50 Hz FREQUENCY MAGNETIC FIELD EFFECTS ON MITOTIC ACTIVITY IN THE MAIZE ROOT

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Abstract. In this paper the author presents experimental results regarding the cellular division rate and the percentage of chromosomal aberrations induced in the root meristem cells of maize (plant with major role in people life), provided by germinated seeds in the presence of extremely low frequency magnetic field with frequency of 50 Hz and 10 mT magnetic induction (ELF-MF). 50 Hz ELF-MF was generated by a laboratory exposure system consisting of a pair of parallel coils in a Helmholtz configuration. Optical microscopy investigation (cytogenetical tests) has resulted in the evaluation of mitotic index and chromosomal aberrations index that appeared increased following extremely low frequency magnetic field exposure times. The level of chromosomal aberration induced by ELF-MF in the root meristem cells of maize was lower than 1%.

Key words: ELF-MF, maize roots, cell proliferation, chromosomal aberrations, mitotic index.

INTRODUCTION

The biological effects of low frequency electromagnetic fields (EMF) have become a topic of considerable scientific interest scrutinized during the past two decades. Extremely low frequency magnetic fields (ELF-MF) are one of ubiquitous factors in the Earth's environment, that may be emanated by various sources: geomagnetic fields, electric potential in the atmosphere or cosmic radiation. All the electrical devices that we use produce a low frequency electromagnetic field (50 Hz). All of the living organisms on the Earth, including plants, have been exposed to the natural electromagnetic field from the beginning of life and they have adapted to it. Numerous studies show an association of the internal electromagnetic field of plants with many physiological processes. The effects of weak magnetic and electromagnetic fields in biology have been intensively studied on animals [24], micro-organisms [2] and humans [16], but comparably less on plants [4, 9].

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From scientific literature, it is known that biological systems give different bio-responses to extremely low frequency magnetic field exposures at different frequencies and intensities [11]. Various living organisms are differently affected from an extremely low frequency magnetic field, and these effects vary according to exposure conditions, genotype of organisms and the biological system [5, 18].

In this way, several such studies have suggested that extremely low frequency magnetic fields may modify plant growth and development [7–8, 19–23], but exposure to magnetic fields induces quite a variety of biological effects and, moreover, knowledge of the effects on living organisms is still not very clear.

In their study, Huang and Wang, 2007 [12] indicate that the 60 Hz sinusoidal magnetic field has an enhancing effect on the early growth of mung beans; however, some morbid state phenomena were observed on the sprout roots. Also, Bitonti and collaborators showed that exposure of *Zea mays* seedlings to a continuous electromagnetic field (DC) for 30 h induced a stimulation by about 30% in the rate of root elongation compared with the controls [6]. Studies on the meristem cells of the plants have shown that magnetic field is an element which affects normal cell metabolism and also has impact on the cell division [3, 10]. In 2002, Aladjadjiyan [1] found out that the magnetic field about 0.15 T stimulated the shoot development and led to the increase of the germinating energy, germination, fresh weight and shoot length in maize treated for 10, 15, 20 and 30 minutes.

Cell metabolism is sensitive to a range of nonspecific weak treatments that do not directly affect receptors or any other specialized cell structures. The lack of evident targets for weak treatments substantially complicates the search of possible physico-chemical mechanisms underlying their effects on cell processes [14]. Therefore, manifold proposed mechanisms in this way remain a matter of discussion. First, the perception mechanisms were attributed to ferromagnetism, but later discoveries required additional explanations like the “radical pair mechanism” and the “ion cyclotron resonance”, primarily considered by Liboff [15]. The last predicts effects by small ions involved in biological processes that occur in definite frequency and intensity ranges (“windows”) of simultaneously impacting magnetic and electromagnetic fields related by a linear equation, which meanwhile is proven by a number of *in vivo* and, respectively, *in vitro* experiments.

In this way, the goal of the present study was to study the effects of 50 Hz magnetic field of 10 mT on the cellular division rate and the percentage of chromosomal aberrations induced in the root meristem cells of *Zea mays*.

