

# Effect of Magnetic Field on InVitro Seedling Growth and Shoot Regeneration from Cotyledon Node Explants of Lathyrus chrysanthus Boiss

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The stimulatory effects on germination of seeds and growth of plants of static magnetic field (MF) pre-treatments depending on MF intensity, exposure time periods, signal form, flux density, and source frequencies on plants are reported. Seed germination frequency is low due to dormancy in Lathyrus chrysanthus Boiss. from Fabaceae family, consisting of 187 taxa. Tissue culture protocol for this plant has already been optimized. This plant is also used as a model for developing alternative methods to overcome dormancy. This study was conducted to determine the effects of MF on in vitro seed germination, seedling growth, and shoot regeneration capacity of cotyledon node explants in Lathyrus chrysanthus Boiss. to obtain healthy seedlings in large quantities. The seeds of an ecotype (Diyarbakir) were subjected to 125 mT MF strength for different exposure time periods (0-untreated, 24, 48, and 72 h). Sterilized seeds were germinated on growth basal medium in Magenta vessels. Seed germination and seedling growth percentages were recorded after 7 and 14 days of culture initiation, whereas seedling and root lengths were noted 28 days after culture initiation. At the end of the culture, shoot regeneration percentage, shoot number per explant, highest shoot height per explant, and total shoot number per petri dish were recorded. According to the results, it could be concluded that MF treatment could clearly be used to improve germination by breaking dormancy not only in Lathyrus chrysanthus Boiss. but also other plant species. Bioelectromagnetics. 39:547–555, 2018. © 2018 Wiley Periodicals, Inc.

Keywords: Lathyrus chrysanthus Boiss; magnetic field; germination; seedling growth; shoot regeneration

# INTRODUCTION

Physical treatments influence physiological and biochemical processes in seeds, thereby contributing to greater vegetative growth and improved crop yield and quality. Magnetic field (MF) pre-treatment is one of the physical treatments that have been reported to enhance the performance of various crops [El-Gizawy et al., 2016]. MF pre-treatments are being used in agriculture, as a new environmentally friendly technique, to improve the germination of seeds and increase crops' yield [Martinez et al., 2009]. This has brought a new interest in MF's role in regulating plant growth and development when seeds or explants are exposed to MF. However, the role of MFs and their influence on functioning of biological organisms are still insufficiently understood, and are actively studied [Belyavskaya, 2004].

The genus *Lathyrus*, which is in the *Fabaceae* (*Leguminosae*) family, is comprised of more than 200

taxa worldwide [Allkin et al., 1986]. The eastern Mediterranean region is the main center of diversity for the genus, which is less diversified in North and South America [Bässler, 1980; Kupicha, 1983; Kahraman et al., 2012]. In Turkey, 66 species and 76 taxa of *Lathyrus* have been identified [Davis, 1970; Davis, 1988; Günes and Özhatay, 2000]. Some *Lathyrus* taxa are economic and agricultural plants [Davis, 1988];

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this genus includes a range of grain, forage, pasture, and ornamental crops. *Lathyrus chrysanthus* is an ornamental plant with big, colored, and fragrant and non-fragrant flowers [Davis, 1970; Telci et al., 2011]. *Lathyrus chrysanthus* seeds are not cultivated extensively because they have low germination frequency [Beyaz et al., 2016]. Telci et al. [2011] have reported that dormancy in *Lathyrus chrysanthus* Boiss. seeds could be overcome in in vitro culture conditions to obtain rapid seed germination and healthy seedling growth in large quantities. It has also been reported that cotyledon nodes are the most suitable explant type in Lathyrus species [Malik et al., 1992; Barik et al., 2004].

Recently, the stimulative effects of MF strength on seed/tuber vigor and germination, seedling growth, and root development by overcoming dormancy have been reported. Many studies revealed that exposing seeds/tubers to an MF pre-treatment resulted in significant effects on germination/sprouting of seeds/ tubers in lentil (Lens culinaris Medik.), grass pea (Lathyrus sativus L.), chick pea (Cicer arietinum L.) [Vashisth and Nagarajan, 2008], sunflower (Helianthus annuus) [Vashisth and Nagarajan, 2010], maize (Zea mays L) [Vashisth and Nagarajan, 2007], rice (Oryza sativa L.), tomato (Solanum lycopersicum L.) [Florez et al., 2004; Torres et al., 2008; Martinez et al., 2009], soybean (Glycine max) [Shine et al., 2011], barley (Hordeum vulgare L.) [Martinez et al., 2000], and potato (Solanum tuberosum L.) [Yildiz et al., 2017]. However, the impact of MF on seed germination and seedling growth of Lathyrus chrysanthus has not been reported before, according to our knowledge. Thus, in this study, our aim was to evaluate the effects of different MF exposure time periods (0-untreated, 24, 48, and 72 h) at 125 mT MF strength on in vitro seed germination, seedling growth, shoot regeneration capacity of cotyledon node explants in Lathyrus chrysanthus Boiss., and dormancy breaking in in vitro conditions.

