EFFECT OF MAGNETIC FIELD ON PEROXIDASE ACTIVITIES OF SOYBEAN TISSUE CULTURE

Ç. Atak[1], Ö. Çelik[2], A. Olgun[1], S. Alikamanoğlu[3], A. Rzakoulieva[4]
Istanbul Kültür University, Faculty of Science and Letters, Istanbul, Turkey[1]
Halic University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Istanbul, Turkey[2]
Istanbul University, Faculty of Science, Department of Biology, Istanbul, Turkey[3]
JINR, Laboratories of Nuclear Problems, Magnetic Research Group, Dubna, Russia[4]
Correspondence to: Özge Çelik
Email: ocelik@halic.edu.tr

ABSTRACT

In this study, the aim was to determine the effect of magnetic field on peroxidase activities of soybean tissue culture. Shoot tips were put into petri dishes and exposed to a magnetic field for a period of 2.2 and 19.8 s at a magnetic flux of 2.9-4.6 mT. The shoot and root formation rate, fresh weights, chlorophyll quantities, total RNA concentrations and peroxidase activities of regenerated shoots from control and treated shoot tips were determined.

While the rate of shoot formation was 28.57% in the control group, this rate was increased to 94.33% and 78.18%, respectively, in the explants that were exposed to a magnetic field for a period of 2.2 and 19.8 s. While the percentage of root formation in controls was 4.76%, this rate increased to 47.17% and 54.54%, respectively, in those that were exposed to a magnetic field at the same periods. When the fresh weights were determined, we found that the fresh weights of plantlets regenerated from treated explants were increased relative to controls. Chlorophyll a, chlorophyll b and total chlorophyll contents increased 21%, 13% and 18%, respectively, relative to control groups at 2.2 s. Peroxidase activity significantly increased in all magnetic field treatments ($p<0.05$). The total RNA concentration of seedlings regenerated from treatment explants significantly increased relative to controls ($p<0.05$).

The regeneration and plant growth of shoot tips exposed to a magnetic field with a 2.2 s period were positively affected by the MF and increased with respect to controls and the length of time exposed.

Keywords: Soybean, magnetic field, in vitro regeneration, peroxidase activity

Introduction

It has been shown that both low frequency electromagnetic field (EMF) and magnetic fields (MF) have an effect on microorganisms, plants and animals (14). There are many studies showing that magnetic field flux and exposure time affects different features of plants positively or negatively.

Today we know that magnetic fields have a positive effect on plant characteristics such as seed germination, seedling growth, agronomic traits and seed yield (1, 4, 5, 11, 29, 30, 32, 34, 36, 39). In addition, shoot and root regeneration was increased in cultured explants exposed to magnetic fields (6, 41). *In vitro* research has shown that EMF changes cell membrane characteristics, cellular functions and growth. Magnetic field experiments showed that gene expression, protein biosynthesis, enzyme activity, cell reproduction and cellular metabolism increased relative to controls (7, 17, 18, 33, 37, 38). In other studies, which were made in order to show the effects of the magnetic field on plants, researchers used the conditions of the geomagnetic field (gmf) and on its 10^5-10^6 fold screening. Significant changes in the duration of the G_1 phase of the cells of the root meristem were observed and during this period, RNA and protein synthesis under the gmf conditions were intensive (12, 13). Biochemical reactions that have more than one unpaired electron were affected by magnetic fields (23). More than 50 enzymes (like heme enzymes), which produce free radical products during catalysis were affected by magnetic field treatment (19).

Peroxidases (POX) are heme-containing glycoproteins encoded by a large multigene family in plants. Studies have suggested that POX plays a role in lignification, suberization, auxin catabolism and self-defense against pathogens, salt tolerance and senescence (21).

It is known that auxin has a role in root formation. There is a relationship between an increase in IAA and root induction. Peroxidase has a role in the formation of the connection between auxin metabolism (IAA-oxidation) and cell wall complex. Many studies have also shown that peroxidases play a role in the plant growth process. Cytokinins play an important role in axillary bud growth, chloroplast development and shoot formation and the delay of senescence. Cytokinins also regulate the expression of plant peroxidase genes (22, 24).

