

Effects of Magnetic Field on Activity of Superoxide Dismutase and Catalase in *Glycine max* (L.) Merr. Roots

Ö. Çelik^{1*}, N. Büyüksulu², Ç. Atak¹, A. Rzakoulieva³

¹Istanbul Kültür University, Faculty of Science and Letters,

Department of Molecular Biology and Genetics, 34156 Ataköy-Istanbul, Turkey

²European University of Lefke, Department of Genetics and Biotechnology, Mersin10, Turkey

³JINR, Laboratory of Nuclear Problems, Magnetic Research Group, Dubna, Russia

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Abstract

Under a magnetic field the activities of superoxide dismutase (SOD) *in vitro* and *in vivo* and accompanying activities of catalase activity *in vivo* were investigated in soybean roots. In plant cells a magnetic field creates a stress condition as other environmental stress factors do. To respond to the stress conditions, the occurred reactive oxygen species are scavenged by defense systems. In this study, two enzymes of the defense system, SOD and catalase activities were investigated under magnetic field. Enzyme and soybean seeds exposed to a magnetic field for a period of 2.2, 19.8. and 33s at the magnetic flux of 2.9-4.6 mT. SOD activities data were compared with magnetized enzyme and soybean roots. While the absorbance values of enzyme that passed through the magnetic field with a period of 19.8s for 24 hours were measured and SOD activity was significantly increased. At the same time, magnetic field SOD activity of the soybean roots was increased 21.18 % relative to control ($P<0.05$). After soybean seeds were treated by various magnetic fields and time periods, the activities of superoxide dismutase and catalase were significantly increased ($P<0.05$) during germination. At the 19.8s for 72 hours, SOD and catalase activities were increased 21.15% and 15.20% relative to control, respectively. Thus, it is indicated that the function of defense enzymes in seedlings was intensified due to the treatment of magnetic field. The increases of magnetic field exposure times do not cause linear increases in enzyme activities *in vitro* and *in vivo* studies.

Keywords: magnetic field, soybean, antioxidant enzyme

Introduction

The application of a magnetic field creates a stress condition for growth of plants just as environmental stresses such as drought, salinity, mineral deficiency, UV light, heat and chilling cause. All those conditions cause the production of reactive oxygen species (ROS) in the cells.

Superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^\cdot) and singlet oxygen (1O_2) are the major reactive oxygen species produced by the metabolic reactions in plant cells which have multiple defense systems against such oxidative stresses [1-5]. Indeed, ROS are necessary for inter- and intracellular signaling [5], but at high concentrations they can cause damage at various levels of organization, including chloroplasts [6,7]. These ROSs are highly reactive and can alter normal cellular metabolism through

*e-mail: ocelik@iku.edu.tr

oxidative damage to lipids, proteins and nucleic acids [1]. Therefore, excessive ROSs are scavenged by protective systems [2, 8-10]. Under unstressed conditions, the formation and removal of O_2 are in balance. When plants are stressed, the steady state level of ROS usually increases, and it has been hypothesized that ROS (specifically hydrogen peroxide) might also act as messengers turning on stress-related genes. However, the defense system, when presented with increased ROS formation under stress conditions, can be overwhelmed.

Plants have their own enzymatic resources such as the metalloenzymes superoxide dismutase (SOD) and polyphenol oxidase (PPO) to prevent oxidative damages. In plants, Cu/Zn-SOD and Fe-SOD isoforms are found primarily in chloroplasts and in the cytosol. Cu-Zn SOD is the most abundant and the most widely distributed enzyme in the cell [2]. Despite current knowledge on this enzyme, it is still doubtful whether its real catalytic activity is the same as that described *in vitro*. The sequence of reactions in which it takes part would mean *in vivo* eliminating a weak oxidizer and forming a strong one, requiring the involvement of a second enzyme, a catalase (CAT), for neutralization. In principle, this mechanism seems somewhat complex and not very effective for neutralizing an agent that would affect only strong reducers [5].

