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Treatment of Winter Wheat (*Triticum aestivum* L.) Seeds with Electromagnetic Field Influences Germination and Phytohormone Balance Depending on Seed Size

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Abstract: Electromagnetic field (EMF) and its effect on crop plant growth and their quality parameters is increasingly gaining the interest of researchers in agronomic science. However, the exact mechanism of EMF action in plant cells is still unclear. Among the completely unexplored parameters is the relationship between the EMF effects and the seed size. Thus, the EMF effect was analyzed in winter wheat seeds categorized into two size groups, small and big. The study focused on the germination kinetics, early growth parameters, and phytohormone concentrations (indole-3-acetic acid, IAA and abscisic acid, ABA) in seeds, roots, and coleoptiles after exposure to EMFs (50 Hz, 7 mT) and their controls. EMF exposure resulted in faster germination and the more rapid early growth of organs, especially in big seeds in dark conditions. The faster germination and seedling growth of small seeds in control conditions, and of big seeds after EMF exposure, corresponds largely to the decline in IAA and ABA levels. This study confirms that presowing treatment with an EMF is a promising tool for sustainable seed crop improvement, but detailed studies on the EMF mechanism of action, including phytohormones, are necessary to better control future crop yield, especially considering the factor of seed size.

Keywords: wheat; seed priming; seed size; germination; electromagnetic field; phytohormones

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1. Introduction

Seed crops are important sources of food and feed all over the world, and it is important to ensure the quality of their nutritive value and yield improvement. The size and quantity of seeds are important from an evolutionary point of view for the continuation of plant species. Seed size is genetically regulated and is a result of three different growth programs of the diploid embryo, the triploid endosperm, and the diploid maternal ovule [1,2]. Furthermore, epigenetic and environmental factors are also involved in seed development and affect the final agricultural yield [3]. Big seeds have generally better field performance than small seeds, but reports concerning seed germination, emergence, and growth vary among different plant species [4]. For ages in agriculture, seeds have been selected in the search for possibilities to improve seed viability, vigor, and germinability, and to increase the yield of crops. All these parameters are highly influenced by environmental conditions. Developments in modern agriculture and the promotion of principles of rational and sustainable use of natural resources necessitate finding other methods to improve these parameters.

Presowing treatment of seeds is a common method used in modern agriculture. Various types of magnetic fields (MF) have been applied in agricultural research on plants [5–8].

These MFs include the static magnetic field (SMF) and the alternating magnetic field, which we refer to as the electromagnetic field, EMF, due to its electric component. They have been used as presowing treatments that have shown to be useful for most of the studied crop plants [9]. In addition to the type of magnetic field, the duration of exposure, as well as its intensity, are crucial parameters that contribute to the different effects of the SMF and EMF. Moreover, the effect of MFs on plants depends on the plant species and genotype, their physiological state, and environmental conditions [10–12].

A great deal of research has been carried out investigating how the SMF and EMF impact important plant functions. Results from various studies show that the application of different MF frequencies aids in the development of plants such as potato [13] and maize [14]. The use of presowing MF treatment also appears to have a positive effect resulting in: better growth and yield of tomato plants [15]; improved growth rates of the leaves, roots, and bulbs of onions [16]; the enhanced germination and α-amylase activity of rice seeds [17]; and the increased growth and yield, as well as improved general productivity, of soybean [18]. There are several studies in particular which conclude that the use of MFs affects positively the germination rates of seeds [19–21]. The positive effect of the presowing application of MFs on germination and emergence, and the final yield of two varieties of broad beans, have been reported [22].

The exact mechanism of MF action in plant cells is still poorly understood. Currently, it has been proposed that the main entry point for the biological effects of MFs corresponds with specific changes in reactive oxygen species (ROS) and the cytosolic calcium level, which in turn leads to various cellular responses, such as gene expression and enzymatic activities, resulting in final changes in cell metabolism [23]. In addition, the involvement of cryptochromes (blue–light receptors) and the role of auxin signaling in plant growth regulated by MFs have been reported [24]. It is expected that the mechanism of MF action will be different in the case of the SMF and alternating MF [25]. It is already suggested that in the presowing treatment of seeds, a low-frequency EMF may have a positive effect and the application of EMF stimulation may be a good tool for improving the growth and yield of plants for agricultural production [26].

Wheat is a cereal crop that belongs to the Poaceae family and is one of the main crops used for food and feed production in the world [27]. In recent years, the yield of winter wheat has been increasing due to the genetic improvement of cultivars and more intensive agricultural practices [28]. However, due to the expected global demand for food between 2010 and 2050, projected to increase by about 35–56% [29], there is the need to increase the yield of cultivated food crops such as wheat. Additionally, concerns over the environmental impact of intensive agriculture have prompted the search for more sustainable alternative techniques to improve crop yield. Wheat was one of the first plants to be investigated for its response to biomagnetism, and of note is the study in 1963 by Pittman [30] involving the exposure to a MF of 0.065 T. Later studies showed that the exposure of wheat to a weak MF of 16 (2/3) Hz, 20 μT, resulted in a stimulation of the germination rate and root fresh and dry weights [31]. Moreover, the exposure to the SMF with energies of 6217 and 24,868 J/m^3 increased the early growth of wheat [32]. The review articles of Dannehl [33] and Pietruszewski and Martinez [25] provide more information on MF effects on wheat germination, growth, and yield.

Thus, the use of MFs to achieve agronomic goals, such as maintaining seed viability and vigor, ensuring successful seedling emergence, and overcoming seed aging and deterioration, may be a particularly important part of using environmentally friendly techniques to improve crop plant growth. However, before this technique can be widely applied, there is a need to understand the underlying causes of the observed changes. Therefore, the present study was conducted to gain insight into the mechanisms underlying the effect of the physical factor (EMF) on crop plants. The main objective of this study was to examine whether the size of the wheat (*Triticum aestivum* L.) seed can influence the plant response to EMF treatment. The germination kinetics and particular parameters of seed germination in continuous light and dark conditions of the control and treated samples were investi-

gated. Additionally, the effect of the presowing treatment of dry seeds on the growth of the seedlings and the profiles of the level of some selected hormones, indole-3-acetic acid (IAA) and abscisic acid (ABA), have also been investigated.

2. Materials and Methods

2.1. Plant Material

Seeds of winter wheat (*Triticum aestivum*) var. Owacja were received from the IHAR Group (Poland). Undamaged seeds were selected and used for all experiments. The seeds were divided into two groups based on their size (Figure 1): small seeds (length of 5.1–6.0 mm, width of 2.4–3.0 mm, and thickness of 2.0–2.6 mm) and big seeds (length of 6.1–6.9 mm, width of 3.1–4.1 mm, and thickness of 2.7–3.4 mm). A total of 1000 samples each of the small and big seeds weighed 28 g and 43 g, respectively.

Figure 1. Selection of dry seeds of winter wheat (*Triticum aestivum*) based on seed size expressed as length, width, and thickness: (**A**) Small seeds; (**B**) Big seeds. Cross-sections of imbibed seeds 8 h after seeding on moistened filter paper: (**C**) Longitudinal section of a big seed with marked area of cut (gray line), implemented during embryo collection for analysis of phytohormones; (**D**) Longitudinal section of a small seed with marked names of the seed's organs and tissues.

