status, its binding and relationship with viability and vigour during equilibration.

In the present study, seeds of maize, chickpea and sunflower when equilibrated at different RH levels and temperatures, clearly established that moisture absorption pattern of seeds is influenced by their chemical constituents as well as the equilibrium temperature. For the seeds tested, isotherms at 25°C and 35°C exhibited the expected reverse sigmoidal shape, which is attributed to multi-molecular adsorption (Fang et al., 1998). Moisture content increased with increase in relative humidity and decreased with increase in equilibrium temperature. Since protein and carbohydrate absorb more moisture compared to lipids, this equilibrium moisture content of maize and chickpea seeds was higher than that of sunflower seeds at the same RH.

The germination and vigour parameters were evaluated for magnetically exposed and unexposed seeds equilibrated over the wide range of RH at 25°C and 35°C. The germination and vigour reduced at high humidities and at very low humidities in all three crops. Magnetically exposed seeds maintained higher germination and vigour in both temperatures and at all RH in all three crops, which indicated the better quality of the magnetically exposed seeds.

Changes in seed leachate conductivity when equilibrated over different RH showed variation with respect to change in equilibrium RH (Fig. 4.8.1a-b, Fig. 4.8.2a-b and Fig. 4.8.3a-b). The magnetically exposed seed had less seed leachate conductivity than unexposed seeds. This indicates the magnetically exposed seeds had good

membrane stability, which is not affected by extremes of relative humidities. It is widely accepted that there is active participation of membranes in seed hydration and dehydration mechanism. Basra et al. (1988) reported changes in quality and quantity of membrane phospholipid during and after seed priming. The better membrane integrity of magnetically treated seeds may be responsible for the higher vigour and germination of the seeds

5.8 NMR relaxation time T1 and T2 for magnetically exposed seeds at different equilibrium RH and temperatures

The NMR technique provides a novel and sensitive method to characterize the water status in seeds. Ratcliffe (1994) investigated longitudinal and transverse relaxation behaviour of water proton to describe the compartmantation and transport of water in seeds and other plant tissues. The mobility and relative amounts of water molecules can be determined by their relaxation times. In the present study maize, chickpea and sunflower seeds showed difference in the NMR relaxation time T_1 and T_2 at different equilibrium RH. It is expected, since the macromolecular composition could well influence the amount and motion of adsorbed water molecules. NMR relaxation measurement can thus be used to evaluate the seed water status at different RH-levels. The relaxation times decreased with increase in the EMC. In the present study the spin–lattice relaxation time (T_1) for magnetically exposed as well as unexposed seeds of maize (Fig. 4.10.1a-b), chickpea (Fig. 4.10.2a-b) and sunflower (Fig. 4.10.3a-b) equilibrated at high temperature (35°C) was higher than those at low temperature (25°C). A reverse trend was observed in the spin-spin relaxation time (T_2) behaviour of seeds compared to the spin–lattice relaxation time (T_1) (Fig. 4.10.4a-b, 5a-b and 6a-b). Decreases in T_2 with increase in temperature are consistent with the role of exchange process as a T_2 relaxation mechanism (Van As, 1992). This phenomenon is transparent to T_1 processes. As the temperature increases exchange increases and contributes more to T_2 relaxation so that T_2 decreases with increase in temperature. The increase in T_1 with increase in temperature may be attributed to increase randomness of the molecules hence higher T_1 values. The proton spin–lattice relaxation time T_1 of water are directly related to transitional and rotational motion (randomness) of water molecules (Samuilov et al., 1976, 1979).

The maximum T_1 values for both magnetically exposed and unexposed maize seeds was at around 7.5% RH when equilibrium temperature was 25°C and 35°C respectively (Fig. 4.10.1a-b). For chickpea the maximum value was at 8% and 7.5% RH (Fig. 4.10.2a-b) and for sunflower seed at 13% and 11.5% RH when equilibrium temperature was 25°C and 35°C respectively (Fig. 4.10.3a-b). Similar trend was observed for T_2 values in magnetically exposed and unexposed seeds of all three crops (Fig. 4.10.4a-b, 5a-b and 6a-b). This signifies the phase-transition of water during equilibration. The RH corresponding to the T_1 maximum can be identified as the lower limit of RH for seed equilibration at that particular temperature. This observation is further supported by the fall in germination and increase in seed leachate conductivity when equilibrated at RH below these critical values. This may coincide with loss of glassy state, which is responsible for maintaining the integrity of seed membrane (Sun and Leopold, 1993). The trend in seed water spin-spin relaxation time (T_2) with respect to RH at both equilibrium temperatures show a similarity with that of germination percentage. Both of them show nearly a stable higher value for a range of RH, which varied with equilibrium temperature and crop, below and above this range both T_2 and germination reduced drastically. This again confirms that NMR relaxation time of tissue water reflects the cellular membrane structure and integrity (Millerad et al., 1996) and collapse of which will affect the germination adversely. Similar decline in T_2 values in wheat and soybean seeds at 35°C and 45°C has been reported (Krishnan, 1999).

5.9 Water binding characteristics for magnetically exposed seeds at different equilibrium RH and temperatures

Water sorption isotherms constructed for maize, chickpea and sunflower showed reversed sigmoid curves which is generally noticed in all orthodox seeds. Three types of water binding sites, namely strong, weak and multi- molecular water binding sites in seed tissue were previously reported from the studies on water sorption isotherms (Vertucci and Leopold 1987, Walters, 1998). They also observed exchange of strong and multi-molecular sites for the weak binding sites during imbibition of the partially germinated soybean seeds. Water in the strong binding sites is associated with macromolecular surfaces or other biological interfaces by ionic bonding. This type of water is tightly bound and has very negative differential enthalpy. Water in the weak water binding sites

is bound loosely by hydrogen bonding. The analysis of the isotherm using D'Arcy-Watt equation showed that at 25°C and 35^{0}C the unexposed control seeds of maize and sunflower had higher strong binding sites, less weak binding sites and more multimolecular binding sites as compared to the magnetically exposed seeds (Table 4.11.1c-d and Table 4.11.3c-d). At 25°C the unexposed control seeds of chickpea had higher strong and multimolecular binding sites, less weak binding sites as compared to the magnetically exposed seeds (Table 4.11.2c). However at 35°C the magnetically treated seeds had higher strong binding sites and more weak binding sites and less multimolecular binding sites as compared to unexposed control seeds (Table 4.11.2d). The total number of binding sites was greater in unexposed seeds, which is difficult to explain from sorption data.

