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National Academy of Agricultural Science (NAAS) Rating : 3. 03

Acceleration of Germination and Early Growth Characteristics of Soybean and Maize after Pre-Treatment of Seeds with Static Magnetic Field

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ABSTRACT: *Soybean and maize seeds were pre-treated with 200 mT static magnetic field (SMF) for 1 h to evaluate the effect of magnetopriming on germination and early growth charteristics of seeds. The SMF pre-treated soybean and maize seeds showed improved germination related parameters like germination-percentage, water uptake, seedling length, fresh and dry weight of seedlings and vigor indices as compared to untreated seeds. Amylase and protease activities were also higher in SMF treated seeds as compared to untreated seeds in both soybean and maize seedlings. Thus faster hydrolytic activity of enzymes in SMF treated seeds leads to higher rate of germination. Increased levels of hydrogen peroxide and superoxide radical in germinating magnetoprimed seeds of both soybean and maize suggested their role in promotion of germination. SMF treatment influences the physiological and biochemical process in the seeds and thereby contributes to better vigor and improved crop stand.*

Key Words: *Amylase, germination, hydrogen peroxide, superoxide radical, vigour.*

INTRODUCTION

Seed performance is known to improve by the pre-sowing seed treatment including chemical and physical treatments like electrical, microwave and irradiation. Physical methods are not only cost effective but they also significantly improve the crop yield without adversely affecting the environment. They influence the physiological and biochemical process in the seeds, and thereby contribute to greater vigour and improved crop stand. Magnetic Field treatment (MF) is one of the physical presowing seed treatments that have been reported to enhance the performance of crop plants [1,2]. Stimulation of seeds with magnetic fields as a way to increase their quality of germination has caught the interest of many scientists.

MF was shown to induce germination in barley [3], rice [4], oak [5], tomato [6], lettuce [7], corn [8] and chickpea [9]. Magnetoreception in plants depends on magnetic exposure dose, time of exposure and the moisture content that finally increases germination of seeds [10].

The present investigation was carried out in two economically important crops; maize and soybean. Maize *(Zea mays* L.) is considered as queen of the cereals and soybean (*Glycine max* L.) is a major crop of Madhya Pradesh. Pretreatment of imbibed soybean and maize seeds by magnetic fields increased the germination and seedling growth rates [2, 11, 12]. Previous studies on soybean and maize have shown that magnetic field strengths of 200 mT for 1 h was best suited for increasing the germination-related parameters [11, 12]. However, the role of hydrolytic enzymes along with the reactive oxygen species (ROS) in germination of soybean and maize seedlings were not studied after static magnetic fields (SMF) pretreatment. The aim of the present study was to quantify the effects of SMF on germination-percentage, water uptake and vigour of soybean and maize seeds and to establish a relation between the activity of hydrolytic enzymes (amylases and protease) and also to investigate the role of ROS like superoxide radical and hydrogen peroxide.

MATERIALS AND METHODS

Plant Material

The breeder seeds of soybean (*Glycine max* (L.) var: JS-335) were collected from the Directorate of Soybean Research, Indore, M.P., India and seeds of *Zea mays*

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var. JM-216 were obtained from JNKVV, Zonal Agriculture Research Station, Chhindwara, M.P., India.

Magnetic Field Generation

A Testron EM-20 electromagnetic field generator (Testron Instruments, Delhi, India) was fabricated with a variable horizontal magnetic field strength (50–500 mT) and a gap of 5 cm between pole pieces [9]. The pole pieces were cylindrical in shape with a diameter of 9 cm and a length of 16 cm. The number of turns per coil was 3000 and the resistance of the coil was 16 V. A DC power supply (80 V/10 A) with a continuously variable output current was used for the electromagnet. A digital gauss meter (Model DGM-30, Testron Instruments), operating on the principle of the Hall Effect, monitored the field strength produced in the pole gap. The probe was made of indium arsenide crystals and encapsulated in a non-magnetic sheet of 5 mm X 4 mm X 1 mm, which could measure 0–2 T with full-scale range in increments of 5 mT.

Magnetic Treatment

Soybean and maize seeds were exposed to static magnetic field (200 MT for 1 h) in a cylindrical-shaped sample holder of 42 cm^3 capacity, made from a non-magnetic thin transparent plastic sheet. One hundred visibly sound, mature, healthy seeds held in the cardboard container were placed between the poles of the electromagnet under a uniform magnetic field and treated for 1h. The required strength of the magnetic field was obtained by regulating the current in the coils of the electromagnet. A gauss meter was used to measure the strength of the magnetic field between the poles. The local geomagnetic field was <10 mT. All treatments in the experiments were run simultaneously along with untreated control (UT) under similar conditions.

Seed Water Uptake

The water uptake (WU) by seeds of soybean and maize during the imbibition was determined for three replicates of 50 seeds for each treatment. Weighed seeds per replicate were placed between water-saturated cotton in a plastic box and incubated at 25°C. At intervals of 24, 48, 72 and 96 all the seeds were removed, blotted dry, weighed, and immediately returned to the moist cotton to continue imbibition. Changes in weight due to imbibitions were expressed as the amount of water absorbed per seed dry weight, which is calculated by the following formula:-

WU% = (Fresh weight of seed-Dry weight of seed) X 100

Seed Germination

Seed germination was determined by following the method of the International Seed Testing Association [13]. Four replications with 25 seeds each were placed between two layers of moist germination paper, rolled carefully, and wrapped in a sheet of wax paper to reduce surface evaporation. They were placed in a seed germinator (Remi Instruments, Mumbai, India) at 25^{o}C in an upright position. After 8 days, ten seedlings from each replicate were randomly taken for measuring germination parameters. Subsequently, they were dried overnight in an oven at 90^{o}C and their dry weight was measured. Seedling vigour was calculated following Abdul-Baki and Anderson [14] as:

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

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

Amylase and Protease activity

Amylase activity was assayed by the method described by Sawhney *et al.* [15] in germinating seeds (100 mg) of soybean and maize at different times of imbibitions (24 to 96 hrs). Protease activity was measured by the method given by Kunitz [16] in germinating seeds (1 g) of soybean and maize at different times of imbibitions (24 to 96 h).

Determination of superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2)

Free radicals were measured in SMF treated and control of soybean and maize seeds after 72 h of imbibition. Superoxide radical was estimated by the reduction of Na, 3′-[1-[(phenylamino)-carbonyl]-3,4-tetrazolium] (4-methoxy-6-nitro) benzenesulfonic acid hydrate (XTT) (Schopfer [17]. Hydrogen peroxide was estimated by formation of titanium-hydroperoxide complex [18].

Statistical Analysis

The data obtained from germination parameters were carried out in three independent experiments with five replicated measurements and expressed as increase in different parameters of magnetoprimed seeds (MT) over the untreated control (UT). All of the biochemical parameters were carried out within three independent experiments with three replicated measurements.

RESULTS

Germination Percentage: Germination percentage was not much affected, magnetic field treatment caused enhancement was up to 10% in maize and 7% in soybean seeds (Fig. 1).

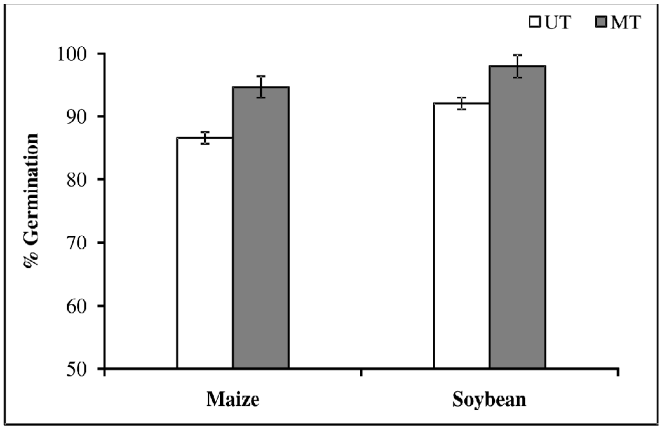

Figure 1: Effect of static magnetic field strength (200 mT for 1h) on germination percentage of (A) soybean and (B) maize seeds at 25°C. Data are the means ±SE of three independent experiments with five replicated measurements.

Water Uptake: Magnetic field strength enhanced water uptake in seeds in comparison to untreated seeds during the time period of 24 to 96 hrs of imbibitions (Fig.2). Seeds treated with a magnetic field showed a significant increase in water uptake, it increases with the increase in the time of seed imbibitions from 24h to 96h. It showed maximum increase of 25% and 51%, respectively in soybean and maize (Fig. 2 A,B) after 96 h of imbibitions.

Seedling length: Seedling length was enhanced significantly after magnetic field treatment due to increase in root and shoot length (Fig. 3A,B). Maximum increase in seedling length obtained was 33 % (soybean) and 27 % (maize) (Fig. 3A,B).

Seedling Dry weight and Fresh weight: Seedling dry and fresh weight were also increased by MF treatment in both soybean and maize (Fig. 3C,D). Magnitude of enhancement was more in dry weight as compared to the fresh weight of seedlings in MF treated seeds. Dry weight of seedlings was significantly enhanced by 75% in soybean and 56% in maize (Fig. 3C,D).

Vigour Index: The trend of increase in vigour index I paralleled to the seedling length and vigour index II paralleled to the dry weight of seedlings (Fig. 3E,F). Enhancement in vigour index-I was maximally enhanced (42%) in soybean as compared to the untreated seeds (Fig. 3E,F). The extent of enhancement in Vigour index-II was more in maize (67%) than in soybean (20%) as compared to the untreated seeds (Fig. 3E,F).

Amylase and Protease activity

α-amylase and protease activity was estimated in seeds treated with SMF and compared with untreated controls at different time intervals after imbibitions (24-96h). Increased hydrolytic activity was observed in seeds after magnetic field treatment. Amylase activity increased up to 72 h after imbibition in germinating seeds of soybean and decreased thereafter. However the maximum increase was observed after 48 h of imbibition (165%) over the

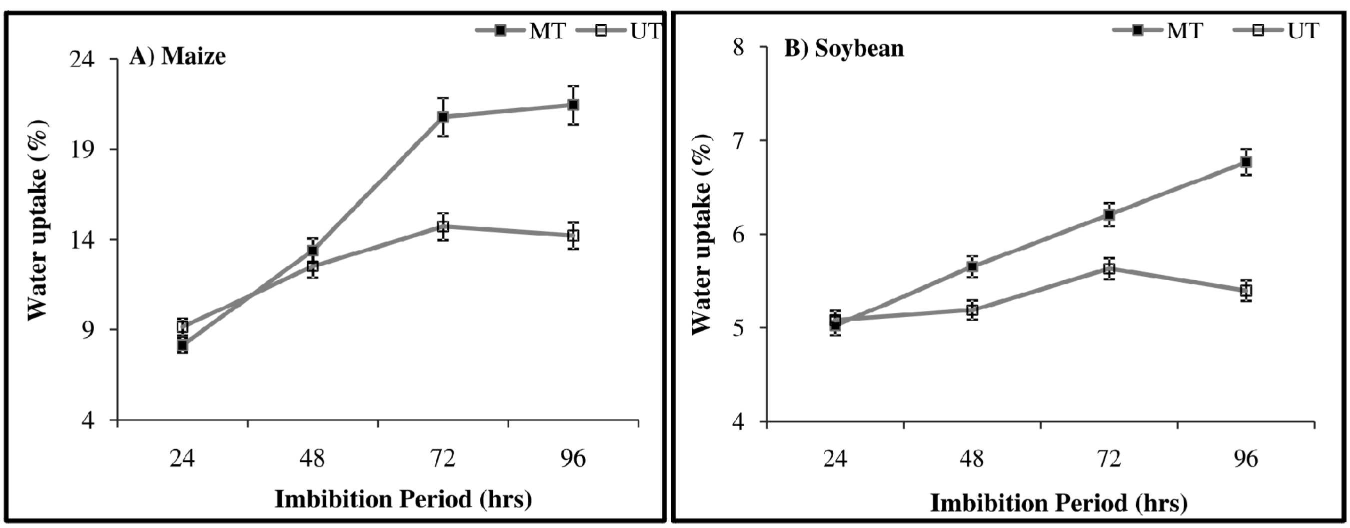

Figure 2: Effect of static magnetic field strength (200 mT for 1h) on water uptake in (A) soybean and (B) maize seeds with different (24 to 96) hours of imbibition at 25°C Data are the means ±SE of three independent experiments with five replicated measurements.

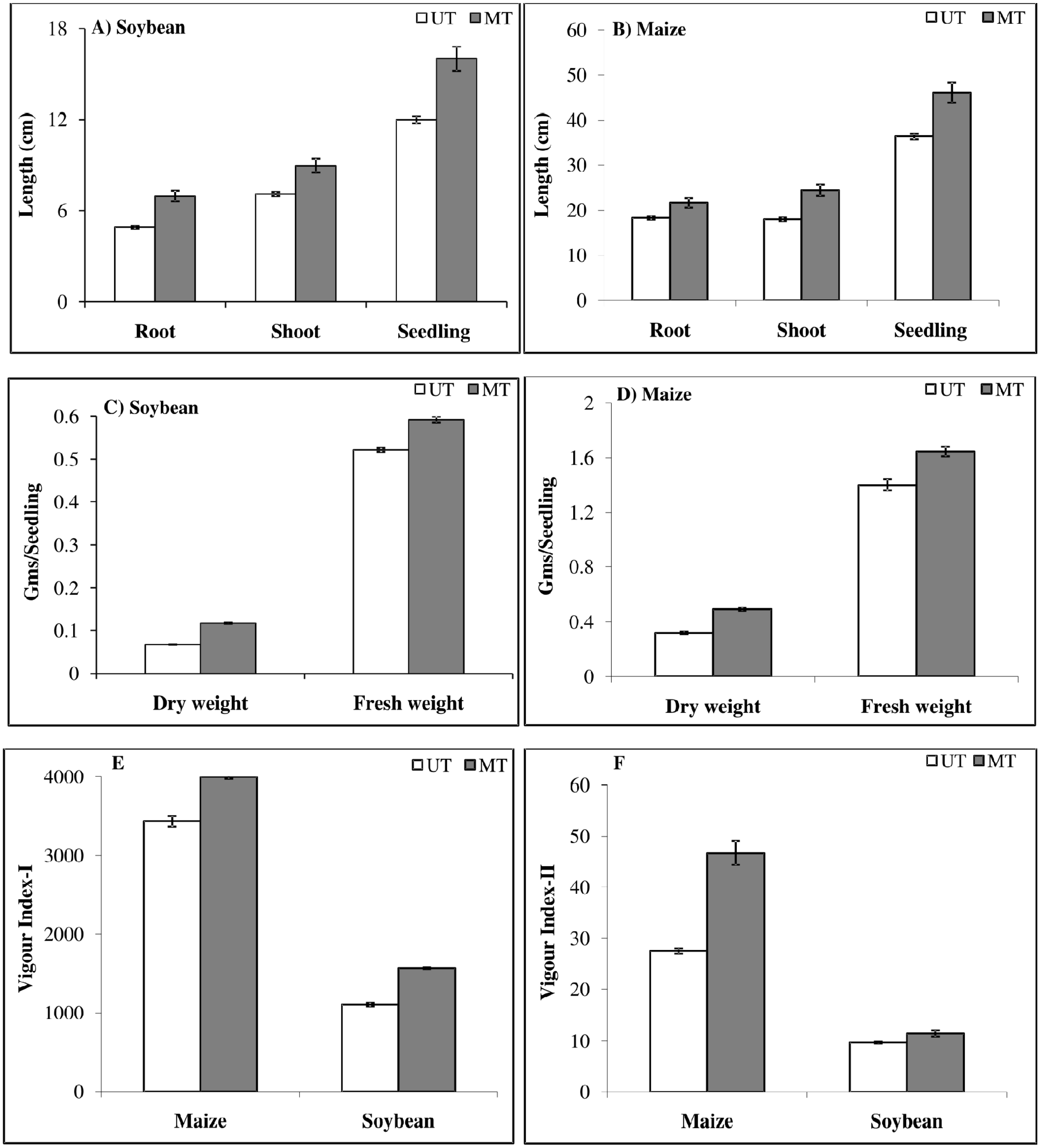

Figure 3: Effect of static magnetic field strength (200 mT for 1h) on (A, B) length of shoot, root and seedling (C,D) dry and fresh weight of seedling (E) Vigour-I and (F) Vigour-II of soybean and maize seeds. Data are the means ±SE of three independent experiments with five replicated measurements.

untreated seedlings of soybean (Fig. 4A). In maize seedlings, activity increased with the the time of imbibition period from 24 to 96h and it showed maximum enhancement of 71% as compared to untreated seedlings at 96 h of imbibition period (Fig. 4B).

The increase in hydrolyzed protein per gram fresh weight of seeds represented an increase in

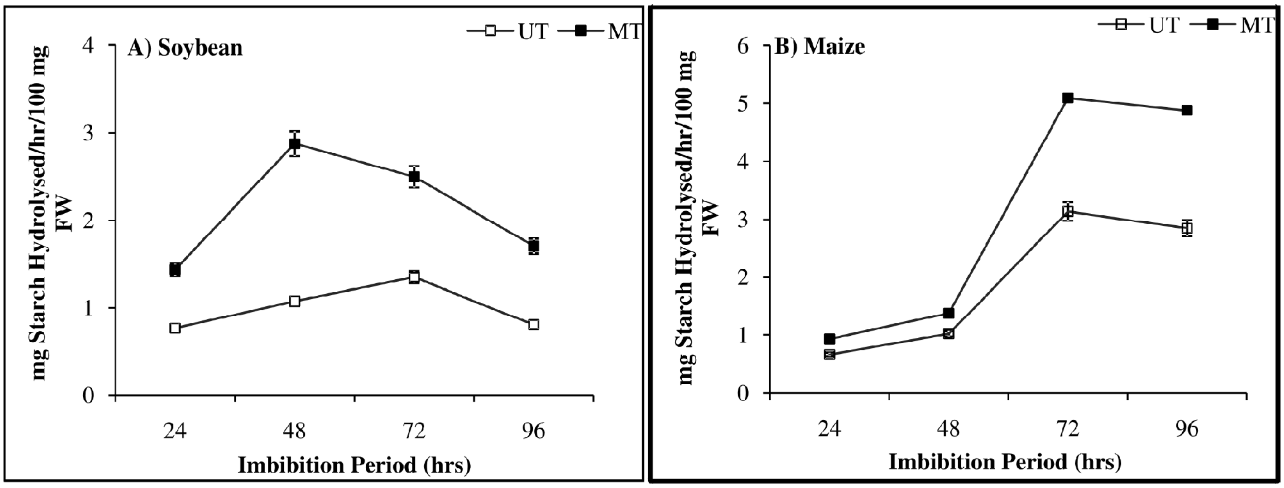

Figure 4: Effect of static magnetic field strength (200 mT for 1h) on á-amylase activity in A) soybean and (B) maize seeds at different intervals of imbibition at 25ºC. Data are the means +SE of three independent experiments with three replicated measurements.

protease activity. Magnetoprimed seeds maintained higher protease activity than the untreated seeds, with the difference being greater at later stages of imbibitions; 48h in soybean as well as in maize (Fig. 5A, B).

Rate of $O_2^{.-}$ production with XTT assay

Production of $O_2^{.-}$ by seedling was demonstrated using the reduction of the tetrazolium salt XTT. A typical time course of this test reaction is depicted in Fig 6A,B. Treatments of seeds with stationary magnetic field increased the rate of production of $O_2^{.-}$ in seedlings of soybean and maize. Magnetic field treatment of seeds with stationary magnetic fields increased the rate of production of $O_2^{.-}$ by 31% in soybean and 80% in maize seedlings as compared to untreated seedlings at 150 min of incubation in XTT (Fig. 6A,B).

H_2O_2 content

H_2O_2 content was higher in the seedlings of soybean and maize after magnetic field treatment (Fig. 7A,B). The enhancement of H_2O_2 in soybean was by 48% in SMF treated seeds over the untreated control. In 8 day old seedlings of soybean, the H_2O_2 was higher after the SMF treatment by 144% over the untreated control at 24h of imbibitions of seeds (Fig. 7A). In 4 day old seedlings of maize the H_2O_2 was enormously increased by 279% after the SMF treatment over the untreated seedlings (Fig. 7B).

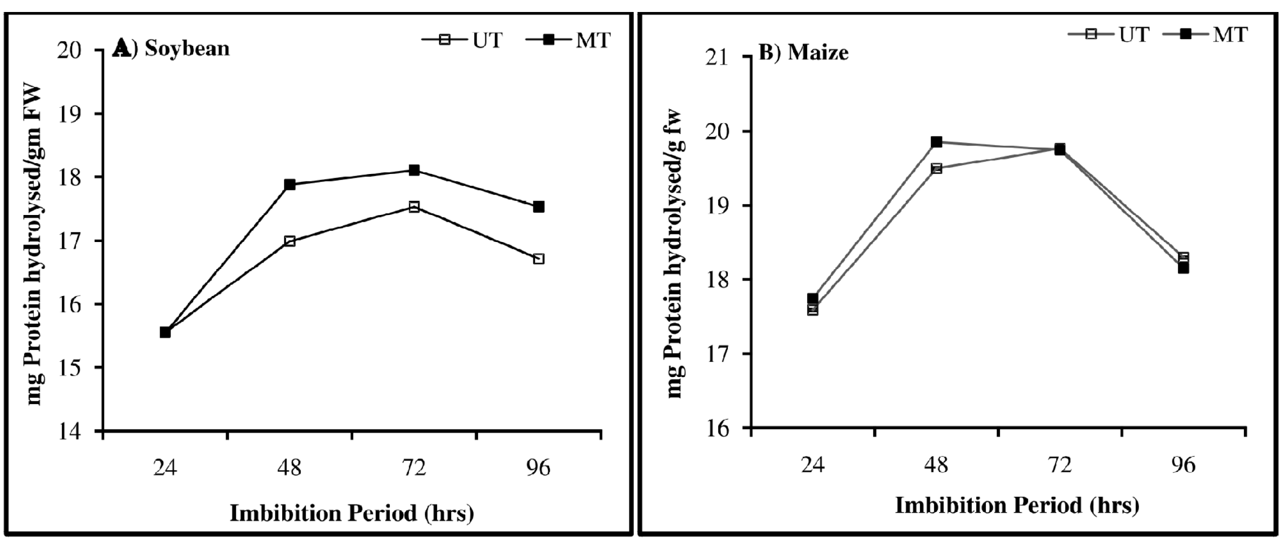

Figure 5: Effect of static magnetic field strength (200 mT for 1h) on the protease activity in (A) soybean and (B) maize seeds at different intervals of imbibition at 25ºC Data are the means +SE of three independent experiments with three replicated measurements.

