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ORIGINAL PAPER

INFLUENCE OF Ca²⁺ CYCLOTRON RESONANCE--TUNED MAGNETIC FIELDS ON GERMINATION AND GROWTH OF WHEAT SEEDLINGS*

Krzysztof Kornarzyński, Siemowit Muszyński

Department of Physics University of Life Sciences in Lublin, Poland

ABSTRACT

In this work, the direct influence of magnetic fields on plants is discussed via the ion cyclotron resonance (ICR) theory, the mechanism for the biological action of magnetic fields in the low-frequency region. Wheat seeds (Triticum aestivum) were exposed to a combination of the local geomagnetic field ($B_{\rm DC}$ = 20.9 µT) and a sinusoidal (AC) extremely low-frequency magnetic field $(B_{AC} = 0.1 \text{ or } 0.3 \text{ mT})$ at one of the three different frequencies, one of which was a resonance frequency for the Ca2+ ions. The persistent exposure to combined static geomagnetic and alternating magnetic fields did not alter the germination of wheat seeds, although under the specified conditions of exposure significant differences in the growth of seedlings were observed. The application of the AC field tuned to the ICR frequency for Ca^{2+} ions $(f_{AC} = 16 \text{ Hz})$ did not affect the germination process of wheat seeds but there was an effect on the growth process of the exposed seedlings. The number of emergent seedlings was significantly modified only for B_{AC} = 0.3 mT field (increase of 43%), while the fresh weight of 10-day-old seedlings was increased for both treatments (increase of 85% and 80% for 0.1 mT and 0.3 mT fields, respectively). After the exposure to $f_{AC} = 11$ Hz field, exposed seeds produced seedlings with significantly decreased fresh weight (decrease of 27% and 38% for 0.1 mT and 0.3 mT fields, respectively). Wheat seeds failed to respond to $f_{AC} = 21$ Hz fields in any of the seedling emergence parameters measured.

Keywords: *Triticum aetivum*, effects of magnetic fields on plants, ion cyclotron resonance theory, calcium, spring wheat.

Siemowit Muszyński, PhD, Department of Physics, University of Life Sciences in Lublin, Akademicka 13 st., 20-950 Lublin, Poland; phone.: +48 81 445 69 05, fax: +48 81 533 35 49, e-mail address: siemowit.muszynski@up.lublin.pl

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INTRODUCTION

Living organisms on the Earth have evolved in the presence of only the geomagnetic field (LIBOFF 2010, MAFFEI 2014). However, over the last century, with the introduction of manmade, wide spectrum magnetic fields, this original environment has rapidly changed. These artificial fields were initially considered too weak to interact with biosystems, and thus incapable of influencing their physiological and biochemical functions. However, biological systems, including plants, are capable to react to man-made magnetic fields, and their response is called magnetotropism. It consists of three phases: reception of a magnetic field, its translation to a biochemical signal that is transported to the response cells and, finally, the stress response (MAFFEI 2014, TEIXEIRA DA SILVA, DOBRÁNSZKI 2016). Most of the discussion has focused on the effects of exposure to electric power distribution lines (SOJA et al. 2003), although there are also many sources of electromagnetic fields in modern farms – fields generated by agricultural machinery or in greenhouses and seedbeds. Thus, whether or not man-made magnetic fields can affect plant systems is a biological question with important agricultural production implications (Pietruszewski, Martínez 2015, Teixeira da Silva, Dobránszki 2016).

The effects of man-made, extremely low frequency magnetic fields have been intensively studied but the mechanism of their action on living systems is still unknown. A clear conclusion which can be drawn from numerous laboratory studies is that interactions observed between man-made magnetic fields and living tissue are not based on tissue heating phenomena (ADEY 1993, PAZUR et al. 2006, NOVITSKII et al 2015). The ion cyclotron resonance (ICR) theory was proposed by LIBOFF (2006) as a mechanism in which the physiological ionic activity of particular ions, i.e. Ca^{2+} , K^+ or Zn^{2-} , can be altered by the biological action of magnetic fields in the low frequency region. This model requires that time varying magnetic (B_{AC}) fields be parallel to the static magnetic field (B_{DC}) (LIBOFF 2006). Numerous ICR experiments have been conducted on crop plants, especially on radish (SMITH et al. 1993, 1995, DAVIES 1996, POTTS et al. 1997, YANO et al. 2004), but also on barley (SMITH et al. 1995, PAZUR et al. 2006), mustard (DAVIES 1996), garden cress (KORDYUM et al. 2007) and bean seeds (SAKHNINI 2007). Experimental layouts were mostly designed to satisfy calcium resonance frequencies but some also also tested combined potassium-tuned (SMITH et al. 1995, DAVIES 1996) and magnesium-tuned (SMITH et al. 1995, YANO et al. 2004) magnetic fields. Many of those researchers have reported positive effects on germination or seedlings' growth, while a comparable number have reported negative or no effects.