The seed water distribution related to strong, weak and multimolecular binding sites were also calculated from the three parts of the D'Arcy –Watt equation for maize, chickpea and sunflower (Fig.4.11.4a-f, 5a-f and 6a-f). With increase in RH there was continuous linear increases in weak binding sites. This might have increased the molecular mobility of seed water and the availability for various biochemical reactions involved in the germination process. A similar increase in weak binding sites has been reported in primed seeds of carrot and tomato (Nagarajan et al., 2003, 2005), which has been related to redistribution of water and its availability for faster germination. This increase was significantly higher for magnetically exposed seeds than unexposed control seeds of all three crops. At the same water activity as compared to control seeds the magnetically exposed seeds had more weakly bound water less strongly bound water or multimolecular bound water. In case of mung bean, Sun et al., 1997 have shown that the osmo priming increased the number of weak binding sites. They have argued that the priming induced water redistribution results in the depression of glass transition temperature (T_g) and enhanced the molecular mobility in the seed tissue, thereby accelerate seed ageing. If we apply the same argument to our result the increased molecular mobility of seed water may be responsible for increased germination characteristics of the magnetically exposed seeds.

5.10 Thermodynamic parameters for magnetically exposed seeds at different equilibrium RH and temperatures

Differential enthalpy (ΔH) is the heat of sorption and is a composite function, which incorporates the strength of water binding and other factors related to the structural changes of the macromolecules. The changes in ΔH values suggest conformational changes with hydration (Luscher and Ruegg, 1982). While it is possible that the macromolecule, particularly the long polypeptides, undergo conformational changes when water is absorbed, the enthalpy of this reaction is likely to be small compared to the enthalpy of water condensation on the molecular surfaces (Vertucci and Roos, 1993). Hence there is change in the differential enthalpy with increase in moisture content in both magnetically exposed and unexposed seeds of all three crops. In present study, the differential enthalpy decreased to a minimum value with increase in relative humidity and then increased with increase in relative humidity in treated and untreated seeds (Fig 4.12.1-3). Differential enthalpy of the absorption of water by the seed macromolecules was reported to change with EMC in soybean and wheat seeds (Krishnan, 1999). A negative peak of ΔH reached at lower moisture content in sunflower seed compared to chickpea and maize. This may be due to the presence of hydrophobic lipids in sunflower seeds.

Gibb's free energy (Fig. 4.12.4a-b, 5a-b and 6a-b) increased with increase in relative humidity for all crops seeds and treatments at both temperatures 25°C and 35°C. The sharp decline in free energy was observed at higher seed water content at 35°C equilibration temperature as compared to 25°C in all three crops. In magnetically treated seed similar trend was observed.

A large negative change in entropy (ΔS) by the seeds equilibrated to different RH and temperature conditions showed the non-random nature of water in the seeds. Negative values for entropy change indicate that the order is increasing for the process (Desmarchelier, 1999). Though the trends in ΔS changes with seed water content are similar at two equilibrium temperatures the minimum value of ΔS was observed at lower seed water content at 35°C as compared to 25°C in all three crops. The behaviour of entropy change with seed water content did not differ significantly between magnetically treated and untreated seeds. The phase transition phenomenon during moisture

equilibration is a function of the chemical constituent of the seed. The large negative ΔS (Fig. 4.12.7a-b, Fig. 4.12.8a-b and Fig. 4.12.9a-b) associated with high negative ΔH, shows that over the moisture range studied dry seeds (with high negative ψ) have a high affinity for water.

Irrespective of magnetic treatment, the maximum germination percentage, seedling shoot / root length and dry weight of seedling were higher at 32.5% RH and 22% RH corresponding to a similar maximum negative differential enthalpy and entropy in maize, chickpea and sunflower seeds. It may be conclude that thermodynamic energy parameters namely ΔH, ΔS and ΔG were not able to explain the observed enhancement in the performance of magnetically treated seeds.

सारांश

विभिन्न रासायनिक संघटकों के बीजों की अकुंरण विशेषताओं में अधिकतम वृद्धि के लिए स्थिर चुम्बकीय क्षेत्र और अवधि के मानकीकरण के लिए एक अध्ययन किया गया। मानकीकृत चुम्बकीय क्षेत्र से उपचारित बीजों का प्रभाव खेत में प्रांकुरण के समय, प्ररोह और जड़ की विशेषताएं, जीर्णता बढ़ने के दौरान गुणवत्तायुक्त परिवर्तन आदि पर अध्ययन किये गये। अंकुरण और भंडारण के दौरान चुम्बकीय क्षेत्र संवृद्धि की यांत्रिकी समझने के लिए चुम्बकीय क्षेत्र से उपचारित बीजों की जैव-भैतिकी और जैव-रासायनिक विशेषताओं पर भी कार्य किया गया। मक्का (किस्म: गंगा सफेद-2), चना (किस्म : पूसा -1053) और सूरजमुखी (किस्म: के.बी.एस.एच-1) का इस्तेमाल परीक्षणात्मक सामग्री के रूप में किया गया और विभिन्न चुम्बकीय क्षेत्र तीव्रता (50 से 500 mT) युक्त विद्युत चुम्बकीय क्षेत्र जनित्र "टेस्ट्रान EM-20" का प्रयोग करते हुए चुम्बकीय क्षेत्र से उपचारित किया गया। सभी क्षेत्रो के लिये 1 घंटें से 4 घंटों के चरणों की विभिन्न अवधि के लिये 0 से 250 mT की तीव्रता के 50 mT के चरणों में विभिन्न चुम्बकीय क्षेत्रों से बीजों को उपचारित किया गया। मक्का, चना और सूरजमुखी के अंकुरण में अधिकतम वृद्धि के लिए चुम्बकीय क्षेत्र और अवधि के मानकीकरण के लिए आरम्भिक परीक्षण किए गए। परीक्षणों से संकेत मिला कि प्रयोगशाला में अंकुरण, अंकुरण की गति, पादपों की लम्बाई और पादपों के शुष्क भार में, नियंत्रित अनुपचरित बीजों की तुलना में चुम्बकीय क्षेत्र से उपचारित बीजों के संपादन में उल्लेखनीय रूप से वृद्धि हुई। क्षेत्र की तीव्रता और उपचारित अवधि में बिना किसी विशेष प्रवृत्ति के इस प्रतिक्रिया में अंतर दिखाई दिया। चुम्बकीय क्षेत्र की तीव्रता और अवधि से सम्बन्धित संयोगो में से सर्वोतम परिणाम, मक्का में 100 mT तीव्रता (2 घंटे की अवधि) और 200 mT तीव्रता (1 घंटे की अवधि), चने में 100 mT तीव्रता (2 घंटे की अवधि) और 150 mT तीव्रता (2 घंटे की अवधि) तथा सूरजमुखी में 50 mT और 200 mT तीव्रता (2 घंटे की अवधि) तक उपचारित करने से बीजों के आवरण झिल्ली समग्रता में सुधार हुआ क्योंकि इससे कोशिकीय रिसाव वैद्युत संवहनीयता में कमी आई। इन चुम्बकीय क्षेत्रों में बीजों को उपचारित करने से

