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Effects of radiofrequency radiation on root tip cells of *Zea mays*

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Abstract

In this study, the mutagenic effects of low power radiofrequency radiation on Zea mays *root tip were studied. Cells in different division phases and chromosomal aberration assay were used to determine the mitotic index and chromosomal aberration frequency of* Zea mays *root tip cells induced by 900MHz radiofrequency radiation. Zea mays seeds, having a uniform genophond, have been exposed to RF field of low power density, for different time intervals, between 1.0 and 36.0 hours. Exposure to RF field was applied to seeds before germination process. Continuous wave on 900 MHz was used for irradiation. Incident field distribution in the irradiation area was characterized, so as an as possible as uniform field to be applied in the volume of the sample. The results showed that the mitotic index and chromosomal aberration frequency showed linear increasing for radiofrequency radiation treatment of increased exposure time.*

Keywords: magnetic nanoparticles, cell proliferation, chromosomal aberrations, mitotic index.

Introduction

Radiofrequency electromagnetic radiations are human survival environmental factors. Radiofrequency energy is a type of non-ionizing radiation, including electromagnetic radiation produced by cellular phones, and is not strong enough to cause ionization of atoms and molecules.

Effects of radiofrequency (RF) irradiation on agricultural plants have scarcely been studied yet, especially their harmful effects on biology. Alongside with the network communication, broad-cast television and electric appliance became common people accept more and more electromagnetic radiation presence, which has already become an environmental pollution problem, affecting directly living conditions. Effects of electromagnetic radiations on biology had been reported of many researchers [1-5]. Tkalec *et al*. exposed *Lemna minor L.* plants to electromagnetic radiations at the frequencies 900MHz and observed that the growth of plants exposed for 2 hours significantly decreased in comparison with the control [6]. Tambiev *et al*. enumerates a series of effects on photosynthetic microorganisms and plants of EHF radiation of low intensity [7].

Recently, Tkalec *et al.* reported that the germination rate and root length did not change significantly after 900MHz electromagnetic field exposure, but modulated field significantly of 4 hours exposure time increased mitotic index compared to corresponding controls in the *Allium cepa* seeds case [8]. The percentage of mitotic abnormalities increased with all the given field conditions.

Cell proliferation is known as rather sensitive to external and internal factors whatever their origin. The cell nucleus may experience the action of electromagnetic radiation as a source of chromosomal aberrations and, further, genetic mutations. In this study, we

investigated the capacity of the 900MHz radiofrequency radiation to influence germination, as well as to influence both the mitotic index and the chromosomal aberration percentage, carried out by means of cytogenetically tests.

Materials and methods

Biological samples were composed by *Zea mays* seeds, harvested from an experimental population with ensured uniform genophond. The seeds have been exposed to RF field of low power density (20mW), for different time intervals, between 1 and 36.0 hours. Exposure to the RF field was applied to seeds before the germination process. The Petri dishes containing seeds were exposed inside a TEM cell (model IFI CC-104SEXX), which was supplied from an RF signal generator (model Hameg HM 3184-3) (Fig.1). Continuous wave on 900 MHz was used for seeds irradiation. Incident field distribution in the irradiation area was characterized as an as possible as uniform field to be applied to the volume of the sample and the absorbed RF power was measured by using a *Luxtron One* probe (SAR<1W/kg).

The seeds were let to germinate in controlled environmental conditions into a laboratory room. From root meristems tissue in early ontogenetic stages of germinated seeds, microscope slides for cell chromosome visualization were prepared by using the *Squash* method combined with *Fuelgen* techniques [9]. Carr modified dye was used to provide selective coloration in plant chromosomes. 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 represent the sum of aberrant cell divisions:

$$\text{M.I.}(\%) = \frac{\text{total cells in division}}{\text{total cells analized}} \cdot 100 \qquad (1)$$

$$\text{A.I.}(\%) = \frac{\text{total chromosomal aberrations}}{\text{total cells analized}} \cdot 100 \qquad (2)$$

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 and discussion

The experimental data about the influence of different exposure times of seeds on radiofrequency field upon the cells in different division phases, are given in Table 1. The results obtained in the frame of the cytogenetic investigation regarding the 900MHz radiofrequency field influence upon both proliferation capacity and abnormal division frequency are further discussed on the basis of the corresponding percentage data.

The microscope analyses have revealed an increased influence of radiofrequency field for increased exposure time at chromosomal level. Furthermore, it is obvious that under the electromagnetic field influence increase of total prophase, metaphases, anaphases and telophases percentage occurred, especially for higher exposure times. The mitotic index is higher for all samples under the radiofrequency field influence in comparison to the control one and the aberration index have relatively low values for all analyzed samples (<3%)

(Fig.2). The highest value of the mitotic index data in the sample corresponding to the highest exposure time increased the control sample value 3 times.

Table 1 . **Cytogenetically investigation results**

t_{RF} (h)	N	M.I. (%)	A.I. (%)	I (%)	P (%)	M (%)	A (%)	T (%)
0	10780	5.371	0.176	94.628	1.280	2.146	25.043	42.028
1	11470	5.666	0.714	94.333	1.665	1.894	16.615	33.507
2	13170	6.218	0.804	93.781	1.913	2.040	22.344	10.317
4	11720	6.672	1.348	93.327	2.141	1.673	13.810	32.669
8	13160	7.758	1.481	92.241	2.606	2.133	14.495	22.157
12	11950	9.924	2.142	90.075	3.121	2.805	15.008	20.643
24	13100	12.167	2.786	87.832	2.954	3.485	16.687	45.219
36	14170	15.998	3.013	84.001	3.260	4.729	25.584	50.865

(t_{RF} – 900MHz radiofrequency field exposure time; **N** –total number of analyzed cells; **M.I.** – mitotic index; **A.I.**- aberration index; **I** - percentage of interphase cells; **P** – percentage of prophase cells; **M** - percentage of metaphase cells; **A** - percentage of anaphase cells; **T** - percentage of telophase cells).

One may observe that mitotic index has a linear increasing rate to the increased radiofrequency field exposure time. In Fig. 2, we supply the linear dependence with correlation coefficient (R^2=0.98) for the mitotic index (M.I.).

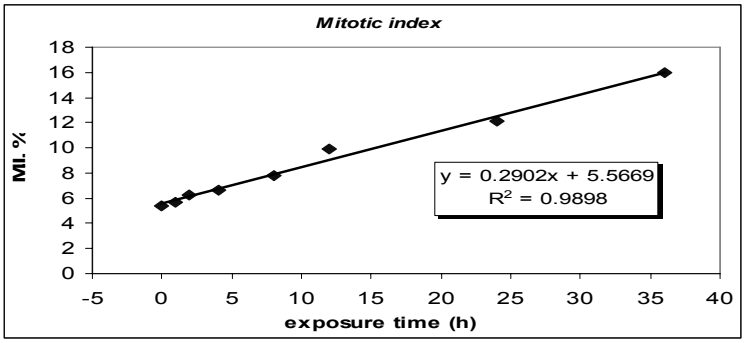

Fig.2. Mitotic index M.I. (%) versus radiofrequency field exposure time (h)

For the radiofrequency field exposed samples, we provide an ascendant dynamics (polynomial dependence) for the chromosomal aberrations index (low values for all samples < 3%) function of the radiofrequency field exposure time, with a correlation coefficient (R^2=0.95), in Fig. 3.