Choosing calcium as the test ion is an appropriate choice since Ca^{2+} ions are a crucial regulator of growth and development in plants (WHITE 2003, HELPER 2005). Moreover, it is well known that suitable combinations of static and time-varying magnetic fields directly interact with the Ca^{2+} channel protein in the cell membrane and transfer of Ca^{2+} into the cytosol from the extracellular medium (BAURÉUS KOCH et al. 2003). On the basis of the previous studies, we considered common wheat (*Triticum aetivum*) to be a suitable experimental subject of our study (PIETRUSZEWSKI, KORNARZYŃSKI 1999, PIETRUSZEWSKI et al. 2001, KORDAS, 2002, MARTÍNEZ et al. 2002, FISHER et al. 2004, PAYEZ et al. 2013). Wheat seeds respond to magnetic field treatments and magnetic fields are known to produce strong influence on many physiological processes in wheat germination and development is well known (AKSENOV et al. 2000, 2001, ROCHALSKA, GRABOWSKA 2007, PIETRUSZEWSKI, MARTÍNEZ 2015, BALAKHININA et al. 2015). Also, an assessment of the impact of the ICR-tuned systems on wheat is of great importance because it is a major crop plant in the world.

The goal of this study has been to investigate effects of the Ca²⁺ ICR frequency on the germination of common wheat seeds and on the growth and development of seedlings under the impact of the combined AC and DC magnetic fields in laboratory conditions.

MATERIAL AND METHODS

Plant material and experimental design

Botanical common spring wheat (*Triticum aetivum cv.* Henika) seeds used in this experiment were supplied by the University of Life Sciences, Lublin, Poland. To partially synchronize the germination and growth of seedlings, seeds of similar size were selected manually.

Magnetic fields treatments

According to the ICR theory, the physiological ionic activity of particular ions can be altered when the ratio of the applied signal radial frequency ω (rad s⁻¹) of the alternating component to the value of magnetic flux density of the static component B_{DC} (µT) is equal to the ionic charge-to-mass ratio (LIBOFF 2006):

$$\omega/B_{DC} = q/m \tag{1}$$

where q is the charge of the ion (C), m is the mass of the ion (kg). For the given n^{th} harmonic frequency of the AC field, where frequency f measured in hertz (Hz) instead of radial frequency ω is used, and $f_n = (2n + 1)\omega/2\pi$, the ICR assumptions can be written as:

$$f_n = (2n+1)\frac{q}{2\pi m}B_{DC} \tag{2}$$

A basic experiment was performed on seeds exposed to the ICR condition for Ca²⁺ ions. The value of the ICR frequency of the AC field for calcium ions (f = 16.0 Hz) was calculated for the following values: $m_{Ca} = 6.655 \ 10^{-26}$ kg, $q_{\rm Ca} = 3.204 \ 10^{-19}$ C, $B_{\rm DC} = 20.9 \ \mu{\rm T}$ (horizontal component of the local geomagnetic field), n = 0 (fundamental cyclotronic frequency). The amplitude (peak-to-peak) of $\rm B_{AC}$ was set at either 0.1 or 0.3 mT. Both values are below the reference levels for general public exposure to time-varying magnetic fields, while the latter value is close to the threshold value of 0.31 mT (International Commision... 2010). In order to verify the calculated value of $f = 16.0 \ {\rm Hz}$, two additional experimental runs were performed, with the seeds germinating in the presence of the same $B_{\rm AC}$ fields, but at different $f_{\rm AC}$ frequencies. They were placed inside the same coil as the ICR-exposed seeds, but were exposed to $B_{\rm AC}$ fields with f = 11.0 or 21.0 Hz frequency.

The homogeneous B_{AC} magnetic fields were generated inside solenoid coils. Each coil was formed from a copper rod wound around a wooden o-ring core with the internal diameter of $\emptyset = 20$ cm and the length of 15 cm. The relatively large diameter and length of a coil ensured sufficient volume of a uniform B_{AC} magnetic field for exposure of the seeds. The coil generated the magnetic field of desired strength without any appreciable heat rise in the area of the Petri dishes. For each exposure treatment, seeds were placed in Petri dishes ($\emptyset = 12$ cm) and inserted into an appropriate coil cell, on the same plane. The area where Petri dishes were placed had a relatively uniform field distribution, manually measured with a teslameter to accuracy ± 0.01 mT. The control samples, spaced from the experimental ones at a distance of 2.0 m, were exposed only to the ambient geomagnetic field and stray 50 Hz electromagnetic fields. The experiments were conducted under natural light conditions, in an air conditioned room, where constant temperature (20°C) was maintained.

