LOW-LEVEL 900 MHz ELECTROMAGNETIC FIELD INFLUENCE ON VEGETAL TISSUE

MIHAELA RĂCUCIU, SIMONA MICLĂUŞ***

*Physics Department, Faculty of Science, "Lucian Blaga" University, 5–7, Dr. I. Rațiu Street, 550012, Sibiu, Romania, mracuciu@yahoo.com
**"N. Bălcescu" Land Forces Academy, 3–5, Revoluției Street, 550170, Sibiu, Romania, miclaus@armyacademy.ro

Abstract. This study is focused on the biochemical changes induced by low level of 900 MHz frequency electromagnetic field in young plantlets (popcorn, i.e. *Zea mays*) provided of irradiated seeds. In this experiment, *Zea mays* seeds have been exposed to RF field of low power density, for different time intervals, between 0.5 and 36.0 hours. Assimilatory pigments and average nucleic acid content were assayed after 12 days of plantlets growth, by spectrophotometric methods. Certain inhibition of chlorophylls biosynthesis was noticed while a stimulatory effect upon the nucleic acid biosynthesis was revealed. Plant development was stimulated by radiation, as revealed by average length compared to controls.

Key words: 900 MHz, biological effect, electromagnetic field, *Zea mays*, photoassimilatory pigments, nucleic acids level.

INTRODUCTION

Increased use of radio and microwave frequencies requires investigations of their effects on living organisms. The current safety standards are based on the thermal effects obtained for short time microwave exposures.

Kwee *et al*. analyzed effects of microwaves at 960 MHz and various specific absorption rate (*SAR*s), on proliferation of human epithelial amnion cells [5]. Tkalec *et al*. exposed *Lemna minor L.* plants to microwaves (MW) at the frequencies of 400, 900, and 1900 MHz [9]. They observed that the growth of plants exposed for 2 h to the 23 V/m electric field at 900 MHz significantly decreased in comparison with the control, while an electric field of the same strength but at 400 MHz did not have such an effect. Tambiev *et al*. enumerates a series of effects on photosynthetic microorganisms and plants of EHF radiation of low intensity [8]. Belyaev *et al*. investigated and synthesized the reported non-thermal biological

Received May 2007;
in final form June 2007.

ROMANIAN J. BIOPHYS., Vol. 17, No. 3, P. 149–156, BUCHAREST, 2007

effects of radiofrequency (RF) and MW and their dependence on several physical parameters and biological variables [1–3].

Effects of RF irradiation on agricultural plants have scarcely been studied yet. An experimental study was designed and accomplished with the goal of getting information upon the energetic modifications induced by low level radiofrequency field on chlorophyll molecules, under controlled exposure conditions. The study of assimilatory pigments from plant green tissue is able to provide information on the capacity of energy transformation of chloroplast membranes, during photosynthesis.

MATERIALS AND METHODS

In this experiment, *Zea mays* seeds have been exposed to RF field of low power density, for different time intervals, between 0.5 and 36.0 hours, and young plantlets developed from exposed caryopsides were used to obtain assimilatory pigments extracts. Exposure to RF field was applied to seeds only, before or during germination. The Petri dishes containing seeds were exposed one by one in the same position inside a TEM cell (model IFI CC-104SEXX), which was supplied from a RF signal generator (model Hameg HM 3184-3) (Fig. 1). Continuous wave (CW) on 900 MHz frequency with an input power of 20mW was transmitted inside the TEM cell. Incident field distribution in the irradiation area was characterized, so an as soon as possible uniform field to be applied in the volume of the samples. Incident power density was 50mW/m^2 (equivalent to approximate 1/100 of reference level given in ICNIRP guidelines for population exposure).