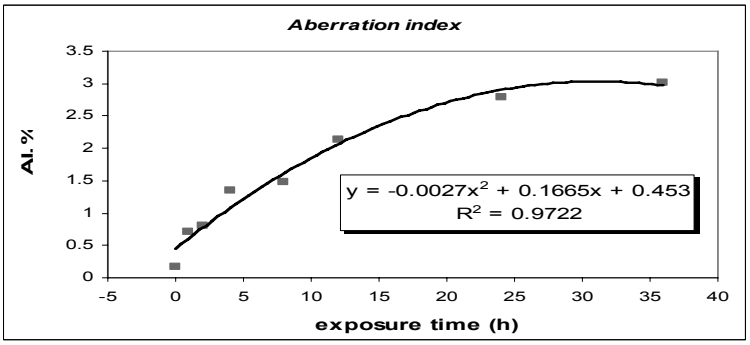

Fig.3. Dependence of chromosomal aberrations index (A.I.) for radiofrequency field exposed samples

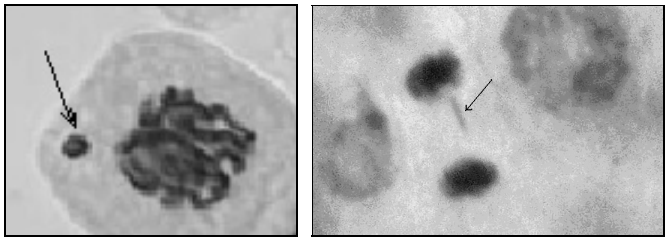

Fig.4. Micronucleus in prophase (4h exposed sample – left), chromosome fragment in telophase (24h exposed sample - right)

Microscope examination found abnormal cell mitosis phenomenon in prophase (micronucleus – Fig.4, left), metaphase, anaphase, telophase (chromosome fragment – Fig.4, right) of *Zea mays* root tip meristem cells in the presence of the 900MHz electromagnetic field. The main types of simple chromosomal aberrations identified in the over 99,000 analyzed cells (micro-photographed using a FUJI – FinePix S5100 digital camera) are: micronucleus, inter-chromatin bridges, retard chromosomes and chromosome fragments (Fig.5).

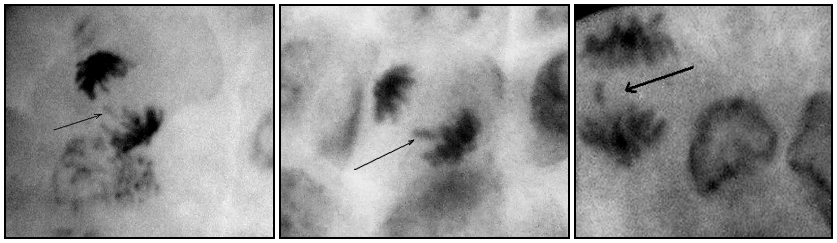

Fig.5. Chromosome fragments in anaphase (8h exposed sample –left, 24h exposed sample – middle, 36h exposed sample – right)

Complex aberrations were observed, such as combinations of retard chromosomes or chromosome fragments with inter-chromatin bridges (Fig.6), the formation of chromosomal bridges was accompanied by the occurrence of the chromosome fragments (Fig.6, right).

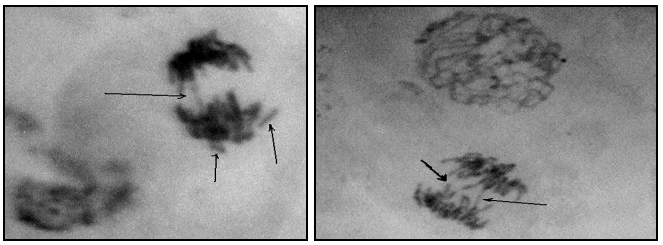

Fig.6. Inter-chromatin bridge and retard chromosome in inaphase (12h exposed sample – left), inter-chromatin bridge and chromosome fragments in inaphase (24h exposed sample – right).

Conclusions

We may conclude that the low intensity 900MHz electromagnetic radiation can provide a low percentage of chromosomal aberrations in *Zea mays* root tip and an increased

mitotic index for increasing the seeds exposure time in the electromagnetic field presence, stimulating the plant proliferation in comparison to the control sample. It may presume that some chromosomal aberrations induced by suitable electromagnetic 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 low power density of the 900MHz electromagnetic field could represent the molecular basis of a putative tool in the biotechnology of *Zea mays* growth with the advantages of being less toxic and easier to manipulate in comparison to ionizing radiation for instance [10].

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