#### MATERIALS AND METHODS

# **Plant Material**

Lathyrus chrysanthus seeds of an ecotype (Diyarbakir) found in southeastern Turkey were used in the study.

#### **Magnetic Field Generation**

MF system was integrated with electromagnets formed by two Helmholtz coils of copper wire (cross-sections: 0.5 mm<sup>2</sup>), 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 3,000 and the resistance of the coil was 16  $\Omega$ . The induced mean MF in the center of the coils could range from 50 to 500 mT (Tesla). Each of the coils was located in a horizontal position. Those coils were connected to a tunable power supply (0-12 A, ref.13506–93, PHYWE Systeme, Gottingen, Germany) to produce a homogeneous MF in the horizontal direction in the central area near the axis of the coils. Additionally, an amperemeter ( $\sim 10$  A AC/DC, ref. EAK-P-3203, PeakTech, Ahrensburg, Germany) was used to measure the current intensity through the coils, which was proportional to the applied MF strength. The coil nuclei were confronted and separated by a distance of about 12 cm to place the gadolinium (GD) anode between them, and both coils were connected in parallel. The accuracy and uniformity of these MF 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 Systeme, Gottingen, Germany) combined with a tanflat-electrode Hall probe (ref.13610–02, gential PHYWE Systeme). The probe was fitted with connecting cable and diode plug to the teslameter and dimensions of it were  $1.2 \times 4 \times 70 \,\mathrm{mm}$ . Also, an capacitor (22,000 µF, ref. 06211–00, electrolytic PHYWE Systeme) was connected in parallel to the power supply to minimize instabilities. Seed exposure was done by placing seeds in the center zone of the coil system. Exposure time duration was controlled by an automatic timer coupled to the MF generator supply.

# **Magnetic Field Treatment**

In our study, a homogeneous static MF of 125 mT was used in accordance with previous studies in seeds of several plant species. These studies revealed that a static MF with a 125 mT value had a stimulatory effect on initial growth stages and increased the germination rate of several seeds including pea, barley, rice, and tomato [Martinez et al., 2000; Florez et al., 2004; Martinez et al., 2009; Carbonell et al., 2011; Maffei, 2014]. For this reason, Lathyrus chrysanthus seeds were exposed to MF strength 125 mT produced in the middle of the gap between the coil nuclei by using an electromagnetic generator system that was fabricated in a laboratory condition. One hundred visibly sound, mature, and healthy seeds held in a plastic container were placed in the area within the electromagnet's coils under a homogeneous MF treated for 24, 48, and 72 h. Static MF between the poles of the coils was measured as 125 mT with a digital teslameter. Moreover, the untreated samples were kept far enough (at least 30 cm away from each other) from the MF-producing coils, to avoid any potential exposure to the MF. The different MF-treated *Lathyrus chrysanthus* were compared to untreated *Lathyrus chrysanthus* seeds in the same growing conditions. The local geomagnetic field (GMF) in the laboratory was less than 60 µT and the direction of the field was north to south. Using the dip needle manual (ref. SF-8619, PASCO Scientific, Roseville, CA), we measured the directions of magnetic north and inclination I was 57 degrees. The direction of static MF intensity (125 mT) between Helmholtz coil pairs was defined by the coil axis which is the vertical direction to the local geomagnetic field in the geomagnetic plane (GP).

In all experiments, temperature levels were monitored and controlled by using a temperature control unit (CC25, Tekon, Bursa, Turkey). The variation in temperature during the course of seed exposure was  $25\pm1\,^{\circ}\text{C}$ . All treatments in the experiments were run simultaneously along with control under similar conditions. The same procedure was applied to the untreated group except for MF exposure.