Germination tests

The germination tests were performed on samples of 100 seeds. Each treatment was repeated three times to confirm the replicability of the results. Seeds were placed in glass Petri dishes lined with three layers of filter paper. Before the experiments, the Petri dishes were sterilized for three hours at 150°C. The dishes were moistened with 10 ml of distilled and deionized water and covered with lids. During the experiment, filter paper was kept at an optimal humidity level by adding fresh distilled water to the Petri dish when needed. Petri dishes were positioned in the region of a uniform field, stacked one on the other, in a completely randomized design and the position of the dishes was randomly changed at every count to minimize the positional effect in the coil. The Petri dishes, both in the experimental coils and in the control position, were covered with black cloth to ensure complete darkness. Prior to the counting, the dishes were removed from the coils. Seeds were checked for the presence of fungus and rotten seeds were removed systematically. The breaking of a seed coat and appearance of a root tip (approximately 4 mm long) was scored as positive germination. The germinated seeds were removed as soon as recorded. Once the counting procedure was finished (3-6 min), the dishes were immediately returned to the coils. Germination was judged to be complete when no further germination occurred for three successive counts.

Growth tests

For analyses of the growth of seedlings, the remaining 90 seeds per treatment were used. The analyses were performed in open glass Petri dishes filled with soil. Each dish contained three replications of 30 seeds each, which were spread uniformly inside the dish. The resulting plant density was about one seed per 1.2 cm². The seeds were placed approximately 0.5 cm beneath the surface of the soil, which had been thoroughly rinsed with distilled and deionized water. Additional portion of distilled and deionized water was added every two days to the dishes. To prevent excessive soil water evaporation, Petri dishes were covered with transparent plastic cups. Seedlings were grown under natural light, but exposition to the direct sunrays was strictly avoided. Dishes were turned around every day at 9 a.m. and 6 p.m. so that seedlings with relatively uniform and straight stems were produced. The time of emergence and position of new seedlings were recorded successively until the 10th day, when the fresh and dry weight of the grown seedlings was measured. Not all seeds formed seedlings, and those that did not were expelled from the analysis. Each manually detached shoot was gently shaken to remove any adhering clods of soil and then wiped with a soft cloth. Total fresh weight of all shoots in each replication was measured with analytical balance with 0.1 mg accuracy. Dry weight was determined after 24 h desiccation in a dryer at a temperature of 105°C. It should be noted that the recorded fresh weight values may be slightly underestimated because of the evaporation during the procedures of sample preparation and weighing.

Statistical analyses

The progress of germination and emergence of seedlings was approximated with the three-parameter Gompertz curve (MUSZYŃSKI, GŁADYSZEWSKA 2008, MUSZYŃSKI et al. 2009) fitted to the seed germination and seedling emergence data using TableCurve 2D software (Systat Software Inc., San Jose, USA). One-way analysis of variance (ANOVA) was used to analyze the differences between treatments within each experiment. The Dunnett's test was used for multiple comparison versus the non-exposed control group, and P < 0.05 was considered as statistically significant in this study. All the statistical analyses were performed with Statistica 12 software (Statistica Inc., Tulsa, USA).

RESULTS AND DISCUSSION

Table 1 presents the effects of the combined magnetic fields on the germination of wheat seeds and development of seedlings, showing mean values and their standard deviation for the studied parameters. The results provide strong evidence of the impact produced by the applied B_{AC} frequency plant biosystems. The overall effect of the B_{AC} magnetic field with the calcium

