

## The effect of magnetic field strength on shoot regeneration and *Agrobacterium tumefaciens*-mediated gene transfer in flax (*Linum usitatissimum* L.)

MURAT AYCAN<sup>1</sup>, RAMAZAN BEYAZ<sup>2</sup>, ANZEL BAHADIR<sup>3</sup>, MUSTAFA YILDIZ<sup>4\*</sup>

<sup>1</sup>Department of Field Crops, Graduate School of Natural and Applied Sciences, Ankara University, Diskapi, Ankara, Turkey

<sup>2</sup>Department of Soil and Plant Nutrition, Faculty of Agriculture, Ahi Evran University, Bağbaşı, Kırşehir, Turkey

<sup>3</sup>Department of Biophysics, Faculty of Medicine, Duzce University, Konuralp, Duzce, Turkey

<sup>4</sup>Department of Field Crops, Faculty of Agriculture, Ankara University, Diskapi, Ankara, Turkey

\*Corresponding author: [myildiz@ankara.edu.tr](mailto:myildiz@ankara.edu.tr)

**Citation:** Aycan M., Beyaz R., Bahadir A., Yildiz M. (2019): The effect of magnetic field strength on shoot regeneration and *Agrobacterium tumefaciens*-mediated gene transfer in flax (*Linum usitatissimum* L.). Czech J. Genet. Plant Breed., 55: 20–27.

**Abstract:** This study was conducted to determine the effects of magnetic field (MF) strength on shoot regeneration and *Agrobacterium tumefaciens*-mediated gene transfer in flax (*Linum usitatissimum* L.). Seeds of flax cv. Madaras were exposed to different MF strengths (0 – control, 75, 150, and 300 millitesla (mT)) for 24 h by using an electromagnetic generator system fabricated in laboratory conditions. After sterilization, seeds were germinated on MS (Murashige and Skoog) medium in Magenta vessels. Hypocotyl explants excised from 7-days-old seedlings were used for regeneration. GV2260 strain of *Agrobacterium tumefaciens* was used in transformation studies. Inoculated hypocotyls were cultured on MS medium containing 1 mg/l BAP (6-benzylaminopurine) and 0.02 mg/l NAA (naphthaleneacetic acid) for 2 days by co-cultivation. Then, they were transferred to MS medium containing the same growth regulators, 100 mg/l kanamycin and 500 mg/l Duocid for selection. The presence of the *nptII* gene was verified by PCR (polymerase chain reaction) analysis in putative transgenic plants. The highest results with respect to shoot regeneration and transformation frequency were obtained from treatments of 75 mT MF strength.

**Keywords:** *Agrobacterium tumefaciens*; *Linum usitatissimum*; magnetic field; regeneration efficiency; transformation

*Agrobacterium*-mediated transformation has been widely used for the introduction of foreign genes into plants and consequent regeneration of transgenic plants (HOOYKAAS & SCHILPEROORT 1992). This bacterium naturally infects the wound sites in dicotyledonous plants and induces diseases known as crown gall (NESTER *et al.* 1984). The production of transformed flax plants from transformed callus is very low. In particular, after inoculation with *Agrobacterium*, the regeneration capacity of explants is somehow decreased dramatically. Possible explanations for this phenomenon are: plant cells may

perceive *Agrobacterium* infection as a pathogenic attack, and the inoculation process may influence plant regeneration negatively (JORDAN & MC HUGHEN 1988; HEBERLE-BORS *et al.* 1990; YILDIZ *et al.* 2002).

It was reported that seed germination and plant development within previous studies were increased by exposing seeds of the various plant species to different magnetic field (MF) strengths (SOLTANI *et al.* 2006; FLOREZ *et al.* 2007; CARBONELL *et al.* 2008). The MF increased seed germination by faster water assimilation and higher photosynthesis (PODLESNY *et al.* 2004). Also, YINAN *et al.* (2005) reported that

<https://doi.org/10.17221/195/2017-CJGPB>

the MF pretreatment had a positive effect on cucumber seedlings by stimulating seedling growth and development. Furthermore, DAYAL and SINGH (1986) exposed tomato seeds to different MF strengths varying from 15 to 155 millitesla (mT) for different exposure times, and they noted an increase in height and number of primary branches in treated plants compared to controls. Besides, FLOREZ *et al.* (2004) also observed an increase in the initial growth stages and an early sprouting of rice seeds when exposed to 125 and 250 mT MFs.

This study was conducted to evaluate the effects of different MF strengths on shoot regeneration and transformation frequencies by *Agrobacterium tumefaciens* in flax (*Linum usitatissimum* L.).

