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Stimulation with a 130-mT magnetic field improves growth and biochemical parameters in lupin (*Lupinus angustifolius* L.)

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Abstract: The influence of magnetic field stimulation (MFS) on plants is a subject of intense research. The influence of MFS on plants varies depending on its intensity, time of exposure, and form of application. Weak MFS has beneficial effects on physiological and biochemical processes in plant tissues. Lupins (*Lupinus* spp.) are economically and agriculturally important plants used mainly in livestock feeding or in human consumption. The effects of a stationary magnetic field (130 mT) on the mitotic activity and selected biochemical parameters of lupin (*Lupinus angustifolius* L.) were evaluated. Nonexposed plants were used as the control. It was noted that the stimulation of plants with a 130-mT magnetic field favored the aboveground parts of the plants, which was manifested by an increase in the average length and fresh weight of shoots and an increase in the photosynthetic pigment content. However, guaiacol peroxidase activity decreased in shoots after their exposure to 130-mT MFS. The development of roots was at the control level. Moreover, an increase in the total protein content in both shoots and roots was observed after the MFS.

Key words: Lupin, magnetic field, mitotic activity, chlorophyll, antioxidant enzyme

1. Introduction

The study of the biological effects of both magnetic and electromagnetic fields is a very popular area of research. Recently, new techniques based on the application of magnetic field stimulation (MFS) have been developed, particularly in agricultural science (Flórez et al., 2004).

The influence of MFS on plants is a subject of intense research. However, there are many factors that determine plant response to stimulation by a magnetic field, e.g., plant species, exposure period, or exposure dose (Pietruszewski et al., 2001). The physiological parameters of plant response were examined under different magnetic conditions (Belyavskaya, 2001; Radhakrishnan and Kumari, 2012). Enhancement of plant growth and development under specific magnetic stimulation has been confirmed by many scientists. The reported beneficial effects of magnetic treatment include increased seed germination (Payez et al., 2013), better plant growth (Payez et al., 2013), and improvement of crop productivity (Radhakrishnan and Kumari, 2012). Moreover, MFS exerts an effect on many physiological and biochemical processes of plant cells. Some experiments have revealed that magnetic treatments can enhance protein and mineral accumulation (Radhakrishnan and Kumari, 2012), improve enzyme activities (Radhakrishnan and Kumari, 2012), or enhance photosynthesis (Rakosy-Tican et al., 2005) and membrane integrity (Payez et al., 2013). Interestingly, MFS can act as a protective agent against heat stress (Ružič and Jerman, 2002), ameliorate soil water stress (Anand et al., 2012), enhance saline-alkali tolerance (Xia and Guo, 2000), or alleviate toxicological effects induced by cadmium (Chen et al., 2011). It was noted that MFS influenced defense response by activation of the antioxidant system (Xia and Guo, 2000). Moreover, an increase in the frequency of protoplast fusion (Nedukha et al., 2007) and a stimulation of growth in in vitro cultures of potato and *Solanum* species (Rakosy-Tican et al., 2005) in magnetic field exposure have been found. In the meristem cells of *Pisum sativum* L. roots exposed to low magnetic fields, ultrastructural changes such as noticeable accumulation of lipid bodies, development of a lytic compartment, and reduction of phytoferritin in plastids were observed (Belyavskaya, 2001).

Nevertheless, the mechanisms of the biological action of MFS in plant tissues are insufficiently understood and remain an open issue; therefore, extensive studies are necessary to elucidate the mechanism(s) and to identify the useful application of MFS (Martinez et al., 2002; Flórez et al., 2004).

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Lupin (*Lupinus* spp.) belongs to economically and agriculturally valuable plants. From the genus *Lupinus*, only four species are of agronomic interest: *L. albus*, *L. angustifolius*, *L. luteus*, and *L. mutabilis* (Reinhard et al., 2006). Lupins are widely cultivated in Europe (Germany, France, Spain, the Netherlands, and Poland), the United States, and Australia. Lupins have three main uses: in livestock feeding (due to their high protein and energy content), human consumption, and crop rotations for their ability to add nitrogen and increase the availability of phosphorus in soils (Kohajdová et al., 2011).

Given the possibility of the use of MFS in agricultural practice, the aim of the present study was to evaluate the effects of weak permanent magnetic field stimulation (130 mT) on the early stages of growth and development and on selected biochemical and physiological characteristics (cell division, photosynthetic pigments, protein content, and guaiacol peroxidase activity) of lupin (*Lupinus angustifolius* L.).