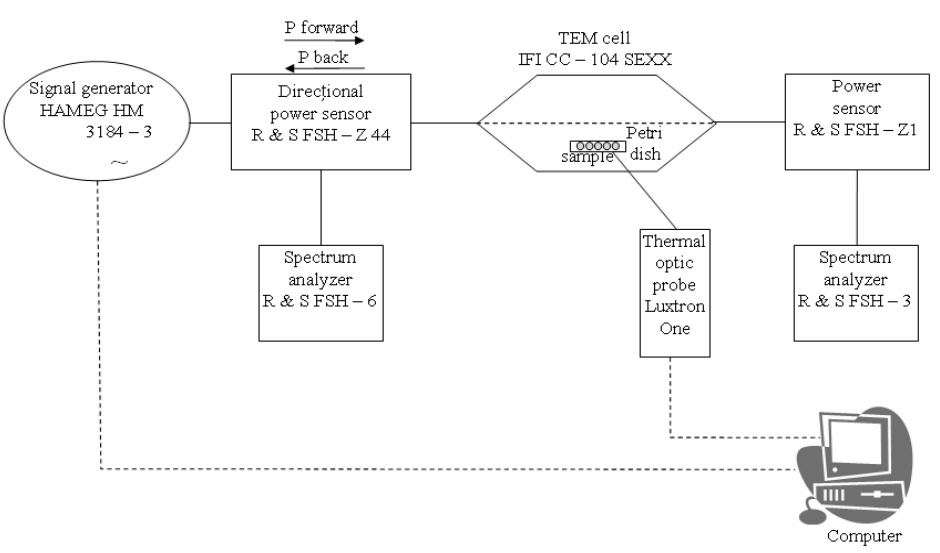

Fig.1. Exposure and dosimetric set-up scheme.

The absorbed RF power (average *SAR*) was both calculated and measured by using a Luxtron One probe. Average *SAR* in the exposed seeds was lower than 1mW/kg. Samples were composed by an equal number of *Zea mays* seeds, harvested from an experimental population, having a rather uniform genomic pool. Germination of irradiated seeds occurred on porous paper support, in darkness and closed Petri dishes, environmental conditions being kept under peer control, temperature and moisture levels, being 23 ºC and 98% humidity respectively. After germination the young plantlets development was conducted in the same controlled laboratory conditions (t = 22±0.5 ºC, illumination – 10 h: 14 h light/dark cycle and 90% humidity) and all plant samples were supplied only with deionized water during the experiment – about 15 ml daily per Petri dish. Plant individual length of the 12 days old plantlets was measured. The average lengths and the standard deviations were calculated for each batch of seeds. After 12 days of plants growth, spectrophotometric assays were accomplished: the content of chlorophyll **a**, chlorophyll **b** and total carotenoid pigments (following the Lichtenthaler & Welburn's method [6]) and the average content of nucleic acids (following a modified Spirin's method [7, 10]) were determined. A CINTRA 5 spectrophotometer UV-VIS provided with quartz cells was used. Biological material consisted of green tissue from seedling leaves, both from exposed samples and controls, for comparison.

Statistical analysis of the experimental data resulted from the five repetitions of the spectrophotometrical analysis was accomplished, by means of average values, standard deviations and t-test. Student t-test (two tailed, pair type) was applied to evaluate reliability of modifications induced by electromagnetic field into the exposed samples in comparison to the control ones, considering the significance criterion of 0.05.

RESULTS

Average *SAR* in the exposed seeds was determined both experimentally and theoretically. Experimentally, the differential power method was used [4] and gave $SAR_{experim}$ = 0.95±0.50 mW/kg value. In this experiment the average *SAR* values in the samples was definitely ≤ 1 mW/kg. The temperature variation during exposure was measured by using a Luxtron One probe, showing no-heating of samples at any time.

The average lengths of plantlets and afferent standard deviations were calculated for each batch of test seeds. The confidence interval was calculated for every batch of plantlets using the Student test, for the confidence level P = 90%. Fig. 2 presents the average plants length for all samples *versus* UHF-CW exposure time. We found that all UHF-CW exposure times of seeds have a stimulating effect on the growth of the plantlets provided by irradiated seeds in comparison with control sample. All results are statistically significant, as resulted from the average comparison with the lengths of the control, using the Student t-test.

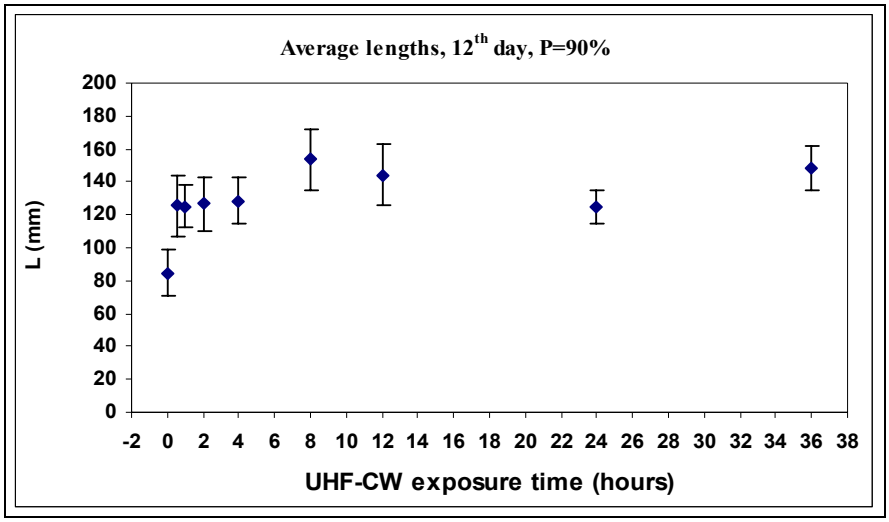

Fig. 2. The average length of plants versus UHF-CW exposure time.