## MATERIAL AND METHODS

**Plant material.** Flax (*Linum usitatissimum* L., cv. Madaras) seeds were obtained from Northern Crop Science Laboratories in North Dakota, USA. Flax seeds were exposed to different MF strengths (0 – control, 75, 150, and 300 mT) for 24 h and then they were surface sterilized with 40% commercial bleach containing 5% sodium hypochlorite at 10°C for 20 min with continuous stirring and then were washed three times with sterile distilled water at the same temperature according to the protocol described by YILDIZ and ER (2002). Sterilized seeds were germinated on a basal medium containing the mineral salts and vitamins of Murashige and Skoog (MS) (MURASHIGE & SKOOG 1962), 3% (w/v) sucrose and 0.7% (w/v) agar.

**Magnetic field generation.** The magnetic field system was integrated with an electromagnet consisting of two Helmholtz coils of copper wire (cross-sections: 0.5 mm<sup>2</sup>) in each, mounted on a wooden frame. The pole pieces were cylindrical in shape with a diameter of 9 cm and a length of 8 cm. The number of turns per coil was 3000 and the coil resistance was 16 Ω. The induced mean MF in the centre of the coils could range from 50 to 500 mT. Each of the coils was located in a horizontal position. These coils are connected to a tunable power supply (0–12 A, ref. 13506-93, PHYWE, Germany) to produce a homogeneous MF in the horizontal direction in the central area near the axis of the coils. Additionally, an ampere meter is used to measure the current intensity through the coils, which is proportional to the applied MF strength. The coil nuclei are confronted and separated by a distance of about 12 cm

to place the GD anode between them, both coils are connected in parallel. The accuracy and uniformity of these magnetic field strengths produced in the middle of the gap between the nuclei (inside the GD anode) were measured by using a digital teslameter (ref. 13610-93, PHYWE) combined with a tangential flat-electrode Hall probe (ref. 13610-02, PHYWE). The probe was fitted with connecting cable and diode plug to the teslameter and its dimensions were 1.2 × 4 × 70 mm. Also, an electrolytic capacitor (22 000 μF, ref. 06211-00, PHYWE) was connected parallelly to the power supply to minimize instabilities.

**Magnetic field treatment.** The flax seeds were exposed to three different MF strengths containing 0 (control), 75, 150, and 300 mT produced in the middle of the gap between the coil nuclei by using an electromagnetic generator system fabricated in laboratory conditions. One hundred visibly sound, mature and healthy seeds held in the plastic container were located in the region within the coils of the electromagnet under a homogeneous MF and treated for 24 h. Static continuous MF between the poles of the coils was measured as 75, 150, and 300 mT with a digital teslameter. Moreover, the control samples were kept far enough (at least 30 cm away from each other) from the MF-producing device to avoid any potential exposure to the magnetic field. The differently MF-treated flax variants were compared with untreated flax seeds (control group) in the same growing conditions. The local geomagnetic field in the laboratory was less than 60 μT and the direction of the field was north to south. In all experiments, temperature levels were monitored and controlled by using a temperature control unit. The temperature during the course of seed exposure was kept at 25 ± 0.5°C. All treatments in the experiments were run simultaneously along with the control under similar conditions. The same procedure was applied to the control group except for the MF exposure.

**Culture conditions.** All cultures were incubated at 25 ± 1°C under cool white fluorescent light (27 μmol per m<sup>2</sup>/s) with a 16 h light/8 h dark photoperiod. The pH of the medium was adjusted to 5.8 prior to autoclaving.

**Tissue culture response.** The hypocotyl sections (20 explants per Petri dish) of the 7-days-old flax seedlings were cultured on MS medium containing 1 mg/l BAP (6-benzylaminopurine) and 0.02 mg/l NAA (naphthaleneacetic acid) in a Petri dish at 1.0 × 1.0 cm distances for 4 weeks. After four weeks, culture initiation, regeneration percentage, number

of shoots per explant, the highest shoot length per explant, total shoot number per Petri dish, number of rooted explants transferred to the soil, number of plantlets growing in soil and length of each plantlet were recorded to determine the effect of different MF strengths on the tissue culture response of flax hypocotyls.

***Agrobacterium tumefaciens* strain.** *Agrobacterium tumefaciens* strain GV2260 harbouring the plasmid p35S GUS-INT was used for inoculation. The binary plasmid p35S GUS-INT contains *neomycin phosphotransferase II (nptII)* gene driven by nopaline synthase (NOS) promoter and  $\beta$ -glucuronidase (*GUS*) gene controlled by *cauliflower mosaic virus* (CaMV) 35S promoter. A single colony of *A. tumefaciens* strain GV2260 was grown overnight in a liquid NB (Nutrient Broth) medium containing 50 mg/l kanamycin and 50 mg/l rifampicin at 28°C in a rotary shaker (180 rpm) ( $OD_{600nm} = 0.6$ ). Then, 100  $\mu$ l of this culture was added to 10 ml NB containing antibiotics and incubated overnight at 28°C in a rotary shaker (180 rpm) ( $OD_{600nm} = 0.6$ ).

