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Original Research

Short-Term Pre-Germination Exposure to ELF Magnetic Field Does Not Influence Seedling Growth in Durum Wheat (*Triticum durum*)

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Abstract

The effects of a short (15 and 30 s) exposure of durum wheat (*Triticum durum*) seeds to extremely low frequency magnetic field (f = 50 Hz, B = 15 mT) on germination and seedling growth under controlled laboratory conditions were studied. Germination rate, fresh weight of seedlings, seedlings height and chlorophyll contents were measured the 4th and 8th day after sowing. Magnetic field did not influence the seed germination process. Fresh weight was affected by applied treatments in the first four days (118% and 89% of control plans for 15 s and 30 s treatment, respectively). In the further period, the action of magnetic field (MF) pre-treatment on growth of seedlings was eliminated, resulting in compensation of growth potentialities. The chlorophyll levels in seedlings were significantly modified, on both the 4th (120% and 87% of control plans for 15 s and 30 s treatment, respectively) and 8th (94% for 15 s treatment and 96% for 30 s) days. However, the chlorophyll a and b ratios remained unchanged after MF treatments. The results show that MF may have, a mostly temporary, negative and positive effect on early growth, which is strongly dependent on the applied exposure time.

Keywords: biological effects of magnetic fields, durum wheat, chlorophyll, germination

Introduction

The mechanism for the stimulating effect of extremely low-frequency magnetic fields (ELF MFs) on seed germination and seedlings development is unknown. Moreover, after years of investigation, experiments and theoretical works, it is still impossible to generate a single dose-response curve. A large number of experimental studies carried out over the last 40 years suggest that even short-term exposure of a plant material to ELF MFs may cause several biological effects [1]. Because ELF MFs are too weak to break chemical bonds in cells, the mechanism of their interaction with living tissue is based on their influence on ongoing biochemical reactions and transport processes [2, 3]. It is assumed that MF interacts with ionic currents in plant cell membranes and changes mainly the transport of calcium ions. Sakhnin [4] applied ELF MF with frequency tuned to the resonance conditions for calcium ions (28.3 Hz), as suggested in the terms of ion cyclotron resonance (ICR) theory [5, 6] and observed in the promotion of germination of bean seeds. Also Smith and co-workers [7] observed an increase of growth rate of radish seedlings when ICR frequencies for calcium and potassium were applied. Thus, the applied frequency is the essential parameter, and the final effect of MF treatment depends on it critically. For example, frequencies up to 160 Hz significantly stimulate the growth on *Zea mays* roots, while when frequencies above 240 Hz were applied, an inhibitory effect was observed [8, 9]. All results show that the effect on growth and germination significantly depends on magnetic

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flux densities, frequencies, plant material and treatment duration. Detailed reviews of other observed effects of MF on plant growth and the physical mechanisms proposed as explanations of the observed phenomena can be found in [10] and [11].

Numerous studies indicate the existence of plant response to short-time MF exposures. Single 20 minute-long exposure to constant MF (250 mT) also significantly increased germination rate and germination percentage of rice [12]. Martinez et al. [13] exposed germinating barley seeds to MF of 125 mT for different exposure periods reported increases in shoot length and weight. De Souza and co-workers [14] reported a positive effect of MF pre-treatments of tomato seeds on root length, fresh and dry root weight, stem length, fresh and dry weight. Moreover, pre-treated seeds produced plants with significantly more fruits with greater fruit weight. Even 2.2 sec exposure to static 2.9-4.6 mT MF increased chlorophyll contents and RNA concentrations in *Paulownia* node cultures [15] and soybean tissue cultures [16], 6.6 seconds-long exposure to 4 mT MF increased the shoot formation rate and fresh weight in regenerated soybean explants [17] and *Paulownia* species [18]. Moon and Chung [19] showed that seeds pre-treated with 60 Hz magnetic field accelerated their germination and the highest germination rates as well as final germination percentages were obtained for the short-time - up to 60s-long exposures.