2. Materials and methods

Lupin (*Lupinus angustifolius* L.) seeds were sown on filter paper moistened with distilled water and placed in tanks with constant access to water. The plant material was divided into two groups: the control group (C) and the experimental group (MFS). The seeds from the control group were grown in a natural magnetic field, whereas the seeds in the experimental group were exposed to a 130-mT permanent magnetic field perpendicular to the plants during the experimental period (14 days). Based on previous studies (Kornarzyński et al., 2004) and literature data (Carbonell et al., 2000; Flórez et al., 2004), we selected the 130-mT magnetic field induction, which gives the best results for the magnetic field. The seeds germinated at 20 ± 2 °C under a 12/12-h photoperiod and an illumination of 300 lx in the vegetation hall for 14 days. After that time, the plant material was used for the analyses. Plant breeding was conducted at the Department of Physics, University of Life Sciences, Lublin, Poland.

2.1. Biometric parameters

After 14 days of the experiment, the basic growth parameters were analyzed. Plant individual average length of roots and shoots was measured with 0.1 cm of precision. The fresh weight (FW) of root, shoot, and leaf samples was measured with 10^{-5} g of accuracy. The biometric measurements were performed on 20 selected plants of the same physiological age.

2.2. Cell division

To study the mitotic activity, 14-day-old root meristems were fixed for 24 h in AA (92% EtOH and CH_3COOH, 3:1). The fixed root meristems were stained with 0.5% acetocarmine. After staining, the plant material was rinsed with dH_2O and macerated for 10 min in a solution of HCl (conc.) and 96% EtOH (1:1). The macerated tissues were placed in dH_2O for 10 min and then in 45% acetic acid for another 10 min (Clark, 1981). The mitotic index (MI) was calculated as the percentage of proliferating cells among 1000 cells. A Leica DM 4000 B microscope was used for the analysis.

2.3. Biochemical assays

The biochemical analyses were carried out on day 14 of the experiment. All measurements were performed in triplicate.

2.3.1. Measurement of photosynthetic pigments

The contents of chlorophyll a (chl a), chlorophyll b (chl b), chlorophyll a + b (chl a + b), and carotenoids (car) were determined with the method of Lichtenthaler and Buschmann (2001). Leaf samples (0.5 g) were homogenized in 5 mL of 80% acetone chilled to 4 °C. The homogenate was centrifuged and the precipitate was washed with cold acetone until complete chlorophyll extraction. The extract was supplemented with cold 80% acetone to 25 mL. The absorbance of the supernatant was read at wavelengths of 663, 645, 652, and 470 nm with an Agilent Cary 60 UV-Vis spectrophotometer. The contents of chlorophyll and carotenoids were calculated according to Lichtenthaler and Buschmann (2001). The extract was diluted with acetone and the wavelength scans were recorded in the range of 800 to 400 nm using an F-7000 Hitachi fluorescence spectrophotometer.

2.3.2. Determination of the protein content

The protein content in the plant material (roots and shoots) was determined according to Bradford (1976) using bovine serum albumin (BSA) as a standard. The measurement was performed 15 min after adding the dye concentrate (Bio-Rad Protein Assay) at a wavelength of $\lambda = 595 \pm 10$ nm with an Agilent Cary 60 UV-Vis spectrophotometer.

2.3.3. Determination of guaiacol peroxidase activity

The measurement of guaiacol peroxidase activity (GPOX; EC 1.11.1.70) was performed according to Velikova et al. (2000). Frozen plant tissues (roots and shoots; 0.5 g) were transferred to a cooled mortar and homogenized with 5 mL of 50 mM phosphate buffer (pH 7.0) with 1 mM EDTA and 1% PVPP. The material was centrifuged at 15,000 rpm at 4 °C for 20 min in an MPW 350-R centrifuge. The reaction mixture contained 2750 mL of 1% guaiacol in 50 mM phosphate buffer at pH 7 and 100 mL of supernatant. The reaction was initiated with the addition of 150 µL of 100 mM H_2O_2 to the reaction mixture. The absorbance was read at 470 nm with the Agilent Cary 60 UV-Vis spectrophotometer. The specific activity of the enzyme was expressed in mM min^{-1} mg^{-1} protein.

