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Impact of MM-Waves on the Different Cellular Levels of Wheat Seedlings

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Abstract. The phospholipids content of the nuclear envelope and the soluble nuclear fraction of the cells of wheat seedlings *T. aestivum* have been investigated. It is shown redistribution in the content of phospholipids in seedlings nuclear subfractions on the third day of germination after a single treatment of wheat seeds by extremely high frequency of electromagnetic irradiation (EHF EMI). It was revealed that under the influence of EHF EMI, total content of anionic phospholipids in the nuclear envelope is reduced, resulting in the changes of its permeability. In contrast to the nuclear envelope, in the matrix of the processing of mm-wave increases the total content of anionic phospholipids that can certainly cause a change in chromatin conformation. In our study have also shown that under the influence of mm-wave increased surface charge, which led to the changes in the electrokinetic potential (EKP) of cell nuclei in the electric-field gradient. Thus, as a result of mm-wave exposure, a potential difference between the outer and inner surfaces of the nuclei is formed due to the difference in content of anionic phospholipids between nuclear membrane and a matrix, which leads to the changes in membrane permeability.

Keywords: nuclear envelope, mm-waves, phospholipid, soluble nuclear fraction, wheat seedlings, EKP-zeta-potential

Introduction

With the development of civilization and technology, our living space is filled with a variety of electromagnetic fields, the sources of which are computers, cell phones, various radiological diagnostic and physiotherapy equipment in medicine, cellular antenna amplifier, etc. The problem of electromagnetic safety becomes extremely relevant, since the most medical devices and technical devices radiate mm-wave in the range of 1-300 GHz. It is expected that many of the structural changes resulting from the impact of the stress mainly are epigenetic in nature [1]. However, most of the loads at the same time carry the membrane structure of the cells, and primarily phospholipids in composition of the cell membrane and the nuclear envelope, in membrane structures of organelles.

The aim of this study was to detect changes in the surface charge of the cellular nucleus and the phospholipid content of the nuclear subfractions of wheat seedlings under the influence of EHF EMI, which can elucidate the mechanisms of the mm-wave influence on living systems at the cellular level.

Materials and methods.

Seed Germination. Seeds of hexaploid wheat: *T. aestivum L.* of Arshaluys variety were soaked overnight in darkness at 26 ^{0} C and then placed on trays with filter paper moistened with tap water and continue growing in a thermostat at temperature 26^{0}C within 72 hours. Presoaked for the night seeds irradiated with non-thermal low-intensive EHF of EMI for receiving irradiated by mm-waves seedlings. The source of irradiation is served by generator of high-frequency signals of EMI G4-141 (made in USSR) in the range of 45 GHz-53 GHz. Radiation was carried out during 20 min at radiation density 0.64 mV/cm^2.

Nuclei isolation and fractionation. Nuclei from 3 day etiolated wheat seedlings were isolated according to the Blobel and Potter method [2] with some modification. Seedlings frozen in liquid nitrogen were ground with a mortar and pestle to fine powder, as described in article [8]. The

pelleted nuclei were repeatedly washed and purified intact nuclei were obtained, which subjected to fractionation on nuclear envelope and soluble nuclear fraction [3, 4].

Phospholipids extraction and quantification. Phospholipids from soluble nuclear fraction and a nuclear envelope were extracted by method of Folch as it was described in [4], and a quantity of separated phospholipids fractions estimated by the content of inorganic phosphorus according to the Ames method [5].

Determination of the nuclear electrokinetic potential. Nuclear electrophoretic mobility was measured by microelectrophoresis. To this end, isolated intact nuclei from wheat embryos were suspended in the medium containing 140 mM NaCl and 10 mM Tris-HCl, pH 7.5 and placed in a horizontal electrophoretic chamber, where using the ocular micrometer of the Biolam microscope (LOMO, Russia) and stopwatch, the rate of nuclear movement was measured. Measurements were performed under the field strength of 35 V/cm, current intensity 10 mA and under different current polarity. The EKP value was calculated using formula taken from the article [3],

$$EKP = 4\pi\eta\omega/DE,$$

where η- is the coefficient of viscosity of dispersion medium, ω – the rate of nuclear movement, D is a water dielectric constant, E – field strength in the electrophoretic chamber. The Table and diagrams presented arithmetic means of 4 independent experiments and its standard deviations.

