Influence of extremely low frequency magnetic field on assimilatory pigments and nucleic acids in *Zea mays* and *Curcubita pepo* seedlings

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Abstract

The effects of extremely low frequency magnetic field on assimilatory pigments and nucleic acids in the seedlings of two agricultural species (*Zea mays* and *Curcubita pepo*) were studied in controlled environmental conditions. During the first 10 days of growth, the plantlets were exposed to 50Hz sinusoidal magnetic field of 10mT intensity generated by a Helmholtz coils system for 1, 2 and 4 hours daily. Plantlets without magnetic treatment were considered as controls. Assimilatory pigments and average nucleic acid level were assayed by spectrophotometric methods in both exposed plants species and control ones. The results showed that the level of chlorophyll a was found 3-fold increased in *Z.mays* seedlings, and 2-fold increased in *C.pepo* seedlings for short exposure periods compared to the controls. The ratio chlorophyll a/chlorophyll b was increased with about 30% in both plant species while for the exposed samples the nucleic acids level was found diminished as 3 times. The chronic exposure of seedlings would appear to influence differently the assimilatory pigments and nucleic acids during early ontogenetic stages so that an increase in both parameters resulted in major average of *Z.mays* seedlings length, while it did not affect *C.pepo* seedlings.

Key words: extremely low frequency magnetic field, assimilatory pigments, nucleic acids, *Zea mays*, *Curcubita pepo*.

Introduction

A large broad of experiments proved that the static or ELF (extremely low frequency) magnetic fields are able to induce detectable responses in various living organisms. Extremely low frequency (ELF) is a term used to describe electromagnetic radiation with frequencies from 3 to 300 Hz. Evidence of various biological effects related to the exposure to the magnetic fields generated either by alternating currents of 50/60 Hz or by the scattered electromagnetic fields around power lines and electric engines were encountered on human and animal organisms (KORPINEN & al. [1], HARRIS & al.[2] and OWEN [3]. Though low frequency magnetic field effects on plants are less studied, the existent scientific reports may contribute to sustain the concept of magneto-sensitivity in the living world. The various effects on growth and seed germination of plants depend in a complex way on magnetic flux densities, frequencies, exposure time and the pre-treatment of the plant material. The magnetic exposure effects on the seed germination and seedling growth during their early ontogenetic stages were first reported by PITTMAN [4], RUZIC & al.[5] [6] and CELESTINO [7] who studied the magneto-sensitivity of tree seed, while ALEXANDER & al. [8] have investigated the response of conserved seed to electromagnetic field. RAJENDRA & al. [9] have studied the magnetic bio-stimulation influence on germination and early growth in agricultural plant species. RUZIC & al. [10] performed an interesting experiment dedicated to tree tissue culture under the influence of pulsed magnetic field, while KHIZHENKOV [11] reported the changes observed in seed membrane permeability following the application of alternating magnetic field. Experimental studies regarding cereal seedling growth after...
magnetic exposure by FREYMAN [12], ALADJADJIYAN [13], MARTINEZ & al. [14], FLÓREZ & al. [15] or PENUELAS & al. [16] were carried out. Variable and contradictory experimental results seem to suggest that the effects of magnetic fields on plants may be species-specific (e.g., stimulate the growth certain plant species, inhibit the growth in some species, and have no effect on the others). FLÓREZ & al. [15] obtained for chronic magnetic field exposure the highest lengths and weights of rice plants. PENUELAS and coworkers reported that magnetic fields of 176 G and 21 G reduce root growth of L. culinaris by 37% and 13 % respectively [16]. These results of PEÑUELAS & al. [16] suggest a new example of possible species-specific effects of moderate magnetic fields on plant growth. The influence of low frequency magnetic field on the seedling growth was reported by DAVIES [17] and FISHER & al. [18] - that applied 16 Hz magnetic field to young plants and YANO & al. [19] – that worked with 60Hz electromagnetic field and assigned the slight perturbation of the early growth of the exposed plantlets to the changes in the photosynthetic CO₂ uptake. Sensitivity of young plantlets to magnetic fields continues to represent a challenging issue of bio-electromagnetism, the diversity of biological material, exposure systems and analysis methods enlarging continuously the frame of this multidisciplinary research field.