2.4. Statistical analysis

The statistical analysis was performed using one-way ANOVA and Tukey's test at a significance level of $P <$

0.05. The results were expressed as mean value ± standard deviation (SD).

3. Results

The analysis of the growth of lupin (*Lupinus* L.) roots showed that the average root length of the control group was 3.79 cm after 14 days of cultivation. The stimulation of the plants with 130-mT MFS did not influence the average length of the lupin roots. On the other hand, there was a significant increase in the length of the shoots after 14 days of exposure to MFS. The analysis showed that the average length of the control shoots was 9.06 cm. However, the average shoot length of the MFS-treated plants was significantly higher by approximately 30% relative to the control level (Table 1). Additionally, the FW of the plant organs was analyzed after 14 days of cultivation. Similar to the results of the root length, the root FW of the experimental plants remained at the control level. On the other hand, MFS significantly improved the FW of the shoot. The analysis showed that the shoot FW of the MFS-exposed plants was significantly higher by approximately 18% relative to the control level. The leaf FW of the MFS-exposed plants remained at the control level (Table 1).

The mitotic index (MI) in the meristematic cells of the control was 13.53%, and the MI value in the MFS-exposed roots was 12.90% (Table 2). There were no statistically significant differences between the mitotic activities of the two plant groups. The frequency of the different phases of mitosis was also analyzed. The cells in the different phases of mitosis in the control plants accounted for 49.62% in prophase, 7.80% in metaphase, 9.57% in anaphase, and 33.01% in telophase. Similar results were obtained in the MFS-exposed roots and the following values were noted: 51.86% in prophase, 9.30% in metaphase, 11.78% in anaphase, and 27.06% in telophase. The exposure of lupin to the 130-mT MFS significantly decreased the percentage of cells by 18% in telophase, whereas the other phases remained at the control level (Table 2).

The measurement of the photosynthetic pigment content in the leaves (chl a, chl b, chl a + b, car) was performed on day 14 of the experiment. The pigment content of the control leaves was 2.06 mg g^{-1} FW (chl a), 1.25 mg g^{-1} FW (chl b), and 3.24 mg g^{-1} FW (chl a + b) (Table 3). The exposure of lupin to the 130-mT MFS for 14 days had a beneficial effect on the chl a and chl a + b content. The chl a content increased by 28% and chl a + b by 15%, in the MFS-exposed plants in comparison to the control. However, the content of chl b remained at the control level and there were no statistically significant differences between these groups. Moreover, the 130-mT MFS had a beneficial effect on the carotenoid content. The measurement showed a 34% increase in the car content of the MFS-exposed plants in comparison to the control. Continuous measurements of chlorophyll fluorescence were also performed and showed that the fluorescence intensity of chlorophyll after 130-mT MFS increased by approximately 40% in comparison to the control (Figure 1).

Additionally, 130-mT MFS improved the total protein content in both roots and shoots (Figure 2). The total protein content was 7.70 mg g^{-1} FW in the control roots

Table 1. Average length [cm] and FW [g] of the primary organs of lupin seedlings (*Lupinus* L.). C: Control conditions, MFS: 130-mT magnetic field stimulation.

Description	Roots [cm]	Shoots [cm]	Roots [g]	Shoots [g]	Leaves [g]
C	3.79 ± 1.02 a	9.06 ± 2.56 A	0.14 ± 0.05 a	0.34 ± 0.07 a	0.10 ± 0.04 a
MFS	3.84 ± 0.72 a	11.80 ± 1.18 b	0.16 ± 0.04 a	0.40 ± 0.05 b	0.08 ± 0.03 a

Mean ± SD, n = 20, $P < 0.05$. Numbers in columns marked with the same letters do not differ significantly.

Table 2. MI and phase percentage in root meristems of lupin (*Lupinus* L.) plants under control conditions (C) and in the presence of 130-mT magnetic field (MFS).

Treatment	MI [%]	Phase percentage			
		Prophase	Metaphase	Anaphase	Telophase
C	13.53 ± 0.94 a	49.62 ± 2.82 a	7.80 ± 2.88 a	9.57 ± 2.83 a	33.01 ± 3.92 a
MFS	12.90 ± 0.71 a	51.86 ± 2.40 a	9.30 ± 2.31 a	11.78 ± 2.72 a	27.06 ± 2.68 b

A total of 9000 cells were scored for each treatment (3 roots × 3 replicates).
Mean ± SD, $P < 0.05$. Numbers in columns denoted with the same letters do not differ significantly.

