THE EFFECT OF EMI EHF ON ELECTRO-KINETIC POTENTIAL OF CELL NUCLEAR MEMBRANES OF WHEAT SEEDLINGS TREATED WITH HYBBERELLIC ACID

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ABSTRACT
The effect of hybbereelic acid and EMI EHF on electro kinetic potential changes of cell nuclear membrane of wheat seedlings has been investigated. It was revealed that electro kinetic potential value depends on functional state of plant organism. It was reported that the treatment of wheat germs with hybbereelic acid and EMI EHF induces a change of nuclear membrane surface charge. It was revealed that the combined influence of chemical as well as physical factors induces more pronounced response on biological systems as compared to separately. It was also observed that the effect of EMI EHF has significant effect on water resonant frequencies as compared to water non-resonant frequencies.
1 Introduction

During vital activity the cellular membranes are exposed to the various external and internal factors viz biologically active compounds, electromagnetic waves, mechanical oscillations and other factors that can affect the structure and functions of membranes. The investigation of pH influence on electro kinetic properties of pea thylakoids showed that acidification results in electro kinetic potential (ζ-potential) magnitude changing (Doltchinkova & Lambreva, 2002). Appearing of different factors in medium or their intensity change may lead to changing of surface charge and cellular membrane structure. At both cell and surrounding medium motion the surface charge creates electric current relative to membrane. The magnitude of this current may indicate the functional state of cellular membranes. That is why ζ-potential created by membrane surface charge is an important parameter reflecting the functional state and activity of the whole cell (Doltchinkova & Lambreva, 2002; Zhang et al., 2008; Chang & Yossifon, 2009; Tajparast & Glavincic, 2012). The investigations carried out on normal and cancerous epithelial cells of human show that ζ-potential changes when cells interact with different nanoparticles (Zhang et al., 2008). The stability of colloid system depends on ζ-potential value (Hunter, 1981; Zhang et al., 2008). It was shown earlier that there is some correlation between ζ-potential value and functional genome activation (Panosyan et al., 1989; Vardevanyan et al., 1993). In cell biology determination of ζ-potential is used for cell vital activity investigation as well as agglutination and adhesion processes of cells. The surface charge plays crucial role in cellular interactions (Hunter, 1981; Veronesi et al., 2002; Lin et al., 2006; Zhang et al., 2008).

Hybberelic acid (HA) is a phytohormone that is important for plant growth particularly for growth of germ’s axial organs. It is known that on early phases of germination germ cells are able to regulate HA synthesis. Due to treatment of seeds by exogenous HA this phase may be absent or short in germs. In cells of germ treated in such way the controlling mechanism of HA synthesis activity starts acting. Many investigations show the controlling action of herb hormones on germ transition to seedling (Holdsworth et al., 1999; Ross et al., 2003; Ho et al., 2003).

Living organisms are intensively exposed to electromagnetic irradiation of extremely high frequencies (EMI EHF) from artificial sources (microwave ovens, high-power lasers and masers, or near long-range radio communication or radar antennas); therefore the investigations of the effect of these waves on biosystems are very actual. It has been revealed that EMI EHF effects on biological systems that are on different levels of living material organization (Babayan et al., 2006; Kalantaryan et al., 2010; Vardevanyan et al., 2013).

The goal of present work is the investigation of combined effect of phytohormone (HA) and EMI EHF on electro kinetic potential value change of nuclear membranes of wheat seedlings that may be quite informative and perspective for functional state estimation of herb cells.

2 Material and method

Preparation of germs from hexaploid wheat (Triticum aestivum) seeds was carried out by the method described by Johnston and Stern (1957). Solution containing 5mMtris-HCl, pH 7.4, 20mM KCland 20mkgl/ml saccharine was the sprouting medium.

Nuclei from isolated germs were obtained by Blobel and Potter method (Blobel & Potter, 1966). Nuclear membranes were determined as described by Minasbekyan et al. (2002). Electrophoretical mobility of nuclei was determined by micro electrophoresis method and ζ-potential value – by formula (Vardevanyan et al., 2012).

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\zeta = 4 \pi \eta w / DE
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where η is dispersion medium viscosity coefficient, w – nucleus motion rate, D – water dielectric constant, E – voltage gradient in electrophoretical cell.

The germination of seeds was carried out in thermostat at 25°C. The irradiation was carried out via G4-141 generator with 37.50-53.57 GHz working interval and 60 mk W/cm² power flux density. Frequency signal stability was ± 0.05%, frequency output signal distortion in constant generation regime does not exceed 6 MHz. The irradiation with 20 min duration was carried out with 49 GHz, 50.3 GHz and 51.8 GHz frequencies. To obtain the seeds treated with exogenous HA, phytohormone was added in flowing water each 125 mg per 1 liter. The statistic treatment of data was carried out.