Plants possess enzymatic systems that protect them against H_2O_2 and other harmful ROSs. These enzymatic systems include superoxide dismutase and catalase. SOD converts superoxide radicals to hydrogen peroxide and CAT converts H_2O_2 to water and oxygen. Plants also contain non-enzymatic ROS-scavengers, e.g. ascorbate, tocopherols, phenolic acids, and flavonoids which are localized in different cellular compartments [2, 3]. CAT is found in peroxisomes, cytosol and mitochondria, the combined action of catalase and superoxide dismutase is critical in mitigating the effects of oxidative stress.

Obviously, SOD is an important enzyme family in living cells for maintaining normal physiological conditions and the most efficient antioxidant enzymes. Gidrol et al. [8] monitored the SOD expression during germination of soybean seeds. Waojtyla et al. showed that SOD, catalase, ascorbate peroxidase, dehydroascorbate reductase and glutathione reductase were present in dry seeds and were activated later during germination, especially in embryo axes [11].

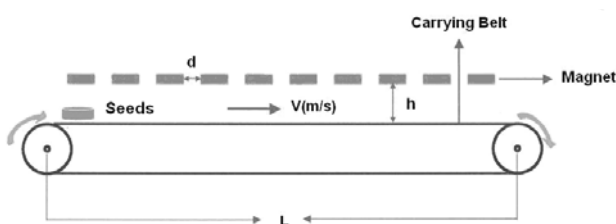


Fig. 1. Magnetic field system plan formed at laboratory.

$L = 2.2$ m	(Carrying belt length)
$h = 0.060$ m	(Distance between sample and magnets)
$d = 0.15$ m	(Distance between magnets)
$n = 10$	(Magnet number)
$v = 1$ m/s	(Passing velocity from magnetic field)

There are many experimental data demonstrating various effects of magnetic field on the plants. The effects of magnetic field on germination of seeds and growth of plants have been the subject of much research [6, 12-16]. In most cases a magnetic field affected the growth processes, cell division and differentiation, induced significant changes at the cellular and subcellular level, altered the Ca^{2+} balance, enzyme activities and various metabolic processes [17].

Seed germination is an important development change in the plant lifecycle. Magnetic field strength is important for stimulation of the germination and a few studies concerning the effects of magnetic field on antioxidant enzymes of germinated seeds have been reported. There is no study concerning the different magnetic field exposure times that shows different biological responses and modulates the enzyme activities. The aim of this work was to investigate the effects of magnetic treatment on SOD and catalase activity during the germination of soybean seeds.

Materials and Methods

Plant Material

Germination tests were carried out under laboratory conditions to study the effect of exposure of soybean seeds to magnetic fields. *Glycine max.* L. Merrill seeds were supplied by the Aegean Agricultural Research Institute (İzmir, Turkey).

Materials and Devices

Bovine erythrocytes SOD, L-methionine, riboflavin, NBT (Nitroblue tetrazolium salt) were purchased from Sigma, Na_2CO_3 was purchased from Merck and EDTA was purchased from Amresco. A Shimadzu 1601 spectrophotometer was used to measure absorbances. For application of a magnetic field, a device prepared by JINR Laboratories and gifted to the University of Istanbul was used [7].

Magnetic Field Experiments

To generate the magnetic fields, we used 10 magnets of $0.45 \times 0.065 \times 0.022$ m dimensions (Figs. 1 and 2). These magnets were prepared by magnetic field group of Joint Institute for Nuclear Research Laboratories (JINR) in Dubna, Russia. At the Magnetic field Laboratory of the Biology Department of Istanbul University, these magnets were mounted onto a belt system that rotated at 1 m/second. The distance between magnets and the belt system was 0.060 m (Figs. 2 and 3). When the magnetic field was applied, samples of enzyme and seeds were put on the belt system that rotated with a rate of 1 m/second at the $21^\circ C$. Magnetic flux density was 2.9-4.6 mT (a portable gaussmeter was used to measure magnetic flux density).

The characteristics of magnets were:

$$B = \mu_0 \mu_R H$$

$$\mu_0 = 4\pi \cdot 10^{-7} \text{ V s / Amp.m.}$$

$$\mu_R = 1 \text{ (Permeability of air)}$$

$$H = 2300\text{-}3700 \text{ amp/m (Magnetic field strength)}$$

The dose applied was obtained by exposing the samples to magnetic field induction 2.9-4.6 mT, at three different times: 2.2, 19.8 and 33 seconds [18, 21].