Table 3. The chlorophyll (chl a, chl b, chl a + b) and car content [mg g^{-1} FW] in the leaves of lupin seedlings (*Lupinus* L.). C: Control conditions, MFS: 130-mT magnetic field stimulation.

Description	chl a	chl b	chl a + b	$R^a/_b$	car	chl a + b/car
C	2.06 ± 0.02 a	1.25 ± 0.07 a	3.24 ± 0.09 a	1.65	0.56 ± 0.05 a	5.85
MFS	2.63 ± 0.05 b	1.17 ± 0.10 a	3.74 ± 0.11 b	2.26	0.75 ± 0.03 b	4.97

Mean ± SD, n = 3, P < 0.05. Numbers in columns marked with the same letters do not differ significantly. chl a: chlorophyll a, chl b: chlorophyll b, chl a + b: total chlorophyll (chlorophyll a + chlorophyll b), $R^a/_b$: chlorophyll a to chlorophyll b ratio, car: carotenoids, chl a + b/car): total chlorophyll (chlorophyll a + chlorophyll b) to carotenoid ratio.

and 9.43 mg g^{-1} FW in the MFS-exposed roots. The 130-mT MFS significantly increased the total protein content in the roots by 22% in comparison to the control. Similarly, a 13% increase in the total protein content of the shoots was noted after the 130-mT MF exposure (Figure 2).

The effect of the MFS on GPOX in the roots and shoots was also analyzed. The GPOX activity in the roots was 69.60 U mg^{-1} protein in the control and 68.31 U mg^{-1} protein in the MFS-exposed plants (Figure 3). There were no significant differences between these groups. However, the lupin shoots were more sensitive to the MFS. In the aboveground parts of the plants, exposure to 130-mT MFS contributed to a significant decrease (15%) in GPOX activity in comparison to the control (Figure 3).

4. Discussion

MFS is currently a subject of intensive research (Pietruszewski et al., 2001; Flórez et al., 2004). So far, many experiments have revealed that MFS produces physical, biochemical, and physiological changes in plant cells (Flórez et al., 2004, 2007). These investigations are important, especially nowadays, when environmentally friendly techniques are promoted in modern agriculture.

In this paper, growth stimulation in lupin (*Lupinus angustifolius* L.) was observed under 130-mT MFS, indicating that the additional MFS could promote plant growth. The results indicate higher sensitivity of the aboveground plant parts than the roots to the application of MFS. We showed that the 130-mT MFS led to a remarkable increase in shoot length and FW. Better growth rates were observed in different plant species after MFS. Maize plants grew higher and heavier after continuous exposure to 125- or 250-mT MFS (Flórez et al., 2007). Similarly, the highest increase of pea plants was noted at a continuous exposure to 125- or 250-mT MFS (Carbonell et al., 2011). Moreover, Dardeniz et al. (2006) obtained a 17% increase in the shoot

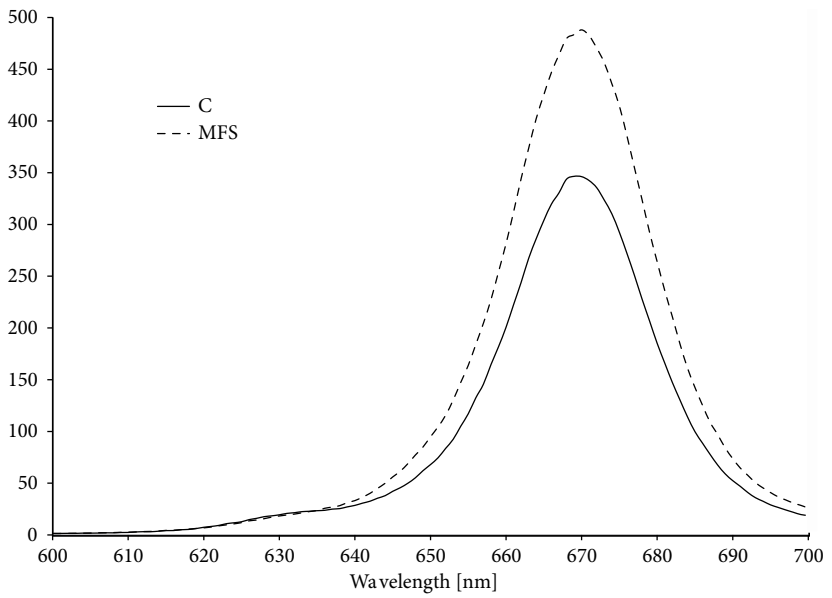

Figure 1. The fluorescence emission spectra of chlorophyll in lupin (*Lupinus* L.) under control conditions (C) and in the presence of 130-mT magnetic field (MFS). The fluorescence excitation was set at 405 nm. The excitation and emission slits were set at 5 nm and the temperature was 22 °C.

