STIMULATION OF RAPID REGENERATION BY A MAGNETIC FIELD IN PAULOWNIA NODE CULTURES

Özge Çelik1*, Çimen Atak1, Aitekin Rzakulieva2

1 Department of Molecular Biology and Genetics, Faculty of Science and Letters, Istanbul Kultur University, Istanbul, Turkıye

2 JINR, Laboratories of Nuclear Problems, Magnetic Research Group, Dubna, Russia.

*Corresponding Author: Tel: +90 212 498 45 64, Faks: +90 212 661 42 71

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ABSTRACT

In this study, the aim was to determine the effect of magnetic fields on regeneration of Paulownia node cultures. Paulownia tomentosa node cultures were used to generate explants and these explants were passed through a 2.9-4.6-mT magnetic flux density 1 and 9 times at 2.2 and 19.8 seconds, respectively. Chlorophyll quantities, total RNA concentrations of shoots and shoot formation rates from control and treated explants were determined. While the shoot formation rate was 61.9% in the control group, this rate was increased in magnetic field experiments and shoot formation was 82.5% in the explants that were exposed to a magnetic field for a 2.2 second period. However, the regeneration percentage of the explants exposed to a MF for a period of 19.8 s was 45%. Chlorophyll a, chlorophyll b and total chlorophyll contents of the 2.2 s group were increased in comparison to the control group. Total RNA concentrations of seedlings regenerated from treatment explants treated for 2.2 seconds significantly increased in comparison to the control ($p<0.05$). Our experiments show that the exposure duration to MFs is an important factor for plant tissue. MFs may be used in in vitro regeneration studies rapid and for a short time.

Key words: Paulownia tomentosa, magnetic field, in vitro regeneration, RNA, chlorophyll content.

INTRODUCTION

There have been many investigations on the effects of low frequency electric and magnetic fields on plants and animals. It has been shown that a magnetic field (MF) has effects on the normal functions of living things. A MF was shown to induce seed germination, shoot development, fresh weight and plant length, fruit yield per plant and average fruit weight [3, 5, 12, 13, 27, 30, 31, 34, 38]. The effects of MFs cause increases in proliferation, gene expression and protein biosynthesis and alterations in cell membrane properties on tissue, cellular and subcellular levels [33, 35, 36]. Investigations of MFs on biological systems have demonstrated generalized increases in gene transcription and changes in the levels of specific mRNAs [18, 32, 33]. The influence of weak electromagnetic (EM) fields on the Ca^{+2} metabolism of living cells was studied. Koch et al. [24] showed that an ELF EM field directly interacted with Ca^{+2} channel proteins in the cell membrane. Fanelli et al. [14] showed that a static MF exerts the strong and reproducible effect of reducing apoptosis in several cell systems. This effect is mediated by the MF's ability to increase Ca^{+2} influx.

However, living organisms have experienced the action of the Earth's magnetic field, which is a natural component of our environment [8]. It was shown that the natural geomagnetic field has an important role on the normal functions of plants. Plant seeds were grown under magnetic screen (ms) conditions which geomagnetic field (gmf) was 10^5-10^6 fold screening by constant component. Under these conditions, it was shown experimentally that the germination of the seeds decreased and seedling growth initiated according to the gmf. Also, the cell reproduction cycle was noted through a lengthening of the G1 phase in these plants, and RNA and protein synthesis were altered [15, 16, 19]. Belyavskaya [8] has determined that phytoferritin in plastids of peas root meristem cells decreased under magnetic screen conditions.

Paulownia is an economically important genus in the family Scrophulariaceae. It has a very wide range of distribution in China. Also, it is cultivated in many parts of Japan, Australia and the USA. Fast growth is one of the important characteristics of Paulownia species. Paulownia wood is widely used for various purposes, and its leaves and flowers are used in medicine [9, 25, 40].