During magnetic field exposure, seeds were put into the paper container and enzyme samples were put into the plastic container. 5 replicates were used in an experimental design and only 10 seeds were used in each replicate. Thus, groups of 50 seeds were subjected to each magnetic treatment and control. A3935 soybean seeds were germinated in a growth chamber at $25 \pm 5^\circ\text{C}$ in dark petri dishes containing filter paper. Seeds were considered germinated when their radicle showed 1mm.

Enzyme Assays

In Vitro SOD Activity

SOD activity was determined by using a spectrophotometric method adapted from the procedure of Sairam et al. [19]. For the experiment of enzyme activity measurement, we used five replicates. 1.0 mg of SOD (3000 unit) was dissolved in 10 ml of dH_2O to prepare stock solution. The magnetic field was applied as mentioned below on the enzyme dissolved in dH_2O . To compare the activities of SOD, one set of the experiment employed the same conditions without a magnetic field. The blank test was set up to include all but the enzyme. For the preparation of samples for the assay of SOD activity, 5 μl of enzyme sample was added into the solution, including 0.1 ml of 1.5 M Na_2CO_3 , 0.2 ml of 200 mM methionine, 0.1 ml of 2.25 mM NBT, 0.1 ml of 3 mM EDTA, 1.5 ml of 100 mM PBS (pH 7.5) and 1 ml of dH_2O . Just after, 0.1 ml of 60 μM riboflavin solution was added. The prepared enzyme samples were kept under fluorescent light for 15 minutes and then the reactions were

stopped by taking the samples to a dark place. Their absorbances were measured at a wavelength of 560 nm against the blank. Enzyme samples were kept on ice until treatment.

SOD Activity of Germinated Soybean

For enzyme activity measurement, we used 5 replicates, including 10 seeds for each. The root tips were homogenized with liquid nitrogen in 0.1 N PBS (pH 7.5). SOD activity measurements were performed according to the *in vitro* SOD assay. 100% inhibition values were calculated according to this formula [20]:

$$100 - [(\text{absorbance} \times 100) / \text{blank}]$$

Catalase Activity of Germinated Soybean

Soybean root tips were homogenized with liquid nitrogen in 0.1 M PBS (pH 7.0). Catalase activity was measured at 25°C by monitoring the consumption of H_2O_2 at 240 nm for 2 min and recording the changes in absorbance every 10 s. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 30 mM H_2O_2 and 2 ml of root tip extract. Catalase activity was determined as $\Delta A / \text{min.g.fresh.weight}$ [19].

Statistics

The results are presented as mean values \pm standard errors. Statistical significance between mean values was assessed by analysis of variance and Duncan's multiple range tests. A probability of (0.05) was considered significant.

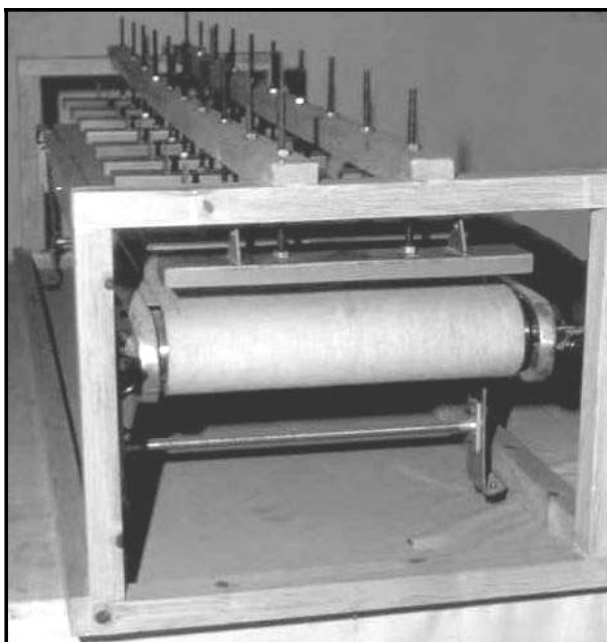


Fig. 2. Magnet system.