Peroxidases have a number of very important roles in plants. They are known as good physiological markers of rooting in many species. POX plays a role in the formation of cofactors, which are necessary for root initiation. Lignification in the cell wall, a process that is catalyzed by peroxidases, may occur during rooting (23, 25). Quiraga et al. (35) proposed that the tomato gene TPX1, which encodes a basic peroxidase product, is involved in the synthesis of lignin and suberin.

Exposure to a magnetic field has been shown to stimulate shoot and root regeneration in explanted soybean tissue cultures (6). The aim of this study was to determine the effect of magnetic field on peroxidase and total RNA activity of shoots regenerated from soybean tissue cultures.

Materials and Methods

Plant material

The *Glycine max* L. Merrill J 357 soybean seeds were used for this research. Seeds were surface-sterilized for 1 minute in 70% ethanol and soaked in 20% commercial bleach (commercial bleach contains about 5% sodium hypochloride) for 20 minutes. Seeds were rinsed three times in sterile distilled water. Sterile seeds were left for germination at 27 °C for 5 days in petri dishes containing 0.8% agar.

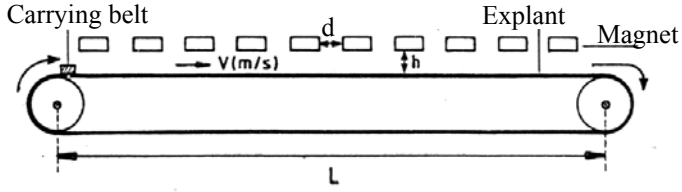
Plant tissue culture

The soybean explants were prepared from young seedlings (5-days-old). The shoot tip explants were incubated on medium containing inorganic nutrients such as Gamborg's medium and vitamins, plus 30 g/l sucrose, 0.8% agar, 40 mg/l adenin sulphate, 0.1 g/l glutamine and 0.1 mg/l 2.4-D at 27°C, with a 16h light/8h dark cycle (6).

Explants were exposed to the magnetic fields in 15x100 mm petri dishes. After magnetic field treatment, explants were immediately transferred to fresh medium. In this study, every treatment had 5 replications and each replication had 10 explants. In these experiments, the fresh weights were determined from plantlets on the 28th day. Also, at this time, the leaves of regenerated plants were used to determine the chlorophyll content, peroxidase activity and total RNA concentration.

Magnetic field experiment

In the magnetic field experiment, we used 10 magnets that were 0.45 x 0.065 x 0.022 m in dimension. These magnets were prepared by the magnetic field group in the Joint Institute of Nuclear Research, Dubna, Russia (JINR) laboratories at the magnetic field laboratory of Istanbul University. These magnets were mounted onto the belt system, which rotated at a rate of 1 m/s. The height of the magnets from the belt system was 0.060 m. The magnet height from the belt system was adjustable (**Fig. 1**). Soybean explants were passed 1 and 9 times through a magnetic flux density of 2.9-4.6 mT for 2.2 and 19.8 s.



L=2,2m (Carrying belt length)
h=0,060m (Distance between sample and magnets)
d=0,15m (Distance between magnets)
n=10 (Magnet number)
V=1 m/s (Passing velocity from magnetic field)

Fig.1.

Chlorophyll content

Chlorophylls were extracted from the leaves. Extraction of the leaf pigment was done with 80% acetone and the absorbance was measured at 663 and 645 nm with a UV-160 Schimadzu spectrophotometer. Chlorophyll a, chlorophyll b and total chlorophyll were calculated in accordance with the Arnon method (3).