Results and Discussion

Over the past decade, it is shown the crucial role of phospholipids in the regulation of processes for the growth and development of plants in the hormonal signal transduction in formation of cellular responses to external stimuli [6, 7].

We have previously shown that the change in physiological state entails changes of electrokinetic (ξ-) potential (EKP) of the seedlings cell intact nuclei and quantitative changes occurring in the phospholipids composed of nuclear sub-fractions [3, 4, 8]. In studies on the influence of mm-waves on the intact nuclei speed in the electric field gradient, we obtained data on the increase ξ-potential of intact cell nuclei isolated from treated 4-day-old wheat seedlings. Research data shown in the diagram (Fig. 1) testify that seed treatment by mm-waves in the range of 45-53 GHz increases the electrokinetic potential at all expositions, which may indicate about changes in the physiological state of cells, as well as changes in morphological and biochemical properties of the cells.

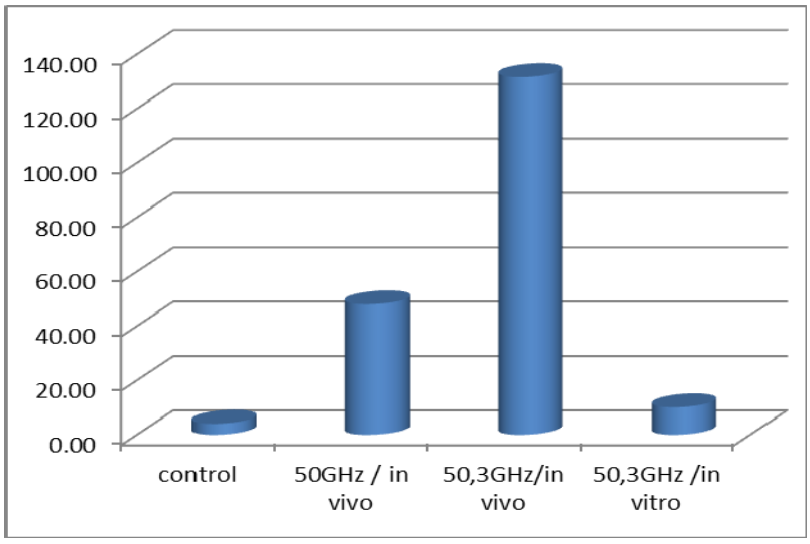

Fig. 1. Change in EKP of wheat seedlings nuclei under influence of EHF EMI.

Such changes have a natural character and indicate on the activation of metabolic processes under the influence of mm-waves. On the diagram also it is shown the value of EKP of nuclei seedlings conducted *in vitro*, since we irradiated the homogenate of a control intact nuclei of the wheat seedlings in the extracellular medium by frequency 50.3GHz during for 20 min. As it is seen, influence of mm-wave at frequency 50.3 GHz on nuclei homogenat *in vitro* increased its EKP to 10.1mV, however this potential is much lower than the value of EKP of intact nuclei *in vivo* (131.6 mV), when study carried by impact of mm-waves on soaked seeds and after that only isolated nuclei from seedlings. Comparison of these data shows that in addition to direct effects of mm-wave on the nuclear envelope of cellular homogenate, a changes occur in the cell itself, in its metabolic activity. We have proposed that such contribution to the change in the surface potential of the nuclear envelope may, primarely, can create the phospholipids in the composition of nuclear membrane.

The study of the nuclear membrane phospholipid composition of wheat seedlings showed that the total phospholipid content is reduced from 1.33 to 0.9 (approximately 33%) after irradiation of mm-wave by frequency of 50 GHz with respect to the control (Fig. 2a). Processing of soaked seeds by mm-wave frequency of 50.3 GHz, increases the total content of the PL in the nuclear envelope to 5.26%, and higher frequencies of EMI - 53 GHz, with the same duration of treatment leads to an increase in the total content of the PL of the nuclear membrane at 118% of control. For example, increasing the proportion of PC and PE on 8% and 9%, accordingly, is relative and close to a control. Have been obtained significant reducing of the share of PA in the nuclear membrane from 15.03% to 5.86% (Fig. 2a), content of which is very important for the structure and permeability of a nuclear membrane [9, 10]. Such a sharp change can lead to significant changes in the properties of the nuclear envelope: a decrease in its permeability, smoothing the surface and a drop in the total surface charge [4].