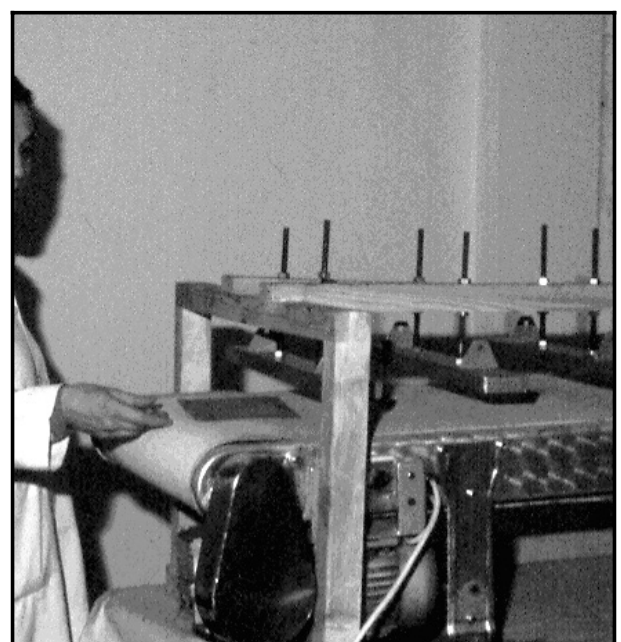


Fig. 3. Magnetic field exposure.

Results

Seed Germination

Germination percentages for soybean seeds exposed to 2.9-4.6 mT magnetic field at 2.2, 19.8 and 33 s are shown in Table 1. The germination percentages were determined at 24, 48, 72 and 144 hours and germination increased for all groups at 24 hours. After 48 hours the germination rates for magnetic-treated seeds were much higher than control seeds. At 72 hours the differences between seedlings for control and magnetic-treated seeds were getting closer. At the end of 144 hours the different germination rates between the treated seeds and control group were not observed, except for the 19.8 s exposure time. Comparing the magnetic-treated seeds with control seeds, the maximum germination rate was obtained at a 19.8 s exposure time.

In Vitro SOD Activity

Absorbance values of magnetized and unmagnetized enzyme (control) samples were measured. Enzyme solutions were subjected to the magnetic field density for 2.2, 19.8 and 33 seconds, respectively. The effects of the magnetic field applied on the SOD were shown on a graph plotting absorbance values (Fig. 4).

Table 1. Germination percentages of soybean seeds exposed to 2.9-4.6 mT magnetic field (%).

Time after MF exposure (hour)	Exposure Time to Magnetic Field (s)			
	Control	2.2	19.8	33
24	15	20	31	26
48	29	45	73	60
72	84	93	95	82
144	92	93	100	94

In vitro SOD activities increased significantly at 2.2, 19.8 and 33 s magnetic exposure compared with the control SOD sample at 0 hour ($P < 0.05$). Maximum activation was at 19.8 s. After 1, 2 and 24 hours, SOD activities increased with respect to control groups for 2.2 and 19.8 s magnetic exposure ($P < 0.05$), and magnetic-treated SOD activities reached their maximum level at 19.8 s. Increasing the magnetic field to 33 s had no effect on the increase of SOD activation. Interestingly, at that magnetic exposure the activity of SOD decreased below the activity of control enzyme for all times except 0 hour. Maximum SOD activity was measured at 19.8 s for 0 hour *in vitro*.

