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INFLUENCE OF MAGNETIC FIELD STIMULATION ON THE GROWTH AND BIOCHEMICAL PARAMETERS IN *PHASEOLUS VULGARIS L.*

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ABSTRACT

Plant response to magnetic field stimulation (MFS) is varied and dependent on many factors such as the intensity, time of exposition, or application form. It is known that high intensity MFS inhibits the growth and development of plants. However, weak MF stimulates many processes in plant cells. This study reports the effects of 130-mT magnetic field stimulation (MFS) on the growth and selected biochemical parameters in the common bean *(Phaseolus vulgaris L.)* in laboratory conditions. Our results indicated that the stimulation of plants by a weak permanent magnetic field (130 mT) increased mitotic activity in meristematic cells of the common bean (*P. vulgaris* L.). There was no influence of the 130-mT MFS on the development of aboveground plant parts; however, there was a marked increase in GPOX activity in the leaves after 130-mT magnetic field stimulation.

Keywords: Bean, magnetic field, growth, assimilation pigment, antioxidant enzyme

INTRODUCTION

Both magnetic and electromagnetic field influence the functioning of biological organisms. The literature describes the effects of electromagnetic field on seed germination **(Aksyonov *et al.*, 2001)** or antimicrobial activity **(Akinyele *et al.*, 2012).** Despite the extensive literature on the effects of magnetic field stimulation (MFS) on plants **(Pietruszewski *et al*., 2001; Belyavskaya, 2004; Çelik *et al.*, 2009; Muszyński *et al*., 2009; Jouni *et al*., 2012)**, recognition and understanding of the mechanisms of action of the magnetic field on the plant organism is still a challenge for researchers. Identification of the mechanism of magnetic field stimulation may be a key in regulating the biological activity of plants **(Cakmak *et al.*, 2012)**. It has been well documented that magnetically treated water **(Alkassab and Albach, 2014)** or magnetic field stimulation exerts an effect on plants **(Belyavskaya, 2004; Çelik *et al*., 2009; Muszyński *et al*., 2009; Jouni *et al*., 2012)**. However, the response of plants to magnetic field stimulation depends on the species, dose, or exposure time **(Pietruszewski *et al*., 2001)**. The optimal magnetic field stimulates plant growth and development or seed germination **(Pietruszewski *et al.*, 2001)**. It was observed that weak magnetic field stimulation had a beneficial effect on seed germination in radish **(Novitskaya *et al.*, 2001)**, root formation in wheat **(Aksyonov *et al*., 2001)**, or formation of somatic embryos in cultures of alfalfa (*Medicago sativa* L.) **(Dijak *et al*., 1986)**. Moreover, studies on the meristematic cells of pea have shown that the magnetic field has an influence on metabolism and cellular division **(Belyavskaya *et al.*, 1992)**. However, **Lebedev *et al*. (1977)** showed a decrease in the fresh and dry weight of shoots and roots in barley seedlings. Moreover, the function of antioxidant enzymes (SOD and CAT) in *Glycine max* (L.) seedlings was intensified due to treatment with the magnetic field **(Çelik *et al*., 2009)**.
Nowadays, the results obtained by researchers on the magnetic field stimulation are particularly promising and important. This is due to the huge interest in the environment-friendly technology and the possibility of obtaining increased yields without the use of fertilizers.
The bean *(Phaseolus vulgaris L.)* is often used for laboratory testing due to its rapid growth, a short period of vegetation, and relatively low cultivation requirements. The bean is an important legume constituting a major source of proteins, fibre, prebiotics, vitamin B, and micronutrients for humans worldwide. In addition, some species of legumes are used as animal fodder **(Câmara *et al.*, 2013)**. These properties have contributed to the selection of these plants for this study.
The objective of our study was to investigate the influence of weak permanent magnetic field stimulation (130 mT) on the growth and selected biochemical parameters of the common bean *(Phaseolus vulgaris L.)*.

