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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Cytogenetic Effects of γ-Radiation in Onion (*Allium cepa* L.) Seedlings

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Abstract—The effect of γ-radiation on the cytogenetic parameters of root meristem cells of onion seedlings was studied in laboratory experiments (Allium-test). An increase in the overall frequency of chromosomal aberrations and micronucleus frequencies in seedling cells at low γ-radiation doses (≤0.1 Gy) was detected for the first time. At a maximum absorbed dose of 13 Gy, chromosomal aberrations were detected in the majority of cells in the anaphase and telophase stages of the cell cycle, and the number of cells with multiple aberrations increased. The main contribution to the overall frequency of chromosomal aberrations, in addition to multiple aberrations, is made by the bridge-type aberrations, fragments, and lagging chromosomes. The data obtained allow using the cytogenetic indices of *Allium cepa* seedlings to assess the biological effects of low-dose γ-radiation.

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As a result of nuclear weapon tests and long-term activity of the nuclear fuel cycle enterprises, including the accidents at NPPs, a considerable amount of artificial radionuclides was discharged into the environment. The Yenisei River floodplain is contaminated with artificial radionuclides, including those in the form of radioactive microparticles, as a result of long-term operation of the Rosatom Mining and Chemical Combine (MCC) [1–3]. Radioactive particles with a high activity of ^{137}Cs (30 MBq) [2, 3] are point sources of external γ-radiation, creating an additional radiation dose load on aquatic and terrestrial organisms. To simulate the effect of γ-radiation of radioactive particles, we have previously performed laboratory experiments with various plant and bacterial bioassays [4, 5], which showed their high sensitivity to low-dose γ-radiation [4, 5]. In some toxicological studies, we used the onion bioassay (Allium-test) [6], which has worked well earlier for the assessment of chemical and radiation toxicity of environmental samples [7, 8]. In our experiments, the Allium-test showed stimulation, rather than inhibition, of root growth at the doses used [6]. The standard Allium-test is usually performed on both onion bulbs and seeds [9, 10]. Seeds are a convenient test object because they are in a dormant state and are characterized by physiological and genetic homogeneity. Bioassays using onion seed showed good results in the assessment of cytotoxicity and genotoxicity of electromagnetic radiation [9] and soils from the Chernobyl exclusion zone [10]. However, the dose dependences of the cytogenetic parameters of onion seedling cells exposed to γ-irradiation were not determined in these studies.

The purpose of this study was to evaluate the effect of γ-radiation (at low doses in particular) on the cytogenetic parameters of onion seedling cells in the Allium-test.

The experiments on biotesting of ionizing radiation were performed with the onion (*Allium cepa* L.) cultivar Stuttgarter Risen seeds. The seeds were pregerminated in polypropylene containers on filter paper moistened with distilled water. In experiments, we used seedlings 2–3 mm in length. Onion seedlings were irradiated with a γ-radiation source (^{137}Cs; activity, 14 GBq) at the Budker Institute of Nuclear Physics (Novosibirsk) for 24 h. In total, we performed seven experiments in 2016–2017. In the experiments performed in 2016, the absorbed dose for onion seedlings was 0.02, 0.05, 0.1, 1, 3, and 5 Gy, which corresponded to a dose rate of 0.8, 2.1, 4.2, 42, 125, and 208 mGy/h, respectively. In the experiments performed in 2017, the absorbed dose for onion seedlings was 0.1, 1, 2.6, 4.5,

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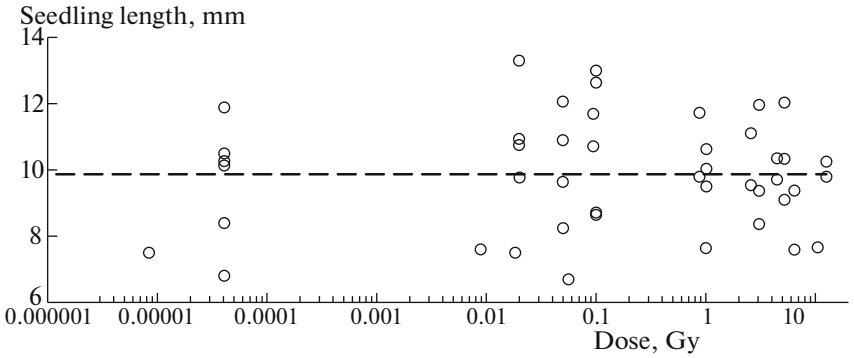

Fig. 1. Dependence of the length of onion seedlings (in mm) on the absorbed dose in the experiments performed in 2016–2017. The dashed line shows the mean seedling length (9.9 ± 1.7 mm, $n = 45$).

