PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2022, **56**(3), p. 225–230

Biology

WHEAT PROTEIN INCREASING THROUGH RADIATION TECHNOLOGIES

L. A. MINASBEKYAN [1*], M. A. PARSADANYAN [2**]

[1] *Scientific-research Laboratory of Biophysics of Subcellular Structures, YSU, Armenia*
[2] *Chair of Biophysics, YSU, Armenia*

The effect of various doses of γ-irradiation on the biochemical composition of the nuclear fractions of wheat seedlings has been investigated. The research results indicate a direct dependence of changes in the biochemical composition of nuclear membranes and soluble nuclear fraction on the magnitude of the radiation dose. A change in the protein content in the fraction of the nuclear membrane and in the soluble nuclear fraction of the cell nuclei of the seedlings of irradiated seeds was observed. The data obtained indicate that ionizing radiation as a result of chain reactions changes the content of nuclear fractions, which in turn leads to a change and disruption of the functional ability of the nucleus.

https://doi.org/10.46991/PYSU:B/2022.56.3.225

***Keywords*:** γ-irradiation, wheat seedlings, the composition of the nuclear membrane, the composition of the soluble nuclear fraction.

Introduction. All biological objects on Earth are constantly under the influence of natural and artificial radiation. Sources of natural radiation are the decay of radioactive isotopes contained in soil, water, and air. The main sources of natural radiation are radon and uranium contained in rocks and soil. However, with the development of modern technologies, radiation from scientific sources such as accelerators and generators, as well as medical equipment for radiation diagnostics and therapy, also increases. The impact of ionizing radiation on living organisms occurs in several successive stages. Comparative studies of irradiation of the cytoplasm and the nucleus showed a higher radiosensitivity of the nucleus, which was the basis for the study of biochemical changes in the membrane and the contents of the nucleus. Immediately upon irradiation of seeds, energy is absorbed at the physical level in a fraction of a second, followed by breaks in chemical bonds, a change in the properties of damaged molecules, and the chemical composition of the cell, which is expressed in biomolecular damage to the cell, which is less studied and require detailed study. Ionizing radiation (IR) processing is of interest to agriculture, horticulture, ecology, and space science. There is evidence that the stage of plant development affects both the expression of genes sensitive to ionizing radiation in plants and the general response of plants to γ-irradiation [1]. High doses of IR in

[*] E-mail: minlia@ysu.am [**] E-mail: marine.parsadanyan@ysu.am

plants alter the profiles of proteins and metabolites. The use of atm and atr gene mutants made it possible to detect changes in ATM and ATR dependent pathways and in protein phosphorylation patterns [2]. In rice varieties obtained by mutagenesis, IR causes changes in the metabolism of carbohydrates and proteins [3], often such changes concern antioxidant systems [4, 5].

The purpose of this study was to study the effect of γ-irradiation on the germination of wheat cv. Amby and biochemical changes in the composition of the nuclear fractions: nuclear membrane and soluble nuclear fraction.

Materials and Methods.

Seed Processing and Germination. In this study, seeds of the soft wheat variety Amby ($d_1D_2D_3$; $ch_{1r}Ch_{2r}$) containing the genes for hybrid dwarfism and hybrid red chlorosis were used. Control 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 and continued to germinate in a thermostat for 72 *h* at a temperature of 26°C. To obtain seedlings of irradiated seeds, dry wheat seeds were subjected to one-time γ-irradiation, which was carried out at the Institute of Physical Research of the National Academy of Sciences of the Republic of Armenia on a K120.000 ^{60}Co isotope emitter with a quantum energy of 1.17 *MeV*, at 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 transferred to trays and then germinated for the next 72 *h* in a thermostat.

Isolation of Cell Nuclei. The kernels of 4-day-old wheat seedlings were isolated according to the Blobbel method with some modifications [6]. The seedlings frozen in liquid nitrogen were crushed with a porcelain mortar to a fine powder, and then the nuclei were isolated as described previously [6]. Precipitated nuclei were repeatedly washed in TKM buffer containing 25 *mM* KCl, 15 *mM* $MgCl_2$, and 10 *mM* Tris-HCl, pH 7.4. As a result, purified intact nuclei were obtained for subsequent study. The purity of the resulting nuclei was determined by biochemical composition and under a microscope.