MATERIAL AND METHODS

Seeds (20 seeds per 1 replication) of the common bean (*Phaseolus vulgaris* L.) were sown in seed trays on filter paper moistened with distilled water. The plant material was divided into two groups: the control (C) and experimental group (MF). Seeds from the control group (C) were growing in a natural magnetic field while the seeds of the test group (MF) were grown during the experimental period (14 days) in the presence of 130-mT permanent magnetic field (MF) perpendicular to the plants. Based on previous studies, we selected the induction of 130 mT, which gives the best results, for the magnetic field **(Kornarzyński *et al.*, 2004).** The seeds germinated at 20±2°C, at a 12/12 h photoperiod and an illumination of 300 lux in the vegetation chamber for 14 days. After this time, the plant material was used to the analyses. Plant breeding was conducted in the Department of Physics, University of Life Sciences in Lublin.

The biometric parameters

After 14 days of the experiment, the basic growth parameters: fresh weight, average length of roots and shoots, and the average length and width of leaves were analysed. Plant individual average length of roots and the aboveground parts of plants was measured with 0.1 cm precision. The fresh weight (FW) of seedling samples was measured with 10^{-5} g accuracy. The biometric measurements were performed in triplicate for selected 10 plants at the same physiological age.

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) and stained in 0.5% acetocarmine. After staining, the plant material was rinsed with dH_2O and macerated for 10 minutes in a solution: HCl (conc.) : 96% EtOH (1:1). The macerated tissues were placed in dH_2O for 10 minutes and then in 45% acetic acid for 10 minutes **(Clark, 1981).** The mitotic index (MI) was calculated as the percentage of proliferating cells among 1000 cells. For the analysis, a Leica DM 4000 B microscope was used.

The biochemical assays

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

Measurement of assimilation pigments

The level of chlorophyll a (chl a), chlorophyll b (chl b), chlorophyll a+b (chl a+b), and carotenoids (car) was determined by the **Lichtenthaler and Wellburn** method **(1983)**. 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 nm, 645 nm, 652 nm, and 470 nm in an Agilent Cary 60 UV-Vis spectrophotometer. The contents of pigments were calculated according to **Bruinsma (1963).** The extract was diluted with acetone and the absorption spectra were recorded in the range of 800 to 200 nm using the Agilent Cary 60 UV-Vis spectrophotometer.

Determination of the protein content

The protein content in the plant material (roots, shoots, and leaves) was determined according to **Bradford (1976)** using BSA as a standard. The measurement was performed after 15 min. of adding the dye concentrate (Bio-Rad Protein Assay) at a wavelength λ = 595±10 nm on the Agilent Cary 60 UV-Vis spectrophotometer.

Determination of guaiacol peroxidase activity (GPOX)

The measurement of guaiacol peroxidase activity GPOX (EC 1.11.1.70) was performed according to **Velikova *et al.* (2000)**. Frozen plant tissues (roots, shoots, and leaves) (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 supernatant. The reaction was initiated by addition of 150 µl 100-mM H_2O_2 to the reaction mixture. The absorbance was read at 470 nm on the Agilent Cary 60 UV-Vis spectrophotometer. The specific activity of the enzyme was expressed in $mM.min^{-1}.mg^{-1}$ protein.

Statistical analysis

The statistical analysis was performed using one-way ANOVA and Tukey's post hoc analysis for determination interaction significance at $p < 0.05$. The results were expressed as mean values ± standard deviation (SD).

RESULTS

The analysis of root growth of common bean (*Phaseolus vulgaris* L.) showed that the average length of the control roots was 16.72 cm and that of the MF-stimulated plants was 16.80 cm after 14 days of the experiment. There were no significant differences between these groups. Moreover, no significant influence of the 130-mT MF on the shoot and leaf growth was observed. The average shoot length and the average length and width of leaves of the MF-stimulated plants persisted at the control level. Additionally, the fresh weight (FW) of the MF-stimulated plants remained at the control level and there were no statistically significant differences between the analysed plant groups (Table 1).

