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Research Paper

Enhancement of maize seeds germination by magnetopriming in perspective with reactive oxygen species

M. B. Shine[1] • S. Kataria[1]* • K. N. Guruprasad[1] • Anjali Anand[2]

[1]School of Life Sciences, Devi Ahilya University, Khandwa Road, Indore, India.
[2]Plant Physiology, Indian Agricultural Research Institute, Pusa, New Delhi, India.

*Corresponding author. E-mail: sunita_kataria@yahoo.com.

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Abstract. The utility of stationary magnetic field (SMF) for biostimulation of seeds and associated physio-biochemical changes in maize (*Zea mays* L.) var. HQPM.1 was studied under laboratory conditions. Magnetopriming improved germination related parameters like percentage of germination, speed of germination, seedling length, fresh weight, dry weight and vigour indices. Among the various parameters seedling length and vigour Index I were the most improved parameters (up to 72 to 59% respectively) after magnetopriming. Two doses (200 mT for 60 min and 100 mT for 120 min) were further taken to understand the involvement of reactive oxygen species ($O_2^{\cdot-}$, $^{\cdot}OH$ and H_2O_2) and antioxidant enzymes in the magnetoprimed seeds as they were best in improving germination related parameters. Enhanced germination in magnetoprimed maize seeds was coupled with enhanced reactive oxygen species content. Peroxidase (both cytosolic and wall bound) activity was enhanced and superoxide (both cytosolic and wall bound) activity was reduced in the embryo and the eight-day-old seedlings from magnetoprimed seeds. Obtained results indicated that magnetopriming can be effectively used as a biostimulant for seed germination and the impact of SMF is biochemically identified in context with reactive oxygen species.

Keywords: Biostimulation, magnetopriming, reactive oxygen species, seedling growth.

INTRODUCTION

The pursuit for an efficient and economical technique to develop quality-sowing seed has been an interest of industry and academics. Among the various priming techniques explored, magnetopriming has attracted an increased attention in the past few decades, as it is ecofriendly and cost effective. The majority of the obtained results show that the magnetic field acts as a biostimulant and causes enhanced germination and growth. Mung beans and water convolvuluses seeds when treated with electromagnetic field enhanced germination (Jinapang *et al.*, 2010). Germination enhancement was optimum for the mung beans at 100 mW/1h power duration level, while for water convolvuluses the optimum germination power-duration level was 1 mW/2 h. Enhanced germination rate, seedling growth and development in cucumber by magnetic field pre-treatment was reported by Yinan *et al.* (2005). The effects of electromagnetic irradiation on seed vigour of maize hybrids, as well as the response of each genotype were assessed by Zepeda-Bautista *et al.* (2009) and reported that pre-sowing treatment increases corn seed vigour through emergence rate, establishment percentage, and dry mass of seedling aerial part, according to the combination of radiation intensity, time and genotype. Reduced mean germination time and enhanced germination were obtained by exposing tomato

seeds to stationary magnetic field of 125 and 200 mT for different duration (Martinez *et al.*, 2009). Plethora of literature is available on the effect of magnetic biostimulation of seeds using stationary magnetic field (Shine *et al.*, 2011[a,b]; Bhardwaj *et al.*, 2012; Thomas *et al*., 2013; Kataria *et al.*, 2015; Kataria *et al.*, 2017).

Extensive research has revealed that the effects of magnetopriming treatments depend on the magnetic field strength, duration of exposure (Wittekind *et al.*, 1990), the physiological condition of the organism involved, and the environmental conditions (Weaver, 1993; Gutzeit, 2001). Pertinent literature on magnetopriming shows that most of the work in this field is limited to studying only the germination related parameters of primed seeds with a few exceptions. Under laboratory and field conditions, SMF strength of 200 mT (60 min) was proved to be best for improving different seedling parameters of maize (Shine and Guruprasad, 2012; Vashisth and Joshi, 2016); however biochemical and biophysiological changes associated with enhanced germination after magnetopriming has not been addressed in case of a cereal crop like maize. Therefore the present study was carried out with the objective of understanding the biochemical and biophysiological changes associated with magnetic biostimulation of maize seeds during germination.

MATERIALS AND METHODS

The breeder seeds of maize (*Zea mays* L.) var. HQPM.1 were collected from Indian Agricultural Research Institute, New Delhi, India. Seeds of uniform size and shape without visible defects and malformation were selected and stored in a desiccators having anhydrous calcium chloride. Seed moisture content was determined by oven drying seeds at 95°C to constant weight (Walters, 1998). Moisture content (%) was calculated as $(W_1\text{-}W_2/W_1) \times 100$, where W_1 was the initial weight of the seed and W_2 was the final weight of the seed after drying. Moisture content was 8.2 % in the seeds used for all treatments and experiments.

Magnetic field generation

An electromagnetic field generator "Testron EM-20" (Testron India, Delhi, India) with variable horizontal magnetic field strength (50 to 500 mT) and a gap of 5 cm between pole pieces was used for seed priming treatment (Vashisth and Nagarajan, 2008). The pole pieces were cylindrical in shape, with 9 cm diameter and 16 cm length. The number of turns per coil was 3000 and the resistance of the coil was 16 ohms. A DC power supply (80 V/10 A) with continuously variable output current was used for the electromagnet. A digital Gauss meter model DGM-30 (Testron India) operating on the principle of Hall Effect monitored the field strength produced in the pole gap. The probe made of Indium Arsenide crystal and encapsulated to a non-magnetic sheet of 5 mm × 4 mm × 1 mm could measure 0-2 T with full scale range in increments of 5 mT.

Magnetic treatment

Maize seeds were exposed to a magnetic field of 50 to 250 mT in steps of 50 mT for 30 to 120 min at an interval of 30 min in a cylindrical shaped sample holder of 42 cm^3 capacity, made from a non-magnetic thin transparent plastic sheet. Hundred mature and healthy seeds held in the plastic container were placed between the poles of the electromagnet under a uniform magnetic field. 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. At low field (50 mT), from the centre to end of the poles, the variation was 0.6% in the horizontal direction and 1.6% in the vertical direction of the applied field. At high field (500 mT), they were 0.4 and 1.2% of the applied field, respectively. The variation in temperature during the course of seed exposure was 25 ± 0.5°C.

Seed germination

Seed germination was determined by following the method of ISTA (1985). Four replications each with 25 seeds 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 Ltd., India) at 25°C in an upright position. After 8 days, germinated seeds were grouped as normal, abnormal seedling, and dead seeds. Normal seedlings were the seedling with well-developed coleoptiles, mesocotyl and radicle, whereas abnormal seedlings were designated to seedling with stunted radicle with weak/no mesocotyl. Germination percentage was calculated on the basis of normal seedlings. Ten such seedlings from each replicate were randomly taken for measuring seedling length. Subsequently, they were dried overnight in an oven at 90°C and dry weight was measured. Seedling vigor was calculated following Abdul-Baki and Anderson (1973), as:

Vigor index I = Germination % × Seedling length (Root + Shoot)

Vigor index II = Germination % × Seedling dry weight (Root + Shoot)

Speed of germination

Hundred seeds in four equal replications were placed on moistened filter paper in Petri dishes and kept in an incubator at 25°C. Radicle emergence from the seed was scored as positive germination. A daily germination count

of the incubated seeds was taken until no more seeds germinated. The speed of germination was calculated following Maguire (1962), as:

Speed of germination = Σ (n/t)

Where *n* is the number of seeds newly germinating at time *t* and *t* is days from sowing.

