



## Stimulation of regeneration by magnetic field in soybean (*Glycine max* L. Merrill) tissue cultures

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### Abstract

The determination of the effects of magnetic field on tissue cultures will be helpful for the magnetic field treatments. In this study which was begun with this purpose, the seeds belonging to J 357 soybean variety were used. The soybean seeds were germinated in sterile conditions and cultures were initiated from the shoot tips. The explants in petri dishes were exposed to 2.9-4.6 mT magnetic fields for 2.2, 6.6, and 19.8 seconds periods. Shoot and root formation rate, fresh weights and chlorophyll quantities of regenerated shoots from control and treated explants were determined. While the shoot formation was 61.91% in control group, this rate increased in all magnetic field experiments and this rate raised up to 86.96% and 74.36% respectively in the explants which were exposed to magnetic field at 2.2 and 6.6 seconds periods. Again, while the percentage of root formation in control was 14.29%, this rate raised up to 26.08% and 35.90% respectively in these which were exposed to magnetic field at the same periods. When the fresh weights were determined, the fresh weights of seedlings regenerated from treatment explants from 6.6 seconds significantly increased in accordance with control ( $P<0.05$ ). Chlorophyll a, chlorophyll b and total chlorophyll quantities of shoot leaf were increased at the treatment magnetic field for 2.2 seconds

**Key words:** Soybean, magnetic field, cytokinin, auxin, regeneration

### Soya (*Glycine max* L. Merrill) doku kültürlerinde rejenerasyonun manyetik alan tarafından stimülasyonu

#### Özet

Manyetik alanın doku kültürlerindeki etkilerinin belirlenmesi bu konuda yapılacak çalışmalara yardımcı olacaktır. Bu amaçla başlanılan çalışmada J-357 soya çeşidine ait tohumlar kullanılmıştır. Soya tohumları steril koşullarda çimlendirilmiş ve sürgün ucu kültürleri kurulmuştur. Petri kutuları içerisindeki eksplantlar 2.9-4.6 mT'lık manyetik alandan 2.2, 6.6 ve 19.8 saniyelik sürelerde geçirilmiştir. Kontrol ve manyetik alana maruz bırakılan eksplantlardan rejenerasyon olan sürgün sayıları, kök oluşumları, yaş ağırlık ve klorofil miktarları belirlenmiştir. Sürgün oluşumu kontrol grubunda %61.91 iken bu oran tüm manyetik alan uygulamalarında artmış ve 2.2 ve 6.6 saniyelik sürelerde manyetik alana maruz bırakılan eksplantlarda sırasıyla %86.96 ve %74.36'ya yükselmiştir. Yine kök oluşum yüzdesi kontrolde %14.29 iken aynı sürelerde manyetik alandan geçirilenlerde bu oran sırasıyla %26.08 ve %35.90'a yükselmiştir. Rejenerasyon olan bitkilerin yaş ağırlıkları belirlenmiş ve 6.6 sn süre için manyetik alan uygulanan eksplantlardan rejenerasyon olan fidelerde yaş ağırlıkları kontrole göre önemli olarak artmıştır ( $P<0.05$ ). Manyetik alanın klorofil miktarları üzerine etkileri belirlenmiş ve 2.2 sn süreyle manyetik alandan geçirilenlerde klorofil a, klorofil b ve toplam klorofil miktarlarında bir artış saptanmıştır.

**Anahtar sözcükler:** Soya, manyetik alan, sitokinin, oksin, rejenerasyon

## Introduction

Nowadays, the changes determined at the living systems which were exposed to different magnetic field strength and periods of magnetic field (MF) and electromagnetic field (EMF) with the lowest frequency have also been drawn the attention of the biologists, molecular biologists and chemists as physicists. For recent 30 years, studies have been done concerning the effects of magnetic field on microorganism, tissue, cell and subcellular structures of the plants and animals (Polk et al., 1995).

The researchers have shown that magnetic field changed the characteristics of cell membrane, effected the cell reproduction and caused some changes in cell metabolism. At the same time, it was put forward that magnetic field effected the growth characteristic and various cellular functions like mRNA quantity, gene expression, protein biosynthesis and enzyme activities and caused the changes concerning the various functions at the organ and tissue levels (Stein et al., 1992; Goodman et al., 1995).

It was also shown that MF effected the various characteristics of the plants like germination of seeds, root growth rate, seedling growth, reproduction and growth of the meristem cells and chlorophyll quantities. In addition to, there were magnetic field studies done with yield and yield parameters of crops like cereal, sunflower and soybean. In these studies the crop yield were increased (Pittman, 1972; Gubbels, 1982; Vakharia et al., 1991; Pietruszowski, 1993; Namba et al., 1995; Atak et al., 1997; Özalpan et al., 1999; Yurttaş et al., 1999; Reina et al., 2001; Oldaçay, 2002).