Quantitative insight into the molecular mechanisms involved in the complex phenomena of plant growth was carried out by means of spectrophotometrical assays. The assimilatory pigment levels have been comparatively studied on the basis of graphical representations of chlorophyll **a** (Chla), chlorophyll **b** (Chlb) and total carotenoids (Car) contents versus UHF-CW exposure times.

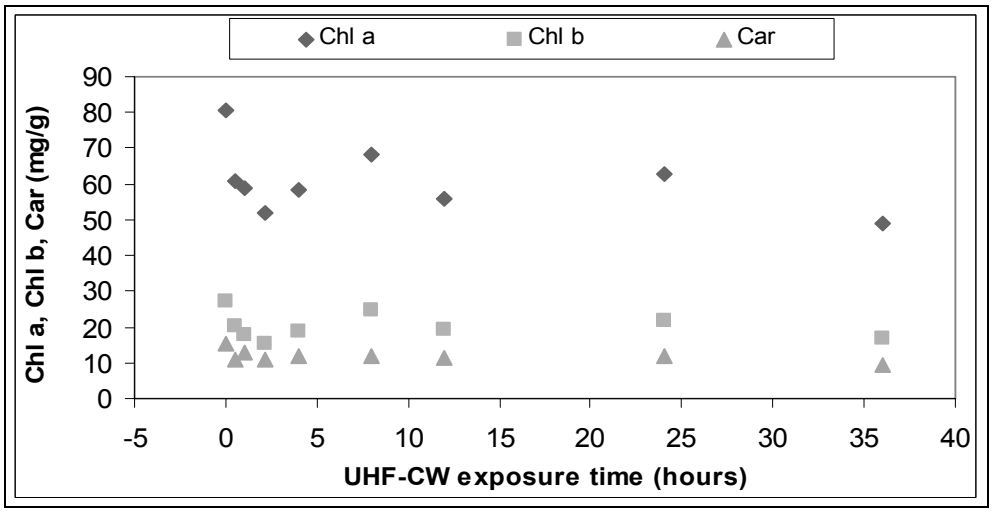

Fig. 3. Assimilatory pigments level in *Zea mays* plantlets versus UHF-CW exposure time (Chl **a** –the content of chlorophyll **a**, Chl **b** – the content of chlorophyll **b**, Car – the content of total carotenoid pigments).

The data provided by the assimilatory pigments levels offered the main information upon the photosynthesis complex process since they are able to reveal the response of the Light Harvesting Complex II (located in the chloroplast membranes) to the external constraints. The photosynthesis pigments contents (chlorophyll **a**, **b** and total carotenoids) in the green tissue of young plantlets (age of 12 days) belonging to the analyzed plant species are presented in Fig. 3. Student *t*-test (two tailed, pair type) was applied to evaluate reliability of modifications induced by electromagnetic exposure of seeds in assimilatory pigments level in the plantlets developed from exposed seeds, for exposed samples in comparison to the control. Statistical analysis results are presented in Table I: all modifications induced by 900 MHz electromagnetic field exposure have statistical significance in comparison with the threshold of 0.01.

Table 1

Results of statistical analysis of assimilatory pigment content in young plants after UHF-CW seeds exposure

UHF –CW (900 MHz) exposure times (h)										
	0	**0.5**	**1**	**2**	**4**	**8**	**12**	**24**	**36**	**Pigment**
Standard deviation (mg/g)	0.168	0.068	0.0401	0.0624	0.017	0.075	0.0777	0.0805	0.0412	**Chl a**
	0.034	0.192	0.0123	0.0564	0.176	0.195	0.0531	0.1993	0.1106	**Chl b**
	0.103	0.172	0.0849	0.0595	0.128	0.131	0.0569	0.0307	0.1296	**Car**
P(*t*-test)		1.302 ×E–05	1.801 ×E–05	3.137 ×E–05	2.386 ×E–05	2.824 ×E–05	4.794 ×E–05	9.545 ×E–05	1.944 ×E–05	**Chl a**
		2.000 ×E–04	1.169 ×E–05	2.990 ×E–05	2.000 ×E–04	0.002	6.085 ×E–05	0.0008	2.749 ×E–05	**Chl b**
		1.000 ×E–04	3.978 ×E–05	5.484 ×E–05	0.002	2.000 ×E–04	9.210 ×E–05	0.0005	1.235 ×E–05	**Car**