2.2. Exposure to Electromagnetic Field

Before seeding, the seeds kept in falcon tubes were exposed for 24 h to electromagnetic fields, EMFs (50 Hz, 7 mT), generated by a coil of 19 cm (inner diameter) and 21 cm in length ("Elektronika i Elektromedycyna" Sp., Otwock, Poland) (Figure 2A). A detailed description of the set-up has been published by Bienkowski and Wyszkowska [34]. The uncertainty in the homogeneity determination of the EMF within the area containing the grains was approximately 4% (Figure 2B). Measurements of the EMF were made using an RX–25 Teslameter (Resonance Technology, Puszczykowo, Poland). A sham exposure setup was constructed to provide the same experimental conditions of light, temperature, and humidity, but without EMFs (thus, affected by only local geomagnetic field) for the control groups. The temperature during the experiments for both the control and EMF-exposed groups was set at 24 ± 1 °C.

2.3. Germination Assay

Immediately after treatment in the exposure system, the seeds were evenly distributed on Petri dishes (diameter of 9 cm) lined with filter paper and moistened with 3 mL of sterilized deionized water (0.05 μS cm^{-1}). For every experimental variant, three replicates of 50 seeds each were used, with 10 seeds for each Petri dish. The seeds were not sterilized so to avoid any effect of the process on seed germination kinetics [35]. Petri dishes with seeds were kept in cultivation rooms with continuous light (25–28 μmol m^{-2} s) or continuous darkness at 24.5 ± 0.5 °C, with 60% humidity.

Over a 3-day period after seeding (AS), nine time points (0, 4, 8, 12, 16, 20, 24, 48, and 72 h) were selected to observe the germination parameters. The germination process was analyzed by observing: (1) coleorhiza emergence over a period of 0–24 h AS, and (2) radicle emergence of at least 2 mm in length (complete germination) over a period of 0–72 h AS.

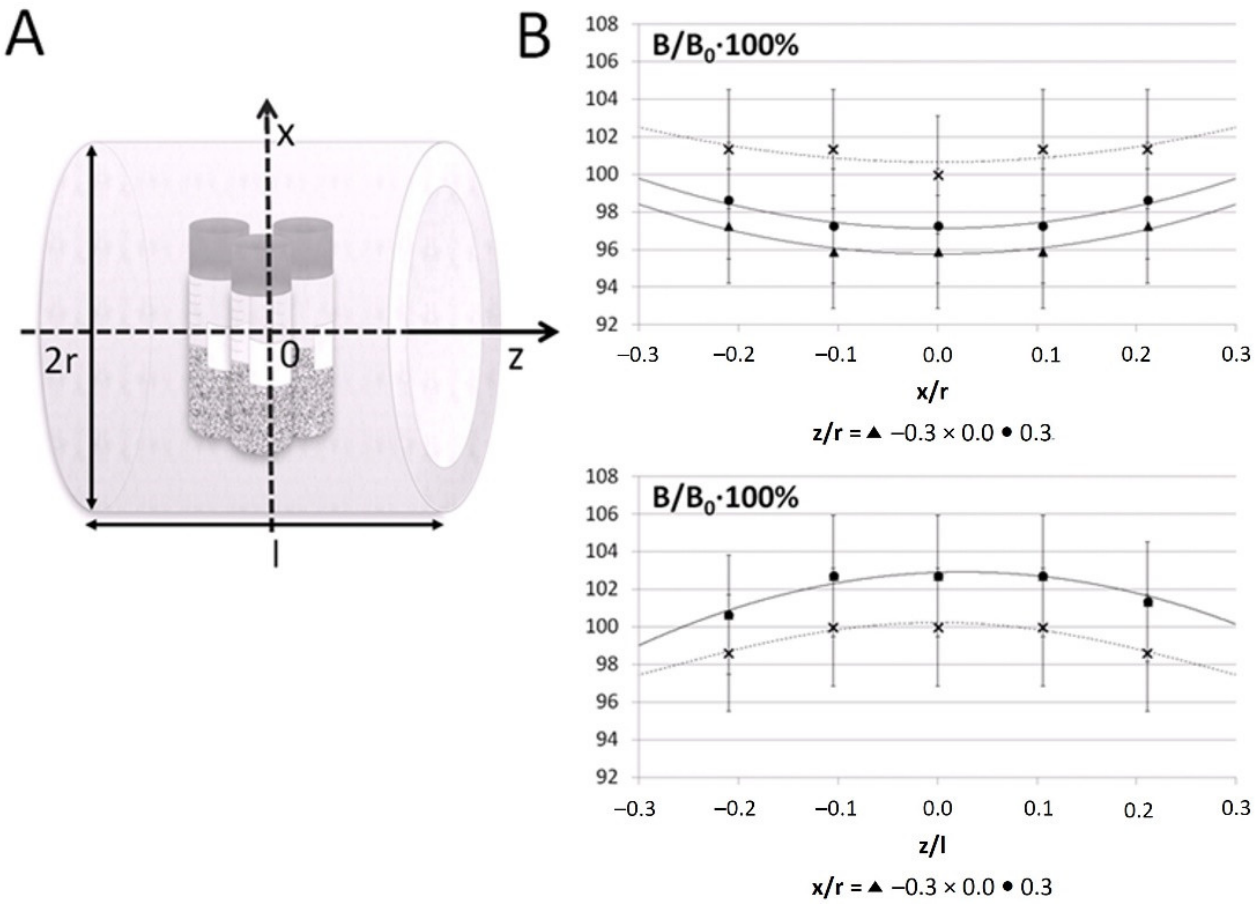


Figure 2. Exposure system: (**A**) The coordinate system; (**B**) The magnetic flux density distribution in solenoid along the *z* and *x* axes. B/B_0 is the normalized magnetic flux density relative to the center point of the solenoid, x/r is the normalized distance from coils center along *x* axis, and z/l is the normalized distance from the coils center along the *z* axis.

To assess the rate of germination process during coleorhiza emergence and completed germination (radicle emergence), six different germination parameters were used. Parameters 1–4 and 6 were calculated following the methodology reported by Ranal and Santana [36], while parameter 5 was calculated using the formula reported by Scott [37]:

$$\text{Germinability, G} = 100\ (N/S) \tag{1}$$

where N is the number of total seeds germinated at the end of the experiment and S is the number of initial seeds used;

$$\begin{gathered}\text{Germination index,}\\ \text{GI} = (|([T_x + 1] - T_1) \times N_1| + |([T_x + 1] - T_2) \times N_2| + \ldots + |([T_x + 1] - T_x) \times N_x|)/S)\end{gathered} \tag{2}$$

where T is a time point in hours until the last hour ($_x$), N is the number of germinated seeds until the last hour ($_x$), and S is the total number of seeds germinated for the experiment at the last time point;

$$\begin{gathered}\text{Coefficient of velocity of germination,}\\ \text{CVG} = 100\ [(N_1 + N_2 + \ldots + N_x)/(N_1\text{T}_1 + N_2\text{T}_2 + \ldots + N_xT_x)]\end{gathered} \tag{3}$$

where N is the number of germinated seeds on the first hour, second hour, and so on, until the last hour ($_x$), and T is the number of hours between seeding and the first count, between seeding and the second count, and so on, until the last ($_x$) count;

$$\text{Mean germination time, MGT} = (N_1T_1 + N_2T_2 + \ldots + N_xT_x)/(N_1 + N_2 + \ldots + N_x) \quad (4)$$

where N is the germination count on any counting period and T is the time point in hours until the last hour ($_x$);

$$\text{Median germination time, the time to reach 50\% of final/maximum germination,} \quad \text{t50} = Ti + [(([N + 1]/2 - Ni) \times (Tj - Ti))/Nj - Ni] \quad (5)$$

where N is the final number of germinated seeds, and Ni and Nj are the total number of seeds germinated in adjacent counts at time Ti and Tj, respectively, when $Ni < N + 1/2 < Nj$;

$$\text{Coefficient of variability of germination time, CV}t = 100\ (st/\text{MGT}) \quad (6)$$

where st is the standard deviation of the germination time and MGT is the mean germination time.