खेत में अंकुरण में वृद्धि प्रदर्शित हुई और एक माह के पौधो में पादप शुष्क भार में उल्लेखनीय वृद्धि दिखाई दी। पौधो की जड़ सम्बंधी विशेषताओं में जड़ो की लम्बाई, जड़ो का बाह्य क्षेत्रफल, और जड़ो के आयतन में अप्रत्याशित वृद्धि प्रदर्शित हुई।

कृत्रिम जीर्णन अवधि के दौरान नियंत्रित बीजों की तुलना में चुम्बकीय उपचारित बीजों में निक्षालन संवहनीयता निम्न थी, नियंत्रित बीजों की तुलना में चुम्बकीय द्वारा उपचारित बीजों के ओज I, ओज II, पादप शुष्क भार, प्ररोह/जड़ लम्बाई में हानि कम थी, जीर्णित नियंत्रित बीजों की तुलना में मक्का, चना और सूरजमुखी के जीर्णीत बीजों पर चुम्बकीय क्षेत्र के उपचार से अंकुरण विशेषताओं में सुधार हुआ। विभिन्न चुम्बकीय क्षेत्र तीव्रता से उपचारित बीजों में नियंत्रित बीजों की तुलना में जीर्णित बीजों में अनआक्सीकारक एंजाइम की उच्च सक्रियता बनी रही। अनउपचारित जीर्णीत बीजों की तुलना में चुम्बकीय क्षेत्र से उपचारित बीजों में जीर्णिता के बाद भी प्रोटीन की उच्च मात्रा थी। जल योजन के चरण III में, तीनों फसलों में, नियंत्रित बीजों की तुलना में चुम्बकीय क्षेत्र से उपचारित बीजों में, स्पिन-लेटिस रिलक्सेशन टाइम (T_1) और स्पिन-स्पिन रिलक्सेशन टाइम (T_2) में उच्च मान था। चुम्बकीय क्षेत्र से उपचारित बीजों तथा नियंत्रित बीजों के प्रोटीन-नाभिकीय चुम्बकीय अनुनाद (एन.एम. आर) स्पिन-स्पिन रिलेक्सेशन टाइम (T_2) के अन्तःशोषण की आरम्भिक अवधि में धीमी गति से जल बदलाव के दो भिन्न-भिन्न घटक दर्शाए गये। अंतशोषण की निरन्तर प्रक्रिया के दौरान बीज जल प्रोटीन को तीन घटकीय प्रणाली में वर्गीकृत किया जा सकता है। और प्रस्फुटीकरण के बाद जल प्रोटीन, दो विभिन्न घटकों में पुनः गठित किए जा सकते है। चुम्बकीय क्षेत्र से उपचारित बीजों में जलयोजक से सम्बन्धित तीसरा घटक, नियंत्रित बीजों की तुलना में पहले दिखाई दिया। अंकुरण अवधि के दौरान नियंत्रित बीजों की तुलना में चुम्बकीय क्षेत्र से उपचारित बीजों में एमाइलेज, डिहाइड्रोजेनेस और प्रोटिएज की सक्रियता उल्लेखनीय रूप से अधिक थी।

विभिन्न आपेक्षिक आर्द्रताओं से समतुल्यता करने पर नियंत्रित बीजों की अपेक्षा चुम्बकीय क्षेत्र से उपचारित बीजों में निक्षालन संवहनीयता निम्न थी। जल शोषक समताप रेखाओं के विश्लेषण से पता

चला कि नियंत्रित बीजों की तुलना में, चुम्बकीय क्षेत्र से उपचारित बीजों में ठोस योजक स्तल या बहुआण्विक योजक स्तल कम और मन्द योजक स्तल अधिक था। तीनों फसलों में अधिकतम अंकुरण विशेषताएं, इस समय की अधिकतम नकारात्मक विभेदक एन्थैल्पी और एन्ट्रॉपी के अनुरूप उच्च थे।

मक्का, चना और सूरजमुखी की बीजों के एक विशिष्ट चुम्बकीय क्षेत्र में उपचारित करने से पादप वृद्धि दर में बढ़ोतरी और जीर्णन के दौरान बीज क्षय मे कमी आई। उन्नत क्रियात्मक जड़ प्राचलों से पता चलता है कि बरानी अवस्थाओं के दौरान चुम्बकीय क्षेत्र से उपचारित बीजों को लाभदायक ढंग से प्रयोग में लाया जा सकता है।, चुम्बकीय क्षेत्र से उपचारित, चने के बीजों में शाखाओं की संख्या में वृद्धि, उपज बढ़ोतरी का संकेत हो सकती है। कोशिकीय जल वितरण के आधार पर तथा अंकुरण सम्बंधित और अनआक्सीकरण एंजाइयों की क्रियाशीलता से, बढ़े हुए प्रभाव की आंशिक रूप से व्याख्या की जा सकती है।