While the studies are going on the subject of interaction of magnetic field with the living systems, some important unsolved problems have been seen. In magnetic field studies, it has been known that magnetic field effect never increased linearly with the increase of strength and period of exposed to magnetic field. Consequently, a simple dose-response relationship has not been found for magnetic field frequency, intensity or duration of exposure. Also, some uncertainties have been seen during the interaction of the living systems with magnetic field and at the beginning of the magnetic field effect mechanism. So, the studies regarding this subject have been carried on (Rafferty et al., 1992; Goodman et al., 1995).

It is important to carry on the determination of the

biological effects formed in plants by magnetic field studies *in vitro* conditions. In the present study, we investigated the effects of magnetic field on the regeneration of soybean (*Glycine max* L. Merrill) shoot tip cultures.

## Material and Methods

### *Plant material*

The J 357 soybean seeds used for this research provided from Samsun Blacksea Agriculture Research Institute. 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 having 0.8% agar.

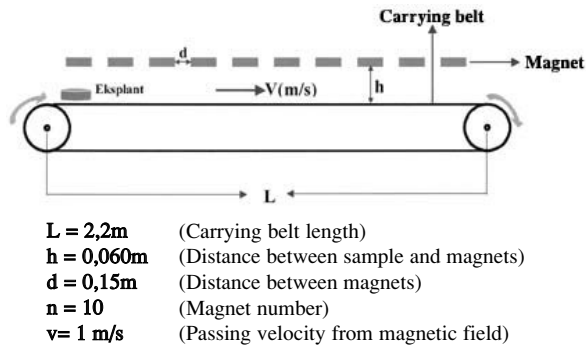
### *Plant tissue culture*

The soybean explants were prepared from young seedlings (5 day-old). The explants were incubated on the medium consisted of inorganic nutrients as Gamborg's medium and vitamins, plus 30 g/l sucrose, 0.8% agar, 40 mg/l adenine sulfate, 0.1 g/l glutamine (6.8 mM) and 0.1 mg/l 2,4-D (Wright et al., 1987; Atak et al., 1994; 1999).

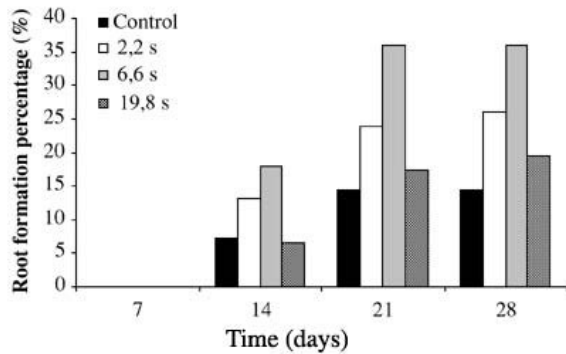
Explants were exposed to magnetic field in 15x100 mm petri dishes. After the exposed of magnetic field, explants were immediately transferred to fresh medium. In these experiments, each treatment had 3 parallels and 15 explants were employed for each parallel. Explants for shoot tip cultures were incubated on the medium. At this culture on the 28<sup>th</sup> day the fresh weights of regenerated plants were determined. We applied Duncan's multiple range test as the method to compare the fresh weight of the groups exposed to magnetic field and control.

### *Magnetic field experiment*

At the magnetic field experiment, we used 10 magnets of 0.45x0.065x0.022 m dimensions (Figure 1). In JINR Laboratories, these magnets prepared by magnetic field group were mounted onto the belt system which rotated with a rate of 1 m/second in our laboratories. The height of the magnets from belt



**Figure 1:** Magnetic field system plan formed at laboratory.

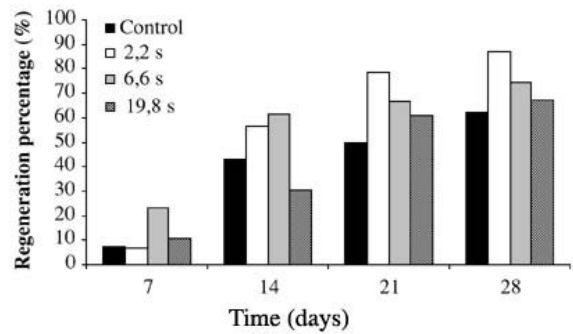


**Figure 3:** The root formation of the explants exposed to 2.9-4.6 mT magnetic field at various periods in accordance with days.



**Figure 5:** Shoot and root development of the explants exposed to MF with control and 6.6 second period.

system was 0.060 m. Explants have been passed at 2.9-4.6 mT magnetic flux density 1, 3 and 9 times at 2.2, 6.6 and 19.8 seconds.