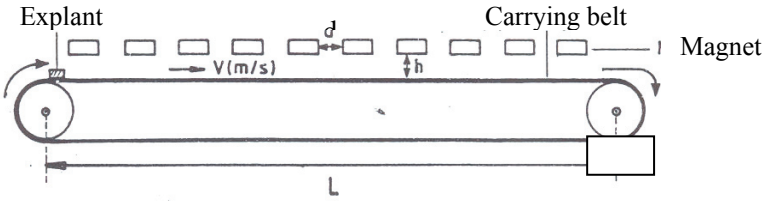
Paulownia can be cultivated from seed, stem and root explants. The germination rate from seed is slower, and it takes a longer time for development in comparison to growing plants from stem and root explants. Vegetative growth is a better way to produce genetically uniform plants [9, 22]. Paulownia node culture is an alternative system for regeneration and micropropagation. Moreover, a low frequency of somaclonal variation was observed in node cultures [21, 23].

The objective of this study was to determine the effect of MFs on shoot regeneration. In these experiments, P. tomentosa node cultures were used in the micropropagation procedure to produce plants and P. tomentosa tissue cultures were exposed to MFs for highly efficient shoot regeneration. The regenerated plants were examined to determine the effects of the MFs on regeneration percentage, chlorophyll content and total RNA concentrations.

MATERIAL AND METHODS

Plant Material:

Paulownia tomentosa seeds were used for this research. Paulownia seeds were obtained from UNITEK. For in vitro regeneration, wet Paulownia seeds were soaked in warm water (40°C) for 10 minutes. Then, the seeds were



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)

Figure 1. Magnetic field system plan.

Table 1: Effect of MF on chlorophyll a, chlorophyll b, total chlorophyll contents (mg/g fresh weight)

Periods exposed to 2,9-4,6 mT MF (sec)	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Control	0.362±0.020[a*]	0.215±0.018[a]	0.577±0.025[a]
2.2	0.645±0.017[b]	0.328±0.035[b]	0.973±0.038[b]
19.8	0.313±0.017[d]	0.220±0.042[a]	0.533±0.032[e]

* Means which were not shown with same letter, are significantly different by Duncan's multiple tests ($p<0.05$). Each mean represent 3 replications.

soaked for 24 hours in water at normal room temperature. The seeds were germinated on 0.8% agar in petri dishes and 10 day-old seedlings were planted into plastic pods, which were filled with standard experimental soils. Until the seedlings were two months old, they were grown in a growth chamber [5, 21]. Nodal segments (0.5 cm) of two-month-old Paulownia were used as explants. P. tomentosa nodes were surface-sterilized for 1 minute in 70% ethanol and were soaked in 20% commercial bleach (commercial bleach contains about 5% sodium hypochloride) for 20 minutes. Nodes were rinsed three times in sterile distilled water [21, 23].

Plant Tissue Culture:

The nodes were incubated for one day on a medium consisting of MS medium and vitamins, plus 30 g/l sucrose, 0.8% agar and 5 mg/l BA [9, 21, 25]. The following day, explants were exposed to a magnetic field in 15x100 mm petri dishes. After exposure to the magnetic field, explants were immediately transferred to fresh medium. For these experiments, each treatment included 10 explants and was repeated three times.

Magnetic Field Experiments:

In the magnetic field experiment, we used 10 magnets with dimensions 0.45x0.065x0.022 m (Figure 1). In the Joint Institute of Nuclear Research Laboratories, these magnets were prepared by the magnetic field group and were mounted onto a belt system, which rotated at a rate of 1 m/second. The distance of the magnets from the belt system was 0.060 m. Explants were passed through a 2.9-4.6 mT magnetic flux density 1 and 9 times for 2.2 and 19.8 seconds, respectively.

Chlorophyll Content:

Chloroplasts were extracted from the leaves of 28-day-old plants. Extraction of the leaf pigments was done with 80% acetone and absorbance was measured at 663 and 645 nm with a UV-160 Shimadzu spectrophotometer. Chlorophyll a, chlorophyll b and total chlorophyll quantities were calculated in accordance with the Arnon method [4].