The fresh weight and length of the roots and coleoptiles of the wheat seedlings were estimated directly at the end of the 3rd day of seed germination on Petri dishes. For dry weight estimation, the seedlings were dried in an oven for 48 h at 70 °C.

2.4. Determination of Phytohormones

The concentrations of IAA and ABA were determined in whole seeds and isolated embryos (Figure 1), and also in the roots and coleoptiles of small and big seeds germinating in darkness. The level of phytohormones in the whole seeds was investigated at 0, 8, 16, 24, 48, and 72 h AS and in the embryos at 8, 16, 24, and 48 h AS. The roots and coleoptiles were collected at 72 h AS for analysis. Phytohormones were extracted using a modified QuEChERS-based method [38].

2.4.1. Sample Extraction

During extraction at specific time points, the samples contained either 10 whole seeds, 5 embryos, or 15 roots, and coleoptiles where needed. The tissues were homogenized with this extraction solution: acetonitrile (ACN), formic acid (FA), and double-distilled water (80, 5, 15; ($v/v/v$)). After homogenization, 15 mg of butylhydroxytoluene (BHT) and 10 ng/mL of deuterated internal standards, D_6–ABA, and D_2–IAA (OlChemim s.r.o., Olomouc, Czech Republic), were added to the extraction solution. After overnight incubation at 8 °C with continuous shaking, sodium sulfate and $NaCl/MgSO_4 \times 7H_2O$ (1/3 (m/m)) were added to the solution and mixed. The samples were centrifuged for 10 min at 10,000× g. The obtained supernatants were collected for the purification step. The supernatants were dehydrated under nitrogen stream, and afterwards, 1 mL of FA (1 M) was added.

2.4.2. Sample Purification

All samples were purified using Discovery DSC-18, polymer-based SPE cartridges (3 mL) obtained from Supelco (Supelco, Darmstadt, Germany). The cartridges were first washed with 2 mL of 100% methanol and then equilibrated with 4 mL of FA (1 M). After loading the tissue sample, the cartridges were rinsed with 2 mL of FA (1 M) alone and with a mixture of 2 mL of FA (1 M) and 20% (v/v) methanol. The fraction containing the analytes was eluted with 0.5 mL of 80% methanol (v/v). The samples were then dehydrated using CentriVap Centrifugal Concentrators (Labconco Corporation, Kansas City, MO, USA). The obtained pellets were resuspended in 45% methanol (v/v). The samples were stored at −20 °C until analysis. For liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis, 10 µL portions of each sample were used.

2.4.3. Sample Analysis

The total ABA and IAA concentration was determined in triplicate using LC–MS/MS Nexera UHPLC and LCMS–8045 integrated system (Shimadzu Corporation, Kyoto, Japan). The ionization source parameters were optimized in positive ESI mode using pure ABA and IAA dissolved in HPLC-grade water (Sigma-Aldrich, Darmstadt, Germany). Samples were separated using a reversed-phase C18 column (150 × 2.1 mm, 2.6 µm, Kinetex) in 10% methanol with 0.1% (v/v) FA (solvent A = water with 0.1% (v/v) FA; solvent B = methanol with 0.1% (v/v) FA), at a flow rate of 0.4 mL/min. The interface voltage was set at 4.0 kV for positive (ES+) electrospray. Data acquisition and analysis were performed with the LabSolutions workstation for LCMS–8045.

2.5. Statistical Analysis

The statistical analysis of germination kinetics and other germination parameters, as well as the phytohormones, was conducted using two-tailed *t*-test. Seedling growth parameters were analyzed with one-way ANOVA, Tukey's test. For all analysis, the PAST 4.0 program was used [39], with the interval of confidence being 95% ($p < 0.05$).

3. Results

3.1. EMF Effect on Germination under Continuous Light and Continuous Dark Conditions

Continuous light and dark conditions were used to compare the potential impact of light as a factor on plant response to EMFs. The germination process was analyzed separately for coleorhiza emergence (0–24 h AS) and for radicle emergence (complete germination, 0–72 h AS). The existence of the two main emergence events happening during the germination of wheat caryopsis, whereby the emergence of the coleorhiza is the first visible step of the germination process, prompted the research team to divide the germination assay into these two phases. Coleorhiza emergence is in turn followed by coleorhiza rupture and the completion of germination by visible radicle emergence. The coleorhiza epidermis would have produced epidermal hairs at the time when the primary root breaks the coleorhiza (Figure 3). Moreover, coleorhiza is the first embryonic organ that comes into contact with water during seed imbibition, and thus is likely to be the first in perceiving other environmental factors [40], including EMFs. In addition, analyzing coleorhiza emergence could be crucial because it also acts as a barrier to germination in endospermic seeds, and therefore is proposed to play a major role in controlling the seed dormancy of cereal and other grasses [40,41].

The analysis of the germination process of wheat seeds have proven that there is a difference between small and big seeds in the speed of their early (coleorhiza emergence) and subsequent (radicle emergence) stages of the germination process. When considering the emergence of the coleorhiza and radicle, the results showed that, in control conditions, small seeds germinate faster than big seeds in both light and dark conditions (Figure 4A–D).

In the control samples, the speed of germination of the small seeds was significantly faster than that of the big seeds: 62% faster at 16 h AS during coleorhiza emergence in dark conditions (Table 1); 41% and 27% faster at 20 h and 24 h, respectively, during radicle emergence in continuous light; and 34% faster at 24 h AS during radicle emergence in continuous dark conditions (Table 2).

When the influence of different light conditions was analyzed, it was found that, when considering radicle emergence in control conditions, big seeds germinated faster in continuous light compared to darkness at 24 h AS (Table 2).

After EMF exposure, faster coleorhiza emergence was observed in both light and dark conditions compared to the controls (Figure 4A,C). In continuous light conditions, the speed of coleorhiza emergence in big seeds increased by 8% at 20 h AS, while in dark conditions, it increased by 44% at 12 h AS and by 47% at 16 h AS. The condition of radicle emergence (complete germination) in seeds exposed to EMFs was similar to that of coleorhiza emergence. In light and dark conditions, seeds treated with EMFs reached complete germination faster than the untreated controls (Figure 4B,D). The significant

improvement in the speed of radicle emergence was detected again for big seeds, but only in dark conditions (faster by 44% at 20 h AS and 16% at 24 h AS), and for the first time for small seeds in light conditions (faster by 2% at 48 h AS).

Germinating seeds require favorable environmental conditions, including a proper amount of water, temperature, and sometimes light [42], and the final results of germination are mostly expressed in terms of the germination rate and speed. However, from an agronomic, planning, or physiological perspective, other additional parameters of germination are frequently presented [43]. Therefore, in this study, six different germination parameters were analyzed for the coleorhiza and radicle emergence of small and big seeds in control conditions and after exposure to EMFs (Tables 3 and 4).

Figure 3. Morphological characteristics of big seeds of winter wheat (*Triticum aestivum*) during the first 24 h of germination, after seeding (AS) on moistened filter paper, illustrating the visible emergence events: (**A**) Dry seed at 0 h AS; (**B**) Imbibed seed with an expanded embryo at 4 h AS; (**C**–**E**) Seeds with emerged coleorhiza between 8–12 h AS; (**F**) Seed with emerged coleorhiza nearing completion of the germination process, between 12–16 h AS; (**G**) Completely germinated seed with emerged radicle visibly rupturing the coleorhiza, at 16 h AS; (**H**) Completely germinated seed with well-visible emerged radicle of at least 2 mm length, between 20–24 h AS.