Table 1 Average length [cm] of the primary organs, average length and width [cm] of the leaves, and average weight [g] of bean seedlings (*Phaseolus vulgaris* L.).

Description	Roots [cm]	Shoots [cm]	Leaf length [cm]	Leaf width [cm]	Seedling weight [g]
C	16.72 ± 1.40 a	25.63 ± 6.48 a	2.02 ± 0.17 a	1.29 ± 0.30 a	1.54 ± 0.43 a
MF	16.80 ± 2.38 a	25.44 ± 1.83 a	1.87 ± 0.22 a	1.30 ± 0.19 a	1.58 ± 0.26 a

Legend: C - control conditions, MF - 130-mT magnetic field stimulation. Mean ± SD, n = 30, p> 0.05. Numbers in columns marked with the same letters do not differ significantly.

On the contrary, the study showed that in bean plants (*P. vulgaris* L.) the stimulation with the magnetic field had a beneficial effect on the mitotic activity in the root meristem cells. The mitotic index (MI) value was 36.33% in the control roots, while the mitotic activity in the MF-stimulated roots was 57.8%, and this was a statistically significant increase by 59% in comparison to the control level. The average number of cells in the different phases of mitosis in the control plants was 64 in the prophase, 29 in the metaphase, 23 in the anaphase, and 247 in the telophase. In the MF-stimulated root meristems of bean plants, the following values were noted: 83 in the prophase, 35 in the metaphase, 17 in the anaphase, and 443 in the telophase. Importantly, a beneficial effect of MF on the number of cells was observed in the metaphase and telophase stages, and the numbers were increased by 23% and 79 %, respectively, in relation to the control (Table 2).

Table 2 Mitotic index (MI) [%] and the number of cells in the different phases of mitosis in root meristems of bean (*Phaseolus vulgaris* L.).

Description	MI [%]	Prophase	Metaphase	Anaphase	Telophase
C	36.33 ± 3.72 a	64.00 ± 6.39 a	28.67 ± 3.87a	23.33 ± 4.47 a	247.33 ± 26.03 a
MF	57.80 ± 1.31 b	82.67 ± 6.43 a	35.33 ± 3.15b	16.67 ± 5.03 a	443.33 ± 9. 45 b

Legend: C - control conditions, MF - 130-mT magnetic field stimulation. Mean ± SD, n = 1000, p> 0.05. Numbers in columns marked with the same letters do not differ significantly.

Continuous measurements of the chlorophyll absorption spectrum were also performed. The highest absorbance was in the ranges of 500 to 400 nm and 700 to 600 nm (Fig. 1).