Chapter - 1

INTRODUCTION

Magnetobiology addresses the biological reactions and mechanisms of the action of primarily weak magnetic fields. It is a fast developing field of research and its practical and environmental aspects being a topic of number of research papers. At the same time, physically, the biological effects of magnetic fields are still regarded as a paradox. Living organisms have electrical and magnetic characteristics, but it is difficult to present them in biochemical terms. Their structures and processes may get modified under the influence of electromagnetic fields. The influence of the geomagnetic field on the growth of plants was scientifically established for the first time in 1862 by the French chemist Louis Pasteur, during his experiments on fermentation, when he discovered that the Earth's magnetic field had a stimulating effect on that process. The late Dr. Albert Roy Davies, the father of modern bio-magnetic science received a patent in 1950 for magnetically treating seeds to stimulate plant growth. It is well known that factors such as temperature, light, gravity and moisture availability affect the germination of the seeds and that plant and plant parts respond to electrical irradiation and magnetic stimuli.

The potential energy of self-preservation in seeds differs at different stages of development. During the harvest collection, seeds also contain different energy levels, and not all planted seeds will grow. Therefore, it results in the excess of costly seed material being used. Magnetic treatment of seeds before sowing allows spending less on the seed as germination rates are increased substantially in same seed lots.

In the modern agriculture of the 21st century, increasing attention has been paid to the productive growth of cultivated plants, which are also environmentally safe. The application of high quality sowing materials which has been properly pre-prepared is an important yield enhancing factor in plant cultivation. Most often chemical methods consisting seed dressing, priming with various chemical substances are used in the presowing seed treatment. Such methods are considered as very effective but not neutral for the environment. Presently, when the rational use of agricultural land is emphasized, greater importance is attributed to some physical methods of the pre- sowing treatment of seeds, which are commonly regarded as being friendlier to the environment. These

physical treatments like electrical, microwave, laser and irradiation etc modify the course of some physiological and biochemical process in the seeds, which increases their vigour and contributes to the improved development of the plants. Physical methods moreover provide significant yield improvement without hazardous toxic fertilizer and management cost. Therefore the practical applicability of physical presowing seed treatment for enhancing the seed performance should be standardized for commercial use. Magnetic seed treatment is one of the physical presowing seed treatments especially worth our attention since its impact on the seeds can change the processes taking place in the seed and stimulate plant development.

This technique has numerous practical applications in modern agriculture.

1. Enhanced germination rate, seedling growth and yield.
2. Decreases the seed rate per hectare by increasing the germination percentage thus, reduce the cost of cultivation.
3. Environment friendly: No adverse effect on the environment.
4. This treatment provides an earlier ripening of the harvest.

Seeds are resting system of organs of future plants. What the plant will be and what results we will get depend upon the quality of the seed. Magnetic treatment of seeds is necessary while using the non-standard (poor quality) seeds, for the improvement of seeds quality, their germination properties, and for the stimulation of growth during vegetation period.

The changes in seed water status during imbibition and germination of seeds can influence the subsequent development and growth. These changes can be elegantly studied non-invasively using low resolution Nuclear Magnetic Resonance (NMR). The relaxation times of water protons can be used to describe the compartmantation and transport of water in seeds (Ratcliffe, 1994). The water molecules with different mobilities can be distinguished by their different relaxation times and their relative amounts can be calculated (Van As, 1992). The proton transverse relaxation time T_2 of tissue water has been shown to be related to the properties of water in different parts of the tissue and its interaction with macromolecules.

Water and temperature play a significant and fundamental role in determining the storage longevity of orthodox seeds, which are viable even with low moisture content (Walters, 1998). In general the seed-ageing pattern is described in terms of its water content during storage. The importance of different properties of water in seeds has been widely recognized (Williams, 1994). Various properties of water in terms of bound water, water activity and water potential are introduced as factors that determine the mechanisms and kinetics of seed longevity (Vertucci 1992).

The binding characteristics of seed water influence its quality and subsequent germination and emergence characteristics. A simple means of studying water binding in seed tissue involves the use of moisture isotherms. In orthodox seeds, three regions of water binding have been classified by the strength with which water is bound and nature of binding sites. Using thermodynamic principles, the strength of binding and the number of water binding sites in these regions can be calculated from sorption isotherms applying D'Arcy-Watt equation. The physical status of water in mature dry seeds as well as in germinating seeds has been studied (Vertucci and Leopold, 1987).

Pre-germination exposure of seeds to known introduced magnetic field for specific time interval has simulating effect on seeds of different chemical constituents. The results can be exploited in improving vigour and field emergence in high viability seeds. In low viability seeds, the biostimulation of magnetic fields can ameliorate the deteriorating effects of storage and costly seeds can be salvaged. Biophysical and biochemical studies carried out on the treated seeds during germination and storage help in understanding the limiting factors and enhancement effects on seed germination and storability. None of the studies has done so far have taken into consideration the chemical constituent and the water status of the seed materials. It is necessary the protocol for seeds containing predominantly starch, protein or oil need to be worked out separately for optimum performance. There is no information on the influence of magnetic field on the deterioration process of seeds in storage. Also no information on the mid-storage correction with magnetic field treatments to ameliorate the damage caused due to storage. Study of Biophysical and Biochemical changes that occur during germination and during storage of magnetically treated seeds explain the basic mechanism of enhancement in germination, vigour, field emergence characteristics and

storability. So far only limited research has been conducted on this issue concerning mainly cereals. This problem may be very important for legume and oil seed cultivation because these plants are characterized by their low and rather variable seed yield over the years.