**Transformation procedure.** *A. tumefaciens* strain GV2260 was grown overnight and diluted with a liquid NB medium to  $1 \times 10^8$  cells/ml. Hypocotyl explants, 0.5 cm in length, excised from 7-days-old sterile seedlings were inoculated in a liquid regeneration MS medium containing 1 mg/l BAP, 0.02 mg/l NAA. After inoculation, hypocotyl explants were transferred to solid MS medium containing 1 mg/l BAP and 0.02 mg/l NAA for co-cultivation for 2 days in culture room at a temperature of  $24 \pm 1^\circ\text{C}$ . Explants were then transferred to the medium which had the same content as that for co-cultivation, supplemented with 100 mg/l kanamycin and 500 mg/l Duocid for selection during 4 weeks.

**Rooting of shoots and recovery of transgenic plants.** Regenerated shoots were then transferred to a rooting medium containing 3 mg/l indole-butyric acid (IBA) and 100 mg/l kanamycin in Magenta vessels (60  $\times$  60 mm) with three replications (clonally propagated regenerants) and incubated at  $24 \pm 1^\circ\text{C}$  for 3 weeks to induce root formation. Plantlets having roots were then transferred to pots with three replications again (plantlets from each Magenta vessel were transferred to pots) in a growth room for 3 weeks where light (27  $\mu\text{mol}/\text{m}^2/\text{s}$ ), temperature ( $24 \pm 1^\circ\text{C}$ ) and humidity were controlled. Humidity was decreased gradually from 100% to 40% during three weeks.

**Genomic DNA extraction and polymerase chain reaction (PCR).** The confirmation of gene transfer

was performed by PCR method. According to DELLA-PORTA *et al.* (1983) total DNA was isolated from 100 mg of fresh tissue of leaves from plants grown in pots as described in GeneJET Plant Genomic DNA purification mini kit (No. K0791; Thermo Fisher Scientific, Lithuania) for each MF strength. The percentage of the transgene was determined by PCR amplification and agarose gel electrophoresis. The PCR primers were chosen to amplify the coding sequence of the transgenes: 5'-TTGCTCCTGCCGAGA-AAG-3' and 5'-GAAGGCGATAGAAGGCGA-3' for a 0.46 kb portion of the *nptII* gene.

Verification of the putative transgenic plants was checked with the *chv* gene of *Agrobacterium* genomic DNA. The existence of the *chv* virulence gene included in the chromosome of *A. tumefaciens* was investigated by means of primers, using the protocol, reported by YANG *et al.* (2013) to be available in the samples taken during the PCR reaction. If the band belonging to the *chv* virulence gene was not seen in gel, it was understood that there was no bacterial contamination. *chv* control PCR primers were chosen to amplify the coding sequence: 5'-CGAAC-CGCTGTTCGGCCTGTGG-3' and 5'-GTTTCAG-GCCGGCGGCATCCTGG-3' for a 0.85 kb portion of the *chv* gene.

PCR reaction mixtures were prepared using 100 ng/ $\mu$ l DNA, 2 mmol  $\text{MgCl}_2$ , 0.25 mmol dNTP, 0.5 pmol forward (sense) primer, 0.5 pmol reverse (anti-sense) primers and 0.625 U *Taq* DNA Polymerase (No. EP0402; Thermo Fisher Scientific, USA). Reactions were incubated in a programmable thermocycler (Techne-Prime Thermal Cycler) for 35 cycles. Each PCR cycle consisted of denaturation at 95°C for 1 min, annealing at 58°C (*nptII* gene), 62°C (*chv* gene) for 30 s, and extension at 72°C for 1 min. The PCR products were separated by electrophoresis in 1.5% agarose in TAE (tris-acetate EDTA) buffer followed by ethidium bromide staining. The bands were observed under UV after electrophoresis.

**Statistical analysis.** Three replications were tested. Petri dishes (100  $\times$  10 mm) containing 20 explants, Magenta vessels containing 20 plantlets and pots containing 20 plans were considered the units of replication. One-way analysis of variance (ANOVA) was used to test the effect of different MF strengths on shoot regeneration from flax hypocotyls and *A. tumefaciens*-mediated transformation. All experiments were repeated twice. Data were statistically analysed by IBM SPSS Statistics 22 computer program. Duncan's multiple range test was used to

<https://doi.org/10.17221/195/2017-CJGPB>

compare the means. Data presented in percentages were subjected to arcsine ( $\sqrt{X}$ ) transformation before statistical analysis (SNEDECOR & COCHRAN 1989).