Total RNA Analysis:

Total RNA isolation was performed with the Qiagen RNeasy Plant Mini Kit. For each treatment, 80 mg of frozen leaves were used. Absorbances at 260 and 280 nm were measured with a UV-160 Shimadzu spectrophotometer. $A_{260/280}$ values were determined. Total RNA concentrations were calculated according to the following formula [26].

RNA Concentration (μg/ml) = A_{260} x Dilution Factor x 40

Statistical analysis

Statistical analysis of the data was performed using ANOVA. We applied Duncan's multiple range test to compare the experimental results of the groups exposed to a magnetic field and the control group for total chlorophyll content and total RNA concentration ($p=0.05$) [29].

RESULTS

The regeneration percentages of Paulownia tomentosa node explants, exposed to a 2.9-4.6 mT magnetic field for various periods are shown in Figure 2. The distribution of shoot number on the explants at the 28th day is shown in Figure 3.

The shoot formations from explants, exposed to magnetic fields and from control explants, were observed from the 4th to the 28th day. On the 7th day, the regeneration percentage of the 19.8 s treatment group was higher than the control and the 2.2s treatment group. However, on the 28th day, the regeneration percentage of the 2.2 s treatment group was higher than the control and the 19.8 s treatment group. While the regeneration percentage on the 28th day of the explants in control group was 61,9%, the explants passed through the magnetic field for a period of 2.2 s had a regeneration percentage of 82.5%. The regeneration percentage of the explants exposed to the magnetic field for a period of 19.8 s was decreased in comparison to control.

The number of regenerated shoots from explants for the control group and the groups exposed to the magnetic field

are shown in Figure 3. In the control group, the number of explants with 1, 2 and 3 shoots was determined. The shoot number of the treatment groups was increased in comparison to control. While there were no plantlets with 4 or more shoots in the control group, all the explants treated with MFs had 4 or more shoots. The shoot numbers of regenerated explants exposed to the magnetic field for a period of 2.2 s were higher than control groups.

The amount of chlorophyll, contained in control plantlets and plantlets treated with magnetic fields was spectrophotometrically determined. The chlorophyll a, chlorophyll b and total chlorophyll contents of the 2.2 s group were increased in comparison to control group (Table 1) ($p<0.05$). However, the chlorophyll contents of shoots, which were exposed to the magnetic field for a period of 19.8 s, were decreased.

The total RNA isolated from the leaves of plantlets exposed, to a 2.9-4.6 mT magnetic field for various periods and from control plantlets. Total RNA analysis results for the explants, which passed through the

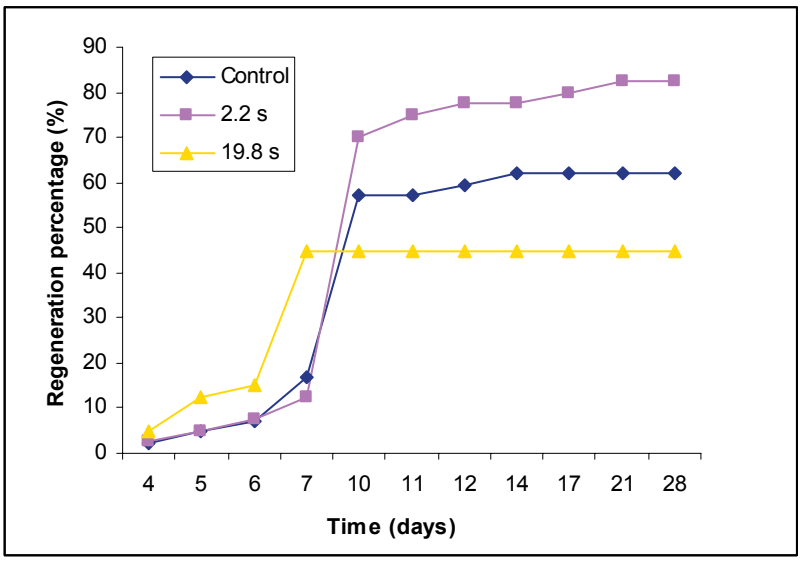


Figure 2. Effect of MFs on regeneration of Paulownia node cultures.