6.4, and 13 Gy or 4, 37, 109, 190, 270, and 530 mGy/h, respectively. The γ-radiation dose rates were determined by the distance of seedlings from the radiation source and were calculated on the basis of the nameplate rating of the exposure dose for the ^{137}Cs source and verified by direct measurements with a DKS-AT1123 dosimeter (SPE Doza, Russia). Non-irradiated seedlings were used as a control (dose rate in the control was 0.002 mGy/h). For irradiation, seedlings were placed in transparent polypropylene containers on two layers of filter paper moistened with distilled water. For each level of exposure and control, 15 seedlings were used. Experiments were performed at a temperature of 18–21°C without illumination. For cytogenetic studies, onion seedlings were fixed in a mixture of ethanol and acetic acid (3 parts 96% ethanol and 1 part of glacial acetic acid) immediately after irradiation. Several days later, the seedlings were stained with 1% aceto-orcein. The stained seedlings were used to obtain squashed preparations, which were analyzed immediately using an Olympus CX31 microscope (Olympus, Japan) at a magnification of 600× and photographed. The cytogenetic abnormalities were studied by the anaphase and telophase methods and the micronucleus test. The frequency of chromosomal aberrations was determined in percent as a ratio of the number of cells with aberrations to the total number of viewed cells in the anaphase and telophase stages.

The main biotest parameters determined in our study were the overall frequency of chromosomal aberrations, the frequency of certain types of aberrations in the anaphase and telophase stages of the cell cycle, the micronucleus rate, and the mean length of grown onion seedlings.

The experimental data were analyzed by variation statistics methods using the STATISTICA 7.0 software package. The significance of differences was assessed using Student's t test.

We found that, in all experiments, γ-radiation at doses ranging from the background to 13 Gy (dose rates up to 530 mGy/h) had no significant effect on the growth of onion seedlings (Fig. 1). As seen in Fig. 1, the length of seedlings in the control and in the irradiated samples was the same. These results for onion seeds differ from the results of our previous experiments on irradiation of onion bulbs, in which we observed the stimulation of root growth in onion bulbs exposed to γ-radiation [6].

The analysis of the overall frequency of chromosomal aberrations in onion seedling cells showed that the number of aberrations in all experiments increased with increasing absorbed radiation dose. At a maximum radiation dose of 13 Gy, we recorded aberrations in the majority of cells in the anaphase and telophase stages of the cell cycle, and the overall frequency of chromosomal aberrations reached 90% (Fig. 2a). Under exposure to low doses (≤0.1 Gy), the overall frequency of chromosomal aberrations was higher than that in the non-irradiated control samples (Fig. 2b). However, only for the absorbed dose of 0.05 Gy we detected a significant difference in this index from the non-irradiated control. With increasing irradiation dose over 1 Gy, all values of the overall frequency of chromosomal aberrations in seedling cells were significantly higher than in the control. At the absorbed dose of 1 Gy, we detected chromosomal aberrations in only 18% of cells in the anaphase and telophase stages, whereas at doses of 6.4 and 13 Gy, the overall frequency of aberrations drastically increased and reached 76 and 90%, respectively (Fig. 2a).