**Figure 2:** The regeneration of the explants exposed to 2.9-4.6 mT magnetic field at various periods in accordance with days.



**Figure 4:** Regeneration of explants exposed to MF at various periods.

*Chlorophyll content*

Chloroplasts were extracted from the leaves of 28-day-plants. An extraction of leaf pigments was done with 80% acetone and the absorbance was measured at 663 and 645 nm with UV-160 Schmadzu spectrophotometer. Chlorophyll a, chlorophyll b and total chlorophyll quantities were calculated in accordance with Arnon method (Arnon, 1949).

**Table 1:** Shoot and root regeneration percentages and fresh weights formed on 28<sup>th</sup> day the shoot tip explants exposed to 2.9-4.6 mT magnetic field at various periods.

Periods exposed to 2.9-4.6 mT MF (second)	Shoot Regeneration (%)	Root Regeneration (%)	Average Fresh Weight (g)
Control	61.90	14.29	0.088b*
2.2	86.96	26.08	0.111ab
6.6	74.36	35.90	0.153a
19.8	67.39	19.57	0.076bc

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

**Table 2:** Chlorophyll a, chlorophyll b and total chlorophyll contents (mg/g fresh weight) on the 28<sup>th</sup> day of the regenerated plants from soybean shoot tip explants exposed to 2.9-4.6 mT magnetic field at various periods.

Periods exposed to 2.9-4.6 mT MF (sec)	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Control	0.0183 ± 0	0.0056 ± 0.00006	0.0239 ± 0.00006
2.2	0.0210 ± 0.00006	0.00664 ± 0.0001	0.0277 ± 0.00003
6.6	0.0148 ± 0.00003	0.00523 ± 0.00004	0.0199 ± 0.00003
19.8	0.0106 ± 0.0001	0.00445 ± 0.00007	0.151 ± 0.00009

## Results

Regeneration (shoot and root formation) and fresh weights of plants of the soybean shoot tip cultures exposed to 2.9-4.6 mT magnetic field at various periods and fresh weights have been shown at Table 1. The change of shoot and root formation percentages in accordance with days from explants exposed to control and magnetic field strength at various periods has been shown at Figure 2 and 3.

The shoot formation from explants which were exposed to magnetic field and control was observed starting from 5<sup>th</sup> to 28<sup>th</sup> day. On the 28<sup>th</sup> day, the regeneration percentages of magnetic explant treatment were higher than control.

While the regeneration percentages of the explant on the 28<sup>th</sup> day was 61.9 in control group, at the explants passed through magnetic field with 2.2 and 6.6 seconds were 86.96 and 74.36 respectively. After shoot regeneration, the root formation from explants which were exposed to magnetic field and control was observed from 11<sup>th</sup> day to 28<sup>th</sup> day.

Root formation percentages were higher than control at all the treatments of the magnetic field from 14<sup>th</sup> to 28<sup>th</sup> day. While root formation was 14.29% in control group on the 28<sup>th</sup> day, as the explants passed through magnetic field with 2.2 and 6.6 seconds

period, this rate increased to 26.08% and 35.90% (Table 1 and Figure 4, 5). The fresh weights of the regenerated plants have increased at the explants pass through magnetic field with 2.2 and 6.6 seconds period in accordance with control group.

The determined increase in average fresh weight exposed to magnetic field with 6.6 second was found significantly important ( $P < 0.05$ ), (Table 1).

With the aim of determination of the magnetic field on chlorophyll quantities, chlorophyll a, chlorophyll b and total chlorophyll contents have been determined. Chlorophyll a, chlorophyll b and total chlorophyll contents have increased 14%, 18% and 15% respectively in accordance with control groups at the lowest period (2.2 seconds) exposed to magnetic field (Table 2).

## Discussion

This research was begun with the aim of determination of the effects of the magnetic field on the soybean shoot tip explants. Shoot and root formation percentage was determined at the control and treated explants which were exposed to magnetic field various periods of 2.9-4.6 mT. Magnetic field increased the shoot and root regeneration rate in soybean organ

cultures. Shoot formation percentage of the explants exposed to magnetic field with 2.2 second period found higher in accordance with those which exposed to long period in this magnetic field flux density.

Investigations of low magnetic field effects on biological systems were shown that natural geomagnetic field (g.m.f) has an important role on the normal functions of the plant cells. At the magnetic screen (m.s.) conditions which g.m.f. was  $10^5$ - $10^6$  fold screening by a constant component, peas, lentil and flax seeds were grown. At this conditions, it was shown with experiments that the germination of the seeds decreased and the seedling growth inhibited according to the g.m.f. Also, cell reproduction cycle was noted through lengthening of the G<sub>1</sub> phase in these plants, and RNA and protein synthesis were affected (Govorun et al., 1992; Fomicheva et al., 1992a; 1992b).