The chlorophylls ratio (chlorophyll **a**/chlorophyll **b**) is known as an indirect indicator of the energetic activity of LHC II system (Light Harvesting Complex II) that is controlling the first stage of solar energy conversion into its chemical form. The chlorophyll ratio (Fig. 4) for samples provided by electromagnetically exposed seeds presents significant variations in comparison with the control value suggesting electromagnetic sensitivity of photosynthesis efficiency. A slight increase for chlorophylls ratio value was obtained for short electromagnetic field exposure time while for long electromagnetic field exposure times a diminished value for chlorophylls ratio was revealed. This ratio being considered an important physiological parameter regarding the photosynthesis efficiency, the results displayed in Fig. 4 might be taken as a premise upon the slight stimulation of the photosynthesis in the

case of short electromagnetic exposure times. The statistical analysis accomplished for the chlorophyll ratio (by applying the *t*-test to compare control and test sample data) revealed statistical significance ($p<0.05$) for all exposed samples.

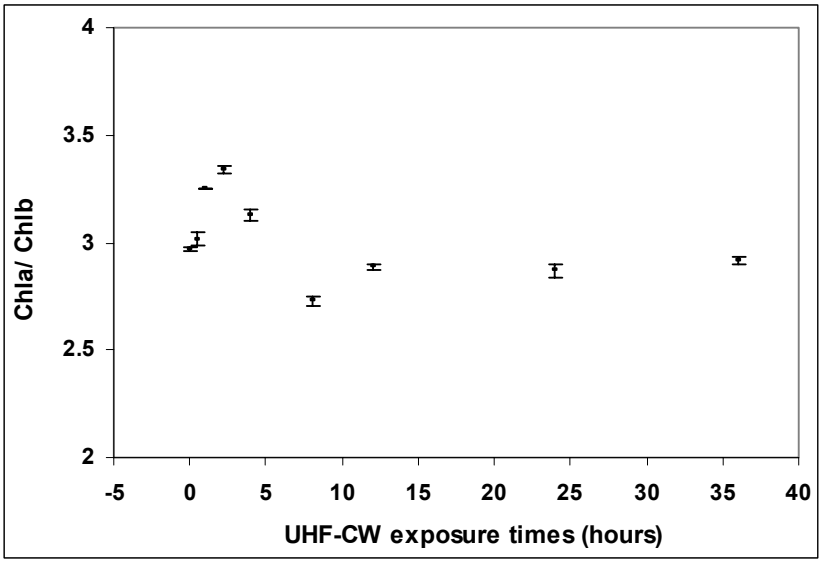

Fig. 4. Electromagnetic field effect on the chlorophylls ratio in *Zea mays* plantlets.

The average content of nucleic acids in *Zea mays* young plantlets provided by electromagnetic exposed seeds is presented in Fig. 5. One can see that for short exposure times the average nucleic acids level is enhanced in comparison to the control sample; an interesting stimulatory effect at 8 hours electromagnetic field exposure was noticed. Applying the *t*-test to compare control and test sample, data for the average nucleic acid level have revealed statistical significance ($p < 0.01$) for all exposed samples. Statistical analysis results are presented in Table II.

Table 2

Results of statistical analysis of total nucleic acid contents in young plants after UHF-CW seeds exposure

UHF –CW (900 MHz) exposure times (h)									
Standard deviation (µg/g)	**0**	**0.5**	**1**	**2**	**4**	**8**	**12**	**24**	**36**
	0.2496	0.3899	0.4363	0.1756	0.5720	0.8422	0.4466	0.3851	0.2430
P(*t*-test)		0.0018	0.0002	2.40 ×E–06	9.98 ×E–06	5.50 ×E–06	2.50 ×E–05	0.0001	7.21 ×E–06