The main types of chromosomal aberrations were bridges, fragments, and lagging chromosomes. Among them, the proportion of lagging chromosomes was somewhat greater that the proportion of other types of aberrations. However, under exposure to the dose of 6.4 Gy, the proportions of lagging chromosomes (25%) and the bridge-type aberrations (20%) were similar. At this absorbed dose, the proportion of fragment-type aberrations did not exceed 10%. Along with these three types of single aberrations, we also recorded multiple aberrations in cells. Under irradiation with maximum doses (6.4 and 13 Gy), multiple aberrations (two or three aberrations of the three types

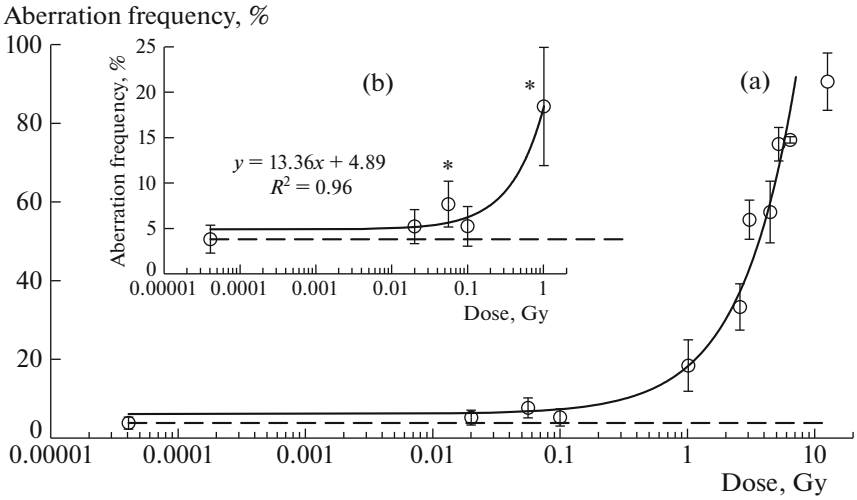

Fig. 2. Dependence of the frequency of chromosomal aberrations in onion seedling cells on the absorbed dose in the experiments performed in 2016–2017 (% of control): (a) results at all radiation doses, (b) results at low doses. Here and in Fig. 3, the dashed line shows the control. Data are represented as $M \pm m$, $n = 7$, $^*p < 0.05$ when compared to the control.

mentioned above) dominated and accounted for 32 to 68%. The radiation dose of 2.6 Gy can be regarded as a threshold dose leading to the occurrence of multiple aberrations: at this dose, the proportion of such aberrations increased by an order of magnitude compared to the dose of 1 Gy (from 0.9 to 9%). In addition to the above-mentioned types of aberrations, other types of disorders such as agglutination of chromosomes, multipolar and asymmetric mitoses, and ring chromosomes were recorded, but their proportion did not exceed 3% of the total frequency of aberrations.

In the experiments performed in 2017, in addition to the analysis of chromosomal aberrations and length of onion seedlings, we performed the micronucleus assay of the apical meristem cells of seedlings. It was found that, at all γ-irradiation doses used, the frequency of micronuclei in seedling cells at the interphase stage significantly differed from the control (Fig. 3). This pattern of micronucleus assay was observed in two experiments performed in 2017, including irradiation at a dose of 0.1 Gy, although the level of chromosomal aberrations at this dose did not differ significantly from the control (Fig. 2b). This was indicative of a greater sensitivity of the micronucleus assay in cells as compared to the determination of the level of chromosomal aberrations under low-dose irradiation. However, the pattern of the dose curves for these two parameters (the frequency of micronuclei and the frequency of chromosomal aberrations) differed significantly under irradiation with doses of 6.4 and 13 Gy. Unlike the frequency of chromosomal aberrations, the micronucleus rate at these doses decreased rather than increased (Fig. 3). These differences are, probably, due to the fact that chromosomal aberrations and micronuclei appear as a result of different processes at the molecular level. For example, micronuclei are formed from chromosomal aberrations, primarily from acentric fragments and lagging chromosomes; however, not all acentrics form micronuclei (part of them pass into the daughter nucleus together with the mass of chromosomes during cell division [11]).

Previously, when using the standard Allium-test (on onion bulbs), discrepant data on the evaluation of ionizing radiation toxicity were obtained [12–14]. For example, Pyatkova et al. [12], who studied the toxicity of radioactive samples collected on the territory of the Semipalatinsk nuclear test site and γ-irradiation of onion bulbs, concluded that significant biological effects (increase in the frequency of aberrant cells of onion root meristem) were observed at exposure levels greater than 0.001 Gy. However, the authