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Evaluation of antioxidative parameters in roots and shoots of pea plant (*Cicer arietinum* L.) in response to static magnetic field stress

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ABSTRACT

Since the magnetic fields (MF) are increasing on the earth, many investigators are studying the effect of MF on the various functions of living things. Also, investigations of MF effects on biological systems have attracted attention of biologists due to planned space flights to other planets. In the present study, we tested the effect of 30 mT static magnetic field (SMF) on some parameters indicative of oxidative stress in intact pea plants (*Cicer arietinum* L. cv. Hashem). Rate of peroxidation of membrane lipids and activities of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) were measured. The results showed that the activities of SOD, CAT and APX were remarkably increased. However, the activity of antioxidative enzymes was not sufficient for scavenging of free radicals, so that the level of membrane lipid peroxidation was significantly increased after exposure to 30 mT SMF. Increased activity of SOD on one hand suggest that exposure to SMF results in enhancement of hydrogen peroxide as a very harmful reactive oxygen species and on the other hand increase of the activity of CAT and APX refers to the strategy of plant against oxidative stress by increasing of the activity of hydrogen peroxide scavengers.

KEYWORDS

Ascorbate peroxidase;
Catalase;
Lipid peroxidation;
Static magnetic field;
Superoxide dismutase.

INTRODUCTION

Magnetic fields (MF) are widely distributed in the environment and their effects are increasing with the burgeoning development of electrical machines[1]. These fields generated by electrical equipment are many times higher than those occurring naturally, and their prevalence is a consequence of technological developments in the second half of the 20th century[2].

Investigations of MF effects on biological systems have also attracted attention of biologists due to planned long-term interplanetary flights. Interplanetary navigation will introduce man, animals and plants in magnetic environment. This brought a new wave of interest in MF's role in regulating plant growth and development[3].

Over many years, the effects of MF on plant life have been the subject of several studies. Recently, many authors have reported the effects of SMF on the metabolism and growth of different plants[4-6]. Some decades ago, strong MF were used[4,7] but more recently, moderate fields, even as small as the geomagnetic field, have been reported to produce striking effects[5,6,8]. It

has also been found that such fields cause an increase in the free radical activity in living organisms[9], which results in the formation of excessive amounts of reactive oxygen species (ROS). An uncontrolled free oxygen radical release, termed oxidative stress, may cause protein oxidation, changes in enzyme activity and lipid peroxidation within the cellular membranes, resulting in structural and functional abnormalities as well as in oxidative damage to the DNA and RNA[10]. So far, few studies on the effects of SMF on plants antioxidative systems have been performed, whereas interest in the biological effects of these fields on vegetable organisms has grown very recently. Thus, the present study was designed to evaluate the effect of exposure to SMF on some parameters indicative of oxidative stress i.e. lipid peroxidation, SOD, CAT and APX in roots and shoots of intact pea plants.

EXPERIMENTAL

Seeds of pea plant (*Cicer arietinum* L. cv. Hashem) were used for the present study. Seeds of uniform size were selected and surface sterilized with 0.1% sodium hypochlorite solution for 10min and then rinsed with double distilled water[11]. The washed seeds were then spread over Petri dishes lined with two-layered wet filter paper. Seeds germinated between wet filter paper at 25^0C in the dark for 4 d. The seedlings of uniform size were then transferred in sand culture in plastic pots saturated with Hoagland nutrient solution. Pots were kept for growth of seedlings in growth chamber (12/12 h photoperiod at 150μmol m^{-2} s^{-1}) providing white fluorescent light with day/night temperature of $25/20^0$C and 60±5% relative humidity. The seedlings were grown in normal growth conditions up to 20d. Then, 20-d old plants were used for treatment.

Exposure to SMF was performed by a locally designed SMF generator (figure 1).

The electrical power was provided using a 220 V AC power supply equipped with variable transformer as well as a single-phase full-wave rectifier. The maximum power and passing current were 1 KW and 50 A DC, respectively. This system designed to generate SMF in range of 0.5μT- 30mT with stable conditions. It consisted of two coil (each 3000 turns of 3mm copper wire) equipped with a U-shaped laminated iron core (to prevent eddy current losses). Using two vertical connectors, the arms of the U-shaped iron core were terminated to four circular iron plates covered with thin layer of nickel (each 23mm thickness, 260cm in diameter). An electronic board was used to stabilize the system so that we always got a uniform SMF. A water circulation system around the coils was employed to avoid the increase of the temperature. The temperature between the circular iron plates (where the samples were located), was measured by a thermometer and was almost the same as other parts of the room (e.g., the location site of the control cells) ±1°C. Since no other electric appliance was working, the control samples were only exposed to the extremely low MF of the earth, as the treatment group was too. Moreover, the control cells were kept far enough from the EMF producing apparatus, to avoid any potential exposure to the magnetic field. Calibration of the system as well as tests for the accuracy and uniformity of the magnetic fields were performed by a teslameter (PHYWE, Germany) with a probe type of Hall Sound. The accuracy of the system was ±0.1% for static field and the range of measurements was 3μT- 30mT. 20-d old pea plants were treated with 30mT, discontinuously for 5 d, each 5h. After treatments, the roots and shoots of pea were harvested and frozen in liquid N_2 and kept at -800C until used for biochemical measurements.