Peroxidase activity

Peroxidases were extracted from the 100 mg 28-day-plant leaves with 0.1 M (pH 7.0) phosphate buffer. Extracts were centrifuged at 8387 g for 30 minutes. Supernatants were treated with 15mM guiaiacol and 5mM H_2O_2 in 0.1 M pH 7.0 phosphate buffer. The enzyme produced a colorful product by using H_2O_2 and guiaiacol as substrates. The absorbance of the product was measured over 3 minutes in 10 s intervals at 470 nm with a UV-1601 Schimadzu spectrophotometer [9, 31]. Peroxidase activity was expressed as dA420/min g fresh weight.

TABLE 1

Effect of MF on shoot and root regeneration and fresh weights

Exposure time (s)	Shoot regeneration (%)	Root regeneration (%)	Fresh Weight (mg) ±S.D.
Control	28.57	4.76	125.8±0.079[a*]
2.2	94.33	47.17	170.2±0.039[b]
19.8	78.18	54.54	167.2±0.047[b]

Values are means of 5 replicates ± SD

* Means which were shown with same letter, are not significantly different by Duncan's multiple tests (P<0.05).

TABLE 2

Effect of MF on chlorophyll a, chlorophyll b and total chlorophyll contents (mg/g fresh weight)

Exposure time (s)	Chlorophyll a (mg/g fresh weight)	Chlorophyll b (mg/g fresh weight)	Total Chlorophyll (mg/g fresh weight)
Control	0.1420±0.04830	0.0573±0.00028	0.1995±0.0796[a*]
2.2	0.1724±0.00248	0.06476±0.00062	0.2372±0.00263[b]
19.8	0.1228±0.00476	0.0495±0.00031	0.1724±0.00478[c]

Values are means of 5 replicates ± SD

* Means which were shown with same letter, are not significantly different by Duncan's multiple tests (P<0.05).

Total RNA isolation

Total RNA isolation was performed with the Qiagen Rneasy Plant Mini Kit. 80 mg of frozen leaves were used for each treatment (26). Absorbances at 260 and 280 nm were measured with a UV-1601 Schimadzu spectrophotometer. Total RNA concentrations were calculated according to this formula:

RNA Concentration (μg/ml): A260 x Dilution Factor x 40

Statistical analysis

Statistical analysis of the data was performed by using ANOVA. We applied Duncan's multiple range test to compare the experimental results of the groups exposed to a magnetic field for fresh weight, total chlorophyll content, peroxidase enzyme activity and total RNA concentration with the control. For the statistical evaluation of the results, significance was defined by a probability level of $p<0.05$ (28).

Results and Discussion

Shoot and root regeneration of the soybean shoot tip cultures exposed to a 2.9-4.6 mT magnetic field at various periods and fresh weights are shown in **Table 1**. The changes in shoot and root formation percentages in accordance with days from explants exposed to control and magnetic field strength at various periods is shown in **Figs**. **2** and **3**.

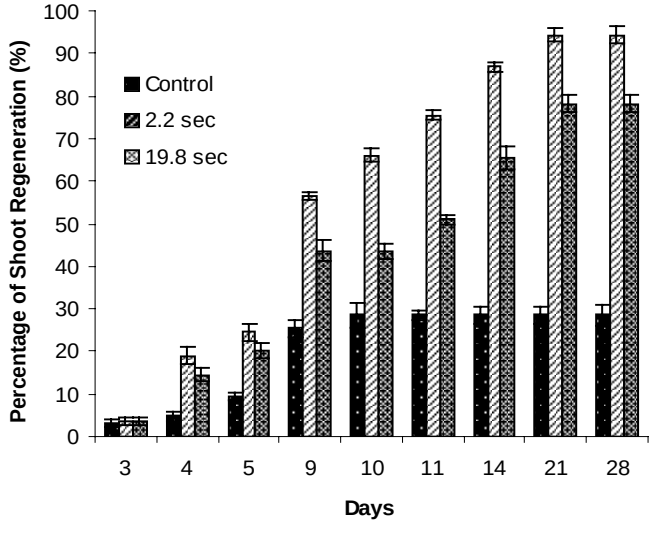


Fig.2.