Table 1

AC magnetic field frequency (Hz)	$\begin{array}{c} {\rm AC} \\ {\rm magnetic} \\ {\rm field} \\ {\rm flux} \ B_{{\rm AC}} \\ {\rm (mT)} \end{array}$	Germination	Seedling development parameters at 10 th day			
		FGP	number of seedlings	fresh weight	dry weight	moisture content
		(%)		(g)	(g)	(%)
11	0 (control)	77.1 ± 1.6	16 ± 2	0.96 ± 0.10	0.10 ± 0.01	89.4 ± 0.5
	0.1	72.4 ± 4.5	15 ± 1	0.70 ± 0.13	0.07 ± 0.03	89.8 ± 1.1
	0.3	76.7 ± 2.3	13 ± 4	$0.59\pm0.19^{*}$	0.06 ± 0.02	89.3 ± 1.0
16	0 (control)	78.9 ± 7.7	14 ± 3	0.89 ± 0.21	0.10 ± 0.06	88.6 ± 0.9
	0.1	83.5 ± 1.9	$20 \pm 2^{*}$	$1.65\pm0.38^{\ast}$	$0.21 \pm 0.01*$	87.3 ± 1.1
	0.3	75.6 ± 3.8	18 ± 5	$1.60 \pm 0.24*$	$0.20 \pm 0.02^{*}$	87.7 ± 1.2
21	0 (control)	64.4 ± 13.5	9 ± 2	0.63 ± 0.11	0.06 ± 0.01	88.0 ± 0.3
	0.1 mT	65.1 ± 5.2	10 ± 3	0.63 ± 0.23	0.07 ± 0.02	88.2 ± 0.5
	0.3 mT	68.9 ± 5.1	13 ± 2	0.71 ± 0.03	0.08 ± 0.01	88.4 ± 0.6

Overview of the measured development parameters of wheat seeds exposed to AC magnetic fields and the corresponding control seeds

FGP – final germination percentage. Moisture content is expressed as percentage of wet basis. Data in the table represent mean values of three replications and their standard deviations. Data for the number of sprouts are rounded up to the whole numbers. Fresh (dry) weight represents average fresh (dry) weight of seedlings in the replication group. An asterisk (*) indicates a significant difference from the corresponding control in the Dunnett's test (P < 0.05).

cyclotronic frequency $f_{AC} = 16$ Hz on the germination and growth of wheat seedlings is plotted in graphs in Figure 1. The cumulative germination and shoot emergence curves represent the mean values of three replicated data sets. The fluctuation ranges (standard deviation) of each point on the plots were not indicated so as to ensure the legibility of the figures. The Gompertz equation was fitted to the experimental data and good approximation of all recorded data with the plotted curves was found ($R^2 > 0.93$).

Possible effects of the exposure to ICR-tuned fields on germination were evaluated by the final germination percentage. In each experimental run, the seeds began to germinate irrespective of the applied magnetic treatment, that is, no acceleration or delay of the germination process was observed, as



Fig. 1. The germination (a) and seedling growth (b) characteristics of wheat as a function of the germination time for a calcium frequency-tuned magnetic field f = 16 Hz. The applied magnetic flux densities B_{AC} of an alternating field: 0.3 mT (+), 0.1 mT (•), non-exposed control (\blacktriangle)

can be seen in Figure 1*a*. Also, there were no differences in the final germination percentages between treatment and control seeds, as presented in Table 1. Concluding, there was no difference in the germination process between all the applied magnetic treatment variants and the control.

Soil experiments revealed that artificial B_{AC} magnetic fields could significantly influence the fresh and dry weight of 10-day-old plants. For both magnetic flux densities and for f = 16 Hz, data in the fourth column of Table 1 show changes in the number of sprouts emerged from the exposed seeds, although the pertinent statistical analysis showed that differences between those seeds and the control ones were only significant for $B_{AC} = 0.1$ mT field (P < 0.05). The significance level was not reached for 0.3 mT field, which was probably due to the increasing variation between replication samples.

A positive and statistically significant effect was recorded for both calciumtuned magnetic fields when the seedling development parameters were measured, as the exposed plants produced seedlings with higher fresh weight. The Dunnett's test applied to the data returned statistically significant differences when comparing the control to exposed seeds, at P < 0.005 and P < 0.05 for 0.1 mT and 0.3 mT B_{AC} fields, respectively. Also, the average dry weight of seedlings exposed to the f = 16 Hz fields was significantly increased (P < 0.05).

Persistent exposure of seeds to f = 11 Hz field inhibited the fresh weight of emerged seedlings at the end of the experiment. The weight difference between the two B_{AC} field experimental variants was not distinct, but significant (P < 0.05) when compared to the control. The stronger field also significantly influenced the dry weight of seedlings (P < 0.05). Wheat seeds failed