The goal of the present study was to determine the effects of 50 Hz magnetic field of 10mT on assimilatory pigments and nucleic acids during the early ontogenetic stages in Zea mays and Curcubita pepo under controlled conditions. Since the magnetic fields represent ubiquitous environmental factors for all plant species belonging to the terrestrial biosphere, the investigation focused on plant magneto-sensitivity need to be enlarged especially in recent decades when the role of the biosphere in balancing electromagnetic pollution became almost a popular issue. The study of assimilatory pigments from plant green tissue following electromagnetic exposure could be able to provide information upon the capacity of chloroplast membranes to gradually transform the energy, during photosynthesis processes. As it is well known the chlorophyll molecules absorb solar part of visible radiation and provide mechanisms for its utilization in photosynthetic reactions while carotenoid pigments contribute to light-harvesting, playing also a photo-protective role, by preventing damage of the photosynthetic systems (DAWSON & al. [20] and MERZLYAK & al. [21]).

2. Materials and methods

2.1. Biological materials.

The biological material was provided by two plant species of agricultural interest: the pumpkin (Cucurbita pepo) and the popcorn (Zea mays). The study was conducted following a completely randomized design, in which the variation cause is the exposure times. Seeds were provided by experimental lots (micro-populations) developed with the aim of ensuring uniform genophond - all plants originating from a single plant and being propagated by auto pollination; initially, the seeds taken for the experiment were harvested from the crop of 2009 year and the first experiment lasted over 30 days during May and June 2010. The whole experiment was repeated during the same vegetation season of the next year, i.e. during May and June of 2011 utilizing seeds harvested from experimental lots - the crop of 2010 year – stored in the same conditions and with the same level of humidity as those used in the first experiment. Each experimental variant - exposed samples (1-2-4 hours) or control ones contain 50 seeds, placed in a glass dish with 80 mm diameter. The coil geometry determined the number of seeds used for each sample and subsequent exposure of the samples in the magnetic field, not simultaneous. Repeating the experiment was limited to one per year - in May and June - as growing different plants during a growing season might induce undesirable variations of measured parameters.
I have been chosen seeds that are of the same size as close as possible (20mm length and 0.24g mass for *C. pepo* seeds, and respectively, 9mm length and 0.27g mass for *Z. mays* seeds); the seeds were kept away from stray electromagnetic fields (in a home-made Faraday cage) for a month prior to the experiment. Seed germination was conducted in darkness, at 22.0°C on watered porous paper support placed in glass Petri-dishes. The moisture content was less than 12% the seeds being stored in dry room in darkness at 5.0±0.5°C until the time of the experiment. The germination was conducted in *Angelantoni Scientifica* climate room in darkness and constant temperature of 20.0±0.5°C on watered porous paper support placed in glass Petri-dishes, the seeds being spread out as uniformly as possible into each dish. When the exposure started the germinated seeds were sprouted (no longer than 10 mm). In the case of *Z. mays* the germination percentage - in the Petri dishes provided with 50 seeds each - ranged between 94% and 100% with an average of 97.6% - while in the case of *C. pepo* the germination percentage resulted in 98.4% - the lowest and the highest values being of 96% and 100%.

During the experiment, the room temperature (23.0±0.5°C), humidity (70%) and lightening (dark/light cycle 14h: 10h) were kept constant. The magnetic field background – in the lack of any magnetic source - was of about 0.30±0.05µT, as measured by means of C.A.40 Gauss meter - five orders of magnitude lower than the magnetic field applied in the frame of the experiment – so, practically, no influences were expected from the terrestrial magnetism. A standard fluorescent lamps (40W) supplied at the industrial net of 50Hz were used for illumination. The magnetic treatment was applied during the 12 first days, in the light period of the day: the samples were exposed in the same order from 9.00h in the morning until 16.00h in the afternoon. In the mean time, the corresponding control sample was let to grow in the same laboratory room placed in a home-made Faraday cage, in the absence of the magnetic field. After 10 days of plants growth, the biochemical assays were carried out in the 11th day starting with 9.00h. For each plant species the experiment lasted for 15 days including the germination time – so that the total investigation of the two species lasted for about 30 days in 2010 and 30 days in 2011.