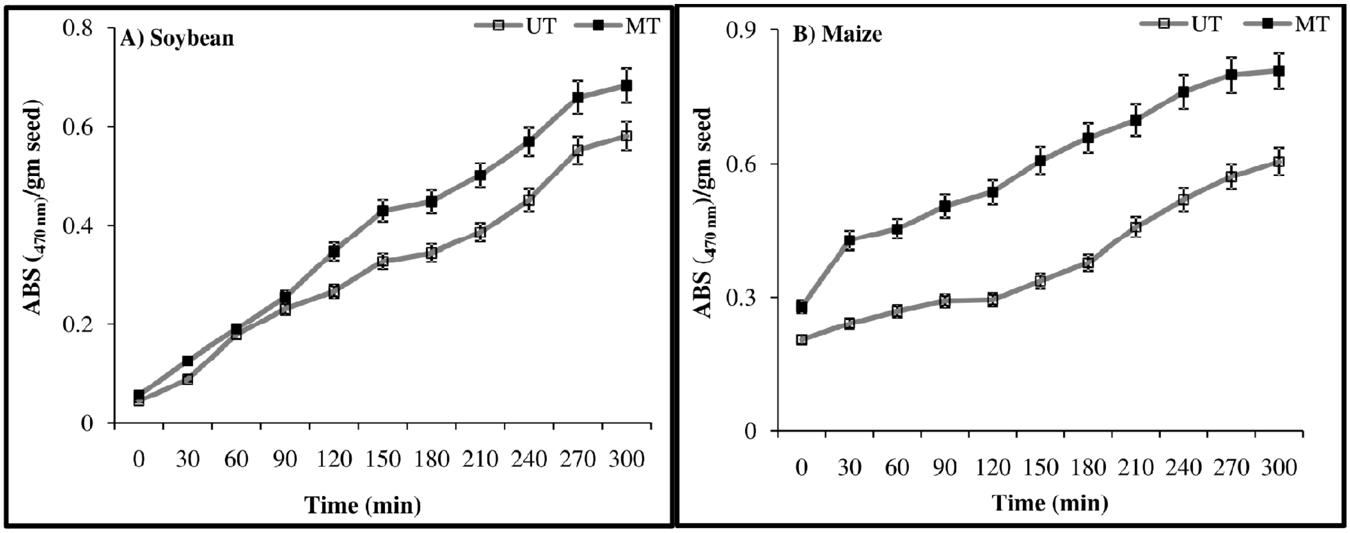

Figure 6: Effect of static magnetic field strength (200 mT for 1h) on the production of superoxide radical in seedlings of (A) soybean and (B) maize seeds. Data are the means ±SE of three independent experiments with three replicated measurements.

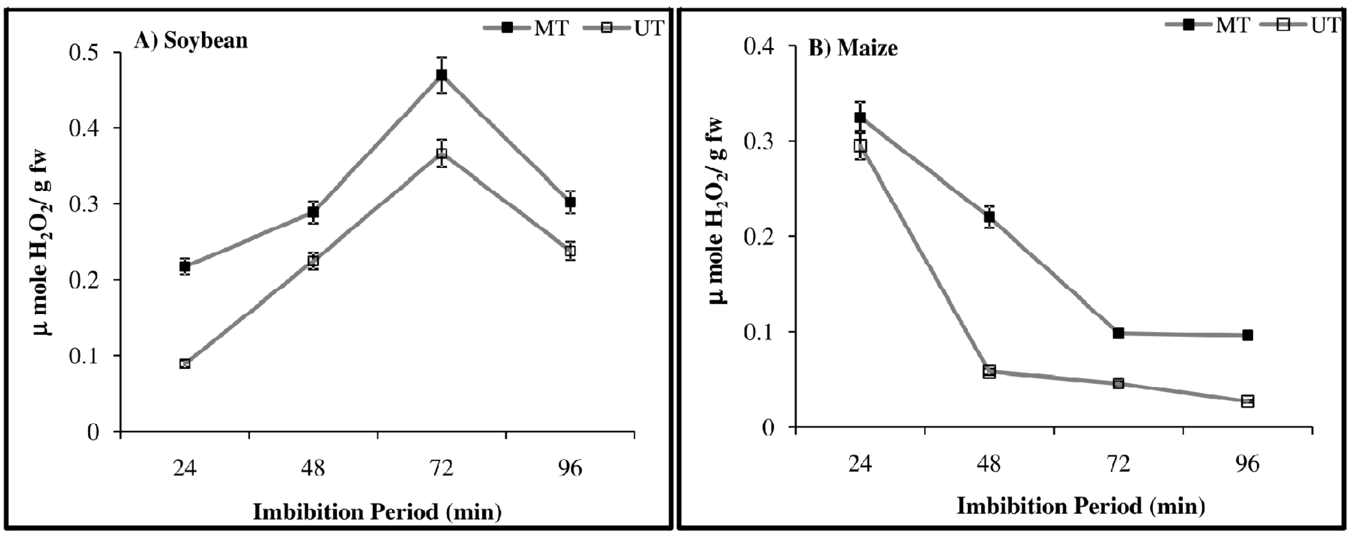

Figure 7: Effect of static magnetic field strength (200 mT for 1h) on the hydrogen peroxide content in seedlings of (A) soybean and (B) maize seeds at different intervals of imbibition at 25°C. Data are the means ±SE of three independent experiments with three replicated measurements.

DISCUSSION

Exposure of soybean and maize seeds to static magnetic field (200 mT, 1h) in the present study showed an overall stimulating effect with respect to all germination characteristics like percent germination, length of seedlings and vigour indices. Such enhanced performance of seeds in their germination characteristics have been reported in other crops also [2,8,9,12]. Magnetoreception in plants depends on magnetic exposure dose, time of exposure and the moisture content that finally, increases germination of seeds. Magnetic field exposure of seeds prior to sowing led to an increase in germination rate and seedling vigour in groundnut, onion, rice, cucumber and chickpea seeds [1,9,19,20]. Several reports also showed that the magnetic field exposure increases germination of low viability seeds and improves their quality and sprouting rate [20,21].

In our study, significant difference in seed water uptake in SMF treated seeds compared to untreated seeds is observed at all the stages of imbibition period (24 to 96 h). It has been suggested that following magnetic field exposure, the increased uptake of water may be due to changes in the intracellular level of Ca^{2+} and ionic current density across the cellular membrane altering the osmotic pressure and the capacity of cellular tissue to absorb water [22]. It is assumed that increased accumulation of water in the seeds after magnetic treatment may activate the germinating enzymes, which accelerate the germination in treated seeds [10]. This shows that metabolic events are triggered in MF treated seeds by faster hydration of associated enzymes.

In the present study we have found that amylase and protease activity of magnetically exposed seeds were higher than the untreated seeds of soybean and maize. The higher activities of hydrolytic enzymes are responsible for the quick seed germination, seedling length and improved seedling vigour in SMF treated seeds. Increased activities of hydrolyzing enzymes was found in *Helianthus annuus*, after exposure of stationary magnetic field strength of 50 mT and 200 mT for 2 h, which were responsible for the quick seed germination, improved seedling vigour and better root characteristics of treated seeds in this plant [10]. In the germinating seeds proteases are involved in the hydrolysis of proteins; the reduction is initiated by endoproteases which convert the water insoluble storage protein into soluble peptides that can be further hydrolyzed to amino acids by exopeptidases [23].

Data presented here indicate a significant enhancement in the production of reactive oxygen species like H_2O_2 and $O_2^{\cdot-}$ after treating the seeds of soybean and maize with static magnetic field. Increased levels of free radicals in seeds after treatment with laser light and magnetic fields, accompanied by enhanced dynamics of seed germination, was also reported in faba bean seeds and pea seeds [24,25]. Elevated ROS may serve as messengers to storage organs to bring about mobilization of reserve material and support rapid axis growth [26]. The positive role of H_2O_2 has been recognized in many processes like stomatal closure, defense reaction, peroxisome biogenesis, cell wall cross-linking and lignin synthesis [27]. Production of free radicals in the germinating seeds has been associated with transition from quiescent stage to a metabolically active state that would account for faster germination [28,29]. Seed germination was enhanced with enhanced ROS production and its scavenging reduced germination [30]. In our study increased ROS production was followed by enhanced seedling growth by magnetic field treatment. Thus higher content of $O_2^{\cdot-}$ and H_2O_2 after SMF treatment are involved in enhanced germination and growth of the seedlings. Shine et al. [2] have also found the EPR spectra of adducts produced with $O_2^{\cdot-}$ and $\cdot OH$ radicals at an early stage (just germinated and 8 day old seedlings) and provided the evidence for the important role of ROS in enhancing the performance of seeds at this stage.

In conclusion, magnetopriming of soybean and maize seeds with 200 mT (1h) increased germination-percentage, rate of germination and seedling vigour. In SMF treated seeds water uptake was significantly increased that helped in faster hydration and activation of germination enzymes and triggered the metabolic events leading to enhanced vigour of treated seeds. Increase in ROS and maintenance of their levels for promotion of rapid growth in magnetoprimed seeds also explains the faster rate of germination. Thus, priming of dry soybean and maize seeds with static magnetic field will generate more vigorous seedlings in the field that can withstand abiotic stresses during sowing and competition with weeds in the field.

ACKNOWLEDGMENTS

Financial support by Department of Science Technology Women Scientists-A Scheme (SR/WOS-A/LS-674/2013-G) and UGC Rajiv Gandhi National Fellowship (F1-17.1/2013-14/RGNF-2013-14-ST-MAD-53993/ (SAIII/Website) are thankfully acknowledged.

REFERENCES

Bhardwaj, J., Anand, A. and Nagarajan, S. (2012), Biochemical and biophysical changes associated with magnetopriming in germinating cucumber seeds. *Plant Physiol. Biochem.* 57: 67–73.

Shine, M.B., Guruprasad, K.N. and Anand, A. (2011), Enhancement of germination, growth, and photosynthesis in soybean by pretreatment of seeds with magnetic field. *Bioelectromagnetics* 32:474–484.

Martinez, E., Carbonell, M.V. and Amaya, J.M. (2000), A static magnetic field of 125 mT stimulates the initial growth stages of barley (*Hordeum vulagare*, L.). *Electro-Magnetobiol.* 19 (3): 271–277.

Carbonell, M.V., Martinez, E., Amaya, J.M. (2000), Stimulation of germination in rice (*Oryza sativa* L.) by a static magnetic field. *Electro Magnetobiol.* 19(1):121–128.

Celestino, C., Picazo, M.L., Toribio, M. (2000), Influence of chronic exposure to an electromagnetic field on germination and early growth of *Quercus suber* seeds: Preliminary study. *Electro Magnetobiol.* 19(1):115–120.

Moon, J.D. and Chung, H.S. (2000), Acceleration of germination of tomato seeds by applying AC electric and magnetic fields. *J. Electrostat.* 48: 103–114.