Therefore, a study was planned with the following objectives:

1. To standardize the magnetic field and duration of exposure for maximum enhancement in germination, vigour and field emergence characteristics of different seeds.
2. To evaluate the effect of magnetic field on storability of different seed.
3. To study biophysical and biochemical parameters associated with germination processes and storability of the treated seeds.

Table 4.12.3a. Water status parameters of sunflower seeds exposed by different magnetic field kept at 25°C and at different RH.

Moisture content (%)			Water activity	Chemical Potential (KJ/mol water)	Water Potential (MPa)
Control	50mT(2h)	200mT(2h)			
0.150	0.144	0.142	0.91	-0.101	-0.56
0.130	0.131	0.130	0.87	-0.150	-0.83
0.117	0.114	0.106	0.85	-0.175	-0.97
0.108	0.106	0.104	0.78	-0.267	-1.48
0.097	0.095	0.090	0.75	-0.310	-1.72
0.094	0.094	0.089	0.64	-0.480	-2.66
0.070	0.068	0.055	0.53	-0.683	-3.79
0.057	0.056	0.053	0.505	-0.735	-4.07
0.050	0.051	0.052	0.46	-0.836	-4.63
0.047	0.045	0.051	0.43	-0.908	-5.03
0.046	0.044	0.044	0.34	-1.161	-6.43
0.039	0.039	0.031	0.325	-1.209	-6.70
0.029	0.025	0.027	0.295	-1.314	-7.28
0.028	0.022	0.023	0.13	-2.195	-12.16
0.026	0.024	0.02	0.08	-2.718	-15.06
0.015	0.021	0.014	0.075	-2.787	-15.44
0.013	0.013	0.013	0.055	-3.121	-17.29
0.008	0.003	0.004	0.01	-4.955	-27.46

Table 4.12.3b. Water status parameters of sunflower seeds exposed by different magnetic field kept at 35°C and at different RH.

Moisture content (%)			Water activity	Chemical Potential (KJ/mol water)	Water Potential (MPa)
Control	50mT(2h)	200mT(2h)			
0.136	0.132	0.133	0.90	-0.117	-0.649
0.127	0.125	0.126	0.87	-0.155	-0.858
0.120	0.116	0.117	0.84	-0.194	-1.074
0.103	0.107	0.108	0.755	-0.313	-1.732
0.099	0.100	0.101	0.75	-0.320	-1.773
0.090	0.089	0.090	0.62	-0.532	-2.946
0.069	0.055	0.057	0.505	-0.760	-4.210
0.05	0.051	0.055	0.465	-0.852	-4.718
0.051	0.053	0.053	0.43	-0.939	-5.201
0.044	0.052	0.052	0.415	-0.978	-5.419
0.042	0.034	0.035	0.325	-1.250	-6.926
0.030	0.030	0.031	0.22	-1.684	-9.330
0.025	0.027	0.028	0.21	-1.736	-9.617
0.024	0.023	0.024	0.115	-2.405	-13.328
0.016	0.016	0.016	0.08	-2.809	-15.564
0.014	0.014	0.015	0.075	-2.881	-15.962
0.012	0.011	0.012	0.055	-3.226	-17.873
0.003	0.003	0.002	0.01	-5.122	-28.378

Table 4.1.2. Effect of pre-germination exposure of different doses of static magnetic field and its duration on germination characteristics of chickpea (on 8th day)

Parameter	Germination (%)	Speed of germination	Shoot length (cm)	Root length (cm)	Dry wt. of seedling (g)	Vigour index I	Vigour index II
Magnetic field (mT)							
50	92.3 (74.2)	26.94	10.41	13.5	0.052	2207	4.82
100	94.0 (76.4)	26.58	9.42	14.12	0.049	2214	4.64
150	91.5 (74.3)	26.32	8.74	13.50	0.045	2043	4.10
200	90.3 (73.4)	25.15	9.52	12.52	0.053	1987	4.75
250	90.0 (72.5)	23.25	9.39	11.71	0.049	1895	4.39
Control	85 .0 (67.2)	21.39	7.77	7.42	0.036	1297	3.10
LSD at 5%	3.46	1.87	0.48	0.93	0.0020	101.4	0.26
Duration of exposure (h)							
1	92.6 (74.7)	25.46	10.02	13.04	0.052	2138	4.78
2	92.2 (75.0)	25.77	10.14	13.43	0.053	2177	4.90
3	89.8 (72.8)	25.45	8.73	13.01	0.046	1956	4.14
4	91.8 (74.2)	25.91	9.08	12.80	0.047	2008	4.34
LSD at 5%	3.09	1.67	0.43	0.83	0.0018	90.7	0.24

Values in parenthesis are arcsine transformed value.

Table 4.2.2.1. Effect of magnetic field on field emergence and growth characteristics of chickpea

Magnetic field (mT)	Field emergence index	Field emergence (%)	Shoot height (cm)	Root length (cm)	Branch No./ Plant	Shoot dry weight (g)	Root dry weight (g)	Root dry weight / Shoot dry weight	Electrical conductivity (m Siemens / cm / 25 seeds
Control	66.9	84 (66.5)	15.22	14.64	4	0.282	0.030	0.106	0.985
50 (2h)	69.8 ^{NS}	85 ^{NS} (67.3)	17.82**	18.11**	5**	0.431**	0.090**	0.208**	0.909**
100 (1h)	70.2 ^{NS}	85 ^{NS} (67.3)	19.77**	20.17**	5**	0.422**	0.098**	0.233**	0.861**
150 (2h)	69.6 ^{NS}	89 ^{NS} (71.2)	19.50**	19.28**	5**	0.430**	0.093**	0.216**	0.927*
LSD at 5%	3.96	7.17	0.896	2.484	0.385	0.0199	0.0065	0.0247	0.0546

NS: Not significant at 5% level of probability, * Significant at 5% level of probability,
** Significant at 1% level of probability.
Values in parenthesis are arcsine transformed value.