## RESULTS AND DISCUSSION

It is quite difficult to assess the effects of electromagnet (EM) or MFs the organisms are exposed to. The main reason for this is the complex structure of biological systems. Many experiments with the effects of electrical or MFs on both complex structured and simple structured organisms have been conducted. Since most of the biological structures are non-homogeneous, when they are exposed to electrical or MFs, they show important cellular changes. This situation can be explained by the examination of the responses of molecules, ions, and membranes under electrical and MFs. The cell level effects of electrical and MFs can be listed as membrane changes, ionic effects ( $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ), nucleic acid and gene expression, enzymatic activity, biorhythms, and hormones, genotoxic effects (GOODMAN *et al.* 1995; SIMKÓ 2004; FUNK *et al.* 2009).

Several theories have been proposed, associated with biochemical changes due to the radical pair mechanism, ion cyclotron resonance mechanisms and ferrimagnetism or enzyme activity (GALLAND & PAZUR 2005). However, there is not yet a sufficient explanation for how exactly MF affects biological systems containing plants during dormancy and seed germination (HARRIS *et al.* 2009).

Alternatively, electrical and MFs may activate naturally occurring ion channels within cell membranes, causing an influx of ions and increasing turgor through osmoregulation (REINA & PASCUAL 2001). This theory is plausible because seed germination and early growth are closely linked to cellular water and solute regulation (WELBAUM *et al.* 1998).

One hypothesis suggests that MF interacts with ionic current in the plant embryo cell membrane and this interaction induces changes in both osmotic pressure and ionic concentrations on both sides of the membrane (REINA & PASCUAL 2001). Several researchers reported that in an array of MF at the level of  $10^{-3}$ – $10^{-2}$  T, intermediate reactions affect the chemical reactions by influencing the electron-spin positions, demonstrating the presence of a potential for biological consequences (BELYAVSKAYA *et al.* 1992). In studies at the cell level, RNA and protein synthesis is influenced by the changes in strengths of MF in G1 phase, and the rate of cell division increased in cells exposed to MF (NEGISHI *et al.* 1999; ATAK *et al.* 2003).

Although there are many reports on the effects of MF on seed germination, plant growth, protein biosynthesis, seedling elongation and root development (SAVOSTIN 1930; DAYAL & SINGH 1986; FLOREZ *et al.* 2004, 2007; PODLESNY *et al.* 2004; YINAN *et al.* 2005; SOLTANI *et al.* 2006; CARBONELL *et al.* 2008), to our knowledge, the effects of MF on *in vitro* shoot regeneration capacity and *A. tumefaciens*-mediated gene transfer in flax (*Linum usitatissimum* L.) have not been reported previously.

**Effect of magnetic field strength on shoot regeneration capacity of flax hypocotyl explants.** According to the results, there were statistically significant differences between control (0 mT) and different MF treatments (Table 1). The highest regeneration percentages were obtained at 75 and 150 mT MF strengths as 84.00% and 85.00% of hypocotyl explant regenerated shoots, respectively (Table 1 and Figure 1). The highest results in the number of shoots per explant, the highest shoot length per explant, total shoot number per Petri dish, the number of rooted explants transferred to the soil and the number of plantlets growing in soil were recorded at 75 mT

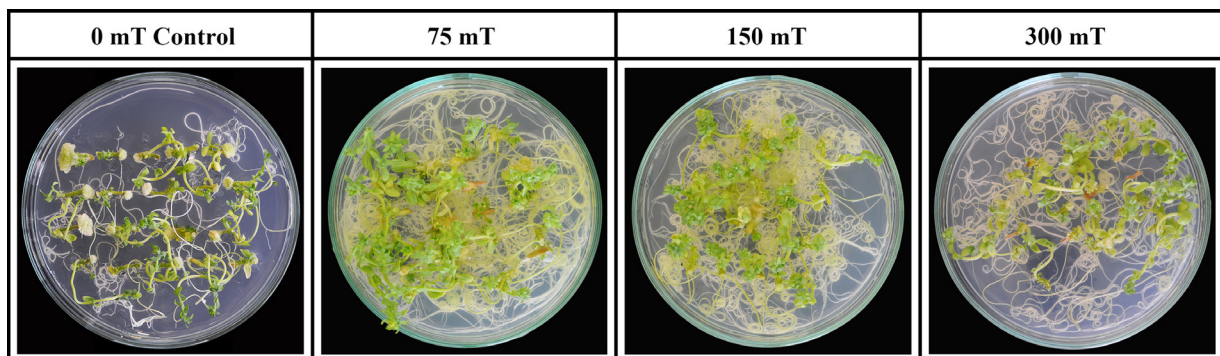


Figure 1. The effects of different magnetic field strengths (millitesla, mT) on the regeneration from flax hypocotyl explants