ELF MF could also reduce or even cancel the negative responses of plants to hostile environmental conditions. Alternating 50 Hz MF pre-treatment reduced the level of biological injuries induced by mutagenic treatments of spring barley seeds with gamma rays or chemomutagens [20] and alleviated the inhibitory effect of heat stress on the growth of cress seedlings [21]. On the other hand, some researchers have noted the negative effects of MF pre-treatments. Seedlings grown from MF pre-treated cucumber seeds were more sensitive to external artificial UV-B radiation, which resulted in a decrease of seedling growth rate and development of pre-treated plants when exposed to UV-B radiation [22]. Short-time exposure (19.8 sec) to MF increased activities of superoxide dismutase and catalase in soybean roots [23].

The aim of the present study was to determine the effects of exposure of dormant seeds to ELF MF on seed germination and seedlings early growth under laboratory conditions. Based on previous works, we considered durum wheat to be a suitable experimental subject for our study [24-26]. Wheat seeds are prone to MF pre-treatment, and the strong influence of MF on many physiological processes of germination is well known [27-31]. Also, the choice of the magnetic field parameters was based on previous experimental results [32].

Experimental Procedures

Seed Material

Real (or botanical) durum wheat (*Triticum durum* cv. Henika) seeds used in this work were supplied by the University of Life Sciences in Lublin, Poland. Moisture content of the seeds was about 10%. Healthy and uniform seeds were hand-selected, counted and divided to 3 groups (960 seeds in each). Each group was subjected to one of the magnetic pre-treatment.

Magnetic Fields Treatment

The pre-sowing magnetic treatments were performed with an electromagnet (Fig. 1). Three coils of electromagnet, assembled from ∅ = 2.2 mm copper wire, were coiled around one part of the iron core. An adjustable air gap was set in the center of the opposite part of the core. The square base of the gap had a side of 100 mm. The coils were connected in series and the multiple-range switch was used to

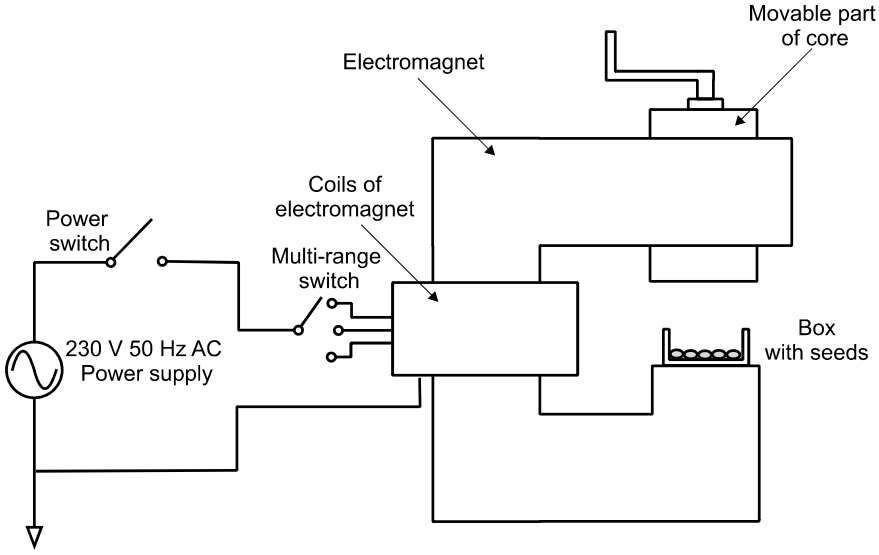

Fig. 1. The electromagnet for the application of magnetic fields. Seeds kept in insulation box are placed in the gap of the core. The movable part of the core and the switch changing the number of attached induction coils are used to generate a magnetic field of particular value inside the gap.

introduce subsequent coils to the circuit. The coils were powered with AC 230 V 50 Hz power supply. When the current passed through the coils, a sinusoidal 50 Hz vertical magnetic field was generated in the air gap in the core. MF flux density was adjusted, both by attaching subsequent coils to the electromagnet's circuit and by shifting an upper movable part of the core using a built-in mechanical crank. The field generated in the gap was measured manually by teslameter (Model TH-27, Aspan, Poland) at 20ºC. The relatively large square base of the gap provided a large volume of uniform magnetic field for exposure of the seeds. The vertical field was uniform to better than 5% to a radius of 8 mm along the middle axes of the gap. Other magnetic fields, except geomagnetism, were undetectable. At a fixed field strength seeds kept in plastic tubes (d = 80 mm) were centrally placed in the gap in the uniform-field region.