MATERIALS AND METHODS

The extremely low frequency magnetic field treatment was applied using a Helmholtz coils system. The experimental system consisted of two coils, each formed by 1,000 turns of 1 mm copper wire, with a mean diameter of 260 mm and

a thickness of 25 mm. The coils were mounted coaxially and placed at a mean distance of 130 mm each other (Fig. 1). The coils were fed through a power terminal of 50 Hz sinusoidal voltage and current intensity was adjusted at 1.76 A. Thus, when a 50 Hz sinusoidal electric current passed through the coils, a vertical sinusoidal magnetic field of 10 mT was generated in the central zone of the coils system. The seeds exposure was done by placing dishes daily in the centre zone of the coils system. Exposure time duration was controlled by an automatic timer coupled to the magnetic generator supply.

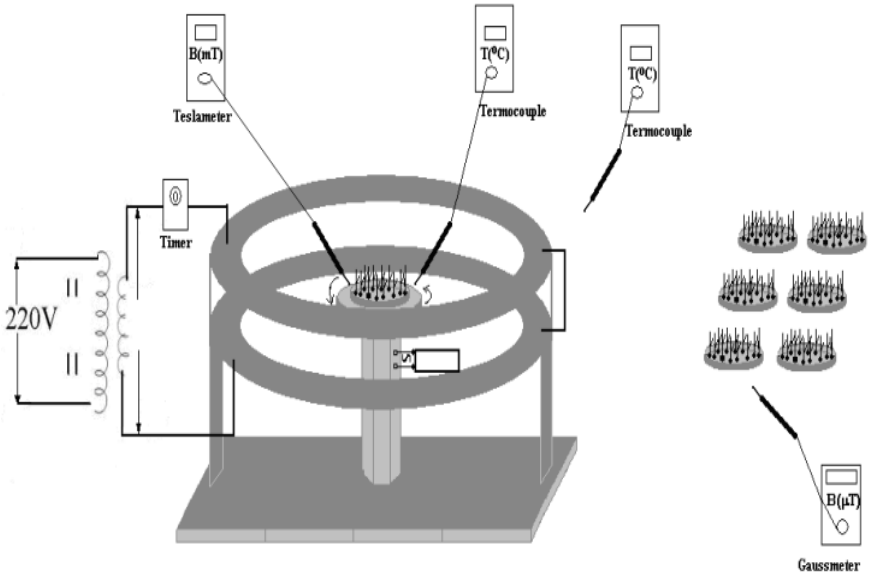

Fig. 1. The schematic representation of the magnetic exposure designs.

The coaxial coil system was supplied from the power transformer connected to the industrial electricity net. Magnetic field and temperature were measured. The measurements of the magnetic field induction evidenced that within the centre of the Helmholtz coils system no significant variations of the value of 10 mT could be detected for a 100 mm diameter area, as measured with a field strength analyzer NARDA EFA-300 model. During the experiment, the ambient temperature was the same for the control and the exposed samples. Magnetic field induction within the experimental room – of about 0.3±0.05 μT, was measured by means of C.A.40 Gaussmeter.

Biological samples were composed of *Zea mays* seeds, harvested from an experimental population with an ensured uniform genofond (lot obtained from Biological Research Institute, Iassy – Romania, in 2007).

The seeds were let to germinate on watered porous paper support, with the same quantity of deionized water, in Petri dishes (each sample was composed of

30 seeds) and in controlled environmental conditions into a laboratory room (darkness and suitable temperature (24.0±0.5 ºC)).

The seeds have been exposed to a 50 Hz frequency magnetic field during the germination process (after the sprouting process of seeds), for different time intervals, between 1 and 36.0 hours. So, the experimental variants were: the control sample (no magnetic field influence – 0 h exposure time) and seven samples corresponding to different exposure times (1, 2, 4, 6, 12, 24 and 36 hours). The whole experiment was repeated twice. For each experimental sample, ten slides (realized from 10 seeds randomly chosen for each sample) were microscopically examined and the final results further presented are the average values on these ten slides per experimental sample.

A statistical analysis was accomplished by means of average values, standard deviations and t-test (two tails, pair type), while the significance was defined by a probability level of $p < 0.05$. The Student test was applied to compare the elements results of the groups exposed to magnetic field with the control.