Changes in the content of the PL were also observed in the composition of the soluble nuclear fraction (Fig. 2b). The PL content of a soluble nuclear fraction of 3-day-old seedlings cells are changes under the influence of disposable processing by mm-wave of frequency 50 GHz during 20 minutes. As follows from the data, the total phospholipid content of the soluble nuclear fraction after treatment with mm-wave is reduced by 71.2%. Treatment EHF EMI leads to an insignificant increase in the share of the PC, PI and PS of about 1-2%, while the share of PA decreased by 6% (Fig. 2b). Total content of the PL at this treatment decreased by 71.2% is relative to control preparations. It is known that EHF irradiation can cause accelerated growth and an increase in the biomass of photosynthetic plants, intensified the photosynthesis processes. [11]. We have also received similar physiological changes under the influence of microwave radiation, but in etiolated seedlings of wheat seed, where the influence of mm-waves on the photosynthetic apparatus and photosynthetic pigments are reduced to a minimum. At the level of the whole organism, we received a positive effect of EHF on physiological processes of the body, possibly temporarily [12].

Processing of seeds by mm-waves with a frequency of 50.3 GHz also has an impact on the content of the PL in the soluble nuclear fraction of seedlings. As it is shown, the total content of the PL and the percentage parts of phospholipids in composition of nuclear soluble fraction is decrease by 25.6% under impact of mm-waves. Our experimental data evidence, that mm-wave with a frequency of 50.3 GHz exert a suppressive effect on the growth of seedling [12] and it leads to decrease in content of the PL in the soluble nuclear fraction (Fig. 2b).

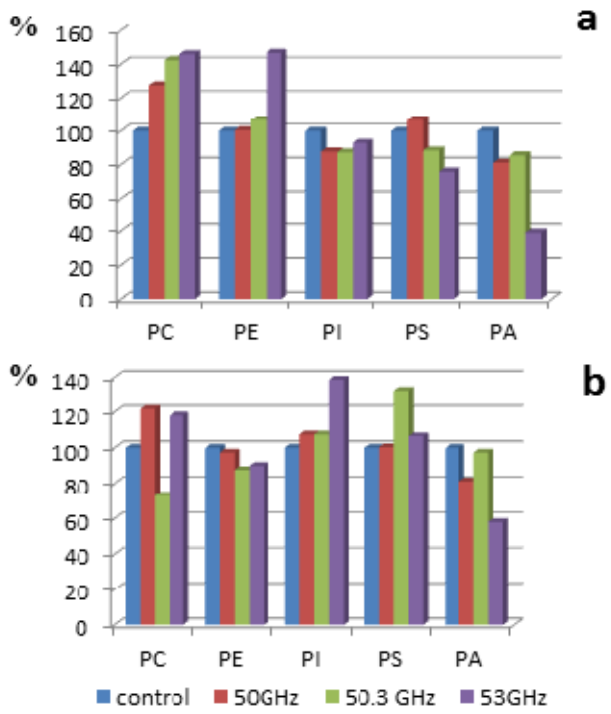

Fig. 2. The content of phospholipids in % of the total content of the nuclear envelope (a) and a soluble nuclear fraction (b) in 3- day seedlings after a single treatment of wheat seeds with different frequencies of mm-wave. PA is a phosphatidic acid; PC-phosphatidylcholine; PE-phosphatidylethanolamine; PI-phosphatidylinosytol; PS-phosphatidylserin

Increasing the frequency of EHF EMI to 53 GHz leads to enhancement of total amount of the seedlings nuclear soluble fraction of the PL at 228%. Such a sharp increase in the content of the PL may cause "liquefaction" the contents of the nucleus, which in turn may lead to conformational rearrangements in the active and inactive chromatin compartments [13, 14]. The results show that changes are occurring as well as in the ratio of individual PL in matrix content (Fig. 2b). Proportion of PC and PS of nuclear matrix is slightly reduced by 2-3%. At the processing EHF EMI by frequency 53 GHz during 20 minutes, proportion of the PA reduced by 12%, while the proportion of PI increased by 2% (Fig. 2b).