seedling emergence parameters measured. The purpose of this study was to determine whether exposure of seeds to combined alternating and constant magnetic fields could trigger a biological response and influence seed germination and development of wheat seedlings. However, the examined indices describing the germination process (germination time, final germination percentage) did not reveal any influence of the applied magnetic field treatment. All significant differences indicated changes in the number or the fresh and dry weight of seedlings in the exposed plants, showing that Ca^{2+} tunings (16 Hz) were generally stimulating to the growth and seedling development. Our results are in partial agreement with the result of another study, where common wheat (Triticum aestivum) and sunflower (Helianthus annuus L.) seeds exposed to a 16.6 Hz magnetic field, showing small, but significant increases in the total fresh weight of seedlings, whereas their dry weight remained unaffected (FISHER et al. 2004). However, wheat demonstrated a marginally higher germination rate (FISHER et al. 2004). On the other hand, ROCHALSKA and GRABOWSKA (2007) showed that the alternating magnetic field of 16 Hz decreased the activity of α -amylase and β -amylase enzymes in common wheat plants, and it is know that α -amylases are activated because of the presence of Ca⁺² ions (BUSH et al. 1989). Further, it was noted that after the exposure of germinating narrow-leaf lupin (Lupinus angustifolius L.) seeds to a 16 Hz magnetic field, a significant decrease in the chlorophyll and carotenoid content was observed, although the seedlings' biometric parameters, mitotic activity, protein content and guaiacol peroxidase activity were not altered (MROCZEK--ZDYRSKA et al. 2016). Nevertheless, it is generally accepted that the effect of a magnetic field depends not only on the field's frequency, but also on the form B_{AC} field's flux density (BAURÉUS KOCH et al. 2003). For example, in our study there was no significant change in the number of sprouts for $B_{AC} = 0.3$ mT observed; likewise, the increase in the seedlings' fresh weight was at a significance level of P < 0.05 in comparison to the control plants, whereas the significance level for $B_{AC} = 0.1$ mT reached P < 0.005.

As double distilled water was used during the germination tests, only the intracellular mineral macronutrient resources deposited in seeds in the course of development were utilized during germination. While germinating and in the early growth stage, seeds utilize mainly seed-derived macronutrient resources but the efficiency of redistribution of these sources in a growing plant may differ among elements. For example, calcium in developing seeds of *Lupinus albus* and L. *angustifolius* was retrieved from the cotyledons to the seedling's axis at around 30% efficiency (HOCKING 1980). Also, the growth of seedlings of common groundsel (*Sencio vulgaris*) was most dependent on the external supply of calcium (FENNER 1986). Thus, the seed content of several essential elements, although constituting only a portion of the total demand, may be essential for seed germination and the formation of seedlings.

For one of the tested non calcium-tuned frequencies (f = 11 Hz), a significant decrease of seedlings weight was observed. This frequency is close to the ICR frequency of ferrous Fe²⁺ ions ($m_{\rm Fe}$ = 9.27 10⁻²⁶ kg, $q_{\rm F}$ = 3.21 10⁻¹⁹ C), which is equal to 11.4 Hz. Iron is a very important element associated with the growth of plants. The active iron fraction, which has been reported to be Fe²⁺, is an important component of plant tissues, which is considered to be that portion of iron participating in metabolic reactions or incorporated into molecular structures (FAGERIA 1992). Adequate concentrations of active Fe^{2+} forms in leaves are able to prevent chlorosis, and the opposite is also true: disorders in the iron distribution in soil can induce plant chlorosis, which can result in a decreased plant growth rate. The observed decrease in the fresh weight of f = 11 Hz in exposed seedlings may have been due to iron-induced chlorosis, which reduced the chlorophyll level in shoots. The negative effects on the seedlings development when the non-calcium ICR frequency was applied were previously reported as the inhibition of the growth of radish seedlings at potassium and magnesium tunings (YANO et al. 2004). Nevertheless, as neither chlorophyll assays in seedlings were performed, nor the total Fe content in shoots was determined, we are unable to verify this hypothesis. We were also unable to find any reports where the ICR theory was tested for ferrous Fe^{2+} ions. We do not intend to discuss any other hypothesis here, therefore the question about the cause and nature of the results obtained for f = 11 Hz remains open.

We hope that this paper highlights the importance of research into the influence of man-made magnetic fields on plant development. The data obtained thus far are still insufficient for drawing a final conclusion about the effectiveness of an application of ICR frequencies on germination and growth, but the mechanism proposed by the ICR theory may help to provide the theoretical basis for at least some of the observed phenomena related to the influence of man-made extremely low frequency magnetic fields on crop plants. The goal of further research should be to study the entire cycle of growth and development of agricultural crops under the influence of manmade magnetic fields of various ICR frequencies. This may lead to practical applications of magnetic fields as a crop growth enhancer.

CONCLUSIONS

The persistent exposure to combined static geomagnetic and alternating magnetic fields did not alter germination of wheat seeds, although under the specified conditions of exposure significant differences in the growth of seedlings were observed. The observed effects depended essentially on the applied frequency. The results indicate that the Ca^{2+} ICR frequency has no statistically significant effect on the germination of wheat seeds. However, the Ca^{2+} tunings were generally stimulating to the growth, as the positive

effect of the 16 Hz frequency on the number of emerged sprouts and final fresh and dry weight of seedlings was observed.

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