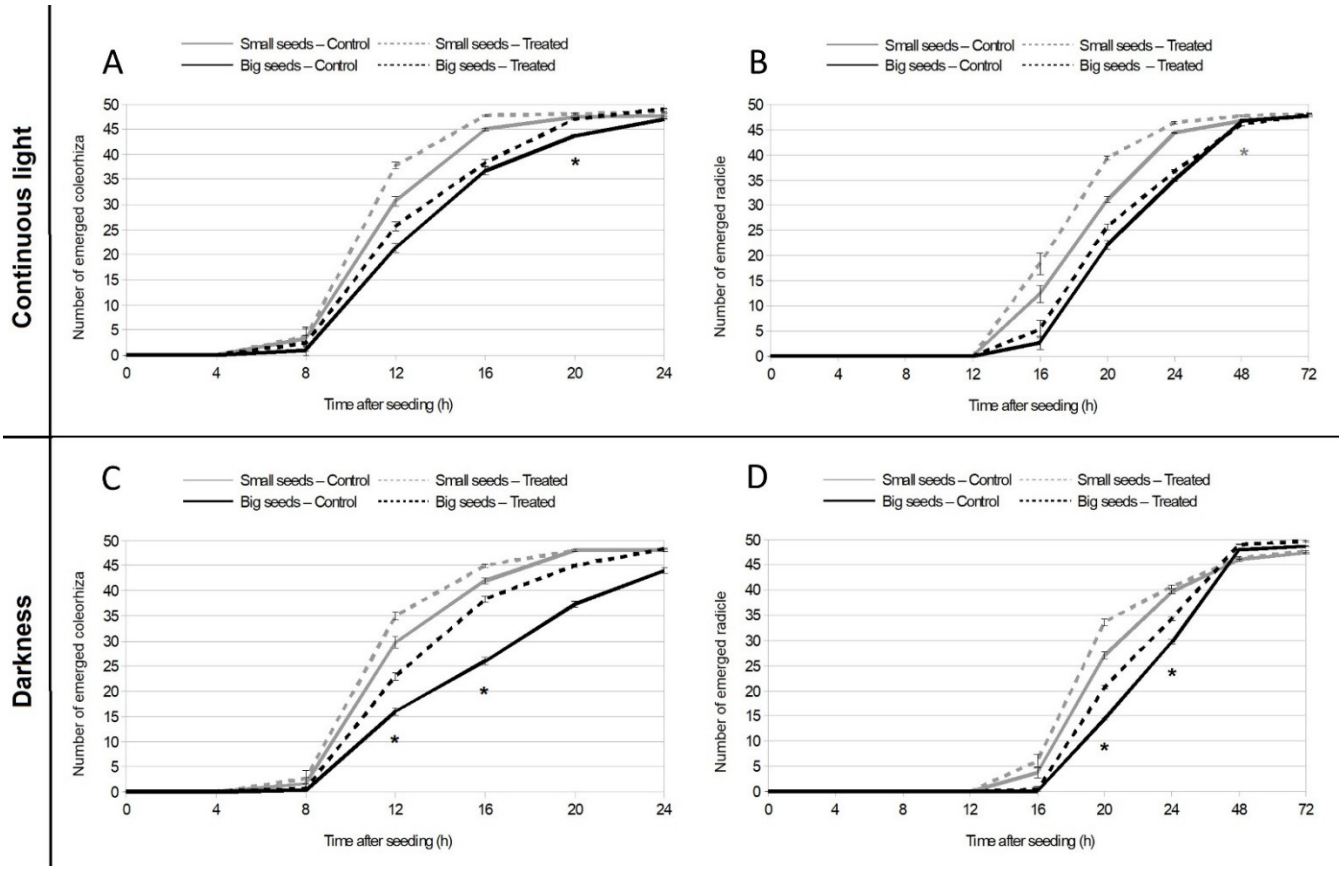


Figure 4. The germination kinetics, in continuous light and darkness, of coleorhiza emergence (**A**,**C**) and radicle emergence (complete germination) (**B**,**D**) of small and big seeds, in control conditions and after EMF treatment. Data are shown as mean values (n = 3), and bars represent standard error ± SE. Gray and black asterisks (*) indicate significant differences (two-tailed t-test) between controls and EMF treatment at $p < 0.05$ for small and big seeds, respectively. Fifty seeds were the total sample size (100%) for each repetition.

Table 1. Effect of seed size of winter wheat and light conditions (continuous light and darkness) on the kinetics (expressed as time in hours) of coleorhiza emergence during germination of control samples (without EMF treatment).

Time after Seeding (h)	Coleorhiza Emergence—Continuous Light		Coleorhiza Emergence—Continuous Darkness	
	Small Seeds—Control	Big Seeds—Control	Small Seeds—Control	Big Seeds—Control
4	0 ± 0	0 ± 0	0 ± 0	0 ± 0
8	3 ± 1.83 a	1 ± 1 a	2 ± 1.29 a	0 ± 0.58 a
12	31 ± 0.97 a	21 ± 0.92 a	30 ± 1.18 a	16 ± 0.75 a
16	45 ± 0.31 a	37 ± 0.81 ab	42 ± 0.56 a	26 ± 0.74 b
20	47 ± 0.1 a	44 ± 0.2 a	48 ± 0.14 a	37 ± 0.61 a
24	48 ± 0.05 a	47 ± 0.08 a	48 ± 0.14 a	44 ± 0.57 a

Note. Data are shown as mean values (n = 3) ± standard error (SE). Different letters indicate significant differences at $p < 0.05$ (two-tailed t-test).

Assessing the germination parameters of seeds in control conditions confirmed the faster rate of germination of small seeds compared to big seeds. Significant differences in the selected germination parameters between the small and big seeds were detected for both emergence events.

Table 2. Effect of seed size of winter wheat and light conditions (continuous light and darkness) on the kinetics (expressed as time in hours) of radicle emergence (complete germination) during germination of control samples (without EMF treatment).

Time after Seeding (h)	Radicle Emergence—Continuous Light		Radicle Emergence—Continuous Darkness	
	Small Seeds—Control	Big Seeds—Control	Small Seeds—Control	Big Seeds—Control
4	0 ± 0	0 ± 0	0 ± 0	0 ± 0
8	0 ± 0	0 ± 0	0 ± 0	0 ± 0
12	0 ± 0	0 ± 0	0 ± 0	0 ± 0
16	12.33 ± 1.77 a	2.67 ± 1.34 a	3.67 ± 0.97 a	0 ± 0 a
20	31 ± 0.58 a	22 ± 0.86 b	27 ± 0.68 ab	14.33 ±0.23 b
24	44.33 ± 0.13 a	35 ± 0.26 b	39.67 ± 0.37 ab	29.67 ± 0.43 c
48	46.67 ± 0.13 a	46.67 ± 0.13 a	46 ± 0.23 a	48 ± 0.22 a
72	47.67 ± 0.17 a	47.67 ± 0.13 a	47.33 ± 0.13 a	48.67 ± 0.13 a

Note. Data are shown as mean values (n = 3) ± standard error (SE). Different letters indicate significant differences at $p < 0.05$ (two-tailed t-test).

Table 3. EMF effects on germination parameters for coleorhiza emergence during germination of small and big seeds of winter wheat, in control conditions, and after EMF treatment.