# **Surface Sterilization and In Vitro Seed Germination**

Treated seeds with  $125\,\mathrm{mT}$  MF and untreated seeds were surface-sterilized using 75% commercial bleach (containing 5% sodium hypochlorite) at  $35\,^\circ\mathrm{C}$  for  $15\,\mathrm{min}$  with continuous stirring and were then rinsed three times with sterile distilled water (sdH<sub>2</sub>0) at the same temperature as reported in previous studies [Yildiz and Er, 2002; Telci et al., 2011; Beyaz et al., 2016]. Sterilized seeds were shaken in sdH<sub>2</sub>0 for 6 h to increase the permeability of seed coat and then were germinated on a basal medium of Murashige and Skoog's (MS) mineral salts and vitamins [Murashige and Skoog, 1962], 3% sucrose, and 0.7% agar in Magenta vessels  $(150\times150\,\mathrm{mm})$ .

#### **Explant Source**

Cotyledon node explants were excised from 28 day-old seedlings and cultured in a petri dish for 4 weeks on MS medium supplemented with 0.25 mg L<sup>-1</sup> 6-benzylaminopurine (BAP) and 0.05 mg L<sup>1</sup> naphthalene acetic acid (NAA) for regeneration. All values given in Table 2 are the mean of three petri dishes with 10 explants in each. Shoot regeneration percentage, shoot number per explant, the highest shoot height per explant, and total shoot number per petri dish were recorded 4 weeks later.

# **Culture Conditions**

All cultures were incubated in a growth chamber (PG34-4, DigiTech, Ankara, Turkey) at  $25 \pm 1$  °C under

cool white fluorescent light  $(27 \,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1})$  with a 16 h light/8 h dark photoperiod. The pH of the medium was adjusted to 5.8 prior to autoclaving.

Seed germination was noted at the end of the 7th day and seedling growth percentage was recorded at the 14th day after culture initiation, whereas seedling height and root length were noted on the 28th day just before explant excision. Cotyledon node explants were excised from 28 day-old seedlings.

# Statistical Analysis

The experimental design was a completely randomized design (CRD). In the study, four replicates for in vitro seed germination and seedling growth and three replicates for shoot regeneration were used. Magenta vessels  $(150 \times 150 \,\mathrm{mm})$  containing 25 seeds for seed germination and seedling growth, and petri dishes  $(100 \times 10 \text{ mm})$  containing 10 explants for shoot regeneration were considered to be the experimental unit. The study was repeated twice in order to verify the accuracy of the trials. Data were statistically analyzed by "Analysis of Variance" in IBM SPSS (Statistical Package for Social Studies) Statistics Version 22.0 (IBM, Chicago, IL) computer program. Values presented in percentages were subjected to arcsine  $(\sqrt{X})$  transformation before statistical analysis [Snedecor and Cochran, 1967].

# **RESULTS**

The effects of 125 mT MF strength for 24, 48, and 72 h on seed germination, seedling growth, seedling height, and root length are shown in Table 1 and Figure 1. The lowest values with respect to seedling growth, seedling height, and root length were determined in the untreated group (0 mT), but the worst result for seed germination percentage value was observed as 20% in 72 h MF exposure time. Only 20 seeds were germinated out of 100 seeds in 72 h MF exposure time. The highest values of seedling growth, seedling height, and root length were recorded at 24 h MF exposure time as 91%, 5 cm, and 7.60 cm, respectively, in our study. At this MF exposure time, seed germination percentage was 50%, although 91% of these seeds were grown out of 100 seeds. However, the highest seed germination percentage was 65% at 48 h MF exposure time which meant 65 seeds were germinated out of 100 seeds; only 81% of these seeds were grown. So, the stimulatory effect of 24h MF exposure time was observed in all measured parameters except for seed germination percentage. Thus, we determined that higher MF exposure time periods decreased seed germi-

TABLE 1. The effect of MF strength 125 mT for 0, 24, 48, and 72 h on in vitro seed germination and seedling growth in *Lathyrus chrysanthus* Boiss.