Frozen samples (200mg fresh weight) were homogenized in 3mL HEPES-KOH buffer (pH 7.8) containing 0.1 mM EDTA. The homogenate was centrifuged at 15000×g for 15 min. All operations were made out at 4^0C. The supernatant was used for SOD activity[12]. Reaction mixture (3mL) consisted of 50 mM HEPES-KOH buffer (pH 7.8), 0.1mM EDTA, 50mM Na_2CO_3

Figure 1: SMF producing apparatus

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(pH 10.2), 12mM L-methionine, 75μM NBT, aliquots of enzyme extract and 1μM riboflavin. One unit SOD activity was defined as the amount of enzyme required to result in a 50 % inhibition of the rate of NBT reduction measured at 560 nm. CAT activity was assayed in a reaction mixture containing 25 mM Na-phosphate buffer (pH 6.8), 10mM H_2O_2 and enzyme extract in a total valium of 1mL. The decomposition of H_2O_2 was followed by the decline in absorbance at 240nm[13]. The activity of APX was measured according to Nakano and Asada[14] by monitoring the rate of ascorbate oxidation at 290nm (ε=2.8mM^{-1} cm^{-1}). In brief, samples were homogenized in 1mL of 50 mM Na-phosphate buffer (pH 7.8) containing 5 mM ascorbate, 5 mM DTT, 5 mM EDTA, 100mM NaCl and 2% (W/V) PVP. The homogenate was centrifuged at 15000×g for 15min at 4°C. The reaction was initiated by adding H_2O_2 to a final concentration of 44μM. The protein content was determined by the method of Bradford[15] using bovine serum albumin as a standard.

The level of peroxidation of membrane lipids was assayed by measuring MDA as final product of lipid peroxidation. Samples were homogenized in an aquatic solution of TCA (10 % w/v). The homogenate was centrifuged at 15000×g for 10 min and 1mL of the supernatant was added to a 1mL 0.5 % TBA. The mixture was incubated at 100 °C in a water bath for 30 min, and the reaction stopped by placing the reaction tubes in an ice-water bath. Then, the absorbance of MDA was read at 532 nm followed by correction for the non-specific absorbance at 600nm. The amount of MDA-TBA complex was calculated from the extinction coefficient of 155 $mM^{-1}cm^{-1}$ [13, 16].

All of the experiments were carried out with at least three independent repetitions in triplicate. All values are shown as the mean ± SD. Statistical analysis was performed using Student's t.test and the differences at level of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Free radicals are very reactive and unstable molecular species that can initiate chain reactions to form new radicals. Although formed as a result of a wide range of normal biochemical processes, they are potentially damaging. Several mechanisms are in place to neutralize their effects, which include a system of nutritional and endogenous enzymatic antioxidant defenses that generally hold the production of free radicals and prevent oxidant stress and subsequently tissue damage[17]. The balance between the oxidants and the antioxidants may be disturbed by an increase in free radical production[18]. This imbalance between the oxidants and the antioxidants can lead to oxidative stress[19].

There were evidences that show certain metabolites and a modulated expression of free radical-scavenging enzymes is needed for an effective defense against the overproduction of toxic oxygen forms in plants exposed to environmental stresses[20,21].

The variations of lipid peroxidation levels and free radicals scavenging enzymes activities in roots and shoots of intact pea plants were investigated under 30mT SMF stress condition. The present study suggests that SMF leads to production of MDA and induces some of the key enzymes of antioxidant defense system in pea plants. Induction in the activities of antioxidative enzymes is a general strategy adopted by plants to overcome oxidative stress due to the imposition of environmental stresses[21,22].

Fatty acid peroxidation is a sensitive marker of cellular membrane injury involving ROS. Thus, we tested the effect of 30 mT SMF on the lipid peroxidation.