2.2. Magnetic exposure system.

The extremely low frequency magnetic field treatment was applied using a Helmholtz coils system. This consisted of two coils, each formed by 1,000 turns of 1 mm cooper wire, with a mean diameter of 260mm and a thickness of 25mm. The coils were mounted coaxially and placed at a mean distance of 130mm each other (Fig.1).
The coils were fed through a power terminal of 50Hz sinusoidal voltage and current intensity adjusted at 1.76A. Thus, when a 50Hz sinusoidal electric current passed through the coils, a vertical sinusoidal magnetic field of 10mT was generated in the centre of the coils system. The seedlings exposure was done by placing dishes daily in the centre of the coils system. Exposure time duration was controlled by automatic timer coupled to the magnetic generator supply. The measurements of the magnetic field induction evidenced that within the centre of the Helmholtz coils system no significant variations of the value of 10mT could be detected for a 100mm diameter area, as measured with a Walker Scientific MG 10D Tesla meter provided with Hall probe (accuracy of 10⁻⁶T). Magnetic exposure intensity was expressed as magnetic energy dose: \( D = w \cdot t \) where \( w \) [J/m³] is the energy density of magnetic field while \( t \) [s] is the exposure time duration. The magnetic field energy density was assessed to \( 39.8 \times 10^{-6} \text{J/cm}^3 \) as resulted by applying the formula: \( w = \frac{B^2}{2\mu_0} \), where: \( B \) [T] is the magnetic field induction and \( \mu_0 \) is the magnetic permeability of free space. Thus, the magnetic dose used during the exposure ranged between 0.143 J·s/cm³ and 0.573 J·s/cm³.

2.3. Analysis methods.

Plants growth – either control non-exposed samples or magnetically exposed variants during the breaks between magnetic exposures - was conducted in the same controlled environment within a certain area where the magnetic measurements indicated that the magnetic field generated by the Helmholtz system practically vanished. During the experiment, all plant samples were watered with 15ml daily per Petri dish. The length of individual plant was measured with 0.1cm precision. Assimilatory pigments and average nucleic acids level in the green tissues of all experimental variants as well as in the control plantlets were assayed by spectrophotometric methods using a Perkin – Elmer 550S UV-VIS device provided with quartz cells of 1 cm width. For both ELF-MF exposed samples and controls, the biological material was harvested from the entire green tissue mass obtained by mixing up all plantlets grown within a Petri dish. The assay of the assimilatory pigments extracts in acetone 80% was performed following the Lichtenthaler & Welburn’s method (LICHTENTHALER & al. [22]), while the assay of nucleic acid level accordingly to a modified Spirin’s method (STRUCHKOV & al. [23], ZHDANOV & al. [24] or ZHDANOV & al. [25]) was carried out: spectrophotometric measurements were performed at the wavelengths of: 663nm, 646nm and 470nm (versus acetone 80%) for the assay of chlorophylls – chlorophyll a (\( \text{Chla} \)) (C₅₅H₇₂O₅N₄Mg), respectively chlorophyll b (\( \text{Chlb} \)) (C₅₅H₇₀O₆N₄Mg) and carotenoid pigments (\( \text{Car} \)) from green tissues and respectively at 260nm and 280nm (versus perchloric acid 6%) in the case of nucleic acids. For the calculation of photosynthetic pigments the formulae below were applied while in the case of nucleic acids calibration curves (based on the spectral readings to the mentioned wavelengths) were used:

\[
\text{Chla} (\mu g/g) = \left( 12.21 \cdot E_{663} - 2.81 \cdot E_{646} \right) \frac{V}{d \cdot w} \tag{1}
\]