We have previously shown [4] that just PA brings the most significant contribution to the formation of the surface charge of the nuclear envelope during seed germination of wheat. Therefore, we assume that the decrease in PA will change the value of the surface charge of the nucleus. Summarizing the results, we can note a regulatory: in the nuclear envelope, there is a clear increase in the content of PC and PE. In contrast to it, decrease in PI and PA obtained in the nuclear envelope for three investigated frequency of mm-wave (Fig. 2a). Since PA is involved in the regulation of important biological processes, such as protein phosphorylation, activation of oxidative processes and modulation of membrane transport, and promotes to curvatures of membrane, formation of bulges and an increase in membrane permeability [9], it naturally follows that these changes may have an impact on the successful performance of the nuclear membrane.

As evident from Fig. 2a, neutral PL (PC and PE) reached to 51.7% in content of nuclear envelope, whereas anionic PL (PI, PS, PA) decrease to 41% under influence of mm-waves. In the content of soluble nuclear fraction in our study obtained accordingly decreasing of PE and PA (Fig. 2b). As a result, changes in content PL of nuclear fractions observed the following changes in the total charge, which is represented below in table.

Table. The anionic and neutral phospholipids content of wheat seedlings, expressed in % of total content.

Nuclear fraction	PL	**Total content of PL (in %)**			
		control	50 GHz	50.3 GHz	53 GHz
envelope	neutral	35.33 %	40 %	43.64	51.72
	anionic	64.67 %	59.9%	56.38	48.28
soluble	neutral	38.04	40.74	31.11	38.46
	anionic	61.95	59.26	68.88	61.54

If we sum all the neutral and anionic phospholipids of the nuclear envelope separately, we will get a definite regularity: increasing of neutral and decrease of anionic PL in the content of the nuclear envelope. In contrary, in the content of the soluble nuclear fraction under the influence of mm-wave obtained decrease of neutral and increase of anionic phospholipids. Such redistribution of the contents in nuclear fractions under the influence of EHF EMI may entail an increase of the potential difference between the inner and outer surfaces of the nuclear envelope and will lead to conformational change of the chromatin [14], as well as changes in permeability of the nuclear envelope. It should also be noted that during germination under normal physiological conditions is registered regular increase of the anionic phospholipid content of the nuclear envelope, and conversely, decreasing of the anionic phospholipids in the soluble nuclear fraction [4] that, according to our physical model [17], will promote to the facilitated transport through NPC directed **out of the nucleus** to cytoplasm in the cells. In this study we observed decreasing of anionic PL in the content of nuclear envelope and increasing of anionic phospholipids in the content of soluble nuclear fraction under the influence of EMI (see. Table). Such redistribution may lead to a change in direction of the facilitated transport of substrates through the NPC **into nucleus**. Increasing of negatively charged content of the anionic phospholipids also leads to condensation of chromatin, as it is known from the literature, that the addition of negatively charged lipids *in vitro* is promote decondensation of chromatin [14, 15]. Over the past few years it has been demonstrated a link between stress and phospholipid signaling, including such as osmotic, temperature stresses and exposure to various pathogens. It was rrevealed a specific role of phospholipase D, inositol polyphosphate phosphatase, PA and other members of phospholipid path in response to abiotic stress [10, 16]. Increasing the total content of the PL and the simultaneous sharp decline in the part of PA in the nuclear membrane (see Table) are indicative of the fact that the nuclear surface smoothing [4] and it general surface charge decreasing.

According to our proposed model for the mechanism of the nuclear envelope permeability [17], a decrease in the surface charge of the nuclear envelope due to reduced content of anionic phospholipids leads to a reduction of the potential difference $\Delta\varphi$ between the surface nucleus and inner layers, which in turn may lead to reduction of membrane transport activity. However, as we have obtained, EKP of seedlings nuclei increases under the action of mm-waves in all exposures (Fig. 1), and consequently leads to increased transport activity into or out of nuclei. The result of present work confirm once more our supposition about the role of PL in the regulation of nuclear membrane permeability via changes in the potential difference between the inner and outer layers of the nuclear membrane, which, in its turn, is one of the regulatory mechanisms of transport across NPCs. From the obtained data it can be concluded that decrease in the content of the anionic PL in the nuclear membrane is a protective reaction of cells in response to mm-wave that enhance transport activity, and the reduction of the anionic PL in content of the nuclear envelope restrains increase transport activity through the nuclear envelope, playing the role of a kind of natural buffer.

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