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EMI EHF-INDUCED CHANGES OF FREE-RADICAL OXIDATION PROCESSES IN *TRITICUM AESTIVUM* L. SEEDLINGS

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The influence of electromagnetic irradiation by extremely high frequencies 41.8 *GHz* and 51.8 *GHz* (EMI EHF) on chemiluminescence (H_2O_2–CL) intensity, malondialdehyde (MDA) content and catalase activity in wheat shoots cells has been investigated. It has been shown that this irradiation impacts on lipids oxidation of cellular membranes of plant seedlings and leads to intensity increasing of free-radical processes, measured by CL. It has also been shown that at multiple irradiation of wheat seedlings the increasing of MDA rate is observed. At the same time EMI-induced oxidative stress was indicated by markedly change of catalase activity depending on EMI frequency used and exposure duration.

Keywords: EMI EHF-irradiation, wheat seedlings, oxidative stress, chemiluminescence, MDA content, catalase.

Introduction. During a long-term period of development all organisms have fully adopted various environmental conditions on our planet. The natural low-intensity electromagnetic fields of the Earth are the constant part of biosphere and many processes of cell normal metabolism, in one way or another, are associated with these natural fields [1, 2]. Nowadays, because of technogenic human activities, intensity of artificial electromagnetic irradiation (EMI) produced by the operation of commercial frequency generators, microwave and extremely high frequency (EHF) devices have been significantly increased. So, the problem of electromagnetic safety of biosystems as sums is extremely important.

Experiments conducted have shown that EMI EHF or electromagnetic radiation in millimeter range (MM waves) interacts with biosystems of different levels of organization, including molecular level [2–4]. A few mechanisms of EMI interaction with living organisms are discussed [2, 5].

According to one of them EMI EHF affects living organisms also by causing oxidative stress: it increases the activity and concentration of reactive oxygen species (ROS), including superoxide radicals ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\bullet}$) [6, 7].

Interaction of the last ones with non-saturated fatty acid residues of membrane lipids induces the formation of peroxide radicals of lipids ($RO_2^{\bullet}$) and as a result of their recombination non-stable tetroxide degrading with chemiluminescent (CL) light quantum yielding is formed [8, 9]. Spontaneous weak luminescence (chemiluminescence of

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biological tissues) is a phenomenon of radiation induced by chemical reactions of electronic excited states, gives a possibility directly to determine concentration of free radicals and dynamics of their transitions in metabolism processes [8]. It was clearly shown that CL of biological objects was caused by free radical processes and by peroxidation of the lipids (LPO) and lipid containing structures [9].

The interest in free radicals is conditioned by the fact that they are participants in the important physiological processes in living organisms as well as in certain response reaction of the organism to MM EMI effect [8, 9]. ROS are highly reactive in the absence of any protective mechanism, they can seriously disrupt normal metabolism through oxidative damage to membrane lipids, proteins and nucleic acids [10, 11].

Plants cells have evolved a defensive, ROS regulating system that consists of enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and non-enzymatic components-glutathione and ascorbate; this system allows cells signaling while avoiding cellular damage [10].

Among enzymes providing antioxidant protection of plants catalase (CAT. EC 1.11.1.6) plays an important role, being the major scavenger of H_2O_2. Catalase was the first antioxidant enzyme to be discovered and characterized in plants cells [11]. This enzyme, which is located in peroxisomes, glyoxysomes and mitochondria, and is apparently absent in chloroplast, catalyzed the dismutation of two molecules of H_2O_2 into water and oxygen [10, 11]. All forms of the enzyme are tetramers with each monomer of 50–70 *kDa* [12].

There is compiling evidence, which shows that the activities of enzymes involved in ROS scavenging were altered by several environmental stresses, including EMI EHF [3, 4, 13–16]. The changes of activity and isoenzyme patterns of POX in *Triticum aestivum* shoots were observed in response to EMI EHF treatment [3]. Payez et al. reported the increase of CAT activity and reduction of POX in wheat seedlings exposed to 10 *kHz* EMF [13]. It has been shown that EMI with 50.3 *GHz* and 64.5 *GHz* frequencies changed enzymatic activities in barley seeds, particularly at the initial stages of their germination and led to rise the activity of amylotic enzymes [14]. Recently, Zare et al. reported that catalase activity and H_2O_2 content increased in response to high frequency EMI in *Zea mays* L. leaves and roots [15], whereas, in the other study, Talei et al. proved that a certain level of microwave power and exposure time can improve the germination of rice seedlings and increase oxidative stress level [16]. However, the specific mechanisms of EMI EHF action on biological systems remained not clear yet.

So, the objective of this study was to test the influence of multiple EMI EHF on free-radical oxidation processes of lipids, i.e. intensity of H_2O_2–CL, accumulation of malondialdehyde (MDA) and catalase activity in growing wheat seedlings.