The 3 days old root meristem tissue samples from germinated seeds (using only roots reached about maximum 3 mm length) were used to prepare ten microscope slides for every experimental sample. Microscope slides for cell chromosome visualization were prepared by using the Squash method combined with Fuelgen techniques [13]. Carr modified dye was used to provide selective coloration in plant chromosomes. For every microscope slide examined counts were performed on all visual fields of the slide. The cell mitotic index and chromosomal aberration percentage were examined and counted microscopically on squashes, and the aberrant cells were micro-photographed. The mitotic index is able to give the percentage of dividing cells in every sample while chromosomal aberration index represents the sum of aberrant cell divisions:

$$M.I.\,(\%) = \frac{total\ \ cells\ in\ division}{total\ \ cells\ \ analyzed} \cdot 100 \tag{1}$$

$$A.I.\,(\%) = \frac{total\ cells\ with\ chromosomal\ \ aberrations}{total\ \ cells\ \ in\ division} \cdot 100 \tag{2}$$

The counting of normal and aberrant dividing cells was carried out (using Nikon microscope) taking into account all cell division phases: prophase, metaphase, anaphase and telophase.

RESULTS

The experimental data about the influence of different exposure times of seeds on an extremely low frequency magnetic field (50 Hz) upon the cells in different division phases, are given in Table 1.

The results obtained in the frame of the cytogenetical investigation regarding 50 Hz frequency magnetic field influence upon both proliferation capacity and abnormal division frequency are further discussed based on the corresponding percentage data.

Table 1

Results of cytogenetical investigation (t_{MF} – 50 Hz frequency magnetic field exposure time; N – average value of total number of analyzed cells per slide; M.I. – average value of mitotic index; A.I. – average value of aberration index; D – average value of total number of divided cells per slide)

t_{MF} (h)	*N*	*D*	*M.I.* (%)	*A.I.* (%)
0 (control sample)	2801.66	102.66	3.37	2.47
1	3160.00	130.00	4.11	6.39
2	2966.66	147.85	5.26	6.42
4	1747.14	124.42	7.45	6.57
6	1576.25	146.87	9.02	6.86
12	2932.85	257.00	11.39	7.35
24	2254.28	200.85	9.36	7.41
36	1941.42	176.28	9.13	9.59

The results obtained in the frame of the cytogenetical investigation regarding 50 Hz frequency magnetic field influence upon both proliferation capacity and abnormal division frequency are discussed further based on the corresponding percentage data. The microscopical analyses have revealed an increased influence of an extremely low frequency magnetic field for an increased exposure time at chromosomal level.

Also, it is visible that under an extremely low frequency magnetic field influence, the total number of divided cells increases (prophase, metaphases, anaphases and telophases percentage), with increasing exposure times.

The mitotic index is higher for all samples under an extremely low frequency magnetic field influence in comparison to the control one, but a remarkable stimulation of the average value of mitotic index was noticed for low exposure times (between 0 and 12 hours) (Fig. 2).

The statistical analysis accomplished for the average value of mitotic index (by applying the t-test to compare control and test sample data) revealed a statistical significance ($p < 0.05$) for all magnetic field exposed samples with one exception (1 h to the 50 Hz magnetic field exposed sample). The highest value of the mitotic index data in the sample corresponding to the 12 hours exposure time increased three times the control sample value. One may observe that mitotic index has a linear increasing rate as to the increased extremely low frequency field exposure time, in the relatively low times cases (following linear equation 0.68 *A.I.* + 3.924, with $R^2 = 0.94$). For the highest exposure time values the mitotic index data have an approximately constant level.

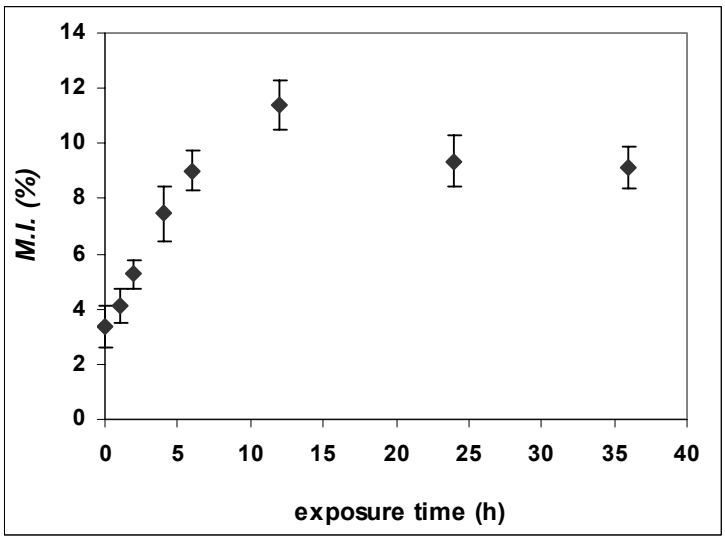

Fig. 2. The mitotic index (*A.I.*) under 50 Hz frequency magnetic field influence.

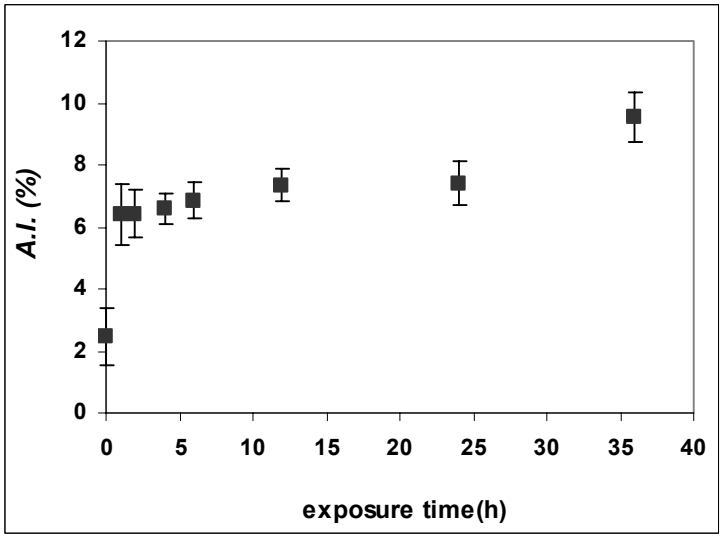

Fig. 3. The chromosomal aberration index (*A.I.*) under 50 Hz frequency magnetic field influence.

The average value of aberration index has increased values for all analyzed samples in comparison to the control one (Fig. 3), but with very low variations between the magnetic field exposed samples. The statistical analysis accomplished for the aberration index (by applying the t-test to compare control and test sample data) revealed statistical significance ($p < 0.05$) for all samples under magnetic

field influence. Aberration index (*A.I.*) linearly increased with magnetic field exposure time, for all exposed experimental samples (following linear equation 0.0785 *A.I.* + 6.26, with $R^2 = 0.89$).

Microscopical examination found an abnormal cell mitosis phenomenon predominant in anaphase (retard chromosome or inter-chromatin bridges) of *Zea mays* root tip meristem cells in the presence of 50 Hz magnetic field, and the low rate detection was found for the micronucleus in interphase. The main types of simple chromosomal aberrations identified in the over 121,000 analyzed cells (micro-photographed using a digital camera FUJI – FinePix S5100) are: micronucleus, inter-chromatin bridges, broken inter-chromatin bridges, retard chromosomes and chromosome ring. Complex aberrations were observed, such as combinations of inter-chromatin bridges with retard chromosome (Fig. 4 left), retard chromosome with micronucleus in metaphase (Fig. 6. right), and chromosome ring with retard chromosome (Fig. 7 right).

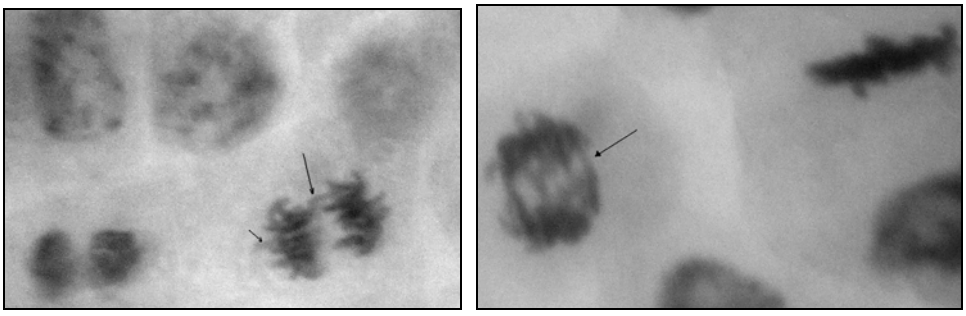

Fig. 4. Inter-chromatin bridges with retard chromosome in anaphase, 1 hour exposure time in the 50 Hz magnetic field sample (left) and inter-chromatin bridges in anaphase, 12 hours exposure time in the 50 Hz magnetic field sample (right).