Obtaining Nuclear Fractions. To separate the nuclear membranes, the pellet of whole nuclei was resuspended in 10% sucrose in 50 *mM* Tris-HCl buffer, pH 7.4, containing 1 *mM* DTT, 1 *mM* $MgCl_2$, and a protease inhibitor. Next, the enzymes deoxyribonuclease-1 (5 *mg*/*mL*) and ribonuclease A (1 *mg*/*mL*) were added and incubated for 15 *min* at 37°C, after which they were centrifuged for 10 *min* at 20,000 *g* to separate the soluble nuclear fraction (supernatant) and fraction nuclear membranes, as described in [6]. The supernatant (supernatant) containing the soluble nuclear fraction was collected, and the precipitated nuclear membranes were then washed 2–3 times by resuspension and subsequent centrifugation for 15 *min* at 27,000 *g* in a TsVR-1 centrifuge (MRTU-42, USSR) in a solution of 0.25 *M* sucrose in TKM buffer.

Determination of the Component Composition of Nuclear Fractions. The content of the nuclear membrane and soluble nuclear fraction was carried out separately. Protein was determined by the Lowry method in samples, as given in [6], preliminarily boiled in the presence of 1 *N* NaOH.

Statistical Analysis. The tables and graphs show the arithmetic mean values from 4 independent experiments and their standard errors. All calculations of means and standard errors were calculated using Microsoft Office Excel.

Results. Radiation effects are used to develop new forms of plants, for pre-sowing treatment and disinfection of seeds. Previously, we also conducted studies of the effect of γ-irradiation on the seeds of soft wheat varieties Frisco L-2, which has hybrid depression. The treatment with such high doses of IR was carried out in order to overcome the hybrid depression of wheat, but we did not achieve mutations in the direction of weakening the genes of depression [7]. In the present work, the seeds of common wheat Amby, which also has hybrid depression, were used as an object of study. The hybrid depression of wheat is especially difficult to overcome in cases of a combination of strong alleles. According to some authors, the most effective method of overcoming depression in highly necrotic hybrids (F1) is γ-irradiation (^{60}Co). Cobalt-60 is the longest-lived radioactive isotope of Cobalt and has important practical applications.

Exposure to ionizing radiation, as well as many other forms of abiotic stress, causes the production of ROS [8], triggers oxidative stress reactions, which can initially lead to changes in the structure of the nuclear membrane and its permeability, after which signal molecules (OH–) penetrate into the nucleus and cause disturbances in gene expression [9, 10].

Earlier, in our studies, changes in germination time, morphometric parameters, and radiosensitivity were observed [11]. In this study, we determined the protein content in fractions of the cell nucleus for the total protein content depending on the radiation dose. Data on the protein content and its changes depending on the radiation dose in the composition of the nuclear membrane are presented in Fig. 1.

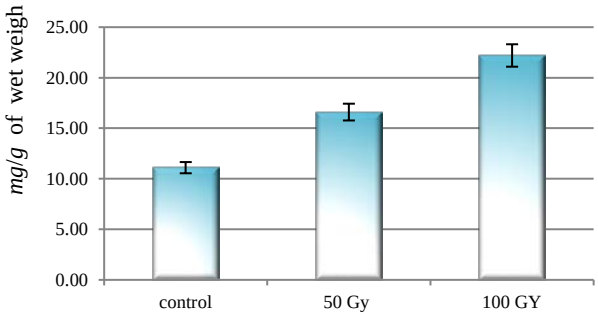

Fig. 1. Changes in the content of proteins in the composition of the nuclear membrane of seedlings depending on the dose of seeds irradiation (n=3, differences are statistically significant at $p < 0.05$).

According to our research data, under the influence of IR at radiation doses of 50 *Gy*, a significant increase in the protein content by almost 1.5 times was observed in the composition of the nuclear membrane. An increase in the total protein content in the nuclear membrane indicates an increase in the density of the nuclear membrane and proteins on the inner sheet of the nuclear membrane, which attach the heterochromatin compartment to it. An increase in protein content in response to stress indicates an intensification of the synthesis of proteins and enzymes involved in starting the processes of cell regeneration and adaptation.

Our data on the increase in protein in fenugreek plants under the influence of IR are consistent with the literature data [12]. Using this technology, an improvement in the drought tolerance of mutant tomato plants was achieved in terms of protein expression profiles [13]. On preparations of the brain of mice and liver of rats, in response to IR at significantly low doses of IR, oxidative modification of proteins was shown, which was carried out by increasing the carbonylation of proteins and the loss of sulfur in the amino acid residues of methionine and cysteine [14]. In rice seedlings, changes in the expression of genes associated with cellular processes and signaling pathways involved in the synthesis of the cell wall and the biosynthesis of secondary metabolites were shown [3].

Increasing the dose of γ-irradiation to 100 *Gy* leads to even more profound changes in the structure of the nuclear membrane. As shown in the diagram in Fig. 2, at this dose of seed irradiation, the protein content also increases, although according to previously obtained data, under the influence of this dose of irradiation, a delay in the growth of seedlings was observed [11]. Thus, in the study, walnut growth suppression was obtained and 23 activated genes associated with reactive oxygen species and 79 genes associated with ribosomal proteins were identified [15]. An increase in the content of total protein in the composition of the nuclear membrane indicates violations of the structure of the nuclear membrane and changes in its functional activity and permeability, an increase in the density of nuclei and, possibly, the removal of part of the phospholipids of the nuclear membrane.