Table 1. The effect of different magnetic field strengths on shoot regeneration from hypocotyl explants of flax

Magnetic field strength (mT)	Regeneration (%)	Shoot No. per explant	The highest shoot length per explant (cm)	Total shoot No. per Petri dish	The No. of rooted explant transferred to soil	The No. of plantlet growing in soil	Length of plantlet (cm)
0 (control)	62.00 <sup>b</sup>	1.54 <sup>b</sup>	2.62 <sup>b</sup>	19.20 <sup>c</sup>	11.80 <sup>b</sup>	3.40 <sup>b</sup>	6.00 <sup>c</sup>
75	84.00 <sup>a</sup>	2.24 <sup>a</sup>	3.36 <sup>a</sup>	37.63 <sup>a</sup>	26.80 <sup>a</sup>	21.20 <sup>a</sup>	7.20 <sup>b</sup>
150	85.00 <sup>a</sup>	2.00 <sup>a</sup>	3.08 <sup>a</sup>	34.20 <sup>b</sup>	26.00 <sup>a</sup>	20.60 <sup>a</sup>	8.70 <sup>a</sup>
300	82.00 <sup>a</sup>	2.00 <sup>a</sup>	2.96 <sup>a</sup>	33.00 <sup>b</sup>	26.40 <sup>a</sup>	20.10 <sup>a</sup>	7.80 <sup>b</sup>

Values within a column followed by different letters are significantly different at the 0.01 level; each value is the mean of 3 replications containing 20 explants per replication; mT – millitesla

Table 2. The effect of different magnetic field strengths on *Agrobacterium tumefaciens*-mediated gene transfer in flax

Magnetic field strength (mT)	Regeneration (%)	Shoot No. per explant	The highest shoot length per explant (cm)	Total shoot No. per Petri dish	The No. of rooted explants transferred to soil	The No. of plants growing in soil <sup>1</sup>	The No. of PCR positive plants <sup>2</sup>	Transformation efficiency (%) (2/1) × 100	Increase in transformation efficiency according to control (%)
0 (control)	41.25 <sup>b</sup>	1.17 <sup>c</sup>	1.24 <sup>c</sup>	9.53 <sup>d</sup>	8.70 <sup>b</sup>	1.86 <sup>d</sup>	0.00 <sup>d</sup>	0.00	
75	82.00 <sup>a</sup>	2.40 <sup>a</sup>	3.40 <sup>a</sup>	39.40 <sup>a</sup>	16.40 <sup>a</sup>	12.60 <sup>a</sup>	8.00 <sup>a</sup>	63.00	63.00
150	78.00 <sup>a</sup>	2.36 <sup>a</sup>	2.76 <sup>b</sup>	36.80 <sup>b</sup>	15.60 <sup>a</sup>	8.40 <sup>b</sup>	5.00 <sup>b</sup>	59.00	
300	46.00 <sup>b</sup>	1.70 <sup>b</sup>	2.54 <sup>b</sup>	15.60 <sup>c</sup>	9.20 <sup>b</sup>	5.50 <sup>c</sup>	3.00 <sup>c</sup>	54.00	

Values within a column followed by different letters are significantly different at the 0.01 level; each value is the mean of 3 replications containing 20 explants per replication; mT – millitesla

<https://doi.org/10.17221/195/2017-CJGPB>

MF strength as 2.24, 3.36, 37.63, 26.80, and 21.20, respectively. The lowest values in all characters were obtained from control (0 mT) where no MF strength was used. From the results, it could be concluded that 75 mT MF strength gave rise to the highest values. Shoot number per explant and total shoot number per Petri dish are the best indicators showing the tissue culture response. In the present study, the number of shoots per explant and total shoot number per Petri dish were recorded as 1.54 and 19.20 in control (0 mT) treatment while they were 2.24 and 37.63 in the above-mentioned treatment, respectively (Table 1). It is well known that regeneration capacity of the tissue decreases significantly in gene transformation studies using *A. tumefaciens* due to the plant defence mechanism against a pathogenic attack. That is why a higher shoot regeneration frequency is a prerequisite for the success in transformation studies. An increase in the shoot regeneration frequency at 75 mT MF strength could be attributed to the higher mobilization of nutrients and growth regulators in the tissue. Higher results at 75 mT MF strength could be due to the higher hormone levels of the tissue as reported by OKUBO *et al.* (1991).