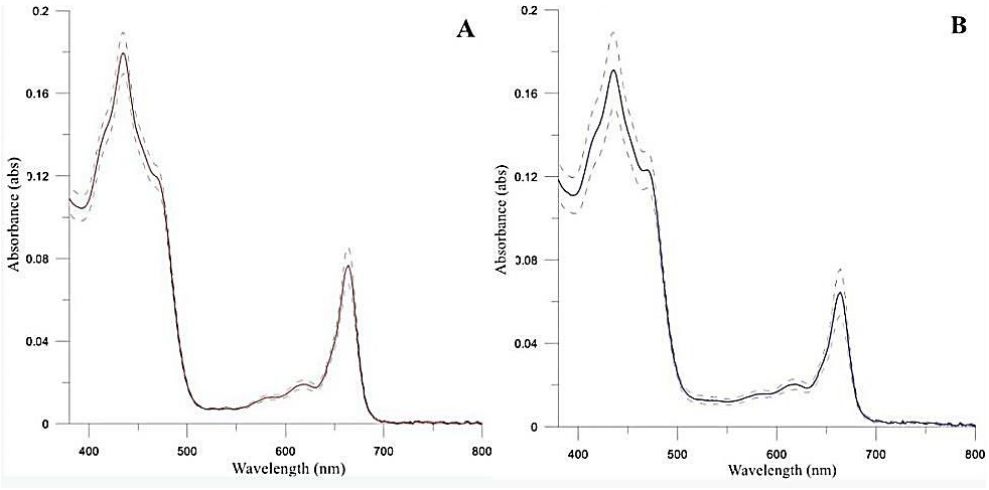

Figure 1 The assimilation pigment absorption spectra in bean (*Phaseolus vulgaris* L.) leaves. A - control conditions and B - in the presence of the 130-mT magnetic field (MF).

The measurement of the assimilation pigment content in the leaves (chl a, chl b, chl a+b, carotenoids) was performed on day 14 of the experiment. In the leaves of the common bean (*P. vulgaris* L.), the pigment content of the control was 0.21 $mg.g^{-1}$ FW (chl a), 0.09 $mg.g^{-1}$ FW (chl b), and 0.33 $mg.g^{-1}$ FW (chl a+b). The pigment content in the MF-stimulated leaves was 0.16 $mg.g^{-1}$ FW (chl a), 0.08 $mg.g^{-1}$ FW (chl b), and 0.26 $mg.g^{-1}$ FW (chl a+b). We noted that MFS did not influence significantly the chlorophyll content (Table 3). Similarly, the carotenoid content in the bean leaves was 0.13 $mg.g^{-1}$ FW in the control and 0.12 $mg.g^{-1}$ FW in the MF-stimulated leaves and this indicated that the carotenoid content in bean leaves exposed to MF remained at the control level (Table 3).

Table 3 The chl a, chl b, chl a+b and carotenoid content [$mg.g^{-1}$ FW] in leaves of bean (*Phaseolus vulgaris* L.).

Description	Chl a	Chl b	Chl a+b	Carotenoids
C	0.21 ± 0.05 a	0.09 ± 0.01 a	0.33 ± 0.10 a	0.13 ± 0.01 a
MF	0.16 ± 0.03 a	0.08 ± 0.01 a	0.26 ± 0.04 a	0.12 ± 0.01 a

Legend: C - control conditions, MF - 130-mT magnetic field stimulation. Mean ± SD, n = 3, p> 0.05. Numbers in columns marked with the same letters do not differ significantly.

Additionally, the effect of MFS on guaiacol peroxidase activity (GPOX) in the roots, shoots, and leaves of the common bean (*P. vulgaris* L.) plants was analysed. The experiment showed that GPOX activity in the bean roots was 17.1 U.mg^{-1} protein in the control and 20.67 U.mg^{-1} protein in the MF-stimulated plants and there were no statistically significant differences between these groups (Fig. 2).

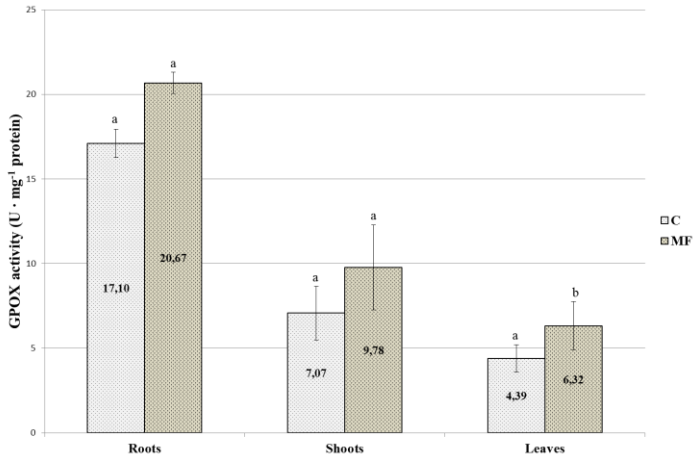

Figure 2 Guaiacol peroxidase activity (GPOX) in bean (*Phaseolus vulgaris* L.) organs. C - control conditions and MF - 130-mT magnetic field stimulation. Mean ± SD, n = 3, p> 0.05. Numbers in columns marked with the same letters do not differ significantly.