Spectrophotometric assay of $O_2^{\cdot -}$ production

$O_2^{\cdot -}$ production was measured by the reduction of Na,3'-(1-((phenylamino)-carbonyl)-3,4-tetrazolium) (4-methoxy-6-nitro) benzenesulfonic acid hydrate (XTT) (Sigma-Aldrich) (Frahry and Schopfer, 2001). Groups of 20 embryos or 20 abraded seedling parts (0.5 cm, from 8 day old dark grown seedling) were incubated in 5 ml of K-phosphate buffer (pH 6.0) containing 500 µm XTT in darkness at 25°C on a shaker. Aliquots were obtained at 15 min interval and absorbance was read at 470 nm by using spectrophotometer (UV-1601: Shimadzu, Nakagyo –Ku, Kyoto, Japan).

Electron paramagnetic resonance spectroscopy (EPR) of $^{\cdot}OH$ radicals

The $^{\cdot}OH$ content in embryos and eight day old seedlings were measured as described by Liszkay *et al*. (2003). coleoptiles/mesocotyl/radicle (0.5 cm) from control and treated seedlings were incubated for 1 h in 15 ml of water and then in 2 ml reaction medium containing 20 mM K-phosphate buffer (pH 6.0), 850 mM ethanol/50 mM α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (POBN) as a spin trapping system. After incubating for 1 h on shaker, EPR spectra of the hydroxylethyl-POBN adduct were measured at room temperature. EPR spectra were recorded using a JEOL JES-FA 300 X-band EPR (Akishima, Tokyo, Japan) spectrometer EPR instruments setting-Microwave power 5 mW, modulation width 0.2 mT, amplitude 8000, field centre 338.092 mT with width +/-5.0 mT, sweep time 4 min and time constant 0.03 s. Hydroxyl radical produced from fenton reaction was recorded as a standard spectra for hydroxyethyl-POBN adduct. All the chemicals used in spin trapping experiments were purchased from Sigma-Aldrich, St. Louis, USA. Hydroxyl radical ($^{\cdot}OH$) production was specifically tested in embryos and eight-day-old seedling using ethanol/POBN spin trap, which reacts with $^{\cdot}OH$ and forms relatively stable hydroxy ethyl/POBN adducts (Ramos *et al.*, 1992).

Hydrogen peroxide assay

Hydrogen peroxide content was determined by measuring the absorbance of titanium-hydroperoxide complex (Mukherjee and Choudhari, 1983). A group of 20 embryos and 0.5 g of 8-day-old dark grown seedling tissue (coleoptiles/mesocotyl/radicle) was crushed in 10 ml of chilled acetone. Concentration of hydrogen peroxide was determined using the standard curve plotted with known concentration of hydrogen peroxide. A standard curve in the range 0 to 10 µM/ml H_2O_2 was used for the calculation of H_2O_2 present in the tissue.

Enzyme extraction and assay

Superoxide dismutase (SOD) (EC 1.15.1.1) and peroxidase (POD) (EC 1.11.1.7) were obtained from maize seedlings as described by Carpita (1984) with some minor modifications (Kukavica *et al*., 2009). One gram of tissue was homogenized in 5 ml Tris-HCl buffer (50mM, pH 7.0) containing 50 mM NaCl, 0.05 % Tween-80, and 1 mM phenylmethyl sulfonyl fluoride (PMSF). The homogenate was filtered through two layers of mira cloth and supernatant was centrifuged at 12,000 rpm for 20 min. The supernatant obtained after centrifugation was used for the estimation of cytosolic SOD and POD. The pellet was washed four times with the above buffer without detergent and salt until the pellet was free of cytosolic SOD and POD (tested with reaction mixture of the respective enzyme) and then suspended in 2 ml of 1 M NaCl. Cell wall isolate obtained after centrifugation was digested for 24 h at 4°C in 2 ml of a mixture containing 0.5 % cellulase and 2.5 % pectinase in 50 mM Tris-HCl (pH 7.2) and centrifuged at 10, 000 rpm for 10 min (Lin and Kao, 2001). POD and SOD bound to the cell wall isolates were released by this procedure. The supernatant obtained through this procedure was used for the quantification of wall bound SOD and POD.

Total SOD activity was assayed by the inhibition of the photochemical reduction of Nitro Blue Tetrazolium chloride (NBT) as described by Beauchamp and Fridovich (1971). One unit of SOD was defined as the amount of enzyme which produced a 50 % inhibition of NBT reduction under the assay conditions (Giannopolitis and Ries, 1977). Peroxidase was assayed by the method of Chance and Maehly (1955). The activity was calculated as change in OD min^{-1}mg $protein^{-1}$.

Protein concentration was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. All the chemicals for enzyme analysis were purchased from Hi-Media, Mumbai and Maharashtra, India.

RESULTS

Germination characteristics at different magnetic field dose

Magnetopriming of maize seeds resulted in the improvement of the following parameters in the range of

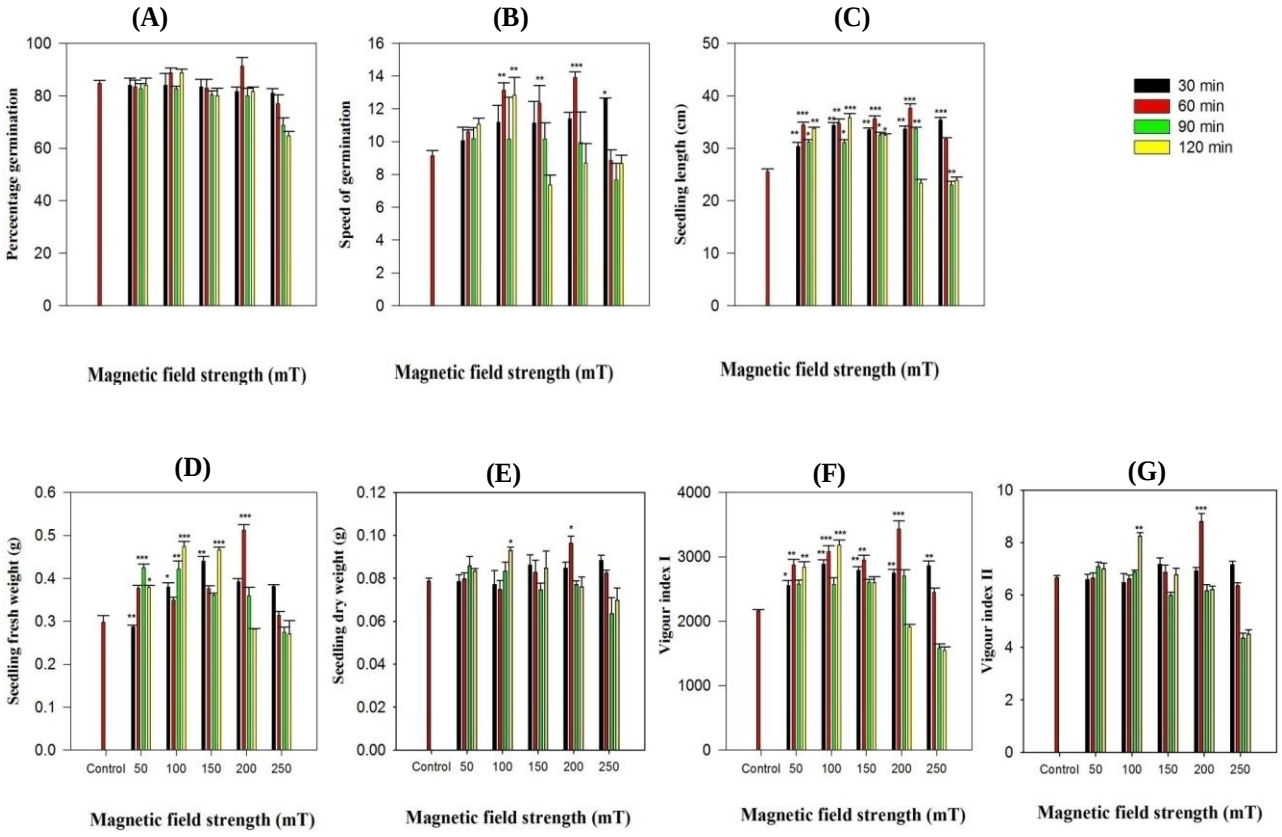

Figure 1. Effect of static magnetic field treatment on different germination parameters of maize seeds. Vertical line above the bar indicates ± S.E.M. Upper symbol indicates the differences vs control. ***, ** and * indicate significance at $p < 0.001$, 0.01, 0.05 respectively.

0 to 10% for germination, 8 to 58% for speed of germination, 19 to 47% for seedling length, 17 to 72% for seedling fresh weight and 5 to 22% for seedling dry weight, 18 to 59% for vigour index I and 0 to 23% for vigour index II (Figure 1A to G). It was also observed that higher field strength for longer duration (in most of the cases 200 mT for 120 min and 250 mT above 30 min) had a detrimental effect on these parameters. Maximum enhancement of seedling parameters were obtained by 200 mT (60 min) followed by 100 mT (120 min), hence these two doses were further used for understanding the different biochemical and biophysical changes associated with magnetopriming.

Effect of magnetic field treatment on reactive oxygen species (ROS) generation

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

Treatment of seeds with SMF increased the production of $O_2^{\cdot-}$ in embryos and eight-day-old seedlings. Magnetic field treatment enhanced $O_2^{\cdot-}$ production up to a 34% in the embryo, 23% in coleoptiles, 57% in the mesocotyl and 31% in radicle over untreated seedlings (Figure 2A to D). The rate of $O_2^{\cdot-}$ production was higher in seeds treated with magnetic field strength of 200 mT (Figure 2A to D).

Electron paramagnetic resonance spectroscopy (EPR) of $^{\bullet}OH$ Radicals

Trend of $^{\cdot}OH$ content after magnetic field treatment paralleled with that of superoxide content. Hydroxyl radical production in the embryo was enhanced up to 26% (100 mT; 120 min) and 39% (200 mT; 60 min) by magnetic field treatment over the control (Figure 3A and Table 1). A similar trend was also observed in the eight-day-old seedlings (coloeptile, mesocotyl and radicle) (Figure 3B, C, D). The maximum difference was observed in mesocotyl (26% by 100 mT (120 min) and 34% by 200 mT (60 min), followed by coleoptile (22 and 31% by 100 mT (120 min) and 200 mT (60 min) respectively) (Figure 3B, C and Table 1).

H_2O_2 content

H_2O_2 content was higher in the embryonic axes and eight-

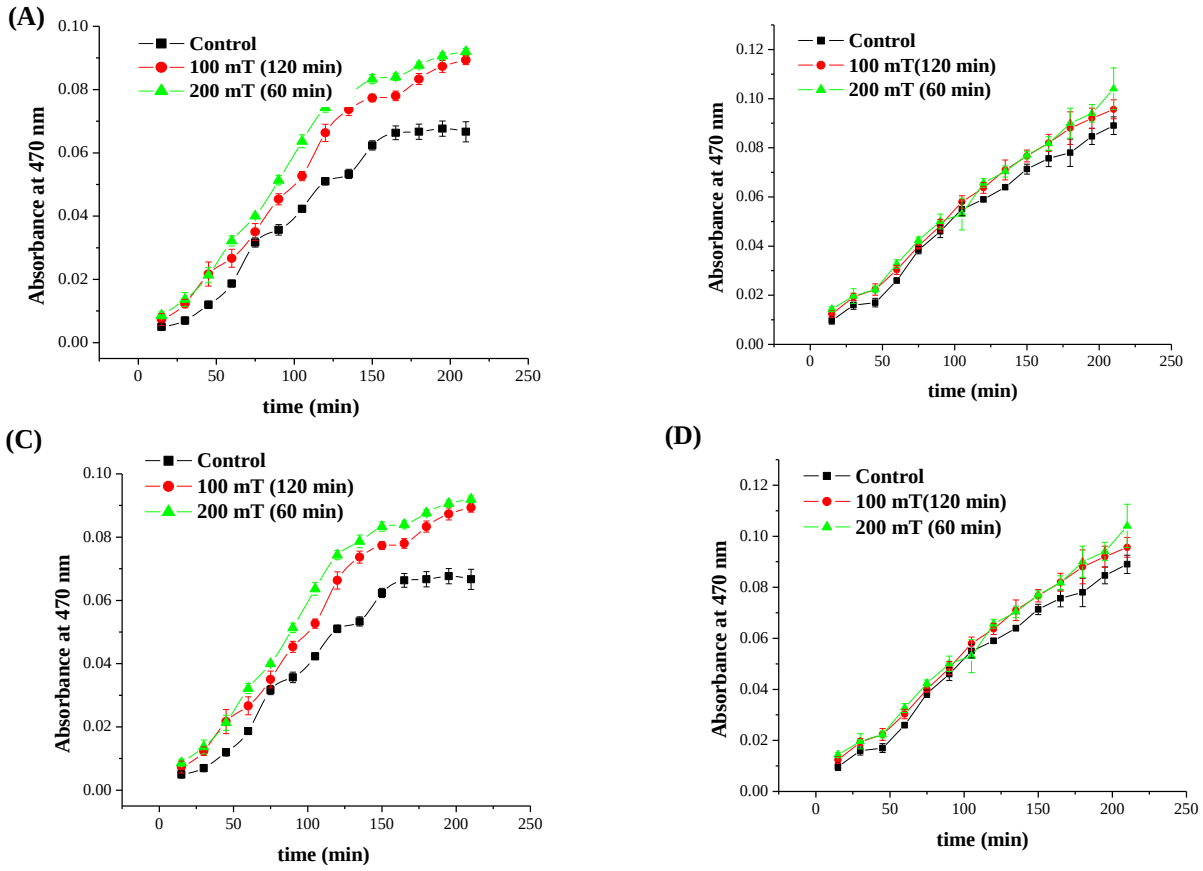

Figure 2. Kinetics of $O^{2.-}$formazan production from Na, 3'-[1-[(phenylamino)-carbonyl]-3,4-tetrazolium] (4- methoxy -6-nitro) benzenesulfonic acid hydrate (XTT) from the maize seeds treated with static magnetic field. (A) embryo (B) coleoptile (C) mesocotyl (D) radicle. Vertical line above the bar indicates ± S.E.M of three independent experiments.

day-old seedlings as in the case of $O_2^{\cdot-}$and $^{\cdot}OH$ after magnetic field treatment. The enhancement of H_2O_2 in embryo was by 13% (100 mT; 120 min) and 48% (200 mT 60 min) over the untreated control (Figure 4).

In eight-day-old seedlings, H_2O_2 was higher by 16 to 20% in coleoptiles, 39 to 38% in mesocotyl and 19 to 23% in radicle by the magnetic field exposure of 100 mT (120 min) and 200 mT (60 min) respectively over the control (Figure 4).

Taken together, this data shows that $O_2^{\cdot-}$, $^{\cdot}OH$ and H_2O_2 are produced in the germinating maize seedlings and their production was higher in the seedlings that emerged from SMF treated seeds followed by enhanced seedling vigour, than control seedlings. Higher reactive oxygen species were observed in seeds treated with 200 mT (60 min) as compared to 100 mT (120 min).

Antioxidant enzyme activity

SOD activity (both cytosolic and wall bound) was reduced in the embryo and eight-day-old seedlings after magnetic field treatment. In case of embryo, cytosolic SOD was reduced by 29% and wall bound SOD was reduced up to 26% after magnetic field treatment (Figure 5A). Amongst the two different treatments, seeds treated with 200 mT (60 min) reduced the SOD content slightly more as compared to 100 mT (120 min). SOD content in eight-day-old seedling followed the same trend as in embryo after magnetic field treatment. The magnitude of reduction in cytosolic SOD content was upto 26% in coleoptiles and 34% in mesocotyl over control seedlings. In case of radicle the magnitude of reduction was much higher (up to 64%) in cytosolic SOD after magnetic field treatment over untreated seedlings (Figure 5B). Wall bound SOD was also reduced in the eight-day-old seedlings after magnetic field treatment. Magnitude of reduction was up to 26, 33 and 20% in coleoptiles, mesocotyl and radicle respectively compared to control. Maximum difference was observed in all cases after treating the seeds with 200 mT for 60 min (Figure 5C).

In contrast to SOD, peroxidase (both wall bound and cytosolic peroxidase) activity was significantly enhanced by SMF treatment (Figure 6A). An enhancement of 10% (100 mT; 120 min) and 30% (200 mT; 60 min) in cytotsolic peroxidase and of 29% (100 mT; 120 min) and

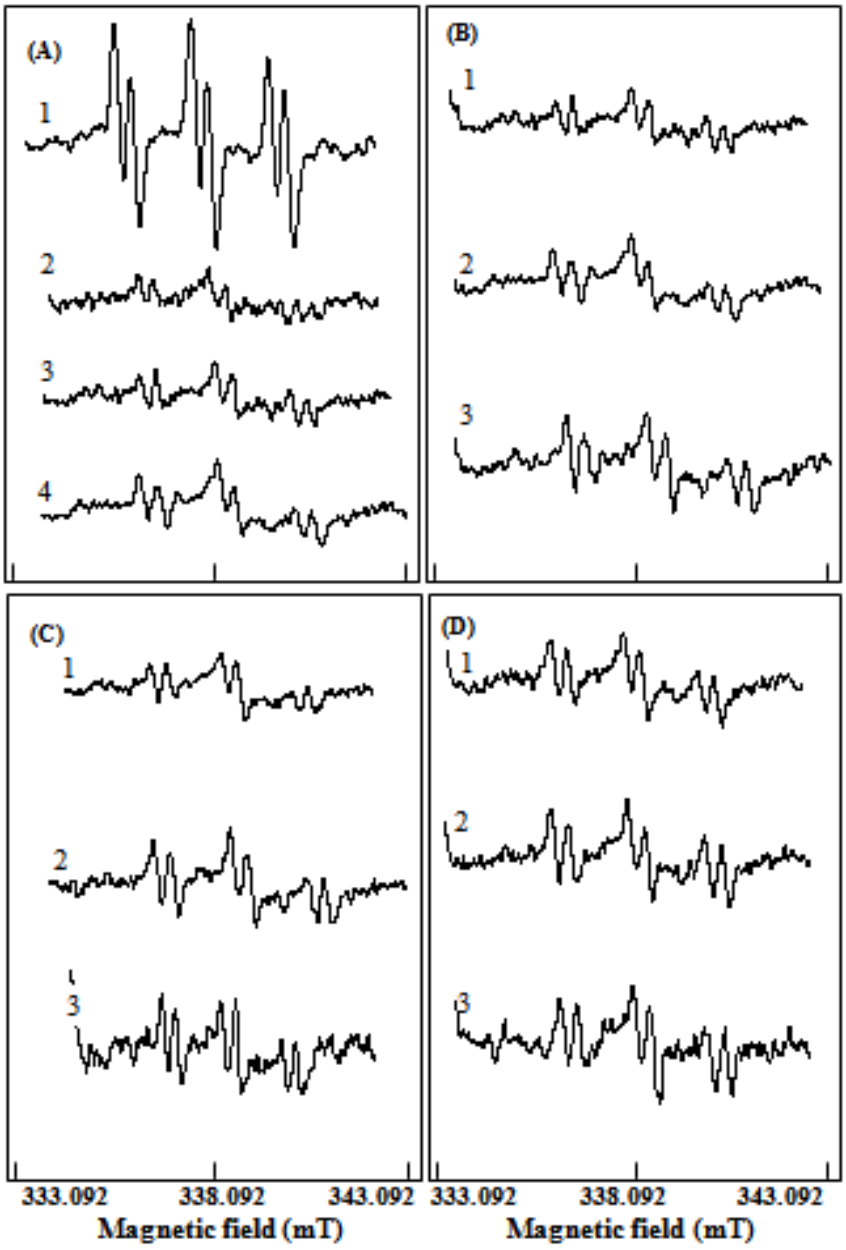

Figure 3. EPR spectra of hydroxyethyl/ α-(4-pyridyl -1-oxide)-N-tert-butylnitrone (POBN) adduct diagnostic for ·OH after magnetic field treatment. Group of 20 embryos or ten abraded hypocotyls (0.5 cm) from control and treated seedlings were used for obtaining spectra (A) embryo: [(1) Spectra from Fenton reaction, (2) control, (3) 100 mT (120 min), (4) 200 mT (60 min)] (B) coleoptiles (C) mesocotyl (D) radicle. [(1) control, (2) 100 mT (120 min) and (3) 200 mT (60 min)]. Each spectra is the representative of eighteen individual spectra.

58% (200 mT; 60 min) in wall bound peroxidase was recorded after SMF treatment at embryonic stage. In the eight day old seedlings the enhancement was up to 22, 44, and 22% in coleoptile, mesocotyl and radicle respectively in case of cytosolic peroxidase (Figure 6B). In case of wall bound peroxidase the enhancement was up to 34% in coleoptiles and mesocotyl and 15% in radicle over untreated seedlings (Figure 6C).

DISCUSSION

Magnetopriming is a promising technique for the biostimulation of seeds. In the present study, the magnetopriming improved seedling parameters in maize under laboratory condition. The effect was dependent on the magnetic field strength and time of exposure without showing any specific trend. Obtained results showed that

Table 1. Percentage enhancement in α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (POBN) spin adduct in maize seedlings after magnetic field treatment.

Treatment	Embryo	Coleoptile	Mesocotyl	Radicle
0 mT	100 ± 00	100 ± 00	100 ± 00	100 ± 00
100 mT (120 min)	126.45 ± 4.092***	122.23 ± 3.45**	126.97 ± 8.97*	113.74 ± 4.78*
200 mT (60 min)	139.13 ± 3.99***	131.13 ± 5.27***	134.33 ± 10.43*	124.56 ± 4.77*

Data are the means of ± S.E.M. of eighteen individual EPR spectra. Upper symbol indicates the differences *vs* control. ***,** and *, indicate significance at $p < 0.001$, 0.01, 0.05 respectively.

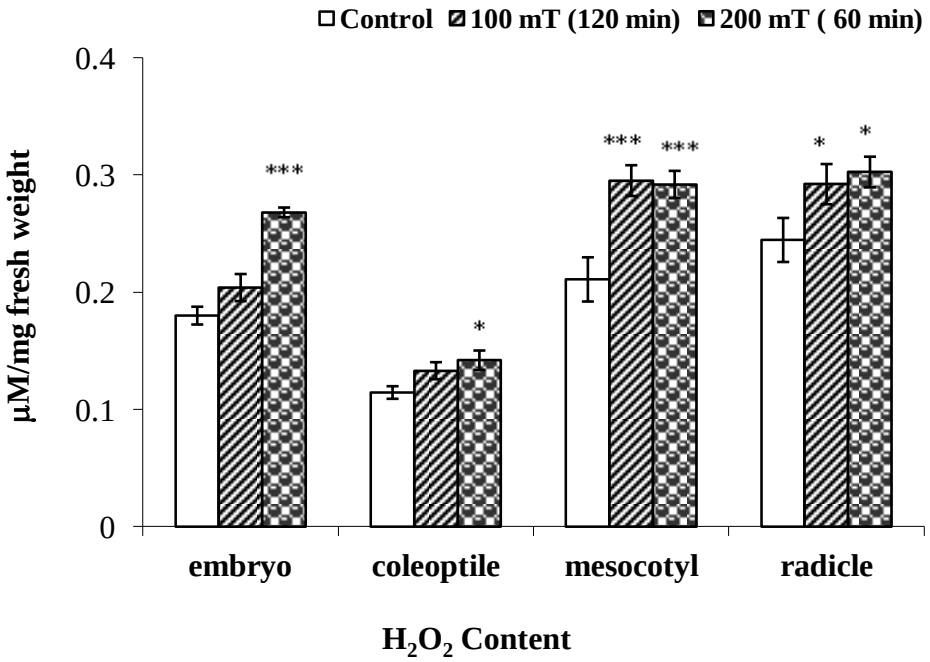

Figure 4. Effect of Static magnetic field treatment on H_2O_2 content in maize. Vertical line above the bar indicates ± S.E.M. Upper symbol indicates the differences vs. control. *** and * indicate significance at $p < 0.001$ and 0.05 respectively.

certain combination of SMF and duration of exposure like 200 mT for 60 min and 100 mT for 120 min are highly effective in enhancing germination related parameters. This observation indicates that the internal energy of the seed responds positively when there is an appropriate combination of magnetic field and exposure time (Bhatnagar and Dev, 1977). Kavi (1983) reported that ragi seeds exposed to 100 mT, changed their internal potential energy and suggested that by selecting a suitable combination of magnetic field and exposure time, it may be possible to obtain higher yield. According to previous reports magnetic field stimulated the shoot development of maize and led to the increase of germinating energy, fresh weight and shoot length (Aladjadjiyan, 2002; Vashisth and Joshi, 2016). Florez *et al.* (2007) reported that exposure of maize seeds to SMF enhanced the germination and early growth of seedling. In the present study enhanced germination in magnetoprimed maize seeds were coupled with enhanced production of reactive oxygen species ($O_2^{\cdot-}$, $^{\cdot}OH$ and H_2O_2) in treated seeds. Similar kind of results were observed in magnetoprimed seeds of soybean by Shine *et al*. (2012). The difference in ROS content was observed only in the imbibed seeds and differences in mean ROS were similar in magnetoprimed and unprimed seeds before imbibition (Data not given).

Enhancement of total free radical content was also observed by priming with laser light, magnetic field (Podlesny *et al*., 2001; 2005) and polyethylene glycol (Naglreiter *et al*., 2005), followed by enhanced germination characters. In the present study, ROS content was specifically studied after magnetopriming. Furthermore, it is reported that pretreatment of seeds with H_2O_2 accelerated the germination in a dose dependent manner in maize seeds (Gondim *et al*., 2010) and scavenging of H_2O_2 by ascorbic acid reduced the

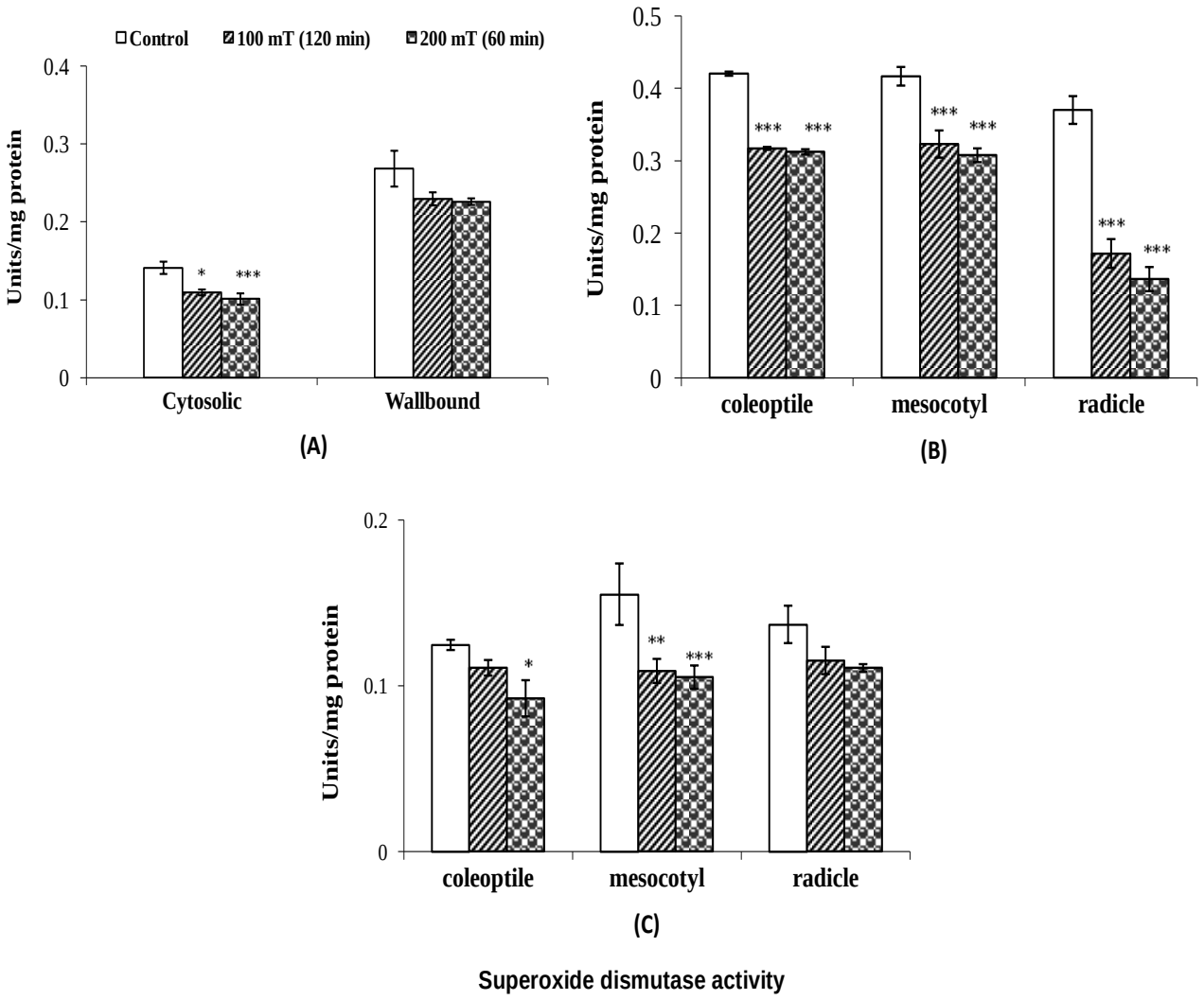

Figure 5. Effect of static magnetic field treatment on superoxide dismutase (SOD) in maize. (A) embryo (B) cytosolic SOD (C) wall bound SOD of eight day old seedling. Vertical line above the bar indicates ± S.E.M. Upper symbol indicates the differences vs control. ***, ** and * indicate significance at $p < 0.001$, 0.01, 0.05 respectively.

germination of wheat seeds (Ishibashi *et al*., 2008). In the present study, enhanced ROS content in the magnetoprimed seeds was beneficial and not detrimental for the maize seeds as in case of ROS accumulation during seed deterioration.

Antioxidant enzymes which are responsible for maintaining cellular homeostasis, respond differently to magnetopriming. SOD which is responsible for the detoxification of $O_2^{\cdot-}$ was reduced, POD which is responsible for detoxification of H_2O_2 was enhanced by magnetopriming. Peroxidase is a bifunctional enzyme which can oxidize various substrates in the presence of H_2O_2 and also produce ROS. It exists either in ferric form (Fe^{3+} - peroxidase) or in a labile perferryl form of peroxidase (Fe^{2+}-$O_2^{\cdot-}$ Fe^{3+}-$O_2^{\cdot-}$ peroxidase), which is also designated as compound III (Yokota and Yamazaki, 1965). Conversion of peroxidase from ferric form to perferryl form requires $O_2^{\cdot-}$ (Chen and Schopfer, 1999), which is probably provided by the NAD(P)H oxidase present in the plasma membrane (Liszkay *et al.*, 2004). Peroxidase transformed in to the perferryl state by $O_2^{\cdot-}$ catalyzes the generation of $^{\cdot}OH$ from H_2O_2 (Chen and Schopfer, 1999). SOD prevents the generation of $^{\cdot}OH$ by scavenging $O_2^{\cdot-}$ and thereby inhibiting the conversion of peroxidase from ferric form to perferryl form. The enhanced $^{\cdot}OH$ in the magnetoprimed maize seeds was apparently due to the enhanced POD and reduced SOD content. Preliminary experiments conducted to establish the role of ROS in seed germination of magnetoprimed seeds also showed, scavenging superoxide using Mn-desferal $^{\cdot}OH$ by urea or that inhibition of peroxidase using KCN reduced the germination related characters of magnetoprimed seeds (Schopfer *et al.*, 2001). $^{\cdot}OH$ generated in the first days of germination contribute to

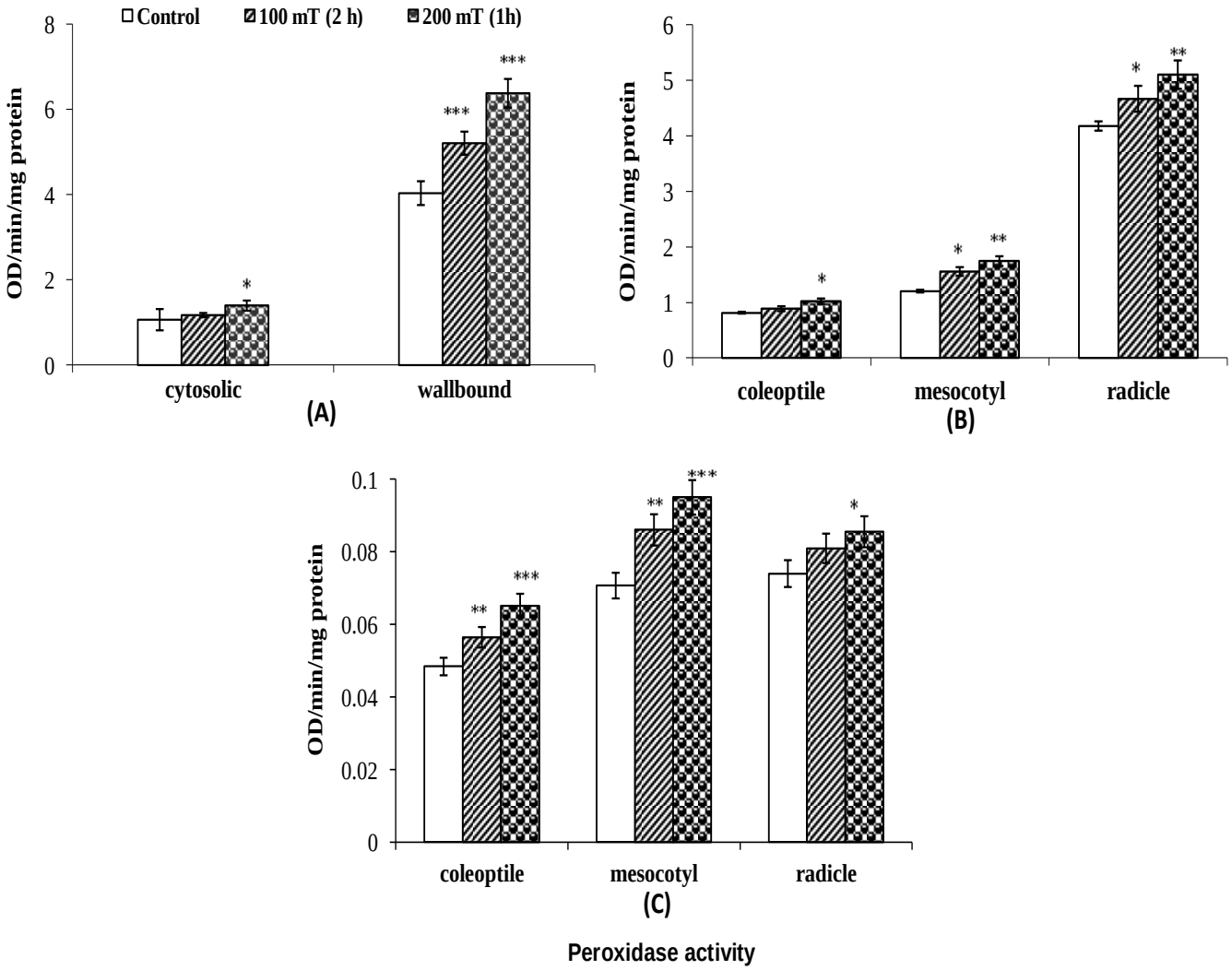

Figure 6. Effect of static magnetic field treatment on peroxidase (POD) in maize. (A) embryo (B) cytosolic POD (C) wall bound POD of eight day old seedling. Vertical line above the bar indicates ± S.E.M. Upper symbol indicates the differences vs control. ***, ** and * indicate significance at $p < 0.001$, 0.01, 0.05 respectively.

the breakage of the seed coat and subsequent cell elongation, cleavage of various polysaccharide and there by the mobilization of stored food (Schweikert *et al.,* 2000) and non-enzymatic cell wall loosening (Chen and Schopfer, 1999). High levels of ROS ($O_2^{\cdot-}$, $^{\cdot}OH$ and H_2O_2) are produced in the leaf expansion and root elongation zone of maize (Rodriguez *et al.*, 2002; Liszakey *et al*., 2004). H_2O_2 acts as a signaling molecule and regulates the catabolism of ABA and biosynthesis of GA for faster germination (Liu *et al.*, 2010). The ROS generated in the seed germination is involved in endosperm weakening, protection against pathogen, Ca^{2+} signaling, gene expression, redox regulation, hormone signaling, cell wall elongation etc. (Bailly *et al*., 2008; El-Maarouf Bouteau and Bailly, 2008). In our study, enhanced germination characters were followed by enhanced ROS production in maize seeds. On the other hand enhanced ROS content followed by enhanced antioxidant enzymes (POD) may also prevent the excess accumulation of ROS, thereby preventing the oxidative damage in germinating seeds.

CONCLUSION

In conclusion, exposure of seeds to stationary magnetic field for a particular duration acts as biostimulant for maize seeds. Maximum enhancement in germination characters were obtained by treating the seeds with 200 mT (60 min) and 100 mT (120 min). The enhanced germination in magnetoprimed seeds was followed by enhanced ROS content, POD activity, but reduced SOD activity. Treatment of seeds with 200 mT (60 min) showed maximum response in germination related parameters and also exhibited maximum free radical content. The different response of antioxidant enzymes after magnetopriming may contribute to the enhanced

level of ROS in these seeds. Increase in ROS and maintenance of their levels for promotion of rapid growth in magnetoprimed seeds also explains the faster rate of germination. SMF treatment influences the physiological and biochemical process in the seeds and thereby contributes to better vigor and improved crop stand.

REFERENCES

Abdul-Baki AA, Anderson JD (1973). Vigour determination in soybean by multiple criteria. Crop Sci. 10:31-34.

Aladjadjiyan A (2002). Study of the influence of magnetic field on some biological characteristics of *Zea mays*. J. Central Eur. Agric. 3:89-94.

Bailly C, El-Maarouf-Bouteau H, Corbineau F (2008). From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology. C. R. Biol. 331:806-814.

Beauchamp CO, Fridovich I (1971). Superoxide dismutase: improved assay and an assay applicable to acrylamide gels. Anal. Biochem. 44: 276-278.

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

Bhatnagar D, Deb AR (1977). Some aspects of pre-germination exposure of wheat seeds to magnetic fields: Germination and early growth. Seed Res. 5:129-137.

Carpita NC (1984). Cell wall development in maize coleoptiles. Plant Physiol. 76:205-212.

Chance B, Maehly AC (1955). Assay of catalases and peroxidases. Methods in Enzymology11, P.S. Colowick, N.O. Kaplan (Eds.), Academic Press, New York pp. 764-775.

Chen SX, Schopfer P (1999). Hydroxyl-radical production in physiological reactions: a novel function of peroxidase. Eur. J. Biochem. 260:726-735.

El-Maarouf Bouteau H, Bailly C (2008). Oxidative signaling in seed germination and dormancy. Plant Signal. Behav. 3:175-182.

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

Frahry G, Schopfer P (2001). NADH stimulated, cyanide resistant superoxide production in maize coleoptiles analyzed with tetrazolium based assay. Planta 212:175-183.

Giannopolitis CN, Ries K (1977). Superoxide dismutases. I. Occurrences in higher plants, Plant Physiol. 59:309-314.

Gondim FA, Gomes-Filho E, Lacerda CF, Prisco JT, Azevedo Neto AD and Marques EC (2010). Pre-treatment with H_2O_2 in maize seeds: effects on germination and seedling acclimation to salt stress. Braz. J. Plant Physiol. 22:103-112.

Gutzeit HO (2001). Biological effects of ELF-EMF enhanced stress response: New insights and new questions. Electromagnetobiol. 20:15-26.

Ishibashi Y, Yamamoto K, Tawaratsumida T, Yuasa T, Iwaya-Inoue M (2008). Hydrogen peroxide scavenging regulates germination ability during wheat (*Triticum aestivum* L.) seed maturation. Plant Signal. Behav. 3:183-188.

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

Jinapang P, Prakob P, Wongwattananard P, Islam NE, Kirawanich P (2010). Growth characteristics of mung beans and water convolvuluses exposed to 425-MHz electromagnetic fields. Bioelectromagn. 31:519-527.

Kataria S, Baghel L, Guruprasad KN (2015). Acceleration of germination and early growth characteristics of soybean and maize after pre-treatment of seeds with static magnetic field. Int. J. Tropical Agri. 33: 985-992.

Kataria S, Baghel L, Guruprasad KN (2017). Pre-treatment of seeds with static magnetic field improves germination and early growth characteristics under salt stress in maize and soybean. Biocatal. Agric.Biotech.10: 83-90.

Kavi PS (1983). The effect of non-homogeneous, gradient magnetic field on the magnetic susceptibility values of in sit ragi (*Eleusine coracana* Gaertn) seed material. Mysore J. Agric. Sci. 17:121-123.

Kukavica B, Mojovic M, Vucinic Z, Maksimovic V, Takahama U, Jovanovic SV (2009). Generation of hydroxyl radical in isolated Pea root cell wall, and the role of cell wall-bound peroxidase, Mn-SOD and phenolics in their production. Plant Cell Physiol. 50:304-317.

Lin CC, Kao CH (2001). Cell wall peroxidase activity, hydrogen peroxide level and NaCl-inhibited root growth of rice seedlings. Plant Soil 230:135-143.

Liszkay A, Kenk B, Schopfer P (2003). Evidence for the involvement of cell wall peroxidase in the generation of hydroxyl radicals mediating extension growth. Planta. 217: 658-667.

Liszkay A, Van Der Zalm A, Schopfer P (2004). Production of reactive oxygen intermediates ($O_2^{\cdot-}$, H_2O_2, and $^{\cdot}OH$) by maize roots and their role in wall loosening and elongation growth. Plant Physiol. 230: 3114-3123.

Liu Y, Ye N, Liu R, Chen M, Zhang J (2010). H_2O_2 mediates the regulation of ABA catabolism and GA biosynthesis in Arabidopsis seed dormancy and germination. J. Exp. Bot. 61 (11):2979-2990.

Lowry HO, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurements with Folin reagent. J. Biol. Chem. 193:265-275.

Maguire JD (1962). Speed of germination-aid in selection and evaluation for seedling emergence and vigour. Crop Sci. 2:176-177.

Martínez E, Carbonell MV, Flórez M, Amaya JM, Maqueda R (2009). Germination of tomato seeds (*Lycopersicon esculentum L.)* under magnetic field. Int. Agrophys. 23:45-49.

Mukherjee SP, Choudhuri MA (1983). Implications of water stress-induced changes in the leaves of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Physiol. Plant. 58:166-170.

Naglreiter C, Reichenauer TG, Goodman BA, Bolhar-Nordonkampf HR (2005). Free radical generation in *Pinus sylvestris* and *Larix deciduas* seeds primed with polyethylene glycol or potassium salt solution. Plant Physiol. Biotechnol. 43:117-123.

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, Misiak LE, Podlesna A, Pietruszewski S (2005). Concentration of free radicals in pea seeds after the pre-sowing treatment of the seeds with magnetic field. Int. Agrophys.19:243-249.

Ramos CL, Pou S, Britigan BE, Cohen MS, Rosen GM (1992). Spin trapping evidence for myeloperoxidase-dependent hydroxyl radical formation by human neutophils and monocytes. J. Biol. Chem. 267: 8307-8312.

Rodriguez AA, Grunberg KA, Taleisnik EL (2002). Reactive oxygen species in the elongation zone of maize leaves are necessary for leaf extension. Plant Physiol. 129:1627-1632.

Schopfer P, Plachy C, Frahry G (2001). Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. Plant Physiol. 125:1591-1602.

Schweikert C, Liszkay A, Schopfer P (2000). Scission of polysaccharides peroxidase-generated hydroxyl radicals. Phytochem. 53:565-570.

Shine MB, Guruprasad KN (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.

Shine MB, Guruprasad KN, Anand A (2011a). Enhancement of germination, growth, and photosynthesis in soybean by pretreatment of seeds with magnetic field. Bioelectromagn. 32:474-484.

Shine MB, Guruprasad KN, Anand A (2011b). Superoxide radical production and performance index of Photosystem II in leaves from magnetoprimed soybean seeds. Plant Signal. Behav. 6:1636-1638.

Shine MB, Guruprasad KN, Anand A (2012). Effect of stationary magnetic field strengths of 150 and 200 mT on reactive oxygen species production in soybean. Bioelectromagn. 33:428-437.

Thomas S, Anand A, Chinnusamy V, Dahuja A, Basu S (2013). Magneto-priming circumvents the effect of salinity stress on germination in chickpea seeds. Acta Physiol. Plant. 35:3401-3411.

Vashisth A, Joshi DK (2016). Growth characteristics of maize seeds exposed to magnetic field. Bioelectromagn. DOI: 10.1002/bem.22023.

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

Walters C (1998). Understanding the mechanism and kinetics of seed ageing. Seed Sci. Res. 8:223-244.

Weaver JC (1993). Combined environmental exposures to chemicals and transient magnetic fields: A hypothesis for possible human health hazards. Proc. Bioelectromagnetics Society, 16th Annual Meeting, 12-16 June, 1993, Copenhagen, Denmark. p. 36.

Wittekind E, Broers D, Kraepelin G, Lamprecht I (1990). Influence of non-thermic AC magnetic fields on spore germination in a dimorphic fungus. Radiat. Environ. Biophys. 29:143-152.

Yinan L, Yuan L, Yongquing Y, Chunyang L (2005). Effect of seed pre-treatment by magnetic field on the sensitivity of cucumber (*Cucumis sativus*) seedlings to ultraviolet-B radiation. Environ. Exp. Bot. 54:286-294.

Yokota K, Yamazaki I (1965). Reaction of peroxidase with reduced nicotinamide-adenine dinuleotide phosphate. Biochimic. Biophysica Acta 105:301-312.

Zepeda-Bautista R, Hernández-Aguilar C, Domínguez-Pacheco A, Cruz-Orea A, Godina-Nava JJ, Martínez-Ortíz E (2010). Electromagnetic field and seed vigour of corn hybrids. Int. Agrophys. 24:329-332.