Germination Parameters	Coleorhiza Emergence—Continuous Light				Coleorhiza Emergence—Continuous Darkness			
	Small Seeds—Control	Small Seeds—Treated	Big Seeds—Control	Big Seeds—Treated	Small Seeds—Control	Small Seeds—Treated	Big Seeds—Control	Big Seeds—Treated
G (%)	95.34 ± 0.05 a	96.66 ± 0.17 a	94 ± 0.08 a	98 ± 0.08 a	96 ± 0.14 a	96 ± 0.22 a	88 ± 0.57 a	96.66 ± 0.13 a
GI (h)	11.69 ± 0.25 a	12.54 ± 0.18 a	9.72 ± 0.27 b	10.25 ± 0.27 c	11.09 ± 0.25 a	11.89 ± 0.19 a	8.19 ± 0.15 b	9.84 ± 0.2 c
CVG (%/h)	7.58 ± 0.18 a	8.07 ± 0.14 b	6.58 ± 0.14 ac	6.82 ± 0.15 c	7.24 ± 0.16 ab	7.67 ± 0.13 c	5.96 ± 0.06 a	6.62 ± 0.1 b
MGT (h)	13.31 ± 0.23 a	12.46 ± 0.18 a	15.28 ± 0.22 b	14.75 ± 0.22 c	13.91 ± 0.23 a	13.11 ± 0.18 a	16.81 ± 0.1 b	15.16 ± 0.16 c
t50 (h)	11.09 ± 0.21 a	10.34 ± 0.13 a	12.87 ± 0.30 a	12.33 ± 0.3 a	11.62 ± 0.29 a	10.77 ± 0.15 a	14.67 ± 0.17 b	12.57 ± 0.23 c
CVt (%)	22.1 ± 0.19 ab	18.99 ± 0.12 a	25.52 ± 0.22 b	25.8 ± 0.65 ab	21.17 ± 0.26 a	19.35 ± 0.74 ab	27.86 ± 0.21 b	25.57 ± 0.36 b

Note. Data are shown as mean values (n = 3) ± standard error (SE). Different letters indicate significant differences at $p < 0.05$ (two-tailed t-test).

Table 4. EMF effects on germination parameters for radicle emergence (complete germination) of small and big seeds of winter wheat, in control conditions, and after EMF treatment.

Germination Parameters	Radicle Emergence—Continuous Light				Radicle Emergence—Continuous Darkness			
	Small Seeds—Control	Small Seeds—Treated	Big Seeds—Control	Big Seeds—Treated	Small Seeds—Control	Small Seeds—Treated	Big Seeds—Control	Big Seeds—Treated
G (%)	95.34 ± 0.17 a	96 ± 0.14 a	95.34 ± 0.13 a	96 ± 0.08 a	94.66 ± 0.13 a	95.34 ± 0.19 a	97.34 ± 0.13 a	99.34 ± 0.05 a
GI (h)	50.49 ± 0.18 a	52.77 ± 0.12 a	44.19 ± 0.21 b	44.95 ± 0.19 b	46.99 ± 0.23 ac	48.06 ± 0.26 a	40.45 ± 0.2 b	42.97 ± 0.21 c
CVG (%/h)	4.47 ± 0.13 a	4.96 ± 0.09 a	3.49 ± 0.09 b	3.58 ± 0.09 b	3.87 ± 0.12 a	4.05 ± 0.15 a	3.08 ± 0.07 b	3.34 ± 0.08 a
MGT (h)	22.51 ± 0.27 a	20.23 ± 0.19 a	28.81 ± 0.26 b	28.05 ± 0.24 b	26.01 ± 0.31 ac	24.94 ± 0.36 a	32.55 ± 0.23 b	30.03 ± 0.25 c
t50 (h)	18.94 ± 0.19 ab	16.64 ± 0.24 a	20.47 ± 0.16 b	19.90 ± 0.18 b	19.60 ± 0.1 ab	18.68 ± 0.1 a	22.84 ± 0.07 c	21.42 ± 0.12 bc
CVt (%)	31.43 ± 0.65 a	28.06 ± 0.95 a	49.51 ± 0.23 a	55.75 ± 0.77 a	40.25 ± 0.16 a	40.12 ± 0.32 ab	44.58 ± 0.2 ab	47.04 ± 0.12 b

Note. Data are shown as mean values (n = 3) ± standard error (SE). Different letters indicate significant differences at $p < 0.05$ (two-tailed t-test).

Under the control conditions and continuous light, the small seeds, compared to the big seeds, obtained the following germination parameters during coleorhiza emergence: 15% lower MGT and 20% higher GI. Similarly, in darkness under the control conditions, the measured germination parameters during coleorhiza emergence of small seeds compared to big seeds were as follows: 21% lower MGT; 26% lower t50; 7% lower CVt; and 35% higher GI (Table 3). Under control conditions and continuous light, small seeds during radicle emergence performed significantly better than the big seeds in the following parameters: 28% lower MGT; 14% higher GI; and 28% higher CVG. Under control conditions, small seeds were significantly better than big seeds during radicle emergence in darkness in the following germination parameters: 25% lower MGT; 17% lower t50; 16% higher GI; and 26% higher CVG (Table 4). These results proved again that small seeds germinate faster than big seeds, which could be the reason for further differences in the germination response of small and big seeds to the EMF treatment.

EMF exposure had a stimulating effect on the selected germination parameters of the wheat seeds. During coleorhiza emergence, significant stimulations were detected for the small and big seeds in light and dark conditions. However, as seen in Table 3, the big seeds in dark conditions compared to the controls were stimulated the most as follows: 20% increase in GI, 1% increase in CVG, 10% reduction in MGT, and 14% reduction in t50. During radicle emergence, significant stimulations were detected only for big seeds in dark conditions (Table 4), where GI and CVG were 6% and 8% higher, respectively, and MGT was 8% lower in big seeds exposed to EMF compared to the untreated controls. When the germinability (G) parameter was investigated, no differences between the EMF-treated and control samples were found (Tables 3 and 4). This, in turn, supports the need for the analysis of the additional germination parameters conducted in this study.

3.2. IAA and ABA Concentration in Germinating Small and Big Seeds, in Control Conditions, and after EMF Treatment

Plant growth reflects responses to multiple internal and external signals, including the net effect of all plant hormones. Each of these hormones participates in particular aspects of seed development and germination [44]. The concentrations of the two phytohormones, IAA and ABA, were analyzed quantitatively during the germination of treated and control samples of both small and big seeds. Additionally, whole seeds and isolated embryos were investigated separately for their IAA and ABA concentrations. Since most of the significant differences in germination kinetics were detected in continuous dark conditions (Figure 4 and Table 4) when analyzing the effect of different seed sizes and treatment with the control, the hormonal analysis was performed only for germination in the dark conditions. The measurement of the IAA levels in the control conditions showed again that small and big seeds differ and express distinct fluctuations of the IAA concentration during the germination process. Significant differences in IAA levels were observed between whole small and big seeds at 8 and 24 h AS (Figure 5A). At 8 h AS, the amount of IAA in the small control seeds was 24% higher than in the big seeds. Conversely, at 24 h AS, the big seeds contained 105% more IAA than the small seeds in the control samples. In isolated embryos, even more pronounced differences in the IAA concentration between small and big control seeds were observed in the control samples (Figure 5B). At the time points of 24 h and 48 h AS, the embryos of big seeds expressed IAA levels significantly higher than that of embryos of small seeds by 118% and 427%, respectively. These results justified the need for the separate analysis of the whole seeds and isolated embryos, which do not contain a large amount of storage tissue. Within the control sample, the gradual decline of the IAA level measured from 8–72 h AS in the small whole seeds and the relatively low IAA amount from 8–48 h of germination in the small isolated embryos (Figure 5A,B) can serve as a biochemical mechanism to describe the faster germination of the small seeds (Figure 4, Tables 3 and 4).