Similarly, there was no significant influence of MFS on the GPOX activity in the shoots. The study showed that the GPOX activity in the shoots of the control was 7.07 U.mg^{-1} protein and in the MF-stimulated plant 9.78 U.mg^{-1} protein. However, the leaves of the bean plants appeared to be highly sensitive to the 130-mT MF stimulation. The activity of GPOX in the control leaves was 4.39 U.mg^{-1} protein, and 6.32 U.mg^{-1} protein in the leaves exposed to 130 mT MF. A statistically significant increase (by 44%) in the activity of GPOX was observed in the bean leaves after stimulation with 130 mT MF (Fig. 2).

DISCUSSION

Magnetic field stimulation (MFS) of plants is one of environmental-friendly techniques used to improve crop yields and seed germination and the results obtained are promising **(Pietruszewski and Kania, 2010; Cakmak *et al*., 2012; Jouni *et al*., 2012)**. In this paper, the influence of permanent magnetic field stimulation (130 mT MF) on the growth and development of the common bean (*Phaseolus vulgaris* L.) was studied. In our experiment, there was no effect of the MF stimulation on the root growth of the common bean. However, **Vashisth and Nagarajan (2009)** found that 50-mT magnetic field stimulation led to a 42.4% increase in root length of sunflower. **Nagy *et al*. (2005)** also observed a 70% increase in the sunflower root length after MF stimulation in 0.01 Hz - 20 kHz and induction of 400 μT. Moreover, in *Lens culinaris*, a permanent magnetic field (120 mT, 10 min.) increased the root length by 58.96% **(Shabrangi and Majd, 2009)**. On the other hand, the results of this study showed that the 130-mT MF stimulation had a beneficial effect on cell mitotic activity in common bean (*P. vulgaris* L.) root meristems, and the mitotic index (MI) increased by 59%. Similar results were obtained by **Răcuciu (2011)**, who observed a 3-fold increase in the number of dividing root cells after 12 h stimulation of maize (*Zea mays*) with a 10-mT and 50 Hz magnetic field. Similarly, **Shabrangi *et al*. (2011)** observed a 3% increase in the mitotic activity in meristematic cells after 4-h exposure of maize (*Zea mays*) to MF stimulation (60 Hz, 3 mT). In our study, an increase in the number of cells in the metaphase or telophase stages by 23% and 79%, respectively, was also observed in bean plants exposed to 130-mT MFS. **Răcuciu (2011)** and **Fomicheva *et al*. (1992)** suggest that the increase in the mitotic activity of root meristems exposed to the magnetic field is caused by prolongation of the duration time of the different phases in the cell cycle and by the number of cells in the different stages of mitosis. This is in agreement with **Fomicheva *et al*. (1992)**, who also noted a prolonged time of the G1 and G2 phases in *Linum usitatissimum* L. by 51% and 33 %, respectively.

We also studied the influence of 130-mT MF stimulation on the growth and development of the aboveground plant parts. We noted that 130 mT MFS did not significantly influence the growth of shoots and leaves or fresh weight in bean plants. However, **Bilalis *et al*. (2013)** reported a 42% and 31% increase in leaf area in cotton (*Gossypium hirsutum* L. cv. *Campo*) after stimulation with a magnetic field of 3 Hz and 12.5-mT induction after 35 and 45 days, respectively. Moreover, **Yaycili and Alikamanoglu (2005)** observed an increase in the number of leaves by 36% in *Paulownia tomentosa* and by 40% in *Paulownia fortunei* after MF stimulation (2.9 mT to 4.9 mT). In turn, **Dardeniz *et al*. (2006)** obtained a 17% increase in shoot length of grapes (*Vitis vinifera*) after stimulation with 50 Hz and 0.15 mT MF.