Explants were exposed to a magnetic field and shoot formation was observed from the 5th to 28th day. On the 28th day, the regeneration percentages of explants, exposed to the magnetic field were higher than control levels. While the regeneration percentage of the explants on the 28th day was 28.57% in the control group, explants that passed through a magnetic field with a period of 2.2 and 19.8 s had a regeneration percentage of 94.33% and 78.18%, respectively (**Fig. 2**).

Root formation was seen on the 9th day in all explants exposed to the magnetic field and on the 11th day to 28th day in the control group. While root formation was 4.76% in the control group on the 28th day, this rate increased to 47.17% and 54.54% in explants that passed through the magnetic field with a period of 2.2 and 19.8 s, respectively **(Fig. 3**).

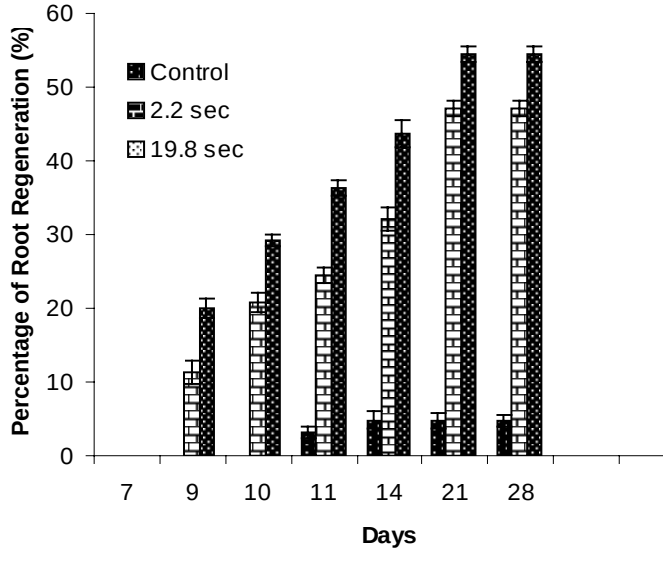


Fig.3.

Fresh weights of regenerated plants showed an increase in all magnetic field treatments relative to the control group. The increase in fresh weights of explants exposed to the magnetic field with a period of 2.2 and 19.8 s were found to be significant (**Table 1**).

On the 28th day of the shoot tip culture experiments, leaves of regenerated plantlets were used to determine the effect of a magnetic field on chlorophyll a, chlorophyll b and total

chlorophyll content. Chlorophyll a, chlorophyll b and total chlorophyll content increased significantly (21%, 13% and 18%, respectively) with respect to control groups at 2.2 s (**Table 2**). Chlorophyll content of plantlets exposed to a MF for 19.8 s was significantly decreased with respect to controls.

Peroxidase activity was determined from leaves from both the control and magnetic field treatment groups. The POX activities of the regenerated plants increased as the explants passed through magnetic field at 2.2 s and 19.8 s periods, relative to the control group (**Table 3**). This increase was found to be significant ($p<0.05$). Also, the POX activity of leaves exposed to magnetic fields for 19.8 s increased relative to those exposed for 2.2 s.

TABLE 3

Effect of MF on peroxidase enzyme activity

Exposure time (s)	Peroxidase Activity (dA/min g fw)
Control	469.8[a*]
2.2	508.32[b]
19.8	638.68[c]

* Means which were shown with same letter, are not significantly different by Duncan's multiple tests ($P<0.05$).

Total RNA concentrations are shown in **Table 4**. The increase in total RNA concentration in samples exposed to a magnetic field with a period of 2.2 and 19.8 s reached significance ($p<0.05$).

TABLE 4

Effect of MF on total RNA concentrations

Exposure time (s)	Total RNA Concentrations (mg/ml)
Control	4.336[a*]
2.2	9.145[b]
19.8	5.024[c]

* Means which were shown with same letter, are not significantly different by Duncan's multiple tests ($P<0.05$).

