PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2020, **54**(2), p. 118–124

Biology

INFLUENCE OF LOW DOSE GAMMA-IRRADIATION ON THE ELECTROKINETIC POTENTIAL OF WHEAT SEEDLINGS CELLULAR NUCLEI

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During ontogenesis, plants are exposed to various stress factors of biotic and abiotic nature. The nucleus and mitochondria are the most sensitive to ionizing radiation cell elements of all living organisms. The effect of ionizing radiation on the electrokinetic potential of isolated nuclei of wheat seedlings was studied, which allows us to judge the changes in the magnitude of the surface charge and its role in the functioning of the cellular nuclei. Changes were obtained in the values of the electrokinetic potential in isolated seedlings of irradiated seeds in a electric field. As a result of chain reactions, ionizing radiation changes the content of nuclear fractions, which in turn leads to a decrease in the surface charge and a decrease in the electromobility of nuclei.

https://doi.org/10.46991/PYSU:B/2020.54.2.118

***Keywords*:** γ-irradiation, wheat seedlings, zeta potential, surface charge.

Introduction. All biological objects on Earth are constantly exposed to the influence of natural and artificial radiation. Sources of natural radiation are the decay of radioactive isotopes contained in soil, water and air. The artificial sources of ionizing radiation are nuclear reactors of nuclear power plants, as well as medical equipment for radiation diagnostics and therapy. The impact of ionizing radiation on living organisms occurs in several successive stages. Ionization radiation primarily leads to the breaking of bonds in double-stranded DNA. In a split second, energy is absorbed at the physical level, followed by breaks in chemical bonds, changes in the properties of damaged molecules and the chemical composition of the cell, which is expressed in biomolecular cell damage, which is less studied and requires detailed investigation.

Studies on human fibroblasts have shown that exposure to γ-radiation at a dose of 1 *Gy* leads to temporary changes in the level of lipids and amino acids, which eventually is restored to the control level, but more intense exposure to 5 *Gy* this time has led to a steady increase in the level of these metabolites, which according

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to the authors, has indicated the degradation of membranes and proteins [1]. It is assumed that the primary target of γ-rays in a cell is chromosomal DNA with a mass of more than $6 \cdot 10^4$ *kDa* due to the spatial structure of the double helix and the wave-like nature of radiation, due to which ionizing radiation is highly effective in creating DNA breaks.

Plants are constantly exposed to various environmental and endogenous stresses due to their motionless lifestyle. All types of biotic and abiotic stresses cause the production of reactive oxygen species (ROS), signaling molecules that control gene expression and activity of anti-stress systems. However, at high concentrations, they can be a source of genotoxic stress for the plant cell [2, 3]. In chloroplasts of plants, ROS are also generated during photosynthesis. In plants, to protect genomic integrity, there is a DNA damage response (DDR) mechanism that regulates cell cycle arrest, DNA repair, and programmed cell death [4]. Many authors still believe that high doses of radiation have a positive effect on seed productivity, affecting the nucleotide composition and content of phosphorus compounds [5, 6] and the chromosomal apparatus of wheat cells [7, 8].

It was shown that, in response to ionizing radiation, the mass of mitochondria generating ROS for ATP synthesis increases in mammalian cells, the membrane potential of mitochondria changes, and the regulation of the permeability of their membranes is disturbed [9]. In plant cells on response to γ-irradiation, a specific transcription factor was found in Arabidopsis thaliana cells: the SOGI suppressor of irradiation (suppressor of γ-irradiation) [4].

At the molecular level, ionizing radiation affects not only the DNA of cells but also changes the physicochemical properties of membranes. Anionic phospholipids that make up the cell and nuclear membranes play an important role in the formation of a negative surface charge, while phosphatidic acid regulates the generation of ROS, protein kinases, etc. [10].

Our research was aimed to study the nature of the effect of γ-irradiation on nuclear membranes, the magnitude of changes in the electrokinetic potential (EKP) of nuclei, the degree of violation of the integrity and electronegativity of nuclei, and changes in the content of protein and nucleic acids in the composition of nuclear fractions.

Materials and Methods.