The following magnetic pre-treatments were provided:

- D0 – reference (control), seeds not affected by artificial magnetic field;
- D15 – seeds exposed to ELF magnetic field (B = 15 mT, f = 50 Hz) for 15 seconds;
- D30 – seeds exposed to ELF magnetic field (B = 15 mT, f = 50 Hz) for 30 seconds.

After treatment, seeds were immediately transferred to the incubation room, where the germination and growth tests were performed. In compliance with statistical analysis all tests were done in six replications with 100 seeds per replication for germination test and in three replications with 60 seeds per replication for growth analysis.

It should be emphasized that the magnetic field used in our experiments (15 mT) is several orders of magnitude greater than the magnetic fluxes the agricultural objects could be accidentally exposed to during the production process. However, applied MF is still too weak to cause any perceptible physical plant tissue damage [11].

Germination Tests

The tests were carried out in accordance with Polish Standard PN-R-65950 [33]. Progress of germination was recorded inside the germination box. Inside the rectangular 500 × 600 × 100 mm plastic vessel a square 500 × 500 mm Plexiglass plate was seated. At the two sides of the vessel, above the water surface, several openings were made to enable uniform air circulation to the interior space. The vessel was covered with a light-tight Plexiglass lid to obtain complete darkness inside. The vessel was filled with distilled water, without reaching the level of the plate. The surface of the plate was covered with four 700 × 500 mm sheets of filter paper. Filter papers were moistened with distilled water. Along the two sides of the plate, the edges of the filter papers were bent and tucked into the vessel so that all edges of filter papers were immersed in distilled water.

A total of 18 samples (six repetitions for each treatment) of 100 seeds each were placed on filter papers in 100 × 100 mm cells. A completely randomized design for experimental samples was ensured. A constant temperature of 21ºC was assured by placing the germination box inside a ventilated thermal chamber. Temperature readings were taken before each observation, and fluctuations of the mean temperature ranged up to 1°C. The temperature measurements precision was 0.2°C. Seeds were germinated in the dark, except for 5 minutes when they were scored. Prior to counting, the germination box was removed from the chamber. Seeds were examined under diffuse green light provided by a fluorescent lamp (Osram Luminux, 310 lm). Seeds were scored as germinated when the radicle had protruded over 3 mm. Counts were made in two-hour intervals. The germinated seeds were withdrawn as soon as recorded. Seeds were checked for the presence of fungus and rotten seeds were systematically removed. Germination was judged to be complete when no further germination occurred for three successive counts.

The germination dynamics were analyzed with a model based on the Gompertz equation [34]:

$$y = y_{\max} \exp\left[-\exp\left(\frac{t - t_0}{b} \right) \right] \qquad (1)$$

This equation is physiologically well founded and requires only three parameter estimates: final germination percentage y_{max} [%], curve inflection point t_0 [h] and a germination rate parameter b [1/h]. The Gompertz equation was fitted to the cumulative seed germination data for each replication separately using SigmaPlot 9 (Systat Software Inc.).

Growth Tests

For growth analyses, the remaining 360 seeds per treatment were used. Each group was divided into 6 samples of 60 seeds each. Seeds were placed on unrolled cellulose wadding towels along the straight line in the 20 mm distance from the towel's upper edge. Used distance of 10 mm between seeds provided sufficient space for growth. Vertical orientation of seeds with the germ pore facing down was maintained. When finished, the towels were rolled. The close contact between the towel's layers and the seeds was ensured. The obtained rolls were put in upright position and placed, with the lower end down, in the vessels. Dry rolls were moistened with distilled water, which was also poured into vessels. Water in vessels was maintained at such a level that the towel rolls were kept saturated. A vessels' order, with the rolled towels, was rotated daily, although a uniform temperature (21±1ºC) and light in the room were maintained. This way the seedlings with relatively uniform and straight stems were produced. Six tubes of each treatment were prepared.