3. Results and Discussion

In present work the effect of EMI EHF and HA on ζ-potential value of wheat seedling nuclear membrane has been investigated. The values of ζ-potential are represented on figure 1 at wheat germ functional state change. Wheat nuclear membranes are charged negatively. As it is obvious from figure 1, a significant difference exists between nuclear ζ-potential values of dry and germinating germs of wheat seeds when the state is changed. Comparing with ζ-potential value of nuclei of dry seed germs, the value of nuclear ζ-potential of three-day germs of germinating seeds is increased by 227.68 % in its absolute magnitude.

The values of ζ-potential of nuclear membranes of three-day germinating seeds irradiated by EMI EHF are represented on figure 2 while in figure 3 combined effect of HA and EMI EHF is exposed. It is obvious from figure 2 that one-time EMI EHF irradiation of germinating seeds results in increasing of ζ-
The effect of EMI EHF on electro-kinetic potential of cell nuclear membranes of wheat seedlings treated with hyberrellic acid

Figure 1 ξ-potential values of nuclear membrane of wheat dry and germinating three-day germs.

Figure 2 ξ-potential values of nuclear membranes of three-day wheat seedlings under effect of external medium factors. The effect of EMI EHF with different frequencies on ξ-potential value.

Figure 3 ξ-potential values of nuclear membranes of three-day wheat seedlings under influence of external medium factors. The effect of HA and combined effect of HA + EMI EHF with different frequencies on ξ-potential value. 1 – Effect of HA; 2 – effect of HA+EMI EHF with 49GHz frequency; 3 – effect of HA+EMI EHF with 50.3GHz frequency; 4 – effect of HA+EMI EHF with 51.8GHz frequency.
potential values of seedling nuclei. Biological system response magnitude depends on EMI frequency.

Thus at irradiation of germinating seeds with 49GHz frequency ξ-potential value increase only by 1.12%, while at 50.3GHz and 51.8GHz ξ-potential value increases by 11.81% and 18.28% respectively. This fact indicates that at irradiation with water resonant frequencies (50.3GHz, 51.8GHz) the effect is more pronounced than at water non resonant frequency – 49GHz. One-time treatment with HA of germinating seeds results in increasing of ξ-potential value by 126.98% (figure 3). The investigation of combined influence of EMI EHF and HA on ξ-potential value was also carried out. It is obvious from represented figure that the irradiation of germs strengthens HA effect, moreover the less strengthening effect is obtained at irradiation with 49GHz as compared to control ξ-potential value increases by 127.5%, at 50.3GHz and 51.8GHz – by 143.1% and 154.3% respectively. Therefore the physical factor enhances biological system response magnitude to the chemical factor action: the irradiation with 49GHz strengthens the effect by 14.8%, with 50.3GHz – by 7.1%, with 51.8GHz – by 12.04%.

The difference between ξ-potential values primordially exists even in relatively inert membrane of intact nuclei. It has been shown that ξ-potential value of nuclear membranes of dry germs in different species of cereal differs (Vardevanyan et al., 2004). Such difference of ξ-potential values of germ intact nuclei probably indicates that cell nuclei initially differ by distribution degree of charge on surface membranes.

Stronger effect of EMI with water resonant frequencies i.e. 50.3GHz and 51.8GHz indicates to water participation in biological system response formation to external physical field stimuli (Babayan et al., 2006; Kalantaryan et al, 2010; Vardevanyan et al., 2013). There is a possibility that the change of water structure under the influence of EMI results in structural as well as functional activity changes of surface layer molecules of membranes, which increases surface charge and permeability of nuclear membranes for different compounds including phytohormone. Moreover the combined effect of EMI EHF and HA is not enough investigated. There are not enough literature data.

Conclusion

Thus it was revealed that the influence of EMI EHF and exogenous HA results in changing of ξ-potential value. It is obvious from the results that at germination when herb genome is active ξ-potential value is higher than in intact wheat nucleus (figure 1). Based on this fact, it may be assumed that these factors also induce an additive activation of genome because in case of their influence the value of ξ-potential is higher. Most probably activation of genome occurs via two phases: interacting with membrane EMI EHF changes nuclear membrane permeability relative to biologically active compounds including to HA; in the second phase HA interacts with DNA not directly resulting in significant genome activation.

References