\[
\text{Chlb} (\mu g/g) = \left( 20.13 \cdot E_{646} - 5.03 \cdot E_{663} \right) \frac{V}{d \cdot w} \tag{2}
\]

\[
\text{Car} (\mu g/g) = \left( 100 \cdot E_{470} - 3.27 \cdot \text{Chla} - 104 \cdot \text{Chlb} \right) \frac{V}{227 \cdot d \cdot w} \tag{3}
\]

where \( w \) (g) is the fresh vegetal sample mass, \( V \) (ml) is the extract volume (in 80% acetone), \( E_\lambda \) is the light extinction to the wavelength \( \lambda \) (nm) and \( d \) (cm) is the quartz cell width.
Statistic analysis of the experimental data resulted from the five repetitions of all measurements was accomplished by means of one-way ANOVA test with Microsoft Excel - to evaluate reliability of modifications induced by low frequency magnetic field into the exposed samples in comparison to the control ones as well as among the samples corresponding to different exposure times, considering the significance criterion of 0.05.

3. Results

The treatment of seedlings with 50Hz sinusoidal magnetic field of 10mT for 4h had a significant effect on Z. mays plants length. The results showed increases of 16.6% in plants length for Z. mays compared to the control (statistically significant), while C. pepo seedlings revealed a slight trend to increase, but statistically non-significant (Fig. 2). It seems that the same constraint is able to induce different effects on different plant species. Since among the biochemical factors that determine the plants growth, the nucleic acids and the chlorophylls are the most involved in the early ontogenetic stages further discussion was focused on the corresponding experimental data extracted during the experiment.

![Graph showing the average length of seedlings of Cucurbita pepo and Zea mays over time.](image)

**Fig. 2.** The average length of the seedlings of: a) Cucurbita pepo plants, b) Zea mays plants; * statistic significant; **statistic no significant (versus the control - non exposed seedlings) (p<0.05 accordingly to ANOVA test).

The nucleic acid levels were significantly decreased (p<0.05) by magnetic treatment for 1, 2 and 4 hours in both plant species. The Z. mays seedling showed decreases of 40.7% for 1h, 48.2% for 2h and 58.8% for 4h in nucleic acid levels (p<0.05), while C. pepo seedlings showed decreases of 8.9% for 1h, 26.4% for 2h and 48.3% for 4h (p<0.05) in comparison with the controls respectively (Fig. 3).
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The cell and tissue development, though strongly determined by the cell genetic information (stored within the nucleic acids), depends not only on the cell division by also on the photosynthesis efficiency. The data from Fig. 4 present the situation of the photosynthetic pigments that play very important role in solar light conversion toward chemical energy stored within organic compounds accumulated in the vegetal organism. The magnetic treatments had a remarkable effects (p<0.05) on the content of chlorophyll a in both crops. The magnetic exposure of *Z. mays* seedlings revealed increases of 205% (3 fold) for 1 h, 271.8% (3.7 fold) for 2h and 242% (3.4 fold) for 4h in the content of chlorophyll a, while *C. pepo* seedlings revealed increases of 17.48% for 1h, 21.33% for 2h and 16.4% for 4h compared to the controls respectively (Fig. 4).
The increases in chlorophyll a content (p< 0.05) for relatively short exposure times could represent an indication upon the putative stimulatory effect of the 50Hz/10mT magnetic field exposure on the plants growth, its amplitude depends on the plant species. On the other hand, the content of chlorophyll b was significantly increased (p<0.05) for magnetically treated seedlings of Z. mays, with increases of 119.8% (2.2 fold) for 1h, 129.7% (2.29 fold) for 2h and 131.94% (2.31fold) for 4h, but it was significantly decreased for C. pepo seedlings, with decreases of 26% for 1h, 18.3% for 2 h and 23.5% for 4h, respectively compared to the control (Fig 4). The influence of the magnetic constraint on the plant growth – discussed inhere by means of the seedling length – is the result of two opposite effects; the first one is the positive effect on the synthesis of chlorophylls, mainly the chlorophyll a that is increased considerably in Z. mays but poorly in C. pepo; the second is the negative effect on the biosynthesis of nucleic acids that are reduced to lower levels comparatively to the controls in both plant species. The extents of the inhibitory effect of the ELF MF action on the nucleic acid synthesis are similar (2.5 fold diminution in Z. mays and respectively 2 fold diminution in C. pepo) while in the case of the stimulatory effect on the chlorophyll a synthesis the increase evidenced in Z. mays is one order of magnitude higher than in C. pepo.

The variations that were recorded in the contents of carotenoid pigments increase slightly (statistically non-significant) in C. pepo case, while in the Z. mays case there is an increasing tendency with 2.4 fold diminution (with statistical signification) (Fig. 4).

Related to the ratio chlorophyll a/ chlorophyll b it is generally accepted that this parameter is an indirect indication on the response of the Light Harvesting Complex II from the chloroplast membranes, where photosynthesis is located – known as particularly sensitive to the external constraints; so, the photosynthesis efficiency seems to be stimulated for short times exposure to 50Hz/10mT magnetic field in both plant species but the higher values of the chlorophyll ratio in Z. mays (due particularly to the remarkable increase of chlorophyll a level) appears as decisive in the plants length growth. The chlorophylls ratio was remarkably influenced by ELF MF treatment in C. pepo and Z. mays seedlings (Fig. 5), thus the magnetic exposure of Z. mays seedlings revealed increases of 37.5% for 1h, 60.1% for 2h and 46% for 4h, while C. pepo seedlings revealed increases of 44.9% for 1h, 46.6% for 2h and 50.5% for 4h compared to the controls respectively.

Fig.5. The effects of ELF MF exposure on the chlorophylls ratio (Chl a/ Chl b) in the seedlings of a) Cucurbita pepo plants, b) Zea mays plants; * statistic significant (versus the control - non exposed seedlings) (p<0.05 accordingly to ANOVA test).
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From Fig. 5 it results obviously that the chlorophylls ratio was increased in both plant species in the same way (with statistic significance related to the significance threshold of 0.05). The slight (but significant) increases in chlorophyll $b$ content in *Z. mays* seems to sustain the positive result of the two overlapped effects as well as the higher values of the chlorophyll ratio do.