Processing and Germination of Seeds. Frisco soft wheat seeds were soaked in Petri dishes overnight in a thermostat at 26°C and then planted on trays with filter paper moistened with tap water (control) and continued to germinate in an incubator for 72 *h* at a temperature of 26°C. To obtain seedlings of irradiated seeds, dry wheat seeds were subjected to one-time irradiation, which was carried out at the Institute of Physical Research of the National Academy of Sciences of Armenia on an isotope emitter K120.000 Co60, with quantum energy of 1.20 *MeV*, and an irradiation power of 0.4 *Gy*/*s*. The treated seeds, as well as the control seeds, were soaked overnight, and then the hatched seeds were reseeded in trays and then germinated for the next 72 *h* in an incubator.

Isolation of Cell Nuclei. The nuclei of 4-day-old wheat seedlings were isolated according to the method from [11] with some modifications. The seedlings, frozen in liquid nitrogen, were crushed with a porcelain mortar to a fine powder, and then nuclei were isolated, as described earlier in [12]. The purity of the obtained nuclei was determined by the biochemical composition [13] and under a microscope.

Obtaining Nuclear Fractions. To separate the nuclear membranes, the whole nucleus precipitate was resuspended in 10% sucrose in 50 *mM* Tris-HCl buffer to separate the soluble nuclear fraction (supernatant) and the fraction of a nuclear membrane [12].

Determination of the Electrokinetic Potential of Nuclei. The electrokinetic potential of nuclei was determined by the magnitude of their electrophoretic mobility [13, 14]. Electrophoresis was carried out with a constant voltage gradient of 70–80 *V* and a current strength of 10–15 *mA*, by following the movement of nuclei under a microscope in the middle layer of the suspension at room temperature. The value of the EKP (ζ, *mV*) was calculated by the Smoluchowski formula:

$$\zeta = \frac{4\pi\eta\omega}{\varepsilon \cdot E},$$

where η is the viscosity coefficient of the dispersion medium, ω is the speed of the nuclei, ε is the dielectric constant of water: ε=81, E is the voltage gradient of the applied electric field in the electrophoretic chamber.

Statistical Analysis. The values for all data were averaged over the results from 4 independent experiments and subjected to analysis of variance: the standard errors and Student’s criteria were used to evaluate the statistical significance of all observed differences between the means of the obtained data and the control values. The confidence level was taken as $p<0.05$.

Results and Discussion. Irradiation with gamma rays is widely used in medicine to sterilize instruments and implantable tissues, in diagnostics and radiotherapy, as well as in agriculture. Radiation effects are used to develop new forms of plants, for pre-sowing treatment and seed disinfection. Earlier, we also studied the effect of γ-radiation in doses of 50, 100, 150 and 200 *Gy* on seeds of soft wheat with hybrid depressiveness [7]. The treatment with such high doses of ionizing radiation was carried out to overcome hybrid depressiveness of wheat, however, mutations by studied depressive genes were not achieved. Moreover, the seedlings of the irradiated seeds sprouted in a way that was not natural for normal seeds, and a tendency to lodging appeared. At the same time, if the etiolated seedlings taken here as control usually have a greenish-yellow color, then the seeds treated with ionizing radiation gave pale yellowish-purple seedlings, which indicated a change in the synthetic activity of the pigments.

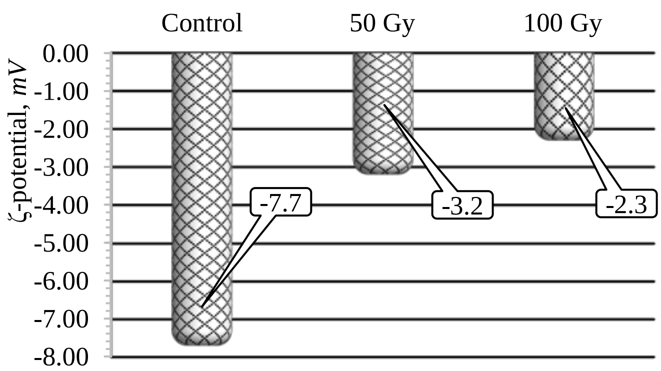
The cell nuclei of seedlings of irradiated seeds upon absorption of high doses of radiation under a microscope looked in general terms the same as after exposure to high temperature or strong poison. Violations of the integrity and smoothness of the surface of the nuclear membrane were observed. The nucleus itself was compacted, or, conversely, liquefied; in some cells, a nuclear breakdown was observed. After absorption of smaller doses of radiation, when the cell is still alive, more or less significant changes occur in its organelles, especially in the cell nucleus. Therefore, we conducted studies of the effects of the above doses on the structure and function of nuclei and the biochemical composition of the nuclear fractions – the nuclear envelope and the soluble nuclear fraction.