**Effect of magnetic field strength on *Agrobacterium tumefaciens*-mediated transformation of flax.** *A. tumefaciens*, a plant pathogen, is commonly used as a vector for gene transfer to plants (JOUBERT

*et al.* 2002). The success of genetic transformation via *A. tumefaciens* is limited in most of the plant species due to the fact that the plant defence mechanism will be active when the pathogen attacks. For this reason, manipulations of the plant, bacteria, and physical conditions have been applied to increase the virulence of bacteria and to increase the transformation efficiency (CHAKRABARTY *et al.* 2002). Pre-culturing explants before inoculation (CHAKRABARTY *et al.* 2002), modification of temperature (CHAKRABARTY *et al.* 2002; DE CLERCQ *et al.* 2002) and medium pH (DE CLERCQ *et al.* 2002), addition of chemicals to inoculation medium such as acetosyringone (CHAKRABARTY *et al.* 2002; DE CLERCQ *et al.* 2002; LOPEZ *et al.* 2004), changing bacterial density and co-cultivation period (LOPEZ *et al.* 2004) and vacuum infiltration (MAHMOUDIAN *et al.* 2002; SPOKEVICIUS *et al.* 2005) have been reported to increase transformation.

The highest shoot regeneration percentage on a selection medium containing 100 mg/l kanamycin and 500 mg/l Duocid was recorded 82.00% as the highest from 75 mT MF strength. It was 41.25% in control treatment in which no MF was used. Shoot number per explant was obtained from 75 mT MF strength as 2.40, while it was 1.17 in control treatment. The highest shoot length, total shoot number per Petri dish, the number of rooted explants transferred to the soil, the number of plants growing in soil and

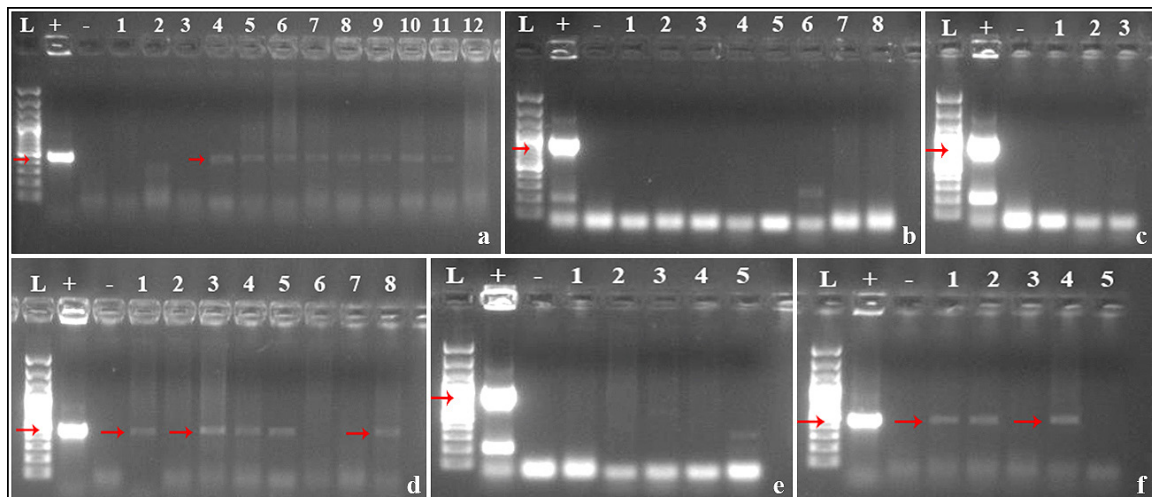


Figure 2. The effects of different magnetic field strengths on gene transfer efficiency in flax: PCR analysis to detect the *nptII* gene in putative transgenic plants exposed to 75 mT magnetic field strength (a), PCR analysis to detect the *chv* gene in putative transgenic plants exposed to 75 mT magnetic field strength (b), PCR analysis to detect the *chv* gene in putative transgenic plants exposed to 300 mT magnetic field strength (c), PCR analysis to detect the *nptII* gene in putative transgenic plants exposed to 150 mT magnetic field strength (d), PCR analysis to detect the *chv* gene in putative transgenic plants exposed to 150 mT magnetic field strength (e), PCR analysis to detect the *nptII* gene in putative transgenic plants exposed to 300 mT magnetic field strength (f)

the number of PCR positive plants were recorded as 39.40, 16.40, 12.60, and 8.00 from 75 mT MF strength, respectively. In control treatment, the lowest results were obtained for the highest shoot length per explant, total shoot number per Petri dish, the number of rooted explants transferred to soil, the number of plants growing in soil and the number of PCR positive plants as 1.24, 9.53, 8.70, 1.86, and 0.00, respectively (Table 2).