The experiment was carried out in the presence of artificial light, provided by a white fluorescent lamp (Osram Luminux, 950 lm) for 24 h a day. To avoid the thermal effect, the lamp was placed 200 cm above the experimental rolls.

On the 4th and 8th day three tubes per treatment were selected and rolled out for measuring different indices. Non-germinated seeds were discounted from analysis.

Table 1. Comparison of parameters and standard error estimates of Gompertz model for each treatment.

Treatment	R^2	y_{max} (%)	SE	t_0 (h)	SE	b (1/h)	SE
D0	0.999	84.8	1.3	34.6	0.1	3.6	0.1
D15	0.999	85.4	1.3	34.1	0.1	3.2	0.2
D30	0.999	85.8	2.3	34.8	0.2	4.0	0.3

y_{max}, t_0, b: model parameters (y_{max}: final germination percentage, t_0: curve inflection point, b: germination rate parameter), SE: standard error of estimation, R^2: coefficient of determination.

Length of shoots, as well as fresh weight of the manually detached shoots were determined. A shoot's individual length was measured with 0.1 cm precision. Total fresh weight of all shoots in each roll was measured with analytical balance (Model WAS 160/X, Radwag, Poland) with 0.1 mg accuracy. After the measurements, the shoots were placed inside light-tight plastic boxes and stored inside the freezer at -18ºC for the chlorophylls assays, for less than 12 hours.

Chlorophyll Content

The chlorophyll tests were carried out under green safety light at 21±1ºC. For analysis, both green and etiolated shoots were frozen in liquid nitrogen and ground to powder. Pigments were extracted with 90% acetone. The resulting macerate was centrifuged at 13,000 rpm for 3 min. Spectra were recorded from 400-720 nm with UV-Vis spectrophotometer (Cray 500, Varian, USA). Concentrations of chlorophyll a (chl **a**) and chlorophyll b (chl **b**) were determined according to [35]:

$$chl.\mathbf{a} = \left(12.7 \cdot A_{663} - 2.69 \cdot A_{645}\right) \cdot \frac{V}{1000 \cdot w} \quad (mg/g) \qquad (2)$$

$$chl.\mathbf{b} = \left(22.9 \cdot A_{645} - 4.86 \cdot A_{663}\right) \cdot \frac{V}{1000 \cdot w} \quad (mg/g) \qquad (3)$$

where A_λ is the light absorbance at the wavelength λ (nm), V is volume of the extract (ml), w – weight of fresh seedlings (g). Three repetitions of pigment extraction and spectrophotometric determinations were carried out for each experimental sample. Thus, nine samples per treatment were prepared and examined. Average values and standard deviations were considered for statistical analysis.

Statistical Analyses

All data were tested for normality with the Kolmogorov–Smirnov test and homogeneity of variance with the Levene test. To determine the effect of magnetic pre-treatments compared to the controls, statistical relevance of growth and chlorophylls content data was checked by the analysis of variance (one-way ANOVA) followed by an all pairwise multiple comparison with the post hoc Holm-Sidak test [36]. For germination test, statistical significance of all data was checked by the analysis of the standard deviations and the two-sided Student's t-test. The significant difference at P = 0.05 was used to distinguish treatment differences in the study. All the statistical analyses were performed with SAS 9.1 software (SAS Institute) and SigmaStat 3.11 (Systat Software, Inc.).