Day 7		Day 14		Day 28	
Total number of seed	Seed germination (%)	Total number of seed	Seedling growth (%)*	Seedling height (cm)**	Root length (cm)
100 100 100	$32.5 \pm 1.6 \text{ b}$ $50 \pm 2.9 \text{ b}$ $65 \pm 1.2 \text{ a}$	100 100 100	$39 \pm 1.5 \text{ b}$ $91 \pm 2.7 \text{ a}$ $81 \pm 2.1 \text{ ab}$	$0.5 \pm 0.1 \text{ d}$ $5 \pm 0.8 \text{ a}$ $3.9 \pm 0.2 \text{ b}$	$1.8 \pm 0.2 \text{ c}$ $7.6 \pm 0.3 \text{ a}$ $6 \pm 0.3 \text{ ab}$ $4.5 \pm 0.2 \text{ b}$
	Total number of seed  100 100	Total number of seed         Seed germination (%)           100 $32.5 \pm 1.6 \text{ b}$ 100 $50 \pm 2.9 \text{ b}$ 100 $65 \pm 1.2 \text{ a}$	Total number of seed         Seed germination (%)         Total number of seed           100 $32.5 \pm 1.6 \text{ b}$ $100$ 100 $50 \pm 2.9 \text{ b}$ $100$ 100 $65 \pm 1.2 \text{ a}$ $100$	Total number of seed         Seed germination (%)         Total number of seed         Seedling growth (%)*           100 $32.5 \pm 1.6 \text{ b}$ $100$ $39 \pm 1.5 \text{ b}$ 100 $50 \pm 2.9 \text{ b}$ $100$ $91 \pm 2.7 \text{ a}$ 100 $65 \pm 1.2 \text{ a}$ $100$ $81 \pm 2.1 \text{ ab}$	Total number of seed         Seed germination (%)         Total number of seed         Seedling growth (%)*         Seedling height (cm)**           100 $32.5 \pm 1.6$ b $100$ $39 \pm 1.5$ b $0.5 \pm 0.1$ d           100 $50 \pm 2.9$ b $100$ $91 \pm 2.7$ a $5 \pm 0.8$ a           100 $65 \pm 1.2$ a $100$ $81 \pm 2.1$ ab $3.9 \pm 0.2$ b

The values represent mean  $\pm$  standard error of the mean (experiments were 4 replicates each with 25 seeds). Values followed by the different letters in a column are significantly different at the 0.01 level.

nation and plant growth, whereas 24 h MF exposure time had a stimulatory effect on seed germination (Table 1).

Similar results were observed for shoot regeneration percentage, shoot number per explant, the highest shoot height per explant, and total shoot number per petri dish 4 weeks after study initiation (Table 2, Fig. 2). The highest values for these parameters were recorded again from 24 h MF exposure time. Additionally, all parameters decreased significantly when the MF exposure time increased over 24 h. However, between the untreated group (0 mT) and 48 h MF exposure time, there were no significant differences observed for all parameters. The number of shoots regenerated per Petri dish was doubled in 24 h MF treatment compared to other treatments, including control where no MF treatment was applied (Table 2).

#### **DISCUSSION**

Nowadays, chemical methods such as some chemicals, fungicides, pesticides, and hormones are often used in pre-sowing seed treatment as an important yield-enhancing factor in plant cultivation [Javid et al., 2011; Zhang et al., 2011; Paparella et al., 2015; Sharma et al., 2015]. These methods are very effective in vigor improvement, but they are not eco friendly and have some handling problems. For this reason, studies have shifted to physical methods such as gamma rays, laser, electron beam, microwave, MF, and radiofrequency energies to bring about biostimulation of seeds, which lead to increased vigor and contribute to the improved development of plants. Moreover, physical methods provide significant yield improvement without the toxic hazards of chemical fertilizers and management costs for enhancing seed



Fig. 1. In vitro seedling growth from Lathyrus chrysanthus Boiss. from seeds pre-treated with 125 mT MF strength at different MF exposure time periods (a) 24 h (treated) and (b) 0 h (untreated) (Bar = 2 cm).

<sup>\*</sup>Seedling growth percentage means the number of seedlings grown out of 100 seeds.

<sup>\*\*</sup>Seedling height means height of above ground part of the plant.

TABLE 2. The effect of MF strength 125 mT for 0, 24, 48, and 72 h on shoot regeneration from cotyledon node explants of *Lathyrus chrysanthus* Boiss.