To know better the molecular mechanisms involved in the plant growth processes after MF stimulation, spectrophotometric measurements of selected physiological and biochemical processes (photosynthesis pigment content or GPOX activity) were carried out. Photosynthesis is the main metabolic process in plant growth and development and thus very sensitive to environmental changes. The differences in chlorophyll content are often observed after treatment with various environmental factors (**Velikova *et al*., 2000)**. In the present study, we indicated that there was no significant effect of the 130-mT MFS on the assimilation pigment content in bean (*Phaseolus vulgaris* L.). The effect of MFS on the assimilation pigment content is unclear. **Atak *et al*. (2007)** showed that stimulation of soybean (*Glycine max* L.) with the magnetic field (2.9 mT to 4.6 mT) decreased the content of chl a, chl b, and chl a+b by 16%, 14%, and 14%, respectively. In contrast, the change in the exposure time resulted in an increase in the assimilation pigment content by 21% (chl a), 13% (chl b), and 18% (chl a+b). Similarly, **Răcuciu *et al.* (2008)** noted that permanent magnetic field stimulation (50 mT) for 14 days contributed to a 3% increase in the content of chl a + b in maize (*Zea mays*), but 100 mT MFS decreased the chl a + b content by 4%. Furthermore, **Muszyński *et al.* (2009)** noted that chlorophyll levels in *Triticum durum* seedlings were significantly modified by extremely low frequency magnetic field (f = 50 Hz, B = 15 mT), however, the chlorophyll a and b ratios remained unchanged.

The ROS scavenging enzymes: superoxide dismutase (SOD), catalase (CAT), and peroxidases (POX) are the main protective agents against ROS formation. SOD is the major enzyme involved in ROS detoxification, CAT is a key enzyme that eliminates H_2O_2, and POX catalyses the reaction of H_2O_2 degradation **(Jouni *et al*., 2012)**. Furthermore, guaiacol peroxidase (GPOX) [EC 1.11.1.7] is an enzyme of the peroxidase class that plays an important role in lignification, ethylene biosynthesis, or defence against pathogens and ROS **(Mika and Lüthje, 2003)**. In the present paper, we have demonstrated that 130-mT MFS has a beneficial influence on GPOX activity, which was manifested by a significant increase in GPOX activity by 44% in the bean leaves. Similarly, **Atak *et al*. (2007)** observed a significant increase in GPOX activity in soybean (*Glycine max* L.) leaves exposed to 2.9-4.6 mT MFS for 2.2 s and 19.8 s, but a better effect was obtained for the longer exposure time. Moreover, 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 peroxidase significantly **(Maziah *et al*., 2012).** The increase in the GPOX activity that we noted in the bean (*Phaseolus vulgaris* L.) leaves may suggest increased potential of the plant defence system **(Jouni *et al*., 2012)**. Furthermore, the studies carried out by **Shabrangi *et al*. (2011)** showed that magnetic field stimulation (60 Hz, 3mT and 10 mT) increased the activity of other antioxidant enzymes, i.e. ascorbate peroxidase (APX) and superoxide dismutase (SOD) in maize (*Zea mays*) roots and shoots. SOD and CAT activity were also intensified in *Glycine max* (L.) seedlings due to the treatment with magnetic field **(Çelik *et al*., 2009).**

CONCLUSION

In conclusion, our results presented in this paper indicate that the 130-mT magnetic field stimulation promoted mitotic activity in meristematic cells in bean (*Phaseolus vulgaris* L.) roots. The development of the aboveground plant parts was slower, but at the control level. Similarly, the assimilation pigment content and GPOX activity in roots and leaves remained at the control level. However, there was a marked enhancement of GPOX activity in leaves after 130-mT MFS. Our results might be useful to expand the general knowledge about the mechanism of the magnetic field action on plants. Currently, these phenomena are still unclear and require further research.

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