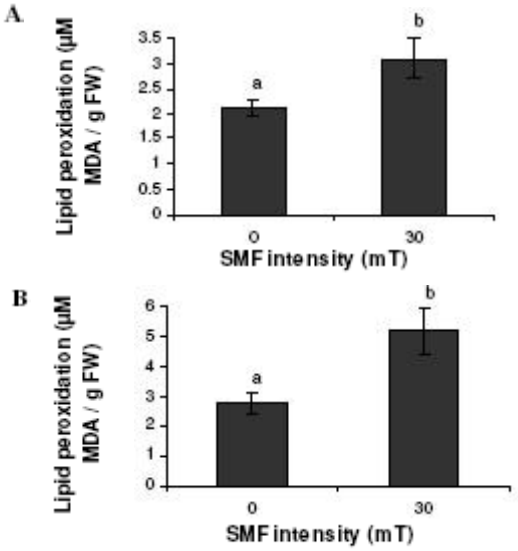

Figure 2: Lipid peroxidation level of roots (A) and shoots (B) of intact pea plants exposed to 30 mT SMF. Data are means ± SD n=3. Bars with different letters are significantly different

The formation of malondialdehyde (MDA) content was considered as a measure of lipid peroxidation. The lipid peroxidation of roots and shoots of intact pea plants increased after exposure to SMF (**Figure** 2A and 2B). The lipid peroxidation was greater in the shoots than in the roots.

Polyunsaturated fatty acid acyl chains of phospholipids are very susceptible to oxidative stress by free radicals, which lead to lipid peroxidation. Production of lipid peroxidation is a toxic process resulting in the deterioration of biological membranes[19].

MDA, as a lipid peroxidation product, is a biochemical marker for the free radical mediated injury[23]. Our results show an increase in the level of lipid peroxidation, indicating that SMF induces oxidative stress in pea plants. Similar to our observations, enhanced lipid peroxidation have been reported under high temperature stress[24], UV-radiation[25] and toxicity by some heavy metals like Ni[26] in different plant species.

The involvement of antioxidative enzymes as regulator of free radical metabolism was determined by measuring the changes in SOD, CAT and APX activities of plant cells during exposure to 30 mT SMF.

The enzymatic components associated with defense against ROS include SOD, CAT, and enzymes of ascorbate-/glutathione cycle. SOD and CAT have been identified as enzymatic protectors against peroxidation reactions[27].

SOD is an essential component of antioxidative defense system in plants and it dismutates two superoxide radicals ($O_2^{\cdot-}$) to water and O_2. Its absence or decreased activity may have noxious metabolic outcomes[28].

As shown in figure 3A and 3B, the activity of SOD was significantly higher in exposed roots of intact pea plants than in controls throughout the experiment. Among the roots and shoots of pea plant, greater level of SOD activity was exhibited in shoots, in response to 30mT SMF.

Our results show increased activity of SOD in pea plants exposed to 30mT SMF. SOD activity has been reported to increase under water stress[29], UV-B radiation[25] and gamma radiation[30]. Increase in SOD activity in response to stress appears to be probably due to de-novo synthesis of the enzymatic protein[31].

Transgenic plants over-expressing SOD, show increased tolerance towards oxidative injury caused due to harsh environmental conditions and among antioxidant enzymes the activity levels of SOD are of more relevance in maintenance of the overall defense system of plants subjected to oxidative stress[32].

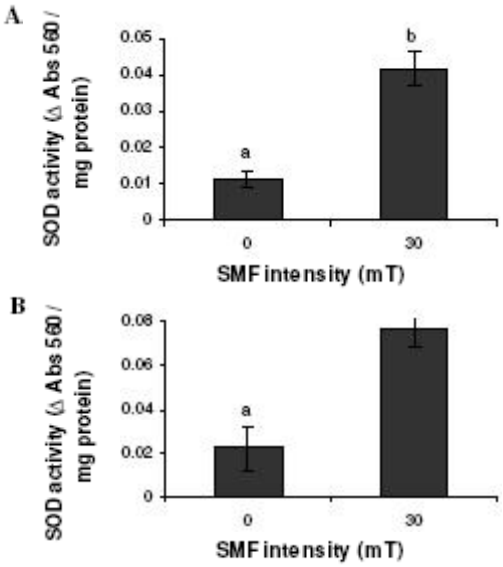

Figure 4: CAT activity of roots (A) and shoots (B) of intact pea plants exposed to 30 mT SMF. Data are means ± SD n=3. Bars with different letters are significantly different