Belyavskaya (2001) has determined that phytoferritin in plastids of peas root meristem cells at magnetic screen conditions has decreased.

By the magnetic field experiments with plants, it has been shown that magnetic field has effected the seed germination, the root elongation and seedling growth (Vakharia et al., 1991; Namba et al., 1995; Atak et al., 1997; Özalpan et al., 1999; Muraji et al., 2000; Atak et al., 2000; Oldaçay, 2002).

Iimoto et al. (1996) showed that a magnetic flux density of 4 mT had beneficial effect on the growth and enhancement of CO<sub>2</sub> uptake of potato plantlets *in vitro*.

During our research which are carried out *in vitro* conditions, at the leaves belonging to regenerated soybean shoots, chlorophyll a, chlorophyll b and total chlorophyll quantities have been determined.

Regenerated shoots and chlorophyll contents were increase in those which were passed through MF with 2.2 second period. Tian et al. (1991) used magnetic field in his investigations. When he was irrigating rice plants with magnetic water, leaf chlorophyll content was increased. Atak et al. (2000) have determined that magnetic field of 3.8-4.8 mT increased the chlorophyll content in *Paulownia* species according to control. The determined increases at chlorophyll a, chlorophyll b and total chlorophyll contents especially appeared in the expose to magnetic field for a short time. Furthermore, chlorophyll content has been investigated in sunflower plants exposed to magnetic field of 3.8-4.8 mT. While Nantio variety of sunflower chlorophyll quantities were increased, AS 508 variety has not shown any effect on chlorophyll quantities according to control (Oldaçay, 2002).

Our results show that fresh weight of regenerated plants from the explants exposed to magnetic field with 6.6 second period was higher according to the control. This increase in the fresh weight has been found significant ( $P < 0,05$ ). At the explants where shoot regeneration was seen, the highest root formation percentage has been seen at soybean explants which were passed through the magnetic field with 6.6 second period.

Liu et al. (1998), before the adventitious root formation *in vitro* soybean cultures, have determined an increase at the fresh weights of the hypocotyl explants. Liu et al. (1996) have told that adventitious roots formation have been stimulated with auxin. During the induction of adventitious roots, levels of IAA increased three days before the appearance of new adventitious roots, indicating that the biochemical changes in the processes of rooting caused by exogenous NAA might occur one day after NAA treatment. The increase in internal IAA level, has provided higher adventive roots. George (1996) reported that an increase was seen at the rooted at *Prunus cerasifera* shoots exposed to EMF and root lengths and fresh weights were increased with EMF .

It has been known that EMF exposure altered key cellular processes such as gene transcription (Phillips et al., 1993). Phillips et al. (1992) have reported the results of studies in CEM-CM 3 T-Lymphoblastoid cells exposed to MF for 1 gauss and 15-120 minutes. It has been showed to alter specific gene transcription in a time dependent and cell density dependent. Magnetic field-induced change in specific gene transcription may play an important role in altering cellular processes. Zhou et al. (2002) investigated the effects on expression of genes relating to cytokinin receptors in HL60 cells exposed to MF at flux density 0.1 or 0.8 mT for durations up to 72 h. MF exposure increased mRNA expression in HL 60 cells.

Stange et al. (2002) have emphasized that 50-60 Hz and 10-100 mT magnetic field has caused some changes at the permeability of plasma membrane at *Vicia faba* tip cell. The seedling root cells exposed to magnetic field can significantly increased <sup>3</sup>H- alanine uptake into the roots of increase plants compared to control plants.

In plant tissue cultures, both auxin and cytokinin are required to induce cell division and growth. Cytokinins play an important role on chloroplast development, shoot formation, axillary bud growth, and induction of number of genes involved in

chloroplast development nutrient metabolism. Auxins have been used in order to induce the rooting on the produced shoots. Apart from this, they also increased the growth of shoot tip explants at the beginning. Cell division has been arranged the interaction of auxin and cytokinins together. Each of these have been effecting the different phases of the cell cycle. While auxins effect on DNA replication, cytokinins are effective on some events causing mitosis. Cell can not enter mitosis when cytokinin are not available (George, 1996; Coenen et al., 1997; Kubo et al., 2000).

In summary, shoot regeneration and chloroplast rate were increased at the explants exposed to magnetic field with 2.2 second period and root formation rate and fresh weight were also increased at the explants exposed to magnetic field with 6.6 second period in accordance with control. As a consequence, the increase in cytokinin and auxin synthesis may be induced by MF and *in vitro* conditions this plant's characteristics have been differently affected by MF exposure of duration.

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