Table 4.2.2.2. Effect of magnetic field on rooting characteristics of one month old chickpea plants

Magnetic field (mT)	Total Length (cm)	Surface area (cm^2)	Projected area (cm^2)	Volume (cm^3)	Average diameter (mm)
Control	94.1	8.29	2.64	0.053	0.280
50 (2h)	197.7**	17.88**	5.69**	0.123**	0.300NS
100 (1h)	170.9**	16.44**	5.23**	0.130**	0.318**
150 (2h)	207.0**	18.38**	5.85**	0.130**	0.282NS
LSD at 5%	13.9	1.32	0.42	0.015	0.0218

NS: Not significant at 5% level of probability, * Significant at 5% level of probability, ** Significant at 1% level of probability.

Table 4.1.1. Effect of pre-germination exposure of different doses of static magnetic field and its duration on germination characteristics of maize (on 7th day)

Parameter	Germination (%)	Speed of germination	Shoot length (cm)	Root length (cm)	Dry wt. of seedling (g)	Vigour Index I	Vigour Index II
Magnetic field (mT)							
50	92.3 (75.0)	14.94	6.25	11.69	0.205	1656	18.87
100	92.0 (73.8)	14.55	9.83	14.19	0.208	2210	19.10
150	93.0 (75.5)	13.64	7.17	10.96	0.196	1685	18.24
200	91.5 (73.8)	14.58	9.01	14.02	0.205	2112	18.71
250	88.5 (71.5)	14.26	7.31	11.36	0.214	1652	18.98
LSD at 5%	3.61	0.91	0.58	0.71	0.02	111.8	1.35
Control	83.5 (66.0)	12.48	5.26	10.62	0.159	1322	13.27
Duration of exposure (h)							
1	92.2 (74.2)	14.59	8.12	13.29	0.207	1975	19.15
2	91.6 (74.7)	14.14	7.70	12.37	0.210	1836	19.20
3	89.2 (72.0)	14.04	8.47	12.06	0.210	1839	18.67
4	92.8 (74.8)	14.81	7.37	12.06	0.195	1802	19.09
LSD at 5%	3.22	0.81	0.514	0.64	0.01	99.9	1.20

Values in parenthesis are arcsine transformed values

Table 4.2.1.1 Effect of magnetic field on field emergence and growth characteristics of maize

Magnetic field (mT)	Field emergence index	Field emergence (%)	Shoot height (cm)	Shoot dry weight (g)	Root dry weight (g)	Root dry weight / shoot dry weight	Electrical conductivity (m Siemens / cm / 25 seeds)
200 (1h)	45.3	94** (76.3)	78.3**	1.119**	0.443**	0.396**	0.498**
100 (2h)	45.9	88* (69.7)	81.0**	1.216**	0.407**	0.335**	0.500**
Control	43.8	83 (65.7)	71.0	0.781	0.028	0.036	0.628
LSD at 5%	NS	4.13	4.67	0.168	0.004	0.025	0.042

NS: Not significant at 5% level of probability, * Significant at 5% level of probability,
** Significant at 1% level of probability.
Values in parenthesis are arcsine transformed value.

Table 4.2.1.2. Effect of magnetic field on rooting characteristics of one month old maize plants

Magnetic field (mT)	Total Length (cm)	Surface area (cm^2)	Projected area (cm^2)	Volume (cm^3)	Average diameter (mm)
200 (1h)	202.5**	27.7**	8.89**	0.320**	0.476**
100 (2h)	200.8**	24.7**	7.87**	0.243**	0.409**
Control	116.0	12.7	4.09	0.115	0.354
LSD at 5%	57.3	5.41	1.62	0.072	0.072

NS: Not significant at 5% level of probability, * Significant at 5% level of probability,
** Significant at 1% level of probability.

Table 4.5.1. Effect of magnetic treatment on germination characteristics of mid storage (partially aged) seeds of maize.

Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Dry wt. of seedling (g)	Vigour index I	Vigour index II
			Fresh seeds			
Control	88 ± 3.65	8.73 ± 0.31	10.84 ± 0.32	0.039 ± 0.0019	1724.8 ± 97.87	3.46 ± 0.307
100mT(2h)	92 ± 2.83	11.63 ± 0.45	15.15 ± 0.45	0.046 ± 0.0013	2463.1 ± 97.32	4.22 ± 0.131
200mT(1h)	92 ± 2.83	12.11 ± 0.26	15.53 ± 0.38	0.046 ± 0.0022	2547.4 ± 29.13	4.27 ± 0.245
			Partially aged seeds			
Control	74 ± 1.15	7.54 ± 0.35	8.20 ± 0.56	0.033 ± 0.0006	1163.7 ±.73	2.46 ± 0.076
100mT(2h)	82 ± 1.15	10.93 ± 0.41	13.79 ± 1.32	0.0411 ± 0.0006	2028.2 ± 97.85	3.37 ± 0.074
200mT(1h)	83 ± 1.00	11.48 ± 0.59	13.00 ± 0.70	0.0421 ± 0.0002	2031.7 ± 34.78	3.50 ± 0.171

± Standard error

Table 4.5.2. Effect of magnetic treatment on germination characteristics of mid storage (partially aged) seeds of chickpea.

Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Dry wt. of seedling (g)	Vigour index I	Vigour index II
			Fresh seeds			
Control	92.5 ± 1.44	8.03 ± 0.36	8.18 ± 0.15	0.040 ± 0.0005	1497.9 ± 43.87	3.69 ± 0.094
100mT(1h)	93.8 ± 1.25	15.88 ± 0.23	15.80 ± 0.75	0.058 ± 0.0028	2970.8 ± 111.45	5.44 ± 0.296
150mT(2h)	93.8 ± 1.25	15.45 ± 0.34	15.65 ± 0.17	0.057 ± 0.0012	2914.6 ± 34.34	5.32 ± 0.120
			Partially aged seeds			
Control	70 ± 2.58	7.23 ± 0.25	6.65 ± 0.29	0.031 ± 0.0024	970.1 ± 29.35	2.18 ±0.218
100mT(1h)	77 ± 1.00	12.20 ± 0.38	11.98 ± 0.43	0.039 ± 0.0002	1862.0 ± 42.43	2.99 ± 0.051
150mT(2h)	78 ± 2.00	12.03 ± 0.24	11.70 ± 0.37	0.039 ± 0.0005	1850.0 ± 42.50	3.02 ± 0.097

± Standard error

Table 4.5.3. Effect of magnetic treatment on germination characteristics of mid storage (partially) aged seeds of sunflower.

Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Dry wt. of seedling (g)	Vigour index I	Vigour index II
			Fresh seeds			
Control	85 ± 1.91	14.15 ± 0.62	11.35 ± 0.38	0.039 ± 0.0009	2168.9 ± 78.21	3.34 ± 0.057
50mT(2h)	90 ± 1.15	19.08 ± 0.28	17.53 ± 0.37	0.045 ± 0.0008	3293.9 ± 52.36	4.06 ± 0.040
200mT(2h)	90 ± 1.15	19.10 ± 0.39	18.05 ± 0.81	0.046 ± 0.0011	3343.6 ± 79.42	4.14 ± 0.106
			Partially aged seeds			
Control	56 ± 1.63	12.53 ± 0.44	8.78 ± 0.74	0.038 ± 0.0005	1191.1 ± 20.28	2.15 ±0.066
50mT(2h)	62 ± 1.15	16.58 ± 0.26	16.05 ± 0.34	0.041 ± 0.0010	2022.4 ± 33.89	2.54 ± 0.064
200mT(2h)	65 ± 3.00	16.30 ± 0.17	16.45 ± 1.09	0.043 ± 0.0026	2129.6 ± 130.69	2.78 ± 0.177

± Standard error

Table 4.1.3. Effect of pre-germination exposure of different doses of static magnetic field and its duration on germination characteristics of sunflower (on 10th day).

Parameter	Germination (%)	Speed of germination	Shoot length (cm)	Root length (cm)	Dry wt. of seedling (g)	Vigour index I	Vigour index II
Magnetic field (mT)							
50	90.1 (72.6)	31.31	14.18	12.23	0.045	2391	4.03
100	87.3 (69.5)	30.49	10.83	6.85	0.044	1529	3.81
150	89.0 (71.9)	31.20	13.10	11.01	0.042	2148	3.78
200	90.3 (72.4)	30.29	14.22	11.65	0.043	2345	3.90
250	87.0 (69.3)	30.63	14.12	12.58	0.043	2313	3.75
Control	82.3 (65.1)	27.68	11.15	7.68	0.040	1554	3.30
LSD at 5%	3.37	1.81	0.63	0.78	0.0019	124.1	0.22
Duration of exposure (h)							
1	87.4 (70.1)	30.70	12.18	8.96	0.044	1843	3.84
2	91.6 (74.2)	31.79	15.69	13.80	0.045	2707	4.12
3	87.2 (69.5)	30.27	13.41	11.14	0.042	2133	3.63
4	88.7 (70.8)	30.36	11.89	9.56	0.043	1898	3.82
LSD at 5%	3.01	1.61	0.57	0.70	0.0017	111.0	0.20

Values in parenthesis are arcsine transformed value.

Table 4.2.3.1 Effect of magnetic field on field emergence and growth characteristics of sunflower

Magnetic field (mT)	Field emergence index	Field emergence (%)	Shoot height (cm)	Root length (cm)	Total seedling length (cm)	Shoot dry weight (g)	Root dry weight (g)	Electrical conductivity (m Siemens / cm / 25 seeds)
Control	45.2	74 (59.4)	91.3	12.5	103.8	3.766	0.278	0.338
50 (2h)	47.7*	81* (64.2)	98.3**	17.8**	115.8**	6.893**	0.486**	0.298**
200 (2h)	47.8*	80* (63.6)	98.8**	16.3**	114.8**	7.094**	0.576**	0.290**
250 (2h)	47.9*	79 NS (62.7)	100.3* *	15.0**	113.3**	7.319**	0.470**	0.318*
LSD at 5%	1.97	5.19	4.81	1.69	5.28	1.321	0.084	0.016

NS: Not significant at 5% level of probability, * Significant at 5% level of probability,
** Significant at 1% level of probability.
Values in parenthesis are arcsine transformed value.

Table 4.2.3.2 Effect of magnetic field on rooting characteristics of one month old sunflower plants

Magnetic field (mT)	Total Length (cm)	Surface area (cm^2)	Projected area (cm^2)	Volume (cm^3)	Average diameter (mm)
Control	224.3	30.81	9.79	0.328	0.379
50 (2h)	385.1**	52.68**	16.77**	0.588**	0.445NS
200 (2h)	387.5**	55.85**	17.80**	0.718**	0.513**
250 (2h)	353.6**	47.96**	15.27**	0.550**	0.460*
LSD at 5%	3.99	5.14	1.63	0.101	0.070

NS: Not significant at 5% level of probability, * Significant at 5% level of probability,
** Significant at 1% level of probability.

Table 3.1 Chemical constituents (% dry wt) of the seed

Chemical constituents	Maize	Chickpea	Sunflower
Starch	74.16 ± 0.833	54.99 ± 0.964	11.50 ± 0.155
Protein	9.93 ± 0.023	21.59 ± 0.028	19.32 ± 0.058
Oil	3.27 ± 0.014	4.71 ± 0.067	43.61 ± 0.208
Moisture content	7.55 ± 0.018	6.99 ± 0.009	5.21 ± 0.006
Germination (%)	90 ± 1.15	90 ± 1.15	88 ± 0.92

± Standard Error

Table 3.3. Relative humidity of various saturated salt solutions and sulfuric acid at different temperatures

Chemicals	Temperature	
	25^0C	35^0C
Con H_2SO_4	1	1
$ZnCl_2$	5.5	5.5
NaOH	7.5	7.5
KOH	8	8
LiCl	13	11.5
$CaCl_2$	29.5	22
$MgCl_2$	32.5	32.5
$Zn(NO_3)_2$	34	21
K_2CO_3	43	41.5
KNO_2	46	46.5
$Ca(NO_3)_2$	50.5	43
$Mg(NO_3)_2$	53	50.5
$NaNO_2$	64	62
NaCl	75	75
NH_4Cl	78	75.5
KCl	85	84
$BaCl_2$	87	87
KNO_3	91	90

Table 4.12.2a. Water status parameters of chickpea seeds exposed by different magnetic field kept at 25°C and at different RH.