Results

Seed Germination

The Gompertz equation was fitted to the experimental, and a good correlation of the fitted curves with the recorded data was found. The parameters of the Gompertz equation for the mean values of germination data of each treatment are shown in Table 1. The effect of MF pre-treatment on the germination of wheat seeds is presented in Fig. 2. The cumulative germination curves represent the mean values of six replicated data. The sprouts began to emerge in the 28th hour after sowing, irrespective of the pre-treatment,

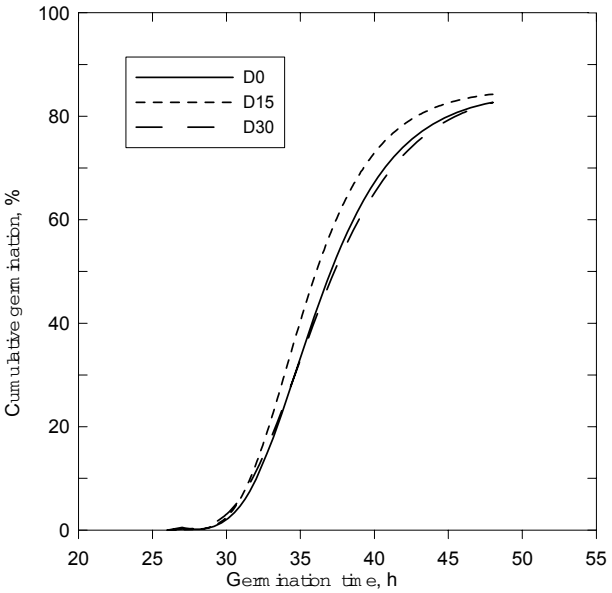

Fig. 2. The germination characteristics of wheat seeds as a function of the germination time for various times of exposure applied. Gompertz equations were fitted to the mean values of 6 repetitions of 100 seeds each per treatment. Exposure times: D15 – 15 s, D30 – 30 s, D0 – control.

Table 2. Effects of 50 Hz magnetic field treatment on morphological indices and chlorophyll contents of wheat seedlings on the 4th and 8th days. Tabled data represent mean values of 3 (seedling height and fresh weight) and 9 (chlorophyll contents) repetitions of 60 seedlings each and their standard errors. Different letters indicate significant differences between treatments (P<0.05, Holm-Sidak test).

Day	Treatment	Seedling height (mm)	Fresh weight (g)	Chl **a** (mg/g)	Chl **b** (mg/g)	Chl (**a**+**b**) (mg/g)	Chl **a**/**b** ratio
4th	D0	40.6 ± 11.9 a	1.69 ± 0.08 a	0.721 ± 0.035 a	0.343 ± 0.047 a	1.065 ± 0.093 a	2.10 ± 0.19 a
	D15	43.0 ± 10.7 a	1.99 ± 0.11 b	0.849 ± 0.026 b	0.431 ± 0.031 b	1.281 ± 0.051 b	1.97 ± 0.10 a
	D30	38.3 ± 11.1 a	1.51 ± 0.08 c	0.616 ± 0.014 c	0.311 ± 0.062 b	0.927 ± 0.086 c	1.98 ± 0.17 a
8th	D0	90.5 ± 17.8 a	4.52 ± 0.24 a	1.478 ± 0.033 a	0.641 ± 0.025 a	2.119 ± 0.031 a	2.30 ± 0.06 a
	D15	93.0 ± 10.7 a	4.56 ± 0.21 a	1.389 ± 0.035 b	0.641 ± 0.029 a	2.004 ± 0.036 b	2.26 ± 0.07 a
	D30	90.8 ± 14.2 a	4.48 ± 0.18 a	1.412 ± 0.039 b	0.612 ± 0.055 a	2.024 ± 0.058 b	2.31 ± 0.12 a

Time of exposure: D0 – 0 s (control), D15 – 15 s; D30 – 30 s. Chlorophyll content is expressed as chl (mg) in a fresh weight (g) of sample.

that is, no acceleration or delay of the germination process was observed. There were no significant changes in the final germination percentage for both treatments when compared to the control seeds. The D15 pre-treated seeds seem to germinate faster, but statistical analysis (Student t-tests performed for data from 32nd, 34th, 36th and 38th hours) showed that differences between D15 pre-treated and control plants during the germination process were not significant (data not shown). In conclusion, there was no difference in the germination process between both treatment variants and control.