MF period (h)	Shoot regeneration (%)	Shoot number per explant	The highest shoot height per explant (cm)	Total shoot number per Petri dish*
0	$70 \pm 5.8 \text{ b}$	2 ± 0.2 b	$2.42 \pm 0.2 \text{ b}$	14 ± 0.6 b
24	$100 \pm 0.0 \ a$	$3.33 \pm 0.3 \text{ a}$	$4 \pm 0.6 \ a$	$33 \pm 1.5 \text{ a}$
48	$75 \pm 2.9 \text{ b}$	$2.25 \pm 0.1 \text{ b}$	$2.5 \pm 0.2 \text{ b}$	$17 \pm 0.6 \text{ b}$
72	$65 \pm 2.9 \text{ b}$	$1.67 \pm 0.1 \text{ c}$	$1.45 \pm 0.2 \text{ c}$	$11 \pm 0.9$ c

The values represent mean  $\pm$  standard error of the mean (experiments were 3 replicates each with 10 explants). Values followed by the different letters in a column are significantly different at the 0.01 level.

performance. MF pre-treatment is one of the physical pre-sowing seed treatments that is worth special attention since its impact on seeds can change processes occurring in the seed and stimulate plant development [Guruprasad et al., 2013]. Manipulation of the physical micro-environment by changing distances among cultured explants has resulted in increased shoot regeneration capacity, root formation, and plantlet establishment by causing positive spacing competition [Yildiz, 2011].

Stress response induction by MF in plants is another hypothesis. Oxidative stress is a major factor that enhances mutation [Galland and Pazur, 2005] and

increases general biological stress [Dhawi and Al-Khayri, 2008]. MF causes stress by contributing free radical induction. This stress caused by MF is stabilized by an increase in photosynthetic pigments such as chlorophyll contents, carotenoids, and amino acids. The increase of these compounds affects osmotic pressure and induces water uptake, leading to an increase of a plant's biomass [Dhawi, 2014]. Many studies have reported that chemical reactions in plants increase under MF [Phirke et al., 1996a, 1996b; Carbonell et al., 2000].

Another hypothesis is the effect of MF on plants' elemental composition due to the interaction

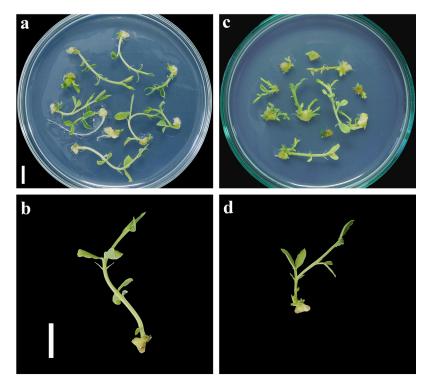


Fig. 2. In vitro shoot regeneration from cotyledone node explants excised from 28-day-old seedlings of  $125 \, \text{mT}$  MF-treated seeds for 24 h (treated) (a,b) (Bar = 1 cm), 0 h (untreated) (c, d) (Bar = 1 cm).

<sup>\*</sup>Total shoot number per petri dish means total shoot number regenerated from 10 explants in a petri dish.

between external and internal MFs resulting from free radicals' non-paired electrons [Goodman et al., 1995; Goodman and Blank, 2002]. Excitation energy of MF accelerates metabolism and consequently leads to better germination [Aladjadjiyan, 2002; Dhawi, 2014]. Induction of molecular transformation via MF provides cells with a better condition for growth and further development. Different plant systems can be affected positively or negatively by flux, intensity, and MF exposure period [Aladjadjiyan and Ylieva, 2003]. Cell permeability and uptake ability are increased by MF due to increased active energy in cellular electrolyte solutions. Furthermore, some studies [Ayrapetyan et al., 1994; Wojcik, 1995; Belyavskaya, 2004] showed that prolonged MF exposure increased uptake of calcium ions by changing electrical conductivity. The increase of calcium ion accumulation can be due to avoiding cellular damage in stress conditions induced by MF, affecting free radical release [Trewavas and Malho, 1998]. Free radical release and element uptake enhance plant seed vigor and stimulate proteins and enzyme activities [Kurinobu and Okazaki, 1995; Stange et al., 2002]. Prolonged MF exposure significantly increased ion content in date palm seedlings by affecting cell membrane permeability, leading to increased element uptake [Dhawi et al., 2009a; Dhawi, 2014].

The last hypothesis is that MF may increase mass and water content in plants [Dhawi, 2014] by acting as an auxin, leading to fruit ripening and increased growth [Krylov and Tarakonova, 1960; Boe and Salunkhe, 1963]. Another explanation of increased water uptake after MF treatment is an increase in ion uptake [Dhawi et al., 2009a] and free radicals [Scaiano et al., 1994; Parola et al., 2006; Ghanati et al., 2007], causing stress and proline accumulation [Dhawi and Al-Khayri, 2008] which change the osmotic [Belyavskaya, 2001] and electric potential.