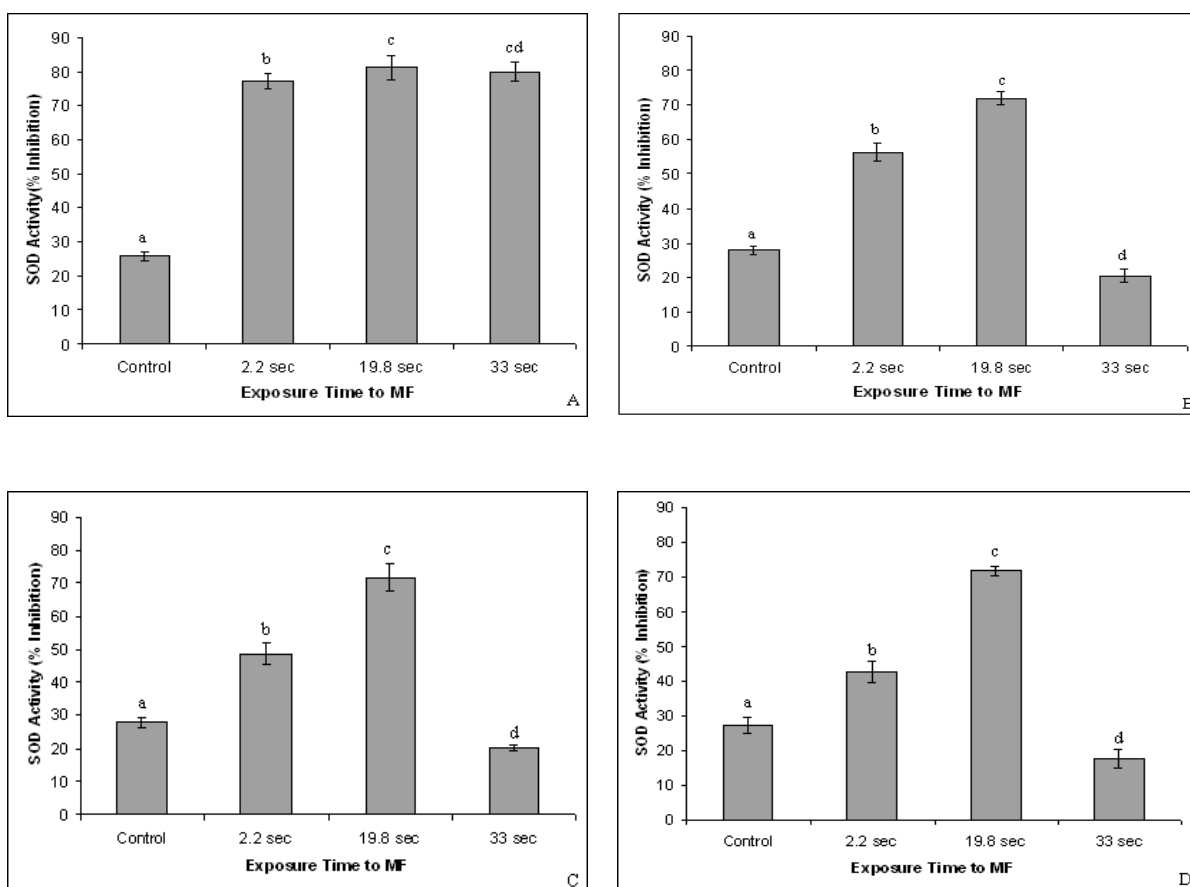


Fig. 4. The effects of magnetic field applied on SOD. (A) 0 hour (B) 1 hour (C) 2 hours (D) 24 hours SOD activities of *in vitro* enzyme assays. Vertical bars indicate standard error. Results are the means of five replicate experiments with standard error. Mean values in columns with different letters are significantly different at the 5% level according to Duncan's Multiple Range Test.

SOD Activities of Soybean Roots

The samples were obtained as explained in methods. SOD activities of magnetic-treated and untreated soybean seeds *in vivo* were measured by the same method used for *in vivo* enzyme activity. The results of measurements were shown in Fig. 5.

In vivo SOD activities significantly increased at 2.2, 19.8 and 33 s magnetic exposure compared with the control SOD sample at 24 hours. At 19.8s magnetic exposure, this activity increased 21.18% relative to control, and this increase was the same for three days. At 72 hours, SOD activities increased significantly (21.15%) at 19.8 s compared with the control ($P<0.05$). However, the maximum values for all cases were at 19.8 s as given for *in vitro* SOD activities. Activity measurements depending on time resulted in the maximum values for control and 19.8 s at 72 hours and for 2.2 s and 33 s at 144 hours. Moreover, the activities of enzyme samples came to a similar level at 144 hours compared to control.