Renia, F.G., Pascual, L.A. and Fundora, I.A. (2001), Influence of a Stationary Magnetic Field on water relations in lettuce Seeds. Part II: Experimental Results. *Bioelectromagnetics* 22:596-602.

Florez, M., Carbonell, M.V. and Martinez, E. (2007), Exposure of maize seeds to stationary magnetic fields: effects on germination and early growth. *Environ. Exp. Bot.* 59:68–75.

Vashisth, A. and Nagarajan, S. (2008), Exposure of seeds to static magnetic field enhances germination and early growth characteristics in chickpea (*Cicer arietinum* L.). *Bioelectromagnetics* 29:571–578.

Vashisth, A., Nagarajan, S. (2010), Effect on germination and early growth characteristics in sunflower (*Helianthus annus*) seeds exposed to static magnetic field. *J. Plant Physiol.* 167:149–156.

Shine, M.B., Guruprasad, K.N. and Anand, A. (2012), Effect of stationary magnetic field strengths of 150 and 200mT on reactive oxygen species production in soybean. *Bioelectromagnetics* 33:428–437.

Shine, M. and Guruprasad, K.N. (2012), Impact of pre-sowing magnetic field exposure of seeds to stationary magnetic field on growth, reactive oxygen species and photosynthesis of maize under field conditions. *Acta Physiol.Plant.* 34:255–265.

ISTA. (1985), International seed testing association. *Seed Sci. Technol.* 13: 299–513.

Abdul-Baki, A.A., Anderson, J.D. (1973), Vigour determination in soybean by multiple criteria. *Crop Sci.* 10:31–34.

Sawhney, S., Toky, K. L. and Nanda, K. K. (1970), Changes in amylase activity during extension growth and floral induction in *Impatiens balsamina* - a qualitative short-day plant. *Indian J. Plant Physiol.* 13: 198.

Kunitz, M. (1947), Crystalline soybean trypsin inhibitor: II. General properties. J *Gen Physiol* 30:291–300.

Schopfer, P. (2001), Hydroxyl radical-induced cell-wall loosening in vitro and in vivo: implications for the control of elongation growth. *Plant J.* 28:679–688.

Mukherjee, S.P. and Choudhari, M.A. (1983), Implications of water stressed induced changes in the levels of endogenous ascorbic acid and H_2O_2 in *Vigna* seedlings. *Physiol Plant* 58:166–170.

Vakharia, D.N., Davariya, R.L. and Parameswaran, M. (1991), Influence of magnetic treatment on groundnut yield and yield attributes. *Indian J. Plant Physiol.* 34:131–136.

Alexander, M.P. and Doijode, S.D. (1995), Electromagnetic field, a novel tool to increase germination and seedling vigor of conserved onion (*Allium cepa* L.) and rice (*Oryza sativa* L.) seeds with low viability. *Plant Genet. Res. Newsl.* 104:1–5.

Carbonell, M.V., Martinez, E., Florez, M., Pintor-Lopez, A. and Amaya, J.M. (2008), Magnetic field treatment improves germination and seedling growth in *Festuca arundinacea* Schreb. and *Lolium perenne* L. *Seed Sci. Technol.* 36:31–37.

Garcia- Reina, F., Arza- Pascual, L. (2001), Influence of a stationary magnetic field on water relations in lettuce seeds Part I: theoretical considerations. *Bioelectromagnetics* 22:589–595.

Callis, J. (1995), Regulation of protein degradation. *Plant Cell* 7:845–857.

Podlesny, J., Misiak, L., Koper, R. (2001), Concentration of free radicals in faba bean seeds after the pre-sowing treatment of the seeds with laser light. *Int. Agrophys.* 15:185–189.

Podlesny, J., Pietruszewski, S., Podlesna, A. (2005), Influence of magnetic stimulation of seeds on the formation of morphological features and yielding of the pea. *Int. Agrophys.* 19:1–8.

Verma, G. and Sharma, S. (2010), Role of H_2O_2 and cell wall monoamine in germination of *Vigna radiata* seeds. *Indian J. Biochem. Biophys.* 47:249–253.

S. Neill and R. Desikan, J. (2002), Hancock, Hydrogen peroxide signaling. *Curr. Opin. Plant Biol.* 5: 388-395.

Puntarulo, S., Galleano, M., Sanchez, R.A. and Boveris, A. (1991), Superoxide anion and hydrogen peroxide metabolism in soybean embryonic axes during germination. *Biochimica et Biophysica Acta* 1074:277–283.

Gidrol, X., Lin, W.S., Degousee, N., Yip, S.F. and Kush, A. (1994), Accumulation of reactive oxygen species and oxidation of cytokinin in germinating soybean seeds. *Eur. J. Biochem.* 224:21–28.

Chaudhuri, A. and Kar, R.K. (2008), Inhibition of seed germination by propyl gallate, a free radical scavenger and recovery of germination by hydrogen peroxide and ethylene in *Vigna radiate*. *World J Agri Sci*. 4: 914-921.