After the EMF treatment, the small and big seeds reacted differently, with changes in IAA levels in the whole seeds and the isolated embryos. In the whole small seeds, EMF exposure caused a significant decrease in the IAA concentration compared to their controls during the germination period of 8–72 h AS (Figure 5A). The biggest declines of 32% and 47% were observed at 8 h and 16 h AS, respectively. The IAA response to EMFs in the samples of whole big seeds was different from that of whole small seeds at the beginning of the germination process. At 8 h AS, EMFs caused a strong and significant increase of 36% in the IAA level in the whole big seeds compared to their untreated control (Figure 5A). At 16 h and 24 h of germination, a significant decrease in the IAA levels in the whole big seeds exposed to EMFs was observed. The IAA level in the small isolated embryos after the EMF treatment did not change significantly during 48 h of germination (Figure 5B). On the other hand, the changes in the IAA level after exposure to EMF in the big isolated embryos followed the pattern of EMF response of the whole big seeds and were more pronounced (Figure 5B). At 8 h AS, the EMF treatment caused a significant increase in the IAA level in the isolated embryos from the big seeds compared to their control. However,

during the subsequent hours of germination (16–48 h AS), the amount of IAA in the treated samples of the isolated embryos from the big seeds was lower than their control, with a 69% reduction recorded at 24 h AS. Moreover, the observed high concentration of IAA at 48 h AS in the isolated embryos from the big seeds (115–220 ng/g), compared to the whole seeds (less than 50 ng/g), both in the control and treated samples, is intriguing, and once more strongly reflects the importance of separate analysis of whole seeds and isolated embryos.

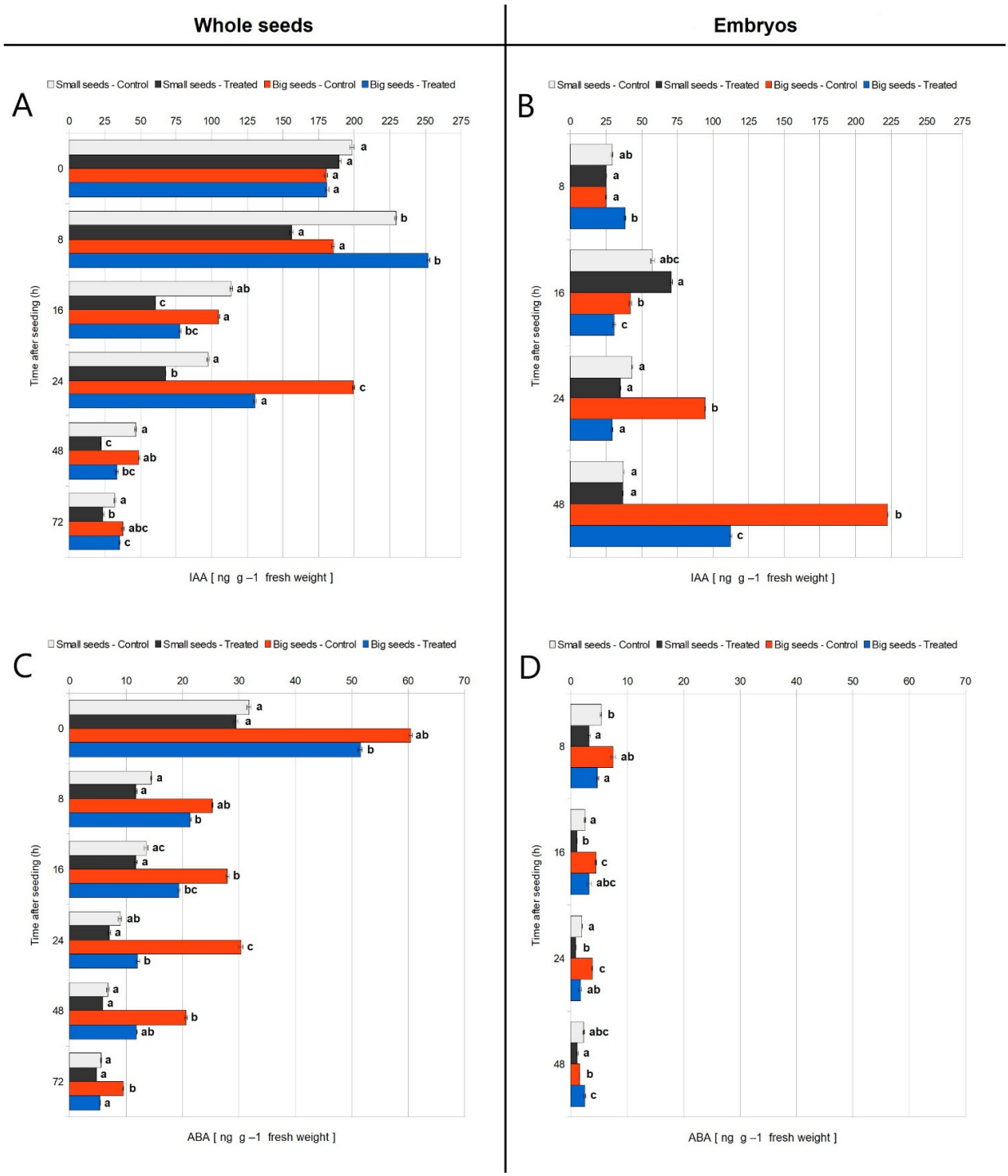


Figure 5. The amount of IAA and ABA phytohormones in control and EMF-treated samples of whole seeds (**A**,**C**) and isolated embryos (**B**,**D**) of small and big-sized winter wheat seeds germinating in darkness. Data are shown as mean values (n = 3), and bars represent standard error ± SE. Different letters above the bars indicate significant differences at $p < 0.05$ (two-tailed t-test).

The concentration of ABA, when analyzing the control samples, was found to be higher in the dry whole seeds (small and big) before seeding, and gradually declined in the next 72 h of germination (Figure 5C). This result confirms the necessity of reducing the ABA levels to achieve the successful germination of wheat caryopsis. Moreover, based on the detected changes in the ABA level during germination in the whole seeds, the differences between the small and big seeds were repeatedly observed. In the control samples, the level of ABA in big whole seeds during the entire germination period was at least two times higher than in the small whole seeds, and the biggest difference of about a threefold increase was noted at 24 h AS (Figure 5C). In contrast, the amount of ABA in the isolated embryos of the small and big seeds was 4–6 times lower than in the whole seeds, and remained at a similar level during the germination process. However, significantly higher levels of ABA were still detected in the embryos of the big seeds compared to the embryos from the small seeds at 16 h and 24 h AS (Figure 5D). Taking into account that ABA is considered as a general inhibitor of the germination process, the lower level of this hormone in the small seeds can be responsible for the observed faster germination of those seeds compared to the big seeds in the control conditions (Figure 4; Table 4).

Subsequently, the EMF treatment led to a decline in the ABA levels in the studied whole seeds and the isolated embryos of both seed sizes (Figure 5C,D). These reduction in the ABA levels were significant in the big whole seeds and the isolated embryos of both the small and big seeds. The biggest reduction in the ABA level in response to the EMF treatment was observed at 24 h AS in the big whole seeds (60%), which occurred also in the isolated embryos of the big seeds at the same time point (Figure 5C,D). The only exception was observed at 48 h AS in the isolated embryos of the big seeds, where the ABA level increased compared to the untreated control. As may be seen, the mechanism of seed response to the EMF exposure expressed in the ABA levels was similar for the big and small seeds, regardless of whether the whole seeds or the isolated embryos were tested, although still more pronounced in the big seeds. This large reduction in ABA levels in the big seeds attributed to the EMF treatment can in turn be a factor for the already noted strong stimulation of the germination rate by the EMF treatment in the big seeds in dark conditions (Figure 4C,D and Table 4).