CAT Activities of the Soybean Roots

CAT activities of samples were measured as described in methods [18]. Results were shown in Fig. 6. At 24 hours, CAT activities of germinating seeds exposed to MF increased significantly (20.19%) with respect to control ($P<0.05$) and the maximum activities were at 19.8 s exposure for all hours (24, 48, 72 and 144 hours). When the changes of activities for the samples at the same magnetic flux and for control samples depending on time are analyzed it is shown that the max value for control and 19.8 s exposure at 72 hours, for 2.2 s and 33 s exposure are at 144 hours. At 19.8s magnetic exposure, this activity increased 15.20% relative to control and this rate decreased depending on time. CAT activities also came to a similar level at 144 hours as seen in the changes for the samples of *in vivo* SOD activities before. Maximum catalase activity was measured at 19.8 s exposure for 72 hours *in vivo*. This increase was found to be significant ($P<0.05$).

Discussion

In our study we aimed to investigate the effect of magnetic field on the activities of two of the oxidative system's enzymes, superoxide dismutase and catalase of germination seeds.

In order to compare the effects of magnetic field and *in vitro*, one of the oxidative enzymes, SOD was analyzed on the base of differentiation of enzymatic activities. SOD activity may follow a similar pathway under magnetic fields for both *in vivo* and *in vitro* system, but a living systems' response may be different. Results obtained from this comparison indicated higher enzymatic activity *in vitro* compared with the *in vivo* system under the same magnetic flux, possibly due to the complexity of a living system. Moreover, the activity of SOD

reached its highest level at 19.8 s for 72 hours *in vivo* and at 19.8 s exposure for 0 hour *in vitro*, which is consistent with a previous work by Buyukuslu et al. [22]. Batcioğlu et al. [23] investigated the magnetic field effect on SOD and CAT enzyme activities and they found that a magnetic field caused a significant increase in SOD activity *in vitro* condition.