Electronegativity, which is measured by determining the EKP values, is considered to be one of the informative biophysical parameters of the functional activity of the nucleus. The study of the EKP of nuclei by their electrical mobility during microelectrophoresis in an electrostatic field gradient can shed light on the question of the influence of γ-irradiation on the electronegativity of the surface of nuclei, as well as on the relationship of EKP with changes in the biochemical composition of nuclear fractions.

Shifts in the values of the EKP of nuclei, thereby changing the electromobility of nuclei in the electric field gradient at a constant current, may be related to the changes in the content of the charge-forming molecules. Therefore, a decrease in the number of negatively charged molecules in the content of the nuclear membrane leads to a decrease in several molecules in the double electric layer, its thinning, and, consequently, to a decrease in the EKP value of the nuclei.

We studied the effects of ionizing radiation on seedlings at doses of 50 *Gy* and 100 *Gy*, since low doses of ionizing radiation are believed to have various biological effects through DNA damage, some of which are favorable for plant growth [5]. In animal cells, ionizing radiation causes a wide range of various biological effects unfavorable for life, such as mortality, infertility, fetal malformation, carcinogenesis, and so on [1]. Among the various types of DNA damage, double-stranded DNA breakage is considered the most critical, since such changes lead to a disruption of genome integrity, which affects the functional activity of cells [15].

The data on the effect of γ-irradiation on the EKP of wheat seedlings nuclei are presented in the diagram below, where changes in the EKP values of isolated nuclei of wheat seedling cells obtained from irradiated seeds are in direct proportion to the absorbed dose of irradiation.



Changes in the electrokinetic potential of intact nuclei of seedlings pretreated with ionizing γ-irradiation.

The data shown in the diagram indicate a decrease in electro-negativity, and thus a decrease in the electromobility of nuclei in a gradient of a static field. So, the exposure to 50 *Gy* leads to a decrease in the absolute value of the potential from 7.7 *mV* to 3.2 *mV*, that is, the electromobility of the nuclei decreases by more than two times. Irradiation of seeds with a higher dose of γ-irradiation of 100 *Gy* leads to even lower values of electronegativity, to a decrease in the electromobility of nuclei and, accordingly, to a decrease in the absolute values of EKP to 2.3, which is only 29.9% relative to that of the seedlings of the control seeds of soft wheat, i.e., there was a drop in EKP by more than 3.3 times.

Such drastic changes in the electromobility of nuclei, a decrease in the EKP value under the influence of ionizing radiation can be a consequence of rather drastic changes in the functioning of the nucleus, the permeability of the nuclear membrane, its integrity, and, finally, changes in the content of its main components. Earlier, we showed a directly proportional relationship between the increase in the electromobility of seedling nuclei and the increase in the functional activity of seeds during germination [13]. Therefore, a decrease in the EKP values under the influence of ionizing radiation indicates a decrease in the electronegativity of the seed nuclei, profound disturbances in the functioning of the nucleus, a change in the permeability of the nuclear membrane up to structural disturbances in the nuclear membrane and soluble nuclear fraction, as well as the destruction of ionogenic molecules on the surface of the cell nucleus.