Some of these studies with different plant species' seedlings placed in MF showed that their growth enhanced [Vashisth and Nagarajan, 2010; Shine et al., 2011; Shine and Guruprasad, 2012], while others showed that development was inhibited [Huang and Wang, 2007]. Hence, it may be predicted that seeds and plants react differently at different frequencies and different intensities of MFs. The MFs have an effect on plants and seeds based on field intensity, exposure time periods, signal form, flux density, and source frequencies [Belyavskaya, 2004; Guruprasad et al., 2013].

In many studies, MF strength that ranged from 125 to 250 mT showed biostimulation on initial growth stages and an increased germination rate in several plant species [Maffei, 2014]. In this context, Martinez

et al. [2000] reported that the application of 125 mT MF for 24 h improved yields of barley. Similarly, positive effects of 125 and 250 mT MF strengths on seed germination and plant growth were noted in the seeds of many plants such as rice [Florez et al., 2004], tomato [Martinez et al., 2009], and pea [Carbonell et al., 2011]. Based on these findings, a static MF of 125 mT was applied to the *Lathyrus chrysanthus* Boiss. seeds at different MF exposure time periods (0-untreated, 24, 48, and 72 h) in the present study. This is the first study demonstrating the effect of MF at different exposure time periods on in vitro seed germination and seedling growth, and also tissue culture response of cotyledon node explants excised from sterile seedlings of *Lathyrus chrysanthus*.

Several chemical treatments have been widely investigated to break the dormancy of Lathyrus chrysanthus seeds. Telci et al. [2011] reported that sodium hypochlorite (NaOCl) solutions used as a chemical method could also be successfully used as a dormancy-breaking agent in seeds of L. chrysanthus Boiss. They showed that the best results were obtained from 3.75% NaOCl concentration (75% commercial bleach containing 5% NaOCl) at 35 °C temperature and a 15-min application period for all parameters examined as seed germination percentage, seedling growth, seedling height, root length, seedling fresh, and dry weights, chlorophyll contents, number of stoma and cell in the field of view area, stoma width and length, and cell width. Also, Yildiz [2012] demonstrated that seedborne contamination increased gradually by decreasing concentrations and application periods of NaOCl below 3.75% (75% commercial bleach containing 5% NaOCl) and 15 min in Lathyrus chrysanthus seeds. Dramatic decreases in seed germination, seedling growth, and seedling length of all cases were observed at 5% NaOCl concentration.

Beyaz et al. [2016] investigated the effects of radiation at different doses (0-control, 50, 100, 150, 200, and 250 Gy) of radioactive cobalt (60Co) gamma rays on seed germination and seedling growth of Lathyrus chrysanthus under in vitro conditions. They found that at 50 Gy gamma radiation, all values were higher than control where no gamma radiation was applied. Thus, they reported that low doses of gamma radiation could simply be used for overcoming dormancy and for obtaining healthy seedlings in Lathyrus chrysanthus Boiss. Our findings were similar to that of Beyaz et al. [2016] in terms of the MF exposure time periods because we determined that higher MF exposure time periods decreased seed germination and plant growth, whereas 24 h MF exposure time had stimulatory effect on seed germination and tissue culture response in our case. Thus, we demonstrated the stimulatory effect of 24 h MF exposure time on all measured parameters except for seed germination percentage. However, 72 h MF exposure time showed an inhibitory effect on all parameters.

From these results, it could be concluded that MF strength and different exposure time periods are closely related to each other. And this relation significantly affected vitro seed germination, seedling growth, seedling height, root length, shoot regeneration capacity of cotyledon node explants in *Lathyrus chrysanthus* Boiss., and dormancy breaking in in vitro conditions. It was found that MF strength and especially exposure time are very significant factors for the germination process of seeds compared with the untreated group (0 h) seeds. Also, opposite behavior between 24 h of MF exposure (stimulation) and 72 h (inhibition of growth) was observed in the current study.

Consequently, we suggest that MF could be used as a convenient physical technique by applying various MF strengths and time periods of MF exposure to improve germination and dormancy breaking Lathyrus chrysanthus Boiss. We also think that combined application of different strengths and exposure time periods of MF in in vitro conditions would have great potential for developing and improving seedling growth and shoot regeneration of various plant species in the future. Besides, further research is needed to determine the positive biophysical effects of MFs and to better understand the molecular and cellular mechanisms of MFs according to the MF source, exposure time period, field intensities, and plant species. These studies should be included in investigating the relationship between MF pre-treatment at different field intensity, different exposure time periods, and several cellular and molecular parameters, especially ion uptake, free radicals, stress reactions, cellular metabolic pathways, DNA content and gene expression levels, plant hormone levels, contents of photosynthetic pigments, and water in different plant systems. In our study, these parameters could not be evaluated due to the limited facilities. We could recommend MF pre-treatment as one of the best methods to improve germination and dormancy breaking of Lathyrus chrysanthus Boiss.

According to the hypothesis revealed, MF affects plant growth and development via biochemical, physiological, genetic, and morphogenetic changes in cells and tissues. Since MF changes water physicochemical properties due to displacement and polarization of water atoms, one of the hypotheses is based on the effect of MF on water and electrolyte solutions [Dhawi, 2014]. These effects include alterations of water surface tension, viscosity, activation of energy water conductivity, strengthening hydrophobic

bounds, and water molecule size due to affecting hydrogen bond formation [Holysz et al., 2007; Cai et al., 2009; Szczes et al., 2011]. Another hypothesis is that MF leads to plant growth enhancement by affecting the molecular [Atak et al., 2003; Carbonell et al., 2011; Dhawi, 2014] and cellular level which leads to an increase in cell viability, organization and differentiation [Vizcaino, 2003; Valiron et al., 2005], cell reproduction and cellular metabolism [Atak et al., 2003; Dhawi, 2014], gene expression [Paul et al., 2006], and enzyme activity [Atak et al., 2007].

Numerous reports indicate that various metobolic pathways including reactive oxygen species (ROS), free radical metabolism [Shine and Guruprasad, 2012; Guruprasad et al., 2013], starch metabolism ( $\alpha$ - and  $\beta$ -amylases, dehydrogenase, protease) [Maffei, 2014], photosynthetic pigment contents (chlorophyll and carotenoids) [Atak et al., 2003; Taia et al., 2007; Telci et al., 2011; Guruprasad et al., 2013; Dhawi, 2014; Maffei, 2014; Yildiz et al., 2017], lipid peroxidation, oxidative metabolism, nitric oxide (NO) metabolism, catalase (CAT) activity, malondialdehyde (MDA) content, H<sub>2</sub>O<sub>2</sub> production, superoxide dismutase (SOD), glutathione reductase (GR), glutathione transferase (GT), peroxidase (PO), polyphenoloxidase (PPO), ascorbate peroxidase (APX), NO, and NO synthase [Atak et al., 2007; Sahebjamei et al., 2007; Shine and Guruprasad, 2012; Guruprasad et al., 2013; Tauati et al., 2013; Maffei, 2014], polyhenols (polyphenols oxidase activity) [Ghanati et al., 2007], amino acid metabolism (proline content) [Stange et al., 2002; Dhawi and Al-Khayri, 2008; Dhawi, 2014], protein metabolism (protein content and cryptochrome proteins: CRY 1 and CRY2) [Guruprasad et al., 2013; Maffei, 2014], and lipid metabolism (phospholipid content) [Maffei, 2014] were affected after exposing plants to MFs by alterating enzymes or metabolites in different plant species.

In addition, MF affects DNA by prolonging the life span of free radical ions and by inducing the singlet-triplet transition of unpaired electrons leading to oxidative stress [Sahebjamei et al., 2007]. DNA content increase or decrease depends on MF exposure level [Dhawi, 2014]. Several scientific reports showed that low-level MF exposure caused a decrease of DNA content in various plant species [Racuciu et al., 2007; Racuciu et al., 2008; Dhawi and Al-Khayri, 2009]. However, a significant decrease in cell number with enhanced DNA content in *Allium cepa* L. root and shoot meristems after artificial shielding of GMF [Nanushyan and Murashov, 2001] was observed. These reports also indicate that MF has genotoxic and mutagenic effects by inducing excitation in cell radical ions, which affect DNA integrity, and by causing

double DNA breaks and spontaneous mutations, and also by increasing cell membrane permeability [Dhawi, 2014]. It has been suggested that MFs interact with DNA processes by inhibiting synthesis or stimulating degradation of DNA on different plant species genotype, or by MF exposure time and exposure doses [Dhawi and Al-Khayri, 2009, Dhawi, 2014].

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