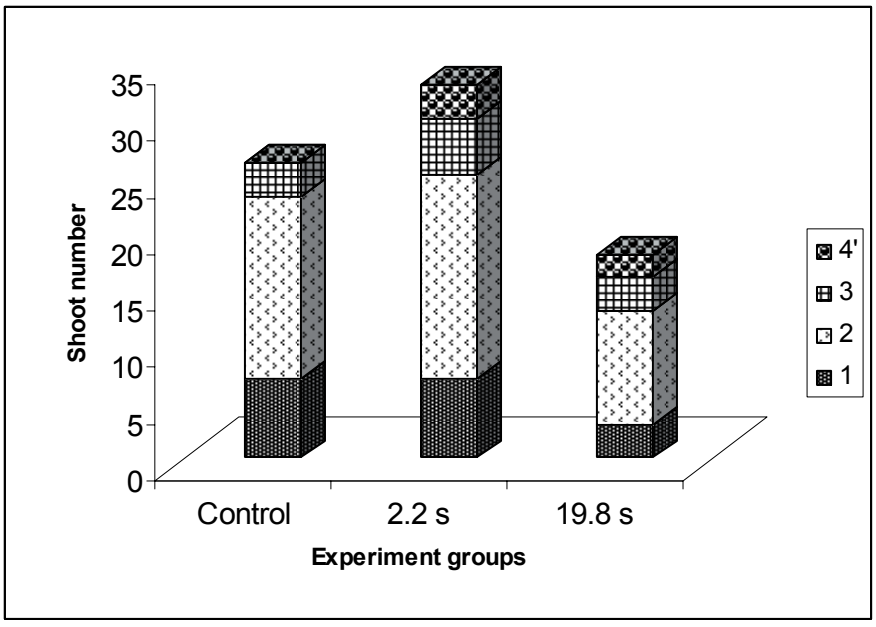


Figure 3. The shoot distribution on the 28 th day of the explants (4' means 4 or more shoots)

Table 2. Effect of MF on total RNA concentrations (μg/g)

Periods exposed to 2,9-4,6 mT MF (sec)	Total RNA Concentrations (μg/g)
Control	3.345 a*
2.2	6.952b
19.8	3.327a

*Means, which were not shown with same letter, are significantly different by Duncan's multiple tests ($p<0.05$). Each mean represent 3 replications.

magnetic field and for control group are shown in Table 2. The total RNA concentration of seedlings that regenerated from explants that were exposed to MFs for 2.2 s was greater than controls ($p<0.05$). Differences in total RNA concentrations between the control explants and those exposed to MFs for 19.8 s were not seen in this experiment.

DISCUSSION

In this study, the positive effect, negative effect or absence of effect of a MF on regeneration percentages, shoot numbers, chlorophyll content and total RNA concentration according to exposed time were determined. The regeneration percentages, shoot numbers, chlorophyll content and total RNA concentration of the explants exposed to a MF for a 2.2 s period were higher than controls. However, the explants exposed to a MF for a period of 19.8 s showed negative effects on regeneration percentage and chlorophyll content. During the same period, the total RNA concentration was not different when compared to the control. Following exposure to a magnetic field for a short time, we showed that plant growth was regulated by the MF.

MFs significantly induce cell metabolism and mitosis in plant meristematic cells [8, 15]. Aladjadjıyan [3] showed that exposure of seeds of Zea mays has a favorable effect on the development of shoots in the early stages. Yaycılı and Alikamanoğlu [39] studied Paulownia tissue cultures and showed the positive effect of magnetic fields on regeneration percentage.