After 4-week cultivation on a selection medium, rooted explants were directly transferred to the soil by skipping an *in vitro* rooting stage. From 75 mT MF strength, on average 16.40 rooted explants were transferred to the soil and finally, 12.60 transgenic plants were grown in soil, reached maturity and all were morphologically normal. On the other hand, only 1.86 plants were grown from the control treatment. Out of 8.70 rooted explants transferred to the soil, only 1.86 putative transgenic plants were grown in soil in the control treatment where no MF was used (Table 2).

After PCR analysis, out of 12.60 plants, 8.00 plants were confirmed to be transgenic at 75 mT MF strength (transformation efficiency 63.00%) while none of the plants out of 1.86 was found transgenic in the control treatment. This meant 63.00% increase in transformation efficiency according to control (Table 2, Figure 2).

Results showed positive effects of magnetic field strength on regeneration and transformation at 75 mT MF strength treatment as compared to control treatment. At MF strengths over 75 mT, shoot regeneration and transformation were hindered significantly.

The results presented in this study clearly indicate that exposing explants to MF strength before culture improved the regeneration capacity and transformation frequency of flax significantly.

**Acknowledgements.** This investigation was supported by Scientific and Technological Research Council of Turkey (TUBİTAK); Grant No. 113O280 to Prof. Dr. M. YILDIZ

## References

- Atak C., Emiroglu O., Alikamanoglu S., Rzakoulive A. (2003): Stimulation of regeneration by magnetic field in soybean (*Glycine max* L. Merrill) tissue cultures. *Journal of Cell and Molecular Biology*, 2: 113–119.
- Belyavskaya N.A., Fomicheva V.M., Govorun R.D., Danilov V.I. (1992): Structural-functional organization of the meristem cells of pea, lentil and flax roots in conditions of screening the geomagnetic field. *Biophysics*, 37: 657–666.
- Carbonell M.V., Martinez E., Florez M., Maqueda R., Lopez-Pintor A., Amaya J.M. (2008): Magnetic field treatments improve germination and seedling growth in *Festuca arundinacea* Schreb. and *Lolium perenne* L. *Seed Science and Technology*, 36: 31–37.
- Chakrabarty R., Viswakarma N., Bhat S.R., Kirti P.B., Singh B.D., Chopra V.L. (2002): *Agrobacterium*-mediated transformation of cauliflower: optimization of protocol and development of Bt-transgenic cauliflower. *Journal of Biosciences*, 27: 495–502.
- Dayal S., Shing R.P. (1986): Effect of seed exposure to magnetic field on the height of tomato plants. *Indian Journal of Agricultural Science*, 56: 483–486.
- De Clercq J., Zambre M., Van Montagu M., Dillen W., Angenon G. (2002): An optimized *Agrobacterium*-mediated transformation procedure for *Phaseolus acutifolius* A. Gray. *Plant Cell Reports*, 21: 333–340.
- Florez M., Carbonell M.V., Martinez E. (2004): Early sprouting and first stages of growth of rice seeds exposed to a magnetic field. *Electromagnetics*, 23: 157–166.
- Florez M., Carbonell M.V., Martínez E. (2007): Exposure of maize seeds to stationary magnetic fields: effects on germination and early growth. *Environmental and Experimental Botany*, 59: 68–75.
- Funk R.H.W., Monsees T., Ozkucur N. (2009): Electromagnetic effects – from cell biology to medicine. *Progress in Histochemistry and Cytochemistry*, 43: 177–264.
- Galland P., Pazur A. (2005): Magnetoreception in plants. *Journal of Plant Research*, 118: 371–389.
- Goodman E.M., Greenebaum B., Marron M.T. (1995): Effects of electromagnetic fields on molecules and cells. *International Review of Cytology*, 158: 279–338.
- Harris S.R., Henbest K.B., Maeda K., Pannell J.R., Timmel C.R., Hore P.J., Okamoto H. (2009): Effect of magnetic fields on cryptochrome-dependent responses in *Arabidopsis thaliana*. *Journal of the Royal Society Interface*, 6: 1193–1205.
- Heberle-Bors E.F., Moreno R.M.B., Alwen A., Stoger E., Vicente O. (1990): Transformation of pollen. In: Nijkamp H.J.J., Van Der Plas L.H.W., Van Aartrijk J. (eds.): *Progress in Plant Cellular and Molecular Biology*. Kluwer Academic Publications: 244–251.
- Hooykaas P.J.J., Schilperoort R.A. (1992): *Agrobacterium* and plant genetic engineering. *Plant Molecular Biology*, 19: 15–38.
- Jordan M.C., Mc Hughen A. (1988): Glyphosate tolerant flax plants from *Agrobacterium*-mediated gene transfer. *Plant Cell Reports*, 7: 281–284.
- Joubert P., Beaupère D., Lelièvre P., Wadouachi A., Sangwan R.S., Sangwan-Norreel B.S. (2002): Effects of phenolic compounds on *Agrobacterium vir* genes and gene transfer