In this study, the aim was to determine the effect of a magnetic field on chlorophyll content, POX activity and total RNA concentration in soybean shoot tip cultures. The number of explants with shoot and root formation and the fresh weights of regenerated plantlets from shoot tip culture which were exposed to magnetic field were greater than in controls. The chlorophyll content of leaves belonging to regenerated soybean shoots was determined for both control and treated explants which were exposed to magnetic field. According to the results, chlorophyll content in a MF with a 2.2 s period increased relative to the control group. Conversely, after a long time in the MF, the chlorophyll content significantly decreased.

In other research, leaf chlorophyll content was also found to increase in response to MF exposure (6, 41). Belyavskaya et al. (8) determined that phytoferritin levels were decreased in the plastids of pea root meristem cells under magnetic screening conditions.

We showed that the magnetic field had effects on POX activity. The increase in POX activity was especially apparent after exposure to the magnetic field for a 19.8 s period. The root formation and POX activity of plantlets that were exposed to the magnetic field for a long period was greater than in controls and those exposed for only 2.2 s.

In this study we determined that chlorophyll content was affected negatively by 19.8s period because of increased POX. Chlorophyll break down was caused by oxidative stress. Following exposure to the magnetic field for a short time, chlorophyll content was increased and plant growth was stimulated. Atak et al. (6) examined the effect of magnetic field on soybean shoot tip explants. They showed that magnetic field exposure increased the shoot and root regeneration, chlorophyll content and fresh weight in soybean organ cultures. Gang et al. (16) found that magnetic fields increased the activity of POX in wheat seedlings. Aouad et al. (2) found a positive correlation between soluble root peroxidases and leaf or root fresh weight. Milavec et al. (27) showed an inverse correlation between soluble and ionically-bound peroxidase activity and chlorophyll content. Soluble peroxidase activity was high, while the amount of chlorophyll content was low. There is a relationship between peroxidase gene expression and plant growth. The expression of the anionic peroxidase gene was suppressed in growing tissue. Anionic peroxidase has been found to be involved mainly in the response to both biotic and abiotic stresses (10). The peroxidase cationic and anionic components showed different sensitivities to the magnetic field. A positive correlation was found between cationic peroxidase and growth rate (7).

In our study, the total RNA concentration of regenerated plantlets exposed to magnetic field with 2.2 s period was found to be significantly different than control and 19.8 s plantlets. The increase in total RNA concentration of seedlings that regenerated from explants that were exposed to the 2.9-4.6 mT magnetic flux at 2.2 s, could be due to the increase in expression of enzymes that play a role in shoot formation, chlorophyll biosynthesis and peroxidase biosynthesis.

RNA and protein synthesis of plant meristem cells were affected at the magnetic screen conditions which geomagnetic field was 10^5 –10^6 screening (13). The total RNA content in various lines of cultured eukaryotic cells increased after exposure to an ELF magnetic field (38). Worczak et al. (40) have studied the effects of high MF on *in vitro* transcription. Their preliminary results with T7 RNA polymerase in fields up to 9 T showed a subtle delay transcription rate.

When plants are exposed to magnetic field, the mechanisms of magnetic field interact with biological systems are not well known yet. But several theories have been proposed. One of these is the 'radical-pair mechanism' consisting of the modulation of single-triplet interconversion rates of a radical pair by magnetic fields. Magnetic field increased the average radical concentration, prolonging their life time and enhancing the probability of radical reaction with cellular components (14, 15, 19). Such effects have the potential to lead to biological consequences.

In summary, *in vitro* experiments showed that both the regeneration and growth of explants was different at the various time periods of 2.9-4.6 mT MF. The growth characteristics of explants exposed to a magnetic field with a 2.2 s period were positively affected and increased relative to controls. When the exposure time of MF was increased (19.8 s), the effects of the MF on growth changed because of the increased POX. While root formation was increased, chlorophyll content of plantlets exposed to a MF for this exposure time was decreased with respect to controls.

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