Under the action of γ-radiation, we also observed changes in the composition of the soluble nuclear fraction. On Fig. 2 presents data on the content of proteins in the soluble nuclear fraction. As evidenced by our data, when exposed to γ-irradiation at 50 *Gy*, a significant increase in protein content was obtained.

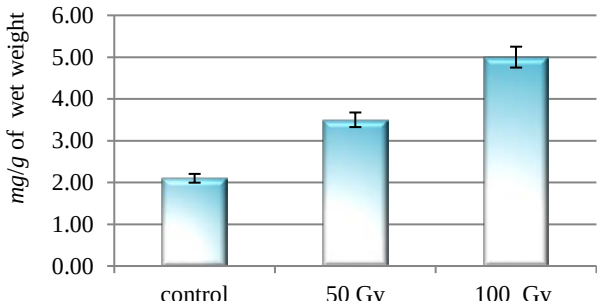

Fig. 2. Changes in the content of proteins in the composition of the soluble nuclear fraction of seedlings depending on the dose of seeds irradiation (n=3, differences are statistically significant at $p < 0.05$).

The observed changes in the protein content also indicate that, under the influence of IR, enzymatic systems are triggered, which require de novo protein synthesis.

Conclusion. In conclusion, summarizing the data obtained by us, we note that as a result of chain reactions that occur during the absorption of radiation energy, the functional activity of subcellular structures changes, and all kinds of metabolic pathways are triggered. Cells use all resources to overcome stress, survive and adapt to new conditions. At the morphological level of cells, this can be expressed both in the suppression of plant growth parameters and in an increase in the mass of

seedlings, an increase in protein content, as evidenced by our studies and literature data [1, 5, 11, 12]. That is why targeted radiation-induced mutagenesis continues to play an important role in improving the most important crops in the world such as rice, wheat, corn, etc. The FAO/IAEA Mutant Varieties Database annually registers many new varieties, many of which are also obtained through the use of the mutagenic effect of γ-irradiation.

Received 20.09.2022
Reviewed 14.11.2022
Accepted 28.11.2022

REFERENCES

1. Hong M.J., Kim D.Y., et al. Comparison of Radiosensitivity Response to Acute and Chronic Gamma Irradiation in Colored Wheat. *Genet. Mol. Biol.* **41** (2018), 611–623. https://doi.org/10.1590/1678-4685-GMB-2017-0189
2. Roitinger E., Hofer M., et al. Quantitative Phosphoproteomics of the Ataxia Telangiectasia-Mutated (ATM) and Ataxia Telangiectasia-Mutated and Rad3-related (ATR) Dependent DNA Damage Response in Arabidopsis Thaliana. *Mol. Cell. Proteomics* **14** (2015), 556–571. https://doi.org/10.1074/mcp.M114.04035
3. Hwang J.E., Hwang S.G., et al. Transcriptome Profiling in Response to Different Types of Ionizing Radiation and Identification of Multiple Radio Marker Genes in Rice. *Physiol. Plant.* **150** (2014), 604–619. https://doi.org/10.1111/ppl.12121
4. Kariuki J., Horemans N., et al. The Responses and Recovery after Gamma Irradiation are Highly Dependent on Leaf Age at the Time of Exposure in Rice (*Oryza sativa* L.). *Environ. Exp. Bot.* **162** (2019), 157–167. https://doi.org/10.1016/j.envexpbot.2019.02.020
5. Ulukapi K., Ozmen S.F. Study of the Effect of Irradiation (^{60}Co) on M1 Plants of Common Bean (*Phaseolus vulgaris* L.) Cultivars and Determined of Proper Doses for Mutation Breeding. *J. Radiat. Res. Appl. Sci.* **11** (2018), 157–161. https://doi.org/10.1016/j.jrras.2017.12.004
6. Minasbekyan L.A., Yavroyan Z.V., et al. Changes in the Composition of Phospholipids in Nuclear Subfractions of Wheat Seedlings Treated with Gibberellin. *Russ. J. Plant Physiol.* **55** (2008), 412–418. https://doi.org/10.1134/S1021443708030138
7. Sadoyan R.R., Minasbekyan L.A. Cytological and Biochemical Changes of Soft Wheat Species of Armenia under Influence of γ-Irradiation. *Ann. Agrar. Sci.* **8** (2010), 32–36.
8. Rosen E., Kryndushkin D., et al. Acute Total Body Ionizing Gamma Radiation Induces Long-Term Adverse Effects and Immediate Changes in Cardiac Protein Oxidative Carbonylation in the Rat. *PLoS ONE* **15** (2020), e0233967. https://doi.org/10.1371/journal.pone.0233967
9. Rejeb I.B., Pator V., Mauch-Mani B. Plant Response to Simultaneous Biotic and Abiotic Stress: Molecular Mechanisms. *Plants* **3** (2014), 458–475. https://doi.org/10.3390/plants3040458
10. Kawamura K., Qi F., Kobayashi J. Potential Relationship between the Biological Effects of Low-dose Irradiation and Mitochondrial ROS Production. *J. Radiat. Res.* **59** (2018), ii91–ii97. https://doi.org/10.1093/jrr/rrx091
11. Minasbekyan L.A., Avagyan I.A. Radiosensitivity of Soft Wheat Seeds to Gamma Radiation. *Proc. of theYSU. Chem. Biol. Sci.* **55** (2021), 165–172. https://doi.org/10.46991/PYSU:B/2021.55.2.165