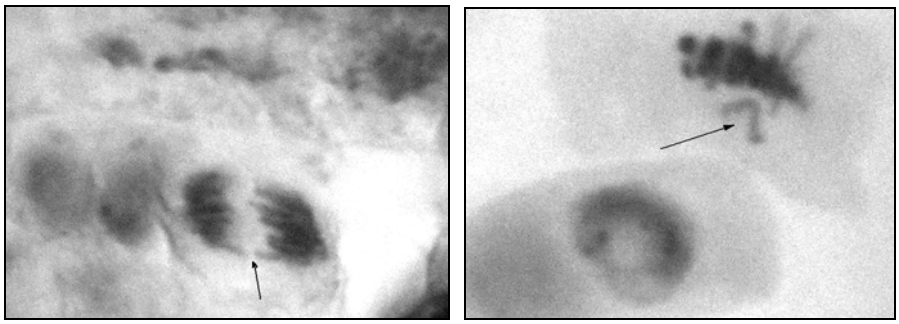

Fig. 5. Broken inter-chromatin bridge in anaphase, 36 hours exposure time in the 50 Hz magnetic field sample (left) and retard chromosome in metaphase, 2 hours exposure time in the 50 Hz magnetic field sample (right).

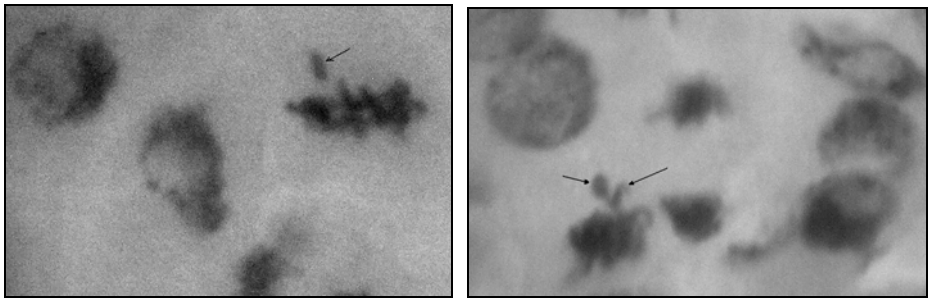

Fig. 6. Retard chromosome in metaphase, 1 hour exposure time in the 50 Hz magnetic field sample (left) and retard chromosome with micronucleus in metaphase, 12 hours exposure time in the 50 Hz magnetic field sample (right).

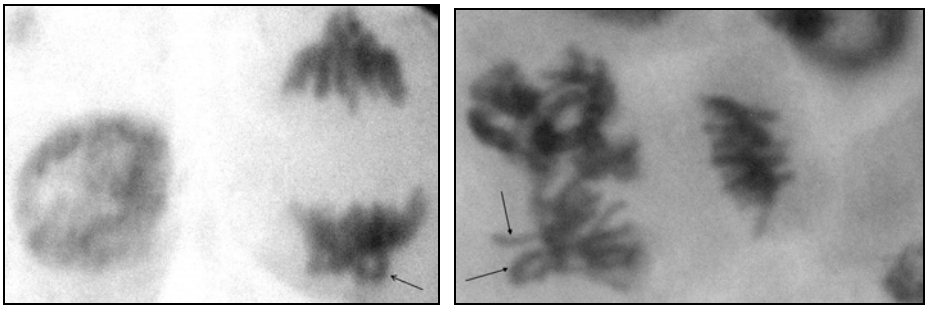

Fig. 7. Chromosome ring in anaphase, 24 hours exposure time in the 50 Hz magnetic field sample (left) and chromosome ring with retard chromosome in anaphase, 12 hours exposure time in the 50 Hz magnetic field sample (right).

Finally, the statistical significance evidenced the differences between the average values corresponding to control and exposed samples which allow us saying that young germinated seeds of maize responded to magnetic exposure within the experimental arrangement presented above.

CONCLUSIONS

We may conclude that the extremely low frequency (50 Hz) magnetic field is able to provide a relatively low percentage of chromosomal aberrations in *Zea mays* root tip and an increased mitotic index for increasing exposure time of the seeds to the magnetic field presence, stimulating the cellular proliferation in exposed seeds in comparison to the control sample. It may presume that some chromosomal aberrations induced by suitable magnetic field exposure time of plant seeds may persist in the next generations so that some phenotypic characters may be modified. These modifications could be observed following the plant development, some of them being benefic for the cultivation of this agricultural

species (*Zea mays*) with major role in people life. This way, the extremely low frequency (50 Hz) magnetic field could represent the molecular basis of a putative tool in the biotechnology of *Zea mays* growth, an important species in the human life, with the advantages of being less toxic and most easy to manipulate in comparison to ionizing radiation for instance [17].

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