Moisture content (%)			Water activity	Chemical Potential (KJ/mol water)	Water Potential (MPa)
Control	100mT(1h)	150mT(2h)			
0.193	0.191	0.189	0.91	-0.101	-0.56
0.174	0.175	0.181	0.87	-0.150	-0.83
0.168	0.172	0.171	0.85	-0.175	-0.97
0.165	0.168	0.167	0.78	-0.267	-1.48
0.161	0.16	0.162	0.75	-0.310	-1.72
0.118	0.123	0.124	0.64	-0.480	-2.66
0.095	0.097	0.098	0.53	-0.683	-3.79
0.092	0.093	0.096	0.505	-0.735	-4.07
0.09	0.089	0.087	0.46	-0.836	-4.63
0.088	0.083	0.085	0.43	-0.908	-5.03
0.077	0.078	0.081	0.34	-1.161	-6.43
0.076	0.075	0.076	0.325	-1.209	-6.70
0.069	0.068	0.069	0.295	-1.314	-7.28
0.041	0.041	0.043	0.13	-2.195	-12.16
0.024	0.023	0.028	0.08	-2.718	-15.06
0.021	0.022	0.026	0.075	-2.787	-15.44
0.02	0.018	0.018	0.055	-3.121	-17.29
0.018	0.016	0.014	0.01	-4.955	-27.46

Table 4.12.2b. Water status parameters of chickpea seeds exposed by different magnetic field kept at 35°C and at different RH.

Moisture content (%)			Water activity	Chemical Potential (KJ/mol water)	Water Potential (MPa)
Control	100mT(1h)	150mT(2h)			
0.152	0.150	0.144	0.90	-0.117	-0.649
0.130	0.140	0.130	0.87	-0.155	-0.858
0.123	0.131	0.123	0.84	-0.194	-1.074
0.119	0.115	0.110	0.755	-0.313	-1.732
0.116	0.113	0.110	0.75	-0.320	-1.773
0.085	0.092	0.098	0.62	-0.532	-2.946
0.073	0.081	0.081	0.505	-0.760	-4.210
0.062	0.069	0.068	0.465	-0.852	-4.718
0.056	0.061	0.065	0.43	-0.939	-5.201
0.052	0.061	0.060	0.415	-0.978	-5.419
0.051	0.055	0.054	0.325	-1.250	-6.926
0.050	0.048	0.046	0.22	-1.684	-9.330
0.047	0.046	0.046	0.21	-1.736	-9.617
0.037	0.035	0.032	0.115	-2.405	-13.328
0.021	0.021	0.024	0.08	-2.809	-15.564
0.020	0.020	0.021	0.075	-2.881	-15.962
0.017	0.018	0.017	0.055	-3.226	-17.873
0.016	0.016	0.015	0.01	-5.122	-28.378

Table 4.12.1a. Water status parameters of maize seeds exposed by different magnetic field kept at 25°C and at different RH.

Moisture content (%)			Water activity	Chemical Potential (KJ/mol water)	Water Potential (MPa)
Control	50mT(2h)	200mT(2h)			
0.189	0.179	0.174	0.91	-0.101	-0.56
0.156	0.146	0.143	0.87	-0.150	-0.83
0.146	0.136	0.139	0.85	-0.175	-0.97
0.123	0.113	0.116	0.78	-0.267	-1.48
0.118	0.108	0.113	0.75	-0.310	-1.72
0.099	0.089	0.089	0.64	-0.480	-2.66
0.089	0.079	0.081	0.53	-0.683	-3.79
0.076	0.075	0.073	0.505	-0.735	-4.07
0.071	0.070	0.067	0.46	-0.836	-4.63
0.069	0.069	0.060	0.43	-0.908	-5.03
0.062	0.062	0.055	0.34	-1.161	-6.43
0.062	0.061	0.051	0.325	-1.209	-6.70
0.054	0.054	0.052	0.295	-1.314	-7.28
0.036	0.035	0.023	0.13	-2.195	-12.16
0.026	0.026	0.021	0.08	-2.718	-15.06
0.025	0.025	0.018	0.075	-2.787	-15.44
0.019	0.018	0.015	0.055	-3.121	-17.29
0.012	0.011	0.013	0.01	-4.955	-27.46

Table 4.12.1b. Water status parameters of maize seeds exposed by different magnetic field kept at 35°C and at different RH.

Moisture content (%)			Water activity	Chemical Potential (KJ/mol water)	Water Potential (MPa)
Control	50mT(2h)	200mT(2h)			
0.193	0.188	0.187	0.90	-0.117	-0.649
0.178	0.168	0.170	0.87	-0.155	-0.858
0.164	0.156	0.160	0.84	-0.194	-1.074
0.137	0.131	0.137	0.755	-0.313	-1.732
0.128	0.127	0.126	0.75	-0.320	-1.773
0.102	0.106	0.107	0.62	-0.532	-2.946
0.084	0.086	0.085	0.505	-0.760	-4.210
0.079	0.076	0.076	0.465	-0.852	-4.718
0.077	0.076	0.074	0.43	-0.939	-5.201
0.073	0.071	0.062	0.415	-0.978	-5.419
0.060	0.060	0.061	0.325	-1.250	-6.926
0.052	0.054	0.056	0.22	-1.684	-9.330
0.049	0.050	0.055	0.21	-1.736	-9.617
0.035	0.035	0.037	0.115	-2.405	-13.328
0.024	0.025	0.027	0.08	-2.809	-15.564
0.024	0.022	0.022	0.075	-2.881	-15.962
0.022	0.020	0.020	0.055	-3.226	-17.873
0.013	0.015	0.014	0.01	-5.122	-28.378