3.3. Seedling Growth Parameters and Concentration of IAA and ABA in Young Organs of Wheat, in Control, and Treatment Conditions

In addition to the 72 h germination analysis, measurements of the length of roots and coleoptiles (enclosing the first leaf), as well as the fresh and dry weight of three-day-old wheat seedlings growing in continuous darkness, were carried out in control conditions and after exposure to EMFs. Afterwards, the IAA and ABA levels were determined in the roots and shoots.

Comparison of growth parameters between the control samples of the small and big seeds showed that, after three days of seeding, the roots and coleoptiles of the small seeds were 11% and 31% longer, respectively, than that of the big seeds (Table 5). This reveals that the control samples of the small seeds do not only germinate faster than the big seeds, but also show faster early growth. In contrast, the fresh and dry weights of the seedlings of small seeds were, respectively, 19% and 42% significantly lower than that of seedlings of big seeds (Table 5). This contradictory result may be attributed to the difference in weight of the remains of the caryopsis of small and big seeds, which were measured together with the seedlings' fresh and dry weight.

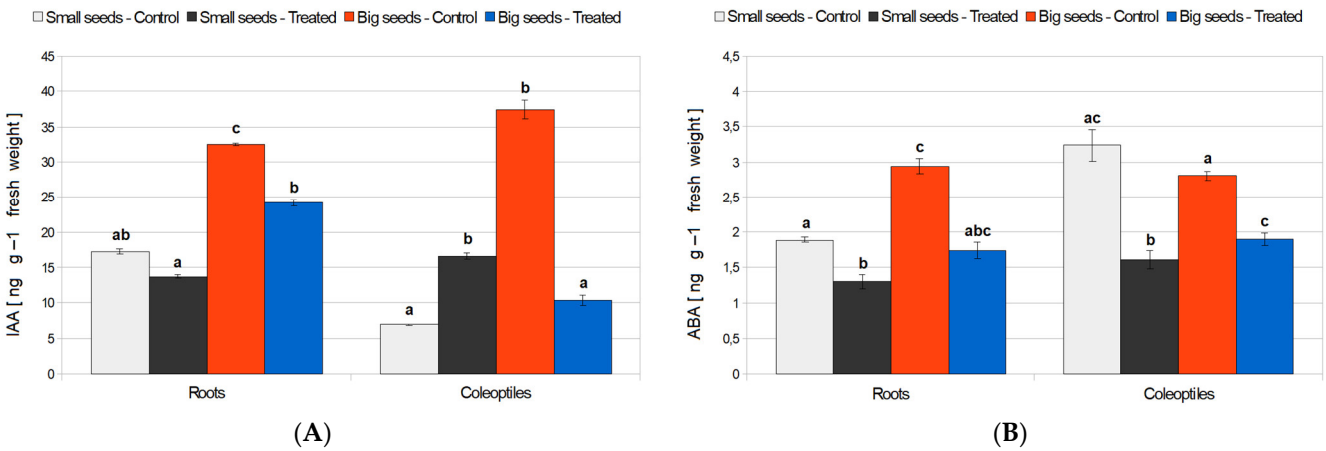
On the other hand, compared to the controls, the EMF treatment led to a significant stimulation of the length of roots (increased by 7%) and coleoptiles (increased by 9%) of the big seeds and the coleoptiles (increased by 1%) of the small seeds. Another observed effect of the EMF exposure is the significant reduction by 4% in fresh weight of seedlings grown from small seeds. The dry mass of the studied seedlings was not affected by the EMF treatment (Table 5). Thus, the strongest stimulation of early growth under the influence of the presowing EMF treatment is related to the organs from the big seeds.

Table 5. Growth parameters of 3-day-old seedlings growing in darkness from small and big seeds of winter wheat, in controls and EMF treatment conditions.

Growth Parameters		Control	Treated
Root length (mm)	Small seeds	71.11 ± 1.45 ac	71.19 ± 1.34 a
	Big seeds	63.94 ± 1.30 b	68.32 ± 1.03 c
Coleoptile length (mm)	Small seeds	34.65 ± 0.65 a	34.85 ± 0.73 b
	Big seeds	26.55 ± 0.82 c	28.84 ± 0.73 d
Fresh weight (mg)	Small seeds	104.11 ± 1.63 a	100.32 ± 1.44 b
	Big seeds	128.70 ± 1.78 c	132.76 ± 1.59 c
Dry weight (mg)	Small seeds	24.83 ± 0.08 a	24.06 ± 0.13 a
	Big seeds	42.61 ± 0.08 b	41.66 ± 0.20 b

Note. Data are shown as mean values (n = 3) ± standard error (SE). Different letters indicate significant differences at $p < 0.05$ (one-way ANOVA, Tukey's test).

The level of IAA in roots and coleoptiles growing from the control samples of small seeds was significantly lower (by 47% and 81%, respectively) compared to that of the roots and coleoptiles of the big seeds (Figure 6A). Similarly, within the control samples, the ABA levels in the roots from the small seeds was 36% lower than that measured in the roots of big seeds (Figure 6B). This shows that lowering the level of IAA and ABA correlates with observed faster early growth of roots and coleoptiles of small seeds in control conditions.



(**A**) (**B**)

Figure 6. Determination of endogenous levels of (**A**) IAA and (**B**) ABA phytohormones in 3-day-old roots and shoots (coleoptiles) growing in darkness from small and big seeds of winter wheat under control and treatment conditions. Data are shown as mean values (n = 3), and bars represent standard error ± SE. Different letters above the bars indicate significant differences at $p < 0.05$ (two-tailed t-test).

Additionally, as we have already shown, the faster growth of roots and coleoptiles of the big seeds after the EMF treatment also correlated with a reduction of the IAA levels. In the big seeds, the EMF exposure caused a significant reduction of 26% and 73% in IAA levels in the roots and coleoptiles, respectively (Figure 6A). However, the coleoptiles of small seeds treated with EMFs expressed higher IAA levels than the untreated ones. Moreover, in response to the EMF exposure, the level of ABA significantly reduced in all but one of the tested samples, with the biggest reduction in the ABA level of 50% observed in the coleoptiles of the small seeds (Figure 6B).

4. Discussion

Growing plants are constantly affected by natural and man-made magnetic fields. Technological advancement and the increasing use of different electric devices have greatly increased the level of electromagnetic fields (EMFs) in the environment [23]. Magnetic fields, especially those at the 50 Hz frequency, are the most common type of low-frequency electromagnetic fields found in regions of industrial activities [45]. Low-frequency EMFs, as part of the broad spectrum of EMFs, are currently also used for successful priming of plant materials, including seeds [26]. Magneto-primed seeds express faster germination, emergence, and vigor, which very often translates into better growing plants with higher yields [46]. Earlier studies showed that wheat seeds respond well to biostimulation with alternating magnetic field (50 Hz), but the positive effects of stimulation could be short-lasting, and appear in the initial phase of germination, thereby indicating the need to study more the early stages of germination to better understand the mechanism of EMF action on wheat germination [47,48]. In this paper, we meet this need by examining the influence of EMFs on the early events of coleorhiza and radicle emergence during the germination of winter wheat caryopsis.

Among the very different factors determining a crop's yield is the size of the seeds, which is an important physical indicator of seed quality that affects vegetative growth [4]. As far as we know, the relationship between seed size, seed germination, and phytohormones (ABA and IAA) under EMF treatment has not yet been reported for wheat or other agronomic crops.

Our current study shows that, among the control samples, small seeds of winter wheat var. Owacja germinate faster than big seeds. This is in line with Zareian and colleagues' [4] research on three different cultivars of bread wheat, indicating that the germination rate significantly decreased with increasing seed size, and was connected with the more rapid absorption of water by the small seeds compared to big seeds. In control conditions in our studies, the length of the roots and coleoptiles from the small seeds were also longer than those from the big seeds. This can also be attributed to a faster full rehydration of the small seeds as a result of their smaller content of colloidal molecules (starch and proteins) compared to the big seeds.