Seedling Growth and Development

On the 4th day the fresh weights of seedlings from both pre-treated groups were significantly different in comparison to the control. The fresh weight of shoots grown from the D30 pre-treated seeds (1.51 g) was significantly lower than the same of the control plants (1.69 g). The D15 pre-treatment seeds produced seedlings of the highest amount of fresh mass (1.99 g). Those results were not maintained on the 8th day, when the total fresh weights were similar for both pre-treated and control plants, and were not significantly different (4.52 g, 4.56 g, 4.48 g, for the D0, D15 and D30, respectively).

For all types of pre-treatment, the percentage change of chl **a**+**b** level (20% and 13% for D15 and D30, respectively) was always greater than the change of fresh weight (17% for D15 and 11% for D30), as can be seen in Fig. 3, where the fresh weight and chl **a**+**b** levels data are presented as data values normalized to the control plants. Surprisingly, none of the applied treatments changed significantly neither chl **b** content nor the chl **a**/**b** ratio, which characterizes the relative amount of the two chlorophyll forms. On the 8th day a significance level is reached only when comparing chl **a** content.

Results show that both experimental variants decreased in the concentration of chl **a** when comparing to the control, with 6% and 4%, respectively.

Thus, at the end of the experiment both applied pre-treatments revealed only detrimental effects on content of one photosynthetic pigment, chl **a**.

Discussion of Results

The purpose of this study was to determine whether extremely-short exposure of seeds to ELF MF could trigger off a biological response and influence development of wheat seedlings. Our aim was to find a direct relationship between ELF MF pre-treatment and evoked response of selected physiological indices of seedling development. Although not identical, the observed changes of the targeted

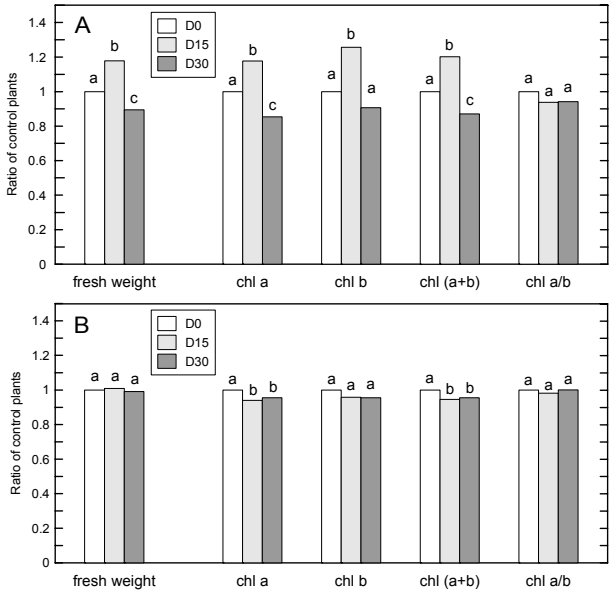

Fig. 3. Weight of shoots, chlorophyll levels and chl **a**/**b** ratio of wheat seedlings on the 4th (A) and 8th days (B), expressed as the ratios of the controls (exposed/control). All bars are normalized for control plants to 1. Different letters in bars show significant differences (P<0.05) according to the Holm-Sidak test.

morphological and physiological indices showed substantial similarities with the previously described physiological responses following MFs treatments.

The organic material of plant tissues has a polar structure resulting from polarized chemical bonds that may, especially in the presence of water molecules, determine its magnetic properties. Thus, recent views of the mechanism of MF action on living systems suggests an involvement of water molecules as a primary receptor of applied MF [37]. Germination of seeds begins with water uptake, followed by many biochemical processes and activation of enzymes. Aksenov and co-workers [29, 31] reported that 30-min. exposure of wheat seeds during their imbibition to an ELF MF caused a still increase in the number of sprouted seeds and increased seedling length. Several other phenomena related to the water uptake process are also observed, like an increase in substance diffusion rate through the seed coat [38], increase in release rate of the products of esterase reaction in the course of swelling of the seeds, and a rapid shift of pH value close to the germ [30]. In our study no significant effect of MF pre-treatment on germination dynamics was observed. This result could be related to applied exposure times, which were short to trigger the direct response of ion channels of dormant seeds to applied MF. The resulted differences in fresh weight of seedlings were not correlated with the differences in shoot heights. This may suggest that applied pre-treatments did not affect growth hormones. However, even 2.2 sec exposure of soybean explants to a static field of 2.9-4.6 mT can affect shoot and root formation rate [17].

This work confirmed the existence of a strong relationship between pre-treatment exposure time and the direction of physiological response of the plant. The results obtained in our study are in accordance with [39], where the 10-min. exposure of grape canes to ELF MF (B = 0.15 mT, f = 50 Hz) increased the weight and length of shoots, while the two-times longer exposure decreased them.

Both chl **a** and chl **b** are associated with light harvesting processes. The quantitative determination of chlorophylls in experimental plant material is recommended as a valuable indicator of plant status under stress conditions.

The results of photosynthetic pigments analysis, especially the fluctuations of chl **a** content and invariable values of chl **b** and chl **a**/**b** ratio, seem to be at variance with several other reports [15-18, 40, 41].

However, there are differences in response of plants for the applied MF even among varieties within the same species, and the same MF could elevate total content of chlorophyll in one variety and, conversely, reliably reduce total chlorophyll content in the second one [17, 42]. Moreover, the interaction of ELF MF and plant response is related with the other applied environmental factors, including light conditions. For example, the resulting effect of permanent exposure to ELF MF of 50 Hz on total content and composition of lipids in radish seedlings was different for seedling grown in light and for seedlings incubated in the dark [43]. This indicates that the mechanisms of ELF MF action on plants could be different in the light and in the dark. Chlorophyll content is strongly affected by light conditions. In our study, seedlings were grown in the constant presence of artificial light, so that the resulting effect of MF pre-treatment on chl **a** level could be related to the response of biochemical reactions involving the light-dependent molecules, particularly MF-sensitive free radicals [11, 41, 44-48].

The changes of chl **a**/**b** ratio could be taken as strong proof of the influence of applied MF on photosynthetic system efficiency [42, 49-52]. Since no significant changes of chl **a**/**b** ratio were observed in our study, the sensitivity of the photosynthetic system efficiency to ELF MF pre-treatment, despite the changes in absolute concentrations of chlorophyll pigments, was not revealed. Therefore, apart from the differences in the development of seedlings caused by applied pre-treatments, the efficacy of the photosynthetic activities seem to remain unaffected.

Our results showed that MF pre-treatment could affect the physiological processes in seeds or plants only in a limited time-period after treatment, which was consistent with many previous studies [12, 53, 54]. The differences in growth rates between both pre-treated and control plants decrease continuously until the 8th observation day and all extraordinary effects of MF pre-treatment are eliminated during further growth. This result corresponds with [55], where the germination percentage of ELF MF pre-treated maize seeds maintained at the increased level only for the first 7 days, and all stimulating effects of MF pre-treatment disappeared on the 14th day. Therefore, at given magnetic field parameters, the effects of MF pre-treatment are strongly determined by the time elapsed since exposure.

Conclusions

The following conclusions have been obtained:

1. An application of alternating magnetic fields of B=15 mT and f=50 Hz on dormant seeds did not influence seed germination, but affected seedling growth. Longer exposure time (30 s) has generally induced slightly negative effects on the phenomena of seedling growth.
2. The resulting differences in fresh weight of seedlings were not correlated with the differences in shoot heights.
3. On the 4th day, for both types of pre-treatment, the percentage change of chl **a** level (increased by 18% and decreased by 15% for D15 and D30, respectively) was always greater than the change of fresh weight (increase by 17% for D15 and decrease by 11% for D30 when compared to the control plants).
4. On the 8th day, total fresh weights were similar for both pre-treated and control plants, and were not significantly different. A significance level is reached only when comparing chl **a** content (decrease by 6% and 4%, for D15 and D30 experimental variants, respectively).
5. Magnetic fields influence chlorophyll **a** pigment, but none of the applied treatments changed significantly neither chl **b** content nor the chl **a**/**b** ratio.

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