Atak et al. [5,6] and Tian et al. [37] showed an increase in chlorophyll content specifically appeared after exposure to a magnetic field for a short time.

Tenforde [36] showed that after exposure to an ELF magnetic field, the total RNA content in various lines of cultured eukaryotic cells increased.

Fomicheva et al. [15] worked with proliferative activity and cell reproduction in the root meristems of the pea lentil and flax under conditions where the geomagnetic field was screened. They found an increase in the total duration of the cell reproduction cycle because of a lengthening of the G_1 phase in all plants. They demonstrated that RNA and protein synthesis of plants meristem cells were affected by magnetic screen conditions where the geomagnetic field was screened 10^5-10^6-fold [16].

Growth regulators are very widely used for micropropagation. Cytokinins, N^6-substituted adenine derivatives, are a class of plant hormones that were first identified as factors that promoted cell division. At the organismal level, cytokinins take part in the control of many biological processes throughout the life of plants. They stimulate protein synthesis and they can promote the maturation of chloroplasts. Cytokinins take part in the control of shoot initiation and growth. At the cellular level, cytokinins act by controlling the expression of many genes [2, 10, 11].

Cytokinins may elevate cell division rates by inducing the expression of CycD3, which encodes a D-type cyclin. D-type cyclins play a role in the G_1-M transition of the cell cycle. CycD3 was expressed in the shoot meristem, leaf pigments, and axillary and its induction was also specific to those tissues [2].

Plants contain the elements required for the transduction of hormonal signals via calcium. In plant cells, the cytosolic calcium concentration is low and very precisely controlled by the activity of regulating proteins such as calcium channels and pumps. Much experimental data shows that cytokinins modulate cytosolic calcium concentrations. Cytokinins could induce calcium penetration into cells [10].

Magnetic fields on the order of 10^{-3} to10^{-2} T can affect chemical reactions by influencing the electronic spin states of reaction intermediates. The other mechanism of MF interaction with cellular systems is through cellular signal transduction (1, 20, 36]. Cells can use intracellular second messengers, such as charged ions like Ca^{2+}. Ca^{2+} has many important roles in all living organisms. Several investigators studying EMF effects have also observed alterations in the intracellular and extra cellular Ca^{2+} environment. Among the effects of ELF MFs that have

been reported alterations in cell membrane properties and the binding of Ca^{2+} in eukaryotic cells and increased uptake of Ca^{2+} in mitogen-activated cells [17, 36]. Fanelli et al. [14] have shown that magnet-generated static magnetic fields enhance capacitate Ca^{2+} influx as an immediate and reversible affect without affecting Ca^{2+} mobilization from intracellular stores. Koch et al. [24] investigated a nonliving biological system consisting of purified membrane vesicles which were exposed to ELF EM fields. They found that the opening of calcium channels is influenced by ELF magnetic fields. In this investigation, they observed both increases and decreases in the Ca^{2+} efflux.

We showed that the growth characteristics of explants exposed to a magnetic field for a 2.2 s period were positively affected. MFs directly interact with Ca^{2+} channel proteins and the movement of Ca^{2+} into the cytosol from the extracellular medium. Calcium is one of the second messengers of cytokinins. As a consequence, an increase in cytokinin synthesis may be induced by MFs and cytokinins may increase in vitro shoot initiation.

When the exposure time to the MF was increased to 19.8 s, the effects of the MF on growth changed because of the stress. Shoot number was increased due to the magnetic field but the chlorophyll content of shoots was decreased. The MF increased the rate of cell proliferation. At the same time MFs are a stress factor for plants. The magnetic field had an effect on POX activity and this activity was increased by MF strength [7]. Milavec et al. [28] 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. In this experiment, the chlorophyll content was decreased by the length of the exposure time to the MF.

In summary, MFs may be used in in vitro studies to increase the percentage of plant regeneration and to shorten the duration of the regeneration because of the effects on cell division and protein synthesis. Our experiments show that the exposure duration to MFs is an important factor for inducing in vitro plant growth.

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