Materials and Methods.

Plant Culture and Electromagnetic Irradiation Procedure. Seeds of wheat (*Triticum aestivum* L.) of the "Bezostaya" cultiver were taken for the experiment. Seed samples were selected for uniform size, sterilized with 0.03% potassium permanganate ($KMnO_4$) solution for 2 *min* and thoroughly rinsed with distilled water. Then these seeds were imbibed in water overnigth and were germinated in Petri dishes on wet filter paper in termostat at 25°*C* in the dark. Ordinary tap water was used for moistening.

The germinated 2-day-old seedlings were treated at EMI with 41.8 *GHz* and 51.8 *GHz* frequensies for 5 days, each 20 *min*. The irradiation was performed using the generator G4-141 type with working interval of 37.50–53.57 *GHz* ("Istok", RF) and power flux density 0.6 $mW{\cdot}cm^{-2}$. Frequency signal stability was ±0.05 and frequency

deviation of output signal in persistent regime of generation did not exceed 6 *MHz*. The distance from the radiating end of the conical antenna to the object of irradiation was 20 *cm*. The choice of applied frequencies was conditioned by the fact that water resonant and near them frequencies are in this range [2].

The non-irradiated germinating seedlings were taken as reference samples.

Plant Extract Preparation. The shoots (500 *mg*) of both control and EMI exposured plants were harvested and homogenized in 5 *mL* cold phosphate buffer (25 *mM*, pH 7.0, containing 1 *mM* EDTA, 0.5% triton X-100) in a mortar and pestle. All procedures were performed in cold conditions. The homogenate was centrifuged at 12.000 *g* for 10 *min* at 4°*C*. The supernatant was used for assessing the protein content and CAT activity using UV-visible Spectrophotometer (model SF-46, USSR). All enzymatic activities were measured at 25°*C*. The method of Lowry [17] was followed to estimate protein content in the shoots using bovine serum albumin as a standard.

H_2O_2–CL Registration was carried out in quantum-metric device, which was assembled by using new developed electronic and amplifier units. As a detector of weak light fluxes a highly sensitive low-noise photoelectronic multiplier PEM-140 (spectral sensitivity diapason 300–700 *nm*) was used.

Chemiluminescent analysis was carried out by computer system of automatic registration and mathematical treatment of the obtained experimental data through the USB 6008 data gathering device and program providing treated in Lab VIEW medium [18]. Device calibration was realized by etalon source of light of known intensity SPCM-1 N46 with absolute light flux $8.43 \cdot 10^5$ quantum/$4\pi s$ [18]. Experiments were performed at room temperature.

Since the wheat leaves extract does not have its own CL at room temperature, because of weak signal, the method of CL activation by hydrogen peroxyde was used. In this case CL was due to the interaction of test sample with H_2O_2. During the decomposition of H_2O_2 the hydroxyl radicals are generated, which give rise to a free-radical chain reaction accompanied by emission of light quanta [9].

For measuring H_2O_2–CL 2.8 *mL* of the sample, 0.2 *mL* of 3% H_2O_2 was added in cuvette and placed against photocatode PEM, which provides high efficiency of registration. CL registration was started via 3–5 *s* after sample insertion.

At registration of radiation from the sample, determination of dark noise background of device was carried out immediately before each measurement. CL of the samples was recorded during 300 *s*. CL intensity (I_{CL}) was measured in conditional units (c.u.) and by CL light-sum.

Assay of MDA Content. Accumulation of products of lipid peroxidation MDA was judged by color reaction with 2-thiobarbic acid, accepting that extinction molar coefficient of dyed complex with MDA is equal to $1.56 \cdot 10^5$ $M^{-1}cm^{-1}$ [19].

Catalase Activity Assay. The supernatant of shoots homogenate was used to assay Catalase activity. The method based on the reaction of H_2O_2 in a mixture with ammonium molibdate ($(NH_3)_2 \cdot MoO_4$) proposed by Korolyuk [20] was used to estimate CAT activity. The change in optical density due to the emergence of complex H_2O_2–ammonium molibdate is measured spectrophotometrically at 1st and 10th *min* at 410 *nm*. The assay mixture contained 1 *mL* Tris-HCl (0.02*M*, pH 7.4) 2 *mL* H_2O_2 (0.03%), and 0.1 *mL* enzyme sample. This was incubated for 10 *min* at 25°*C* in dark, after which the reaction was stopped by adding 1 *mL* of ammonium molibdate (4%). To the blank 0.1 *mL* distillated water was added of the zero time of the same assay mixture. A decrease in the absorbance of H_2O_2 within 10 *min* at 410 *nm* ($\varepsilon = 22.2 \cdot 10^3$ $mM^{-1}cm^{-1}$) was recorded. The CAT activity was expressed in unit activity (u.a.)$\cdot mg^{-1}$ protein; u.a. is defined as the change in absorbance by 1 $min^{-1} \cdot mg^{-1}$ protein.