Conclusion. Summarizing the experimental data obtained by us and available in the literature, we note that as a result of chain reactions that occur during the absorption of radiation energy, the functional activity of subcellular structures, macromolecules (DNA, RNA, proteins), ATP and coenzymes changes, and also there is a change, which stains etiolated seedlings. When irradiated in relatively small doses, a temporary stop of mitosis is observed. When irradiated in large doses, the core swelling and pyknosis (chromatin aggregation on the inner nuclear membrane) occur, which in turn leads to a decrease in the nuclear mobility in the gradient of the electrostatic field. In the cytoplasm, at high doses of radiation, a change in viscosity, swelling of protoplasmic structures, a formation of vacuoles, and an increase in permeability are observed. The impact of ionizing radiation causes damage to cells, but the most important of all is the violation of cell division – mitosis. Large doses can cause complete cessation of cell division or death. Violation of the normal course of mitosis is accompanied by chromosomal rearrangements, the occurrence of mutations leading to shifts in the genetic apparatus of the cell, and therefore to a change in subsequent cellular generations (cytogenetic effect).

In addition to all these processes, redistribution occurs in the nucleus in charged groups on the surface of the nucleus; an increase in RNA and fragments of broken DNA opens up positively charged groups that reduce the overall electronegativity of the nucleus and thus decrease the electromobility of nuclei. Revealing the electrokinetic mobility of nuclei, as well as measuring mitotic activity, are informative methods for studying the effects of ionizing radiation and can be used in practice to quickly obtain information on the strength of an effect and on violations in the genome.

Received 28.02.2020
Reviewed 22.07.2020
Accepted 27.07.2020

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ԳԱՄՄԱ-ՃԱՌԱԳԱՅԹՄԱՆ ՑԱԾՐ ԴՈԶԱՆԵՐԻ ԱԶԴԵՑՈՒԹՅՈՒՆԸ ՑՈՐԵՆԻ ԾԻԼԵՐԻ ԲՋՋԱԿՈՐԻԶԻ ԷԼԵԿՏՐԱԿԻՆԵՏԻԿԱԿԱՆ ՊՈՏԵՆՑԻԱԼԻ ՎՐԱ

Բույսերը օնտոգենեզի ընթացքում ենթարկվում են տարբեր տեսակի բիոտիկ և աբիոտիկ գործոնների սթրեսային ազդեցությանը: Բոլոր կենդանի օրգանիզմներում բջիջների կառուցվածքային տարրերից առավել զգայուն են իոնացնող ճառագայթման նկատմամբ կորիզը և միտոքոնդրիումները: Ուսումնասիրվել է իոնացնող ճառագայթման ազդեցությունը ցորենի ծիլերի մեկուսացված կորիզների էլեկտրոկինետիկական պոտենցիալի (ԷԿՊ) վրա, ինչը թույլ է տալիս դատել կորիզաթաղանթի մակերևութային լիցքի մեծության և վերջինիս դերի մասին կորիզի գործառույթներում: Դիտվել են ճառագայթահարված սերմերի ծիլերի մեկուսացված կորիզների ԷԿՊ-ի արժեքի փոփոխություններ էլեկտրական դաշտում: Իոնացնող ճառագայթումը հանգեցնում է կորիզային ֆրակցիաների կազմի փոփոխմանը, որն էլ իր հերթին բերում է մակերևութային լիցքի նվազմանը և էլեկտրական դաշտում կորիզների շարժունակության նվազմանը:

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ВОЗДЕЙСТВИЕ НИЗКОЙ ДОЗЫ ГАММА-ОБЛУЧЕНИЯ НА ЭЛЕКТРОКИНЕТИЧЕСКИЙ ПОТЕНЦИАЛ КЛЕТОЧНЫХ ЯДЕР ПРОРОСТКОВ ПШЕНИЦЫ

Растения в течение онтогенеза подвергаются воздействию различных стрессорных факторов биотической и абиотической природы. Наиболее чувствительными к ионизирующему излучению структурными элементами клеток всех живых организмов являются ядро и митохондрии. Исследовано влияние ионизирующего излучения на электрокинетический потенциал (ЭКП) изолированных ядер проростков пшеницы, что позволяет нам судить об изменениях величины поверхностного заряда и его роли в функционировании ядра. Получены изменения в величинах ЭКП изолированных ядер проростков облученных семян в электрическом поле. В результате цепных реакций ионизирующее облучение изменяет содержание ядерных фракций, что, в свою очередь, приводит к уменьшению поверхностного заряда и падению подвижности ядер в электрическом поле.