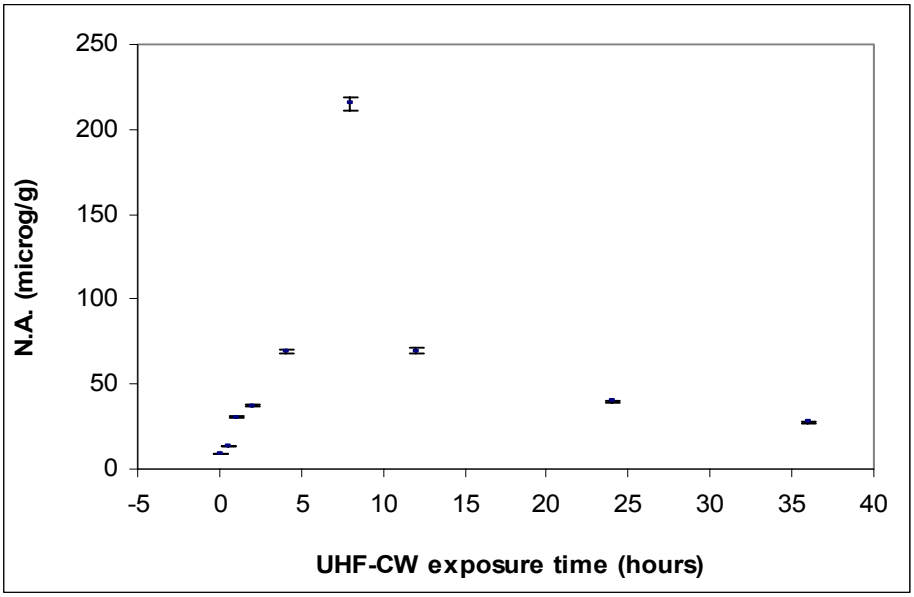

Fig. 5. The level of DNA and RNA for the plantlets provided by electromagnetic field exposed seeds.

CONCLUSION

In summary, in this experimental study regarding the 900 MHz electromagnetic field exposure influences upon plant seedlings developed by exposed seeds, during their early ontogenetic stages, a slight stimulatory influence on the plants growth was gained, the average plants length values being enhanced for all exposure durations. For assimilatory pigments of *Zea mays* plantlets, an inhibitory effect was observed for all exposure durations. A stimulatory effect for short exposure times and a slight inhibitory effect for long exposure times was obtained on the chlorophyll ratio in *Zea mays* plantlets developed from exposed seeds. Also, the total level of DNA and RNA of plantlets developed from exposed seeds was highly enhanced for short exposure durations.

REFERENCES

1. BELYAEV, I.Y., V.S. SHCHEGLOV, E.D. ALIPOV, V.D. USHAKOV, Non-thermal effects of extremely high frequency microwaves on chromatin conformation in cells in vitro: dependence on physical, physiological and genetic factors, *IEEE Transactions on Microwave Theory and Techniques,* 2000, **48**, 2172–2179.
2. BELYAEV, I., Non-thermal biological effects of microwaves, *Microwave Review,* 2005, **11(2)**, 13–29.

3. BELYAEV, I., Nonthermal biological effects of microwaves: current knowledge, further perspective, and urgent needs, *Electromagnetic Biology and Medicine*, 2005, **24(3)**, 345–403.
4. DURNEY, C.H., H. MASSOUDI, M.F. ISKANDER, *Radiofrequency Radiation Dosimetry Handbook*, 4th Edition, USAFSAM-TR-85-73, Brooks AFB, Texas, USA, 1986.
5. KWEE, S., P. RASKMARK, Changes in cell proliferation due to environmental non-ionizing radiation. Microwave radiation, *Bioelectrochem. Bioenerg.*, 1998, **44**, 251–255.
6. LICHTENTHALER, H.K., A.R. WELLBURN, Determinations of total carotenoids and chlorophylls **a** and **b** of leaf extracts in different solvents, *Biochemical Society Transactions*, 1983, **11**, 591 – 592.
7. STRUCHKOV, V.A., N.B. STRAZHEVSKAYA, R.I. ZHDANOV, DNA-bound lipids of normal and tumor cells: retrospective and outlooks for functional genomics, *Bioelectrochemistry*, 2002, **58**, 23–30.
8. TAMBIEV, A.H., N.N. KIRIKOVA, The prospects of use of EHF radiation in photobiotechnology, in: N.D. Deviatkov, O.V. Betskii (eds), *Biological aspects of low intensity millimeter waves*, Moscow Univ. Prospect, 1994, 125–163.
9. TKALEC, M., K. MALARIC, B. PEVALEK-KOZLINA, Influence of 400, 900, and 1900 MHz electromagnetic fields on *Lemna minor* growth and peroxidase activity, *Bioelectromagnetics*, 2005, **26(3)**, 185–193.
10. ZHDANOV, R.I., N.B. STRAZHEVSKAYA, A.R. JDANOV, G. BISCHOFF, A spectroscopic and surface plasmon resonance study of oleic acid/DNA complexes, *J. Biomol. Struct. Dyn*., 2002, **20**, 231–241.