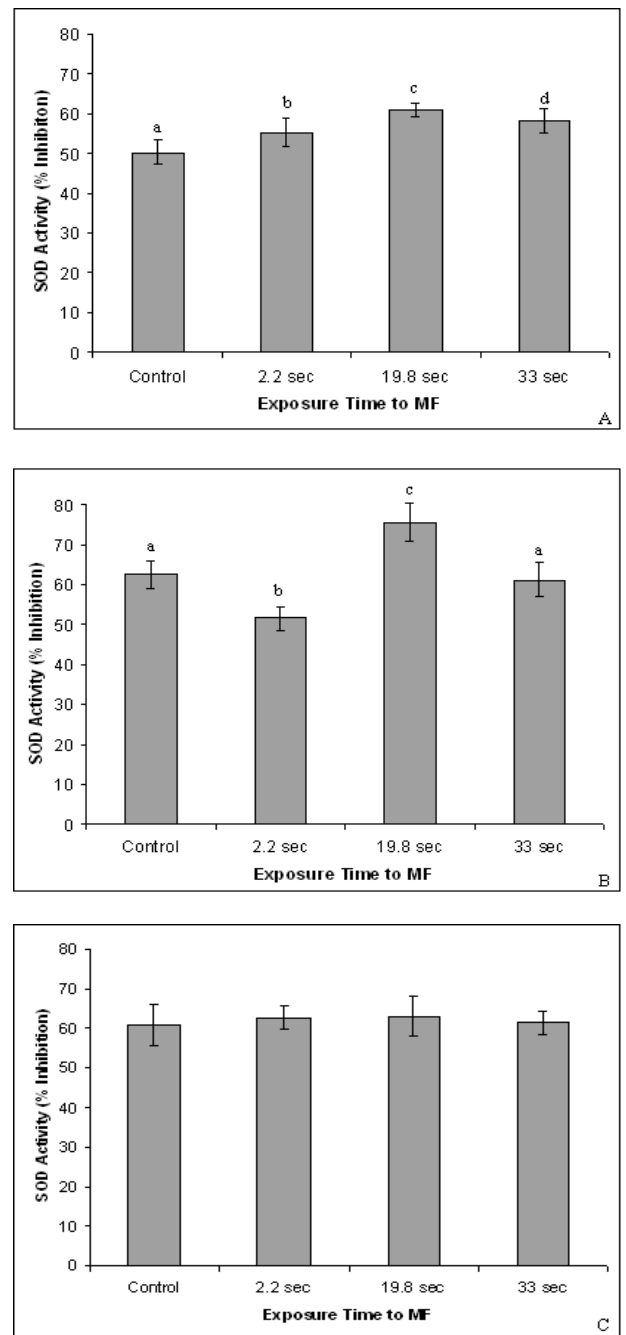


Fig. 5. The effects of magnetic field applied on soybean seeds. (A) 24 hours (B) 72 hours (C) 144 hours SOD activities of the soybean roots. Vertical bars indicate standard error. Results are the means of five replicate experiments with standard error. Mean values in columns with different letters are significantly different at the 5% level according to Duncan's Multiple Range Test. At 144 hours, SOD activities are not significantly increased compared with the control.

In order to obtain a powerful scavenging of toxic oxygen forms, the overproduction of the H_2O_2 -generating SOD must always be combined with increased levels of H_2O_2 metabolizing catalase. Our results are consistent with these findings. Piacentini et al. [24] exposed *Cucumis sativus* etiolated seedlings to an extremely low-frequency magnetic

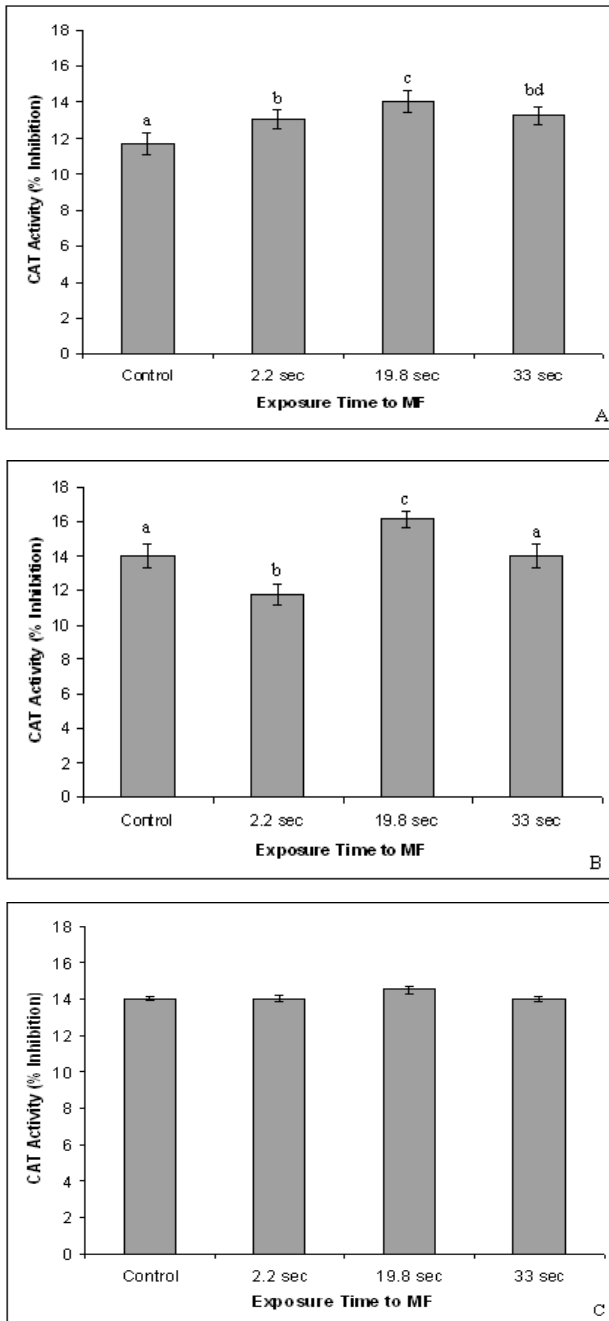


Fig. 6. The effects of magnetic field applied on soybean seeds. (A) 24 hours (B) 72 hours (C) 144 hours CAT activities of the soybean roots. Vertical bars indicate standard error. Results are the means of five replicate experiments with standard error. Mean values in columns with different letters are significantly different at the 5% level according to Duncan's Multiple Range Test. At 144 hours, CAT activities are not significantly increased compared with the control.

field and magnetic-field-induced acceleration of growth. They observed the action of high levels of SOD and catalase activity in *Cucumis sativus* seedlings during the first week of observation.

In the present study, SOD and catalase activities first increased with rising magnetic field intensity, then decreased and after 6 days came to a similar level for all samples. SOD and catalase activities were also brought to the maximum level at 19.8 s for 72 hours. The increase in SOD activity in magnetic-field-treated plants was affected by time. At the same time, 95% germination was achieved at 19.8 s for 72 hours. At 144 hours this rate was 100%.

It may be possible to conclude that 19.8 s magnetic flux density at 2.9–4.6 mT is a noteworthy magnetic dosage in terms of viability and related interactions in our study.

It is known that earth's magnetic field is about $50 \mu T$ and it is a natural component of the environment for living organisms. Adair [25] reviewed that an environmental magnetic field much weaker than the earth's field can affect chemistry, and consequently biology, through the effect on the fields on radical pair recombination. A review by Belyavskaya [17] suggested that prolonged exposure of plants to a weak magnetic field might cause different biological effects at the cellular, tissue and organ levels. Above the earth's magnetic field, moreover, the effects on living systems were found to be more effective. In general, many researchers confirmed the enhancement germination of seeds and growth of plants under magnetic conditions.

Indeed, a previous work by Atak et al. [21] reported an increase of shoot regeneration of soybeans at the magnetic-treated explants compared with control. The same group showed the positive effects of magnetic fields on Paulownia seedlings [7]. Exposure of maize seeds to a stationary magnetic field improved germination rates and the first stages of growth of maize plants [26]. Aladjadjiyan [13] found that the exposure of maize seeds to a MF of 0.15 T led to a germination increase and the maximum effect was 10-min exposure. Aladjadjiyan and Ylieva [6] worked on the influence of a stationary magnetic field on tobacco seed. They found that MF stimulated the development of the germ and led to increasing germination energy and germination. Reina et al. [27] treated lettuce seeds in a stationary magnetic field of 1–10 mT and reported an increase in water uptake rate due to applied magnetic field.

In general, magnetic fields are assumed to enhance germination rates stimulating the activity of proteins and enzymes. We have shown that the increased survival of magnetic-exposed seeds is linked to the maintenance of high SOD and catalase levels.

Metabolically active tissues of plant cells contain free radicals. Waojtyla et al. [11] showed that the highest content of free radicals was observed in embryo axes immediately after the emergence of the radical. On the other hand, magnetic fields increase the average radical concentration, prolonging their lifetime and enhancing the probability of radical reactions with cellular components. These considerations also apply to enzymatic systems that entail radical-pair formation and recombination [28, 29].

Biochemical reactions that involve more than one unpaired electron will be affected by a magnetic field. SOD is one of the enzyme that is involved in the reaction with superoxide radicals. The enzyme has Zn^{2+} and Cu^{+2} metal ions that may be subject to the influence of external magnetic fields due to electron distribution of the mentioned metals. Magnetic field on the enzyme may cause unpaired electrons on metal ions to orient in the same direction with applied magnetic field and so gain more energy. Indeed, this energy may be transferred onto the other molecules that cause more radicals that may form more superoxide radicals via radicalic chain reactions. Then SOD could have more peroxide ions to react with [22].

We have an obvious result that shows that increases of magnetic field intensity and exposure time do not cause linear increases for enzyme activities *in vitro* and *in vivo* studies. At 2.9-4.6 mT magnetic flux density and 19.8 seconds (the suitable combination of magnetic field and exposure time), we found the maximum seed germination rate and maximum level of magnetic-treated SOD and CAT activities. The experiment showed that there were no differences between magnetic-treated and control SOD and CAT activities of germinated soybean seeds at the 144th hour that was the time required for germination.

Abbreviatons

SOD	– Superoxide dismutase;
ROS	– reactive oxygen species;
O ₂ ⁻	– superoxide;
H ₂ O ₂	– hydrogen peroxide;
OH	– hydroxyl radical;
¹ O ₂	– singlet oxygen;
PPO	– polyphenol oxidase;
CAT	– catalase;
NBT	– nitroblue tetrazolium salt;
EDTA	– ethylenediamine tetraaceic acid;
PBS	– phosphate buffered saline;
MF	– magnetic field.

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