4. Discussions

Opposite effects of electromagnetic exposure on different biological parameters of the exposed seedlings were mentioned by HAMADA [26]. He reported that under the influence of microwaves, wheat seedlings exhibited decreases of chlorophyll $a$/chlorophyll $b$ ratio though protein accumulation occurred simultaneously. Other authors found correlated inhibitory effects of electromagnetic exposure. LEBEDEV & al. [27] reported the decrease in the fresh weight of barley plant roots (12%) and shoots (35%) after prolonged exposure (three weeks) to low magnetic field (10nT); simultaneously, the decrease in the dry weight of roots (19%) and shoots (48%) of barley seedlings was also noticed; consequently, the decrease in the water accumulation capacity resulted (as the difference between the fresh mass and the dry mass). The inhibition of biomass production under the effect of weak magnetic field was evidenced also in the case of in vitro tissue cultures of *B. vulgaris* (SYTNIK & al. [28]). The ultrastructural studies carried out on vegetal tissues after magnetic exposure to low magnetic field, mainly the observations of the mitochondria qualitative and quantitative changes, as well as the results of cytochemical investigations (BELYAVSKAIA [29-30]) led to the conclusion that the disruption of Ca$^{2+}$ balance could be caused by weak magnetic fields. Ca$^{2+}$ ions are involved in the control of cell metabolism so that the consequences of calcium balance perturbation could result in various biochemical changes. Ca$^{2+}$ ions transport trough cell membranes seems to be particularly sensitive to the electromagnetic fields: it was found that under the influence of electromagnetic fields the direction of calcium transmembrane flow can be reversed, i.e. calcium ions were induced to flow out of or into cells, depending of the combination of exposure conditions (BAWIN & al. [31]). Accordingly with the original ion cyclotron resonance model (LIBOFF [32]), Ca$^{2+}$ ions moving helically along geomagnetic field lines are accelerated on the extremely low frequency magnetic field superposed on the geomagnetic field, by cyclotron resonance generated. This influences to increase Ca$^{2+}$ influx via calcium channels aligned with the geomagnetic field and thus magnetically induced ion cyclotron resonance can alter equilibrium of biochemical reactions. Nevertheless, physical explanation of plant magneto-sensitivity could be based on the hypothesis that some organelles of plant cells have paramagnetic properties like that found in chloroplasts since 1956 (COMMONER & al. [33]). I don’t exclude the hypothesis that calcium channels respond differently in different plant species exposed to the same electromagnetic constraint (such ion channels were identified in the root cells of wheat by HUANG & al. [34]). The action of weak physical and chemical factors on biological systems is often invoked in the bio-electromagnetism literature, based on the generally accepted statement that the energetic equilibrium states of bio-systems are unstable so that any weak stimulus is sufficient to put the systems in new states, at the expense of their intrinsic energy resources, that is the biological amplification of a weak electromagnetic signal occurs. BINHI [35-38] attempted to formulate several hypotheses on the physical phenomena that could be involved in the explanation of magneto-sensitivity. I can mention some of them: the eddy electric currents induced by alternating magnetic fields; the interference of quantum states of bound ions and electrons; the biological effects of torsion fields accompanying electromagnetic exposure; the
magneto-sensitive free-radical reactions; the stochastic resonance as an amplifier mechanism in magneto-biology. The biochemical reactions that involve spin-correlated radical pairs are sensitive to magnetic fields exposure. A famous biological reaction involving spin selectivity, and thus sensitivity to extremely low frequency magnetic field, is the triplet yield and emission intensity of the photosynthetic reaction centre occur in bacterial medium (HOFF & al. [39]). The electron transfer, radical-pair intermediates and triplet yields, and emission intensity processes generated in photo-systems I and II of spinach plants (SONNEVELD & al. [40]) can be modulated by external magnetic fields. LEELAPRIYA & al. [41] presumed that the positive effect of sinusoidal magnetic field on the seed germination could be the result of enzymatic activity stimulation in enzymes controlling the extension of the cell wall. I believe that at the cellular level an extended hypothesis of the changes induced by magnetic exposure in the electric activity of cell membrane system could be formulated - with possible consequences on the transport phenomena through chloroplast and nucleus membranes: either ions or molecules with roles of growth regulators for instance. Enzyme activity could be also influenced by electromagnetic exposure.

Our days, the interest for further experiments focused on plant magneto-sensitivity is justified by the concerns related to the high and continuous increases in the level of the electromagnetic pollution of the biosphere as well as due to persistent biological effects of electromagnetic exposure at biochemical level of food prepared from plants exposed.

5. Conclusions

This study was performed about two different biological species to point out the influence of ELF electromagnetic energy on the photosynthesis efficiency. The results of the biochemical investigations carried out on Cucurbita pepo and Zea mays plants, during their early ontogenetic stages, after exposure to extremely low frequency (50Hz) magnetic field (about 10mT) have revealed that plant growth could be influenced during such treatment. Positive influence on the photosynthesis efficiency for short exposure times could be presumed as resulting from chlorophylls assay while negative effect on the nucleic acid biosynthesis was also evidenced. The plant length increase in the case of Z. mays seedlings in spite of decreased nucleic acid level could be the result of their higher capacity of water accumulation following electromagnetic exposure. The prolonged exposure time per day induced generally much smaller effects than the short time electromagnetic exposures. Though the electromagnetic energy of the absorbed photons is of many orders of magnitude smaller than that of chemical bonds within the molecules of chlorophylls or nucleic acid however their electromagnetic energy could trigger complex synergetic cellular mechanisms that finally can lead to the growth disturbance. The influence of 50Hz magnetic field exposure appears to depend on the plant species involved in the study. These results could be of interest for the environmental protection studies if one considers the ubiquity of 50Hz magnetic fields in the modern world. In the near future, will be designed such new experiments to support new hypotheses on the mechanism of extremely low frequency magnetic field influence on vegetal organisms.

References

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