All the experiments were performed in triplicates and values presented here are the mean of three values ± standard error.

Results and Discussion. The results of experiments on the effect of EMI EHF on free radical oxidative processes in wheat seedlings are given in Fig. 1–3.

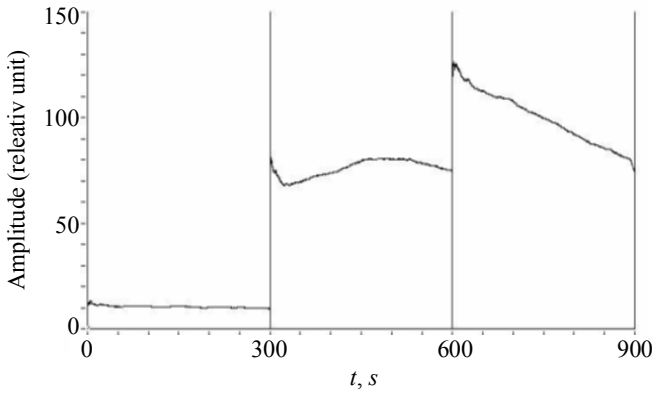

Fig. 1. H_2O_2–CL of 6-day-old wheat seedlings:
1 – background of quantum metric device;
2 – non-irradiated sample;
3 – irradiated sample by EMI with 51.8 *GHz*, 100 *min*.

mean	sigma
10.45	0.5485
76.01	3.955
99.67	12.470

It has been shown that external physical field significantly effects on H_2O_2–CL, MDA accumulation and catalase activity of germinated shoots during their growth.

As it is obvious from Fig. 1, the H_2O_2–CL light-sum of homogenate of control wheat 6 days old seedlings was equal to 76±3.9 c.u. at dark background of device 10.45±0.55 c.u. After multiple irradiation during 5 days (20 *min* per day) of growing seedlings by EMI with 51.8 *GH*z frequency the H_2O_2–CL light-sum increased by 33% compared with control (Fig. 1).

The dependence of MDA rate in control and multiple irradiated wheat shoots cells on irradiation duration is represented in Fig. 2. Studying parameter showed significant differences compared with control, especially at the 51.8 *GHz* frequency (Fig. 2 A, B).Thus, in control samples the MDA content showed trend for slight increasing during growth. Its mean varied from 2.1±0.06 *nM/mg* protein (2^{th} day) to 2.3±0.08 *nM/mg* protein (6^{th} day), increasing by 9.5%.

As it is obvious from Fig. 2, A, the use of one-fold (20 *min*) EMI with both frequencies used of wheat seedlings did not provoke any changes in MDA content, which was practically the same as for control shoots cells. After threefold (60 *min*) and fourfold (80 *min*) irradiation of growing seedlings by EMI with 41.8 *GHz* frequency the MDA content increased by 17 and 19% respectively compared with control ones (Fig. 2, B). On the 5^{th} day (100 *min*) MDA value in multiple irradiated seedlings decreased by 8% comparing with fourfold irradiation, but remained higher than respective control by 11% (Fig. 1 A, B).

In case of two- (40 *min*) and threefold (60 *min*) EMI of wheat seedlings with 51.8 *GHz* the indicator of LPO–MDA value increased by 12 and 25% respectively compared with control (Fig. 1, B). But the further irradiation resulted some decreasing of studying index: after 80 and 100 *min* irradiation the MDA values were higher than control values only by 17 and 8% respectively.

So, as it is obvious from obtained data (Fig. 2 A, B) the greater response of ROS production, which expressed by MDA rate increasing was recognized in case of wheat seedlings EMI by 51.8 *GHz* frequency. According to obtained data MDA content significantly ($p<0.05$) increased in all the multiple EMI and this increase reached its maximum earlier at summary 60 *min* duration for 51.8 *GH*z frequency and at 80 *min* duration for 41.8 *GHz*.

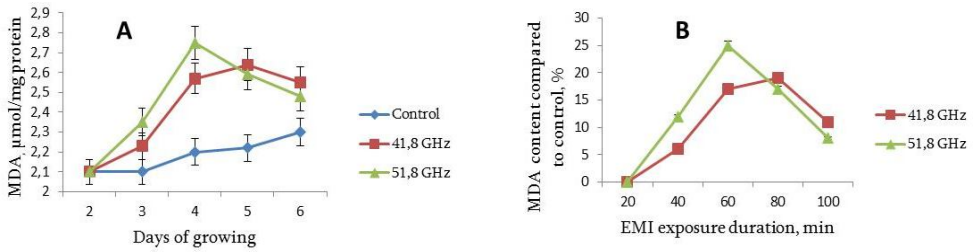

Fig. 2. The dependence of MDA content in cells of EMI EHF irradiated wheat seedlings on EMI exposure duration (A) and this parameter change's value compared with control in percent's (B).