<https://doi.org/10.17221/195/2017-CJGPB>

- induction. A plausible molecular mechanism of phenol binding protein activation. *Plant Science*, 162: 733–743.
- Lopez S.J., Kumar R.R., Pius P.K., Muraleedharan N. (2004): *Agrobacterium tumefaciens* mediated genetic transformation in tea (*Camellia sinensis* (L.) O. Kuntze). *Plant Molecular Biology Reporter*, 22: 201–202.
- Mahmoudian M., Yucel M., Öktem H.A. (2002): Transformation of lentil (*Lens culinaris* M.) cotyledonary nodes by vacuum infiltration of *Agrobacterium tumefaciens*. *Plant Molecular Biology Reporter*, 20: 251–257.
- Murashige T., Skoog F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum*, 15: 473–479.
- Negishi Y., Hashimoto A., Tsushima M., Dobrota C., Yamashita M., Nakamura T. (1999): Growth of pea epicotyl in low magnetic field implication for space research. *Advances in Space Research*, 23: 2029–2032.
- Nester E.W., Amasino R., Akiyoshi D., Klee H., Montoya A., Gordon M.P. (1984): The molecular basis of plant cell transformation by *Agrobacterium tumefaciens*. *Basic Life Science*, 30: 815–822.
- Okubo H., Wada K., Uemoto S. (1991): *In vitro* morphogenetic response and distribution of endogenous plant hormones in hypocotyl segments of snapdragon (*Antirrhinum majus* L.). *Plant Cell Reports*, 10: 501–504.
- Podlesny J., Misiak L., Podlesna A. (2004): Concentration of free radicals in pea seeds after pre-sowing treatment with magnetic field. *International Agrophysics*, 18: 261–267.
- Reina F.G., Pascual L.A. (2001): Influence of a stationary magnetic field on water relations in lettuce seeds. Part I Theoretical considerations. *Bioelectromagnetics*, 22: 589–595.
- Savostin P.W. (1930): Magnetic growth relations in plant. *Planta*, 12: 327.
- Simkó M. (2004): Induction of cell activation processes by low frequency electromagnetic fields. *Scientific World Journal*, 4 (S2): 4–22.
- Snedecor G.W., Cochran W.G. (1989): *Statistical Methods*. 8<sup>th</sup> Ed., Ames, Iowa State University Press: 217–235.
- Soltani F., Kashi A., Arghavani M. (2006): Effect of magnetic field on *Asparagus officinalis* L. seed germination and seedling growth. *Seed Science and Technology*, 34: 349–353.
- Spokevicius A.V., Van Beveren K.S., Bossinger G. (2005): *Agrobacterium*-mediated *in vitro* transformation of wood-producing stem segments in eucalypts. *Plant Cell Reports*, 23: 617–624.
- Welbaum G.E., Bradford K.J., Yim K., Booth D.T., Oluoch M.O. (1998): Biophysical, physiological and biochemical processes regulating seed germination. *Seed Science Research*, 8: 161–172.
- Yang L., Wang C., Wang L., Xu C., Chen K. (2013): An efficient multiplex PCR assay for early detection of *Agrobacterium tumefaciens* in transgenic plant material. *Turkish Journal of Agriculture and Forestry*, 37: 157–162.
- Yinan L., Yuan L., Yongqing Y., Chunyang L. (2005): Effect of seed pretreatment by magnetic field on the sensitivity of cucumber (*Cucumis sativus*) seedlings to ultraviolet-B radiation. *Journal Environmental and Experimental Botany*, 54: 286–294.
- Yildiz M., Er C. (2002): The effect of sodium hypochlorite solutions on *in vitro* seedling growth and shoot regeneration of flax (*Linum usitatissimum*). *Naturwissenschaften*, 89: 259–261.
- Yildiz M., Özcan S., Er C. (2002): The effect of different explant sources on adventitious shoot regeneration in flax (*Linum usitatissimum* L.) *Turkish Journal of Biology*, 26: 37–40.

Received for publication December 12, 2017

Accepted after corrections May 9, 2018

Publish online June 13, 2018