12. Hanafy R.S., Akladious S.A. Physiological and Molecular Studies on the Effect of Gamma Radiation in Fenugreek (*Trigonella foenum-graecum* L.) Plants. *J. Genet. Eng. Biotechnol.* **16** (2018), 683–692. https://doi.org/10.1016/j.jgeb.2018.02.012
13. Celik O., Ayan A., et al. Comparison of tolerance Related Proteomic Profits of Two Drought Tolerant Tomato Mutants Improved by Gamma-Radiation. *J. Biotechnol.* **330** (2021), 35–44. https://doi.org/10.1016/j.jbiotec.2021.02.012
14. Willey N., Caplin N. Ionizing Radiation, Higher Plants, and Radioprotection: from Acute High Doses to Chronic Low Doses. *Front. Plant Sci.* **9** (2018). Article number 847. https://doi.org/10.3389/fpls.2018.00847
15. Liu H., Li H., et al. Mechanism of Early Germination Inhibition of Fresh Walnuts (*Juglans regia*) with Gamma Radiation Uncovered by Transcriptomic Profiling of Embryos During Storage. *Postharvest Biol. Technol.* **172** (2021). Article number 111380. https://doi.org/10.1016/j.postharvbio.2020.111380

Լ. Ա. ՄԻՆԱՍԲԵԿՅԱՆ, Մ. Ա. ՓԱՐՍԱԴԱՆՅԱՆ

ՑՈՐԵՆԻ ՍՊԻՏԱԿՈՒՑԻ ԱՎԵԼԱՑՈՒՄ ՌԱԴԻԱՑԻՈՆ ՏԵԽՆՈԼՈԳԻԱՆԵՐԻ ՄԻՋՈՑՈՎ

Կատարվել է հետազոտություն ցորենի ծիլերի միջուկային ֆրակցիաների կենսաքիմիական կազմի վրա՝ γ–ճառագայթման տարբեր չափաբաժինների ազդեցության ներքո: Հետազոտության արդյունքները ցույց են տվել, որ բջջի կորիզաթաղանթի և լուծվող կորիզային ֆրակցիայի կենսաքիմիական կազմի փոփոխությունները անմիջական կախվածություն ունեն ճառագայթման չափաբաժնի մեծությունից: Դիտարկվել է ճառագայթահարված սերմերի ծիլերի կորիզաթաղանթի և միջուկային լուծվող ֆրակցիայի սպիտակուցի պարունակության փոփոխությունը: Ստացված տվյալները ցույց են տալիս, որ շղթայական ռեակցիաների արդյունքում իոնացնող ճառագայթումը փոխում է կորիզային ֆրակցիաների պարունակությունը, ինչն իր հերթին հանգեցնում է բջջակորիզի ֆունկցիոնալ ունակության փոփոխությանը և խախտմանը:

Л. А. МИНАСБЕКЯН, М. А. ПАРСАДАНЯН

ПОВЫШЕНИЕ БЕЛКОВОСТИ ПШЕНИЦЫ ПОСРЕДСТВОМ РАДИАЦИОННЫХ ТЕХНОЛОГИЙ

Проведено исследование влияния различных доз γ-облучения на биохимический состав ядерных фракций проростков семян пшеницы. Результаты исследований указывают на прямую зависимость изменений в биохимическом составе ядерных мембран и растворимой ядерной фракции от величины дозы облучения. Наблюдалось изменение содержания белка во фракции ядерной мембраны и в растворимой ядерной фракции ядер клеток проростков облученных семян. Полученные данные свидетельствуют, что ионизирующее облучение в результате цепных реакций изменяет содержание ядерных фракций, что в свою очередь приводит к изменению и нарушению функциональной способности ядра.