In this study, wheat germination in two different conditions of continuous light and continuous darkness were analyzed, showing that among the control samples, big seeds germinated better in continuous light compared to darkness. This could be the result of increased water movements under small heat generated by continuous light, which resulted in a faster full hydration of the seeds and, consequently, faster radicle emergence. For early stages of seed germination, darkness is a more natural condition, but further growth, especially of shoots, requires light. Therefore, studying plant responses in continuous light and darkness could help us to better understand the plant adaptation mechanism to unfavorable conditions.

As described in the results, after exposing the seeds of wheat (big and small) to EMFs (50 Hz, 7 mT) under both light conditions, the strongest stimulation of germination was recorded in the big seeds in dark conditions. This increase in germination speed after treatment could be responsible for the subsequent stimulation of the early growth of roots and coleoptiles of the treated big seeds growing in dark conditions. To the best of our knowledge, the effect of the seed size of wheat on its response to EMF treatment has not been investigated before. However, there are many reports of faster germination of seeds under the influence of different doses of EMF and SMF exposure. It has been observed that the exposure to alternating MFs of 30 and 50 Hz, 30 mT [49], of 50 Hz, 30–90 mT [47], and of 50 Hz, 9–35 mT [48] has a positive influence on the germination of wheat seeds. Moreover, high-frequency EMFs (10 kHz) enhanced the speed of wheat germination [50]. Again, the germination of wheat was accelerated by the exposure to the static magnetic field of 30 mT [51] and of 4 and 7 mT [11]. It has been hypothesized that the MF affects the germination of treated seeds by changing the ionic current through their cellular membranes [52]. Other authors have also pointed out that the biological effects of weak

time-varying EMFs are based fundamentally on cell membrane permeability and ionic changes, especially in the intracellular Ca^{2+} level, resulting in alterations in the osmotic pressure and influencing the capacity of a cell tissue to absorb water [18,53]. The findings of this study show that, in control conditions, big seeds germinate slower than small seeds in light and darkness. At the same time, the EMF treatment resulted in a stronger stimulation of germination in big seeds than in small seeds, which can be due to the fact that biological systems during various phases of intensive growth and/or in suboptimal environmental conditions are prone to exhibit greater sensitivity to magnetic fields [11]. Moreover, it has been pointed out that the germination capacity of spring wheat seeds treated with a 16 Hz (5 mT) alternating MF increases when the beginning of the germination process is delayed [54]. Moreover, it has been indicated that the mechanism of action of a MF can be distinct in various light conditions. A study of the composition of lipids in 5-day-old radish seedlings grown in light and darkness under the influence of MFs (50 Hz, ∼500 μT) showed that the amount of lipids doubled in the MF-treated plants. However, in darkness, the effect of the MF on the lipids content was weaker than in light [45]. It is worth pointing out that the processes of early growth in darkness are significantly accelerated due to the ongoing process of tissue etiolation, causing possible differences in EMF response. In darkness, the possible involvement of cryptochromes in plant tissues magneto–perception is also excluded [50].

Currently, knowledge about hormonal changes in plants in response to the MF is limited. Therefore, this study provides a deeper insight into the hormonal regulation at the various early stages of the germination process, and in relation to the size of seeds and EMF treatment, by examining IAA and ABA changes through the LC–MS/MS method. Our investigation showed that small and big seeds manifest differences in the IAA and ABA content and profile during germination process and early growth, both under control conditions and after the EMF treatment. Faster germination of small seeds observed under control conditions was associated with a decrease in the IAA level from 24 h AS. These changes may be associated with the stimulation of coleorhiza rupture and radicle emergence. Thus, this lower IAA level in the whole small seeds and embryos may indicate the earlier breaking of seed dormancy compared to the big seeds. Currently, it has been proven that coleorhiza plays a major role in controlling seed dormancy in cereals [40,41]. Moreover, IAA has been found in some studies to have an inhibitory effect on the germination of *Arabidopsis thaliana* seeds [55] and the changes in sensitivity to IAA during processes after-ripening in wheat seeds support the role of auxin in seed dormancy [56]. Our current research has also revealed that, under control conditions, the faster growth of root and shoot from small seeds is associated with a reduction in the IAA levels in those organs.

In the control conditions of our study, the ABA level is found to be dropping during the germination process. However, the higher level of ABA is recorded in big seeds rather than in small seeds when examining both whole seeds and embryos. This confirms an earlier study of common wheat seeds showing that the ABA content increases as seed dry weight grows [57]. The role of ABA in maintaining seed dormancy is well documented by genetic and physiological studies [58]. It has been shown in studies on barley that the amount of ABA in coleorhiza is a key factor in controlling seed dormancy and germination [40]. In addition, most ABA biosynthetic genes in bread wheat have been found to be lightly upregulated from 0 to 12 h after imbibition due to the breaking of seed dormancy and strongly downregulated during later germination stages [59]. The notable impact of wheat seed size on the ABA content in seeds and seedlings observed in our study can also be a consequence of larger embryo/endosperm ratio in small seeds compared to big seeds. It has been reported that the endosperm synthesizes and continuously releases ABA towards the embryo [60]. Thus, a smaller endosperm could lead to a lower amount of ABA produced in small seeds compared to the big seeds.

As has been shown in our study, the EMF treatment significantly decreases the IAA levels in the whole small seeds during all 72 h of germination, with the biggest reduction compared to the control occurring at 16 h AS when coleorhiza emergence occurs, and

radicle emergence starts. In big seeds, EMFs significantly increases the IAA level at 8 h AS, both, in whole seeds and embryos. Afterwards, between 16–48 h of germination, the EMF decreases the level of IAA. On the other hand, the ABA level was decreased by the EMF treatment, and the biggest reduction in whole seeds was observed at 24 h AS in the big seeds. Moreover, in the roots and coleoptiles from the small and big seeds, the EMF treatment led to a general decrease in the IAA and ABA levels. The existing literature shows that, under MF exposure, changes in the IAA levels are varied depending on the treatment dose and the plant species, while the ABA levels mostly reduce. The presowing treatment of pea seed with alternating MFs of 50 Hz (30 and 85 mT) caused a significant increase in the IAA level in the germinating seeds (end-point analysis), as well as in young stems and roots [61]. Four days after treatment with radio-frequency EMFs of 5.28 MHz (0.74 mT), the IAA level was found to be higher in dry seeds of sunflower and lower in dry red clover seeds compared to their respective controls, while the ABA level was always lower in these EMF-treated seeds [62,63].

5. Conclusions

In this study we present for the first time the relationship between seed size, stages of the germination process and early organ growth of winter wheat, and phytohormones levels in control conditions and after treatment with EMFs (50 Hz, 7 mT). In control conditions, small seeds germinated faster and express more rapid early growth compared to big seeds, which correlates with the reduction of IAA and ABA levels in small seeds and their organs. The EMF treatment is found to mostly stimulate the germination and early growth of roots and coleoptiles from big seeds in darkness. Moreover, EMF exposure causes characteristic changes of IAA and ABA phytohormones at specific stages of the germination process and in young roots and stems growing from small and big seeds. Stimulation of coleorhiza and radicle emergence and the growth of roots and coleoptiles in treated samples of big seeds is associated with a reduction in IAA and ABA levels at certain time points of germination. Therefore, these changes in IAA and ABA levels can be responsible for the faster breaking of dormancy in small seeds in control conditions and in response to EMF treatment in big seeds of wheat.

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