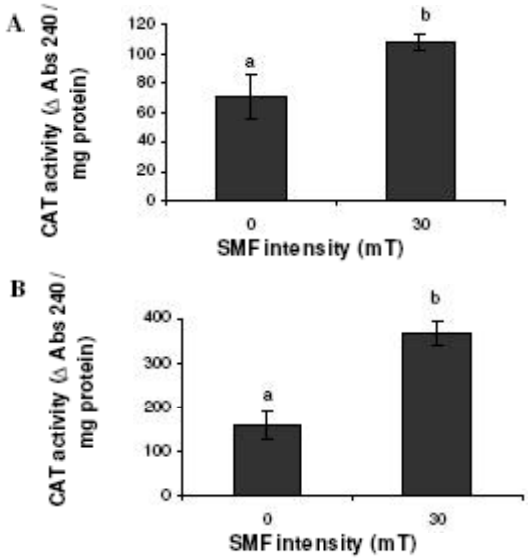

Figure 3: SOD activity of roots (A) and shoots (B) of intact pea plants exposed to 30 mT SMF. Data are means ± SD n=3. Bars with different letters are significantly different

Furthermore, the CAT activity of roots and shoots of intact pea plants increased substantially when compared to their respective controls (figure 4A and 4B).

Similarly, as indicated in figure 5A and 5B, the ac-

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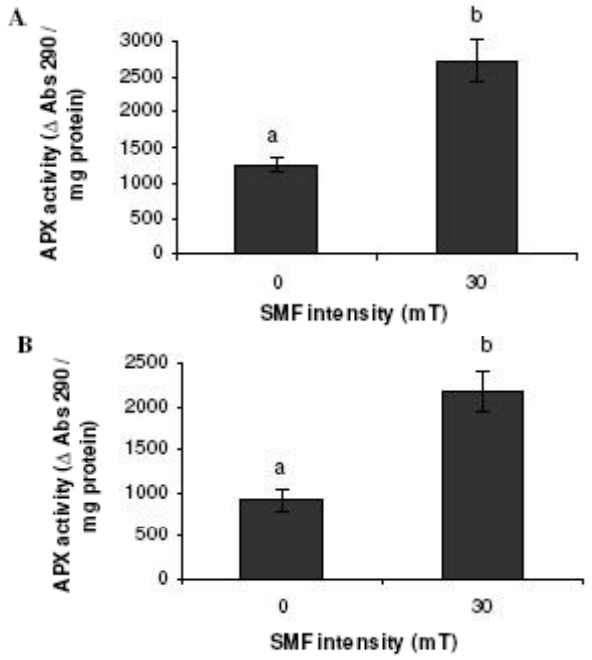

Figure 5:APX activity of roots (A) and shoots (B) of intact pea plants exposed to 30 mT SMF. Data are means ± SD n=3. Bars with different letters are significantly different

tivity of APX in roots and shoots of intact pea plants exposed to 30mT SMF was significantly higher than those of the control groups.

CAT is universally present oxidoreductase that decomposes H_2O_2 to water and molecular oxygen and it is one of the key enzymes involved in removal of toxic peroxides[33]. The present work shows that SMF exposure also increased the activity of CAT. Similar increasing was observed in response to UV-B radiation in leaves of potato plants[34] and sunflower cotyledons[35] and water stress[36].

APX is indispensable component of ascorbate-/glutathione pathway, required to scavenge H_2O_2 produced mainly in chloroplasts and other cell organelles and to maintain the redox state of the cell[37]. APX utilizes the reducing power of ascorbic acid to eliminate potentially harmful H_2O_2. Our results indicate an enhancement in the activity of APX in response to SMF stress. Similar induction was reported in response to ozone toxicity[38], drought[39] and UV-B radiation[40]. APX along with CAT and SOD are considered as key enzymes within the antioxidative defense mechanism, which directly determine the cellular concentration of $O_2^{\cdot -}$ and H_2O_2[37].

Our results suggest that exposition to 30mT SMF causes oxidative stress in roots and shoots of intact pea plants and the enzymes SOD, CAT and APX appear to play a pivotal role in combating oxidative stress in plants.

It is noticeable however, that an increase in the activity of SOD although detoxifies the $O_2^{\cdot -}$ radicals, results in the more production of H_2O_2, in turn. In roots and shoots of intact pea plants exposed to SMF however, the produced H_2O_2 was scavenged, at least in part, by the subsequent increase in the activities of CAT and APX. This may be sufficient for the protection of membranes from H_2O_2-mediated peroxidation. There is also a possibility that during treatment periods of stress situations, the scavenging system may become saturated by the increased rate of radical production[41], and may not sufficient to protect the membrane against potentially deleterious effect of ROS so that become unable to protect membranes from lipid peroxidation. It is the case seen the SMF- exposed pea plants.

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