By analyzing the above data on EMI exposure of wheat seedlings it can be certainly established that depending on the irradiation exposure an intensification of the lipid free radical peroxidation processes takes place.

It has been revealed that the irradiation of germinating seedlings induces catalase activity changes in them the magnitude of which depends on EMI frequency and expose duration (Fig. 3, A, B).

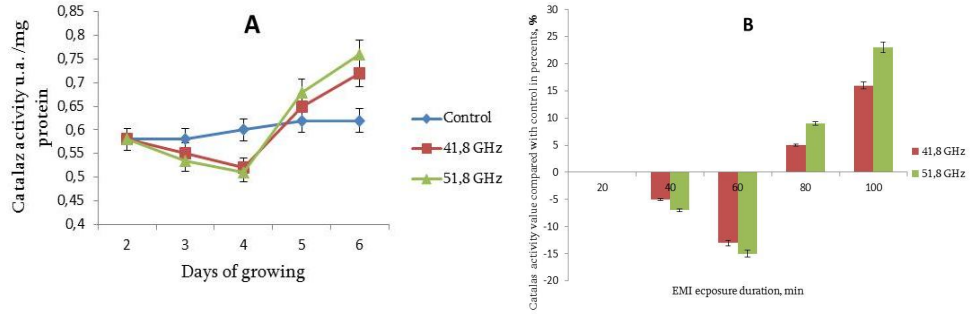

Fig. 3. The dependence of catalase activity in cells of EMI EHF irradiated weat seedlings during their growth (day) on EMI exposure duration (A) and activity change compared with control in percents (B).

According to obtained data, catalase activity is slightly changed during the control plants growth and in 6-day-old shoots it was 6% higher than in 2-day-old shoots (Fig. 3).

From the data represented in Fig. 3, it is obvious that in case of irradiation with 41.8 *GHz* frequency catalase activity decrease was observed for 40 and 60 *min* exposure by 5 and 13% correspondingly compared with control (Fig. 3, B). Interestingly, in case of EMI with 80 *min* duration in 5-day-old shoots the catalase activity increased by 18% compared with previous 60 *min* exposure, so reached the control value and overcame it by 6.4%. The further increase of irradiation duration up to 100 *min* caused a significant activation of catalase: more than 1.16 times compared with 80 *min* duration EMI and by 16% compared with control plants.

As it is obvious from represented in Fig. 3, B, the same regularity was observed during the growth of irradiated shoots with 51.8 *GHz* frequency, moreover in this EMI frequency response was greater. Indeed, 40 *min*. irradiated seedlings had depressed catalase activity. Particularly enzyme activity was only 93% of control (considered as 100%); the inhibition was increased with 60 *min* duration-up to 85% (Fig. 3, B). But, the influence of multiple EMI for 80 *min* and 100 *min* durations caused increase of catalase activity by 9 and 23% compared with control.

Obtained data indicated that there was a significant ($p<0.04$) increase in MDA content of EMI exposed seedlings cell, and this increment was more pronounced at 51.8 *GHz*, compared with control. This fact indicates that the biological effect of MM EMI on organism level is performed mainly at water resonant frequencies that show the certain role of water in biosystem response reaction [2, 3].

In this concern, was stated that the use of EMI at 42.8 and 51.8 *GHz* frequencies leads to an accumulation of free radicals such as $O_2^{\bullet-}$ and H_2O_2 [6, 7].

Increase in MDA content and antioxidant enzyme-catalase activity under EMI-stress in present study is consistent with the studies [14, 15] in which wheat, barley and maize seeds were grown under EMI and antioxidative enzymes activities were found to increase.

Comparing the obtained results of MDA level and catalase activity in irradiated plant shoots we can notice that the comparatively increasing rate of MDA parallel to the duration increase for each applied EMI frequency is correlated to the suppression of catalase activity in the shoots. In plants submitted to external physical field SOD, CAT and GPX act as a defense mechanism, which gets activated [3, 11, 13, 14]. The results obtained in current study also demonstrate that catalase protects wheat plant cells from destructive effects of ROS and constitutes key component of the cellular antioxidant defense system.

According to obtained data the decreasing in MDA content in the seedlings treated with EMI might be due to the increase in the activity of catalase.

This study leads to more clear understanding of EMI action on cellular level. The results of our study revealed the role of antioxidant defense system in organism's response to external physical field.

Conclusion. In the study is shown that increase in catalase activity in *Triticum aestivum* L. seedlings could be attributed to EMI stress. The results may also suggest that the 51.8 *GHz* EMI, the resonant for water frequency, is more effective for increasing the ability of the antioxidant enzyme to protect against free radical oxidative stress.

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