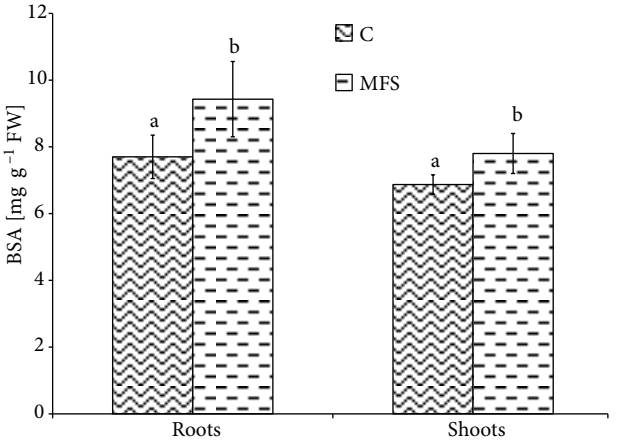

Figure 2. Protein content (BSA) in lupin (*Lupinus* L.) organs under control conditions (C) and in the presence of 130-mT magnetic field (MFS). Mean ± SD, n = 3, P < 0.05. Columns denoted with different letters differ significantly.

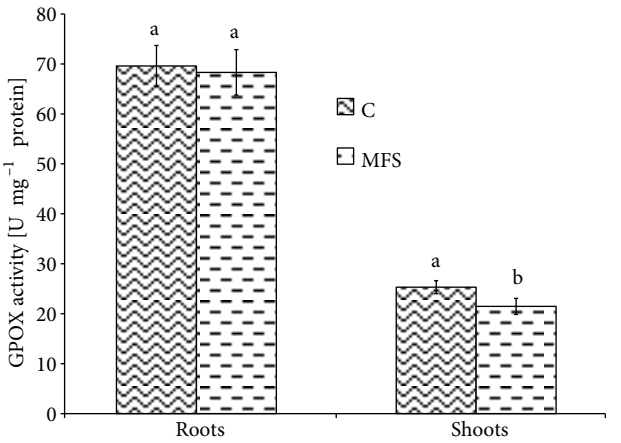

Figure 3. GPOX in lupin (*Lupinus* L.) organs under control conditions (C) and in the presence of 130-mT magnetic field (MFS). Mean ± SD, n = 3, P < 0.05. Columns denoted with the same letters do not differ significantly.

length of grapes (*Vitis vinifera*) after 50-Hz and 0.15-mT MFS.

Quantitative insight into the molecular mechanisms that take part in the processes of plant growth and development after MFS was carried out by spectrophotometric measurements such as photosynthetic pigment content, protein content, and GPOX activity.

Photosynthesis is a basic metabolic process and plays a key role in plant growth and development. Therefore, photosynthesis is very sensitive to environmental factors. The photosynthetic pigments play an important role in photosynthesis by absorbing solar energy and transferring it to chemical energy (Yang et al., 2009). In our study, we showed that better shoot growth contributed to an improvement in the photosynthetic pigment content in plant tissues after MFS. The 130-mT MFS had a beneficial effect on chl a, chl a + b, and car content. chl a is the main photosynthetic pigment directly involved in the conversion of solar energy into chemical energy (Răcuciu et al., 2008). In the present study, a marked increase (28%) in chl a level was evidenced for the plants exposed to 130-mT MFS. Moreover, the total chlorophyll content (chl a + b) increased by 15% in the MFS-exposed plants. A positive correlation between MFS and the photosynthetic pigment level has been documented for maize plants. Răcuciu et al. (2008) reported that permanent MFS (50 mT) for 14 days contributed to a 3% increase in the content of chl a + b in *Zea mays*. However, the content of chl b, which belongs to secondary pigments and is found exclusively in the pigment antenna system (Lichtenthaler and Buschmann, 2001), remained at the control level in the lupin seedlings. Environmental factors severely affect not only the photosynthetic pigment content but also the chlorophyll a/b ($R^a/_b$) and chlorophyll/carotenoid (chl a + b/car) ratio (Yang et al., 2009). In the present study, 130-mT MFS resulted in an increase in $R^a/_b$ and a decrease in the chl a + b/car ratio. $R^a/_b$ is known as an indirect indicator of the energetic activity of the LHC II system, which controls the first stage of the conversion of solar energy into chemical energy (Răcuciu et al., 2008). A marked increase (37%) in $R^a/_b$ was obtained for the 130-mT MFS-exposed plants in comparison to the control value, suggesting magnetic sensitivity of photosynthesis efficiency. This is in agreement with the results presented by Răcuciu et al. (2008), who reported a slight increase (3%) in the $R^a/_b$ value for low magnetic field induction (50 mT). On the other hand, 100-mT magnetic field exposure decreased the value of $R^a/_b$ by approximately 4% in maize plants (Răcuciu et al., 2008). The increase in chlorophyll a/b is possibly due to the lesser sensitivity of chl a compared to chl b (Yang et al., 2009). The ratio of chl a + b to total car (chl a + b/car) is an indicator of the greenness of plants and normally lies between 4.2 and 5 in sunned leaves and between 5.5 and 7.0 in shaded leaves (Lichtenthaler and Buschmann, 2001). In the present study, the chl a + b/car ratio was 5.85 for the control and 4.97 for the MFS group, i.e. no damage to the photosynthetic apparatus was observed in the lupin seedlings. Our observations indicate not only an increase in the chlorophyll content (15%) but also an increase in chlorophyll fluorescence intensity (40%). The noted increase in chlorophyll fluorescence efficiency may suggest that photosynthetic efficiency also increases. Carotenoids belong to isoprenoid compounds commonly found in all photosynthetic tissues, which act as passive light filters that can reduce the light intercepted by chlorophyll and protect from reactive oxygen species (ROS) (Lokhande

and Gaikwad, 2014). We showed that 130-mT MFS resulted in a 34% increase in the car level of lupin leaves. The increase in the car content of lupin leaves might play an important role in the protection against oxidative stress. The increased level of car may help to protect the chlorophylls and maintain better growth and productivity of plants (Lokhande and Gaikwad, 2014). Increase in car content is a common phenomenon after the application of plant growth regulators (Lokhande and Gaikwad, 2014).

Accumulation of ROS is a predominant behavior in plants exposed to stress. A main protective role against ROS is played by ROS-scavenging enzymes such as peroxidases (EC 1.11.1.7). Plant peroxidases are found in vacuoles, tonoplast, and plasmalemma, as well as inside and outside the cell wall. GPOX is an enzyme of the peroxidase class that plays an important role in processes such as lignification, ethylene biosynthesis, defense against pathogens, and inactivation of ROS (Naji and Devaraj, 2009). Experiments performed on several plant species have proved that MFS can change the activities of certain antioxidant enzymes (Xia and Guo, 2000). In the present paper, we noted that the permanent 130-mT MFS had a significant impact on GPOX activity. Our studies showed that GPOX activity significantly decreased in the leaves (15%), whereas in the roots it remained at the control level after exposure to the permanent MFS. On the other hand, an electromagnetic field (275 kV) contributed to a significant increase in GPOX activity in mustard (*Brassica chinensis*) leaves, although a weaker electromagnetic field (33 kV) did not affect GPOX significantly (Maziah et al., 2012).

In our experiment, the root growth and mitotic activity in root meristems of the 130-mT MFS-exposed plants was at the control level. However, De Souza et al. (2005) noted that presowing 120-mT dynamic MFS of tomatoes had a significant effect on root length, which increased by 18%. Moreover, these treatments had a remarkable effect on root fresh and dry weight. Studies on the meristematic cells of pea have shown that MFS affects normal metabolism and has an impact on cellular division (Belyavskaya et al., 1992). Moreover, in broad bean seedlings, 10- and 100-μT MFS at 50 or 60 Hz altered membrane transport processes in root tips (Stange et al., 2002).

In conclusion, the data presented in this paper indicate that 130-mT MFS enhances the growth and development of aboveground parts, which was manifested by an increase in the length and weight of the shoots and an increase in the photosynthetic pigment content of lupin (*Lupinus angustifolius* L.). MFS may prove beneficial for the improvement of the growth and productivity of economically important lupin plants.

The studies undertaken in this paper might be helpful in expanding the general knowledge about the mechanisms of plant response to MFS exposure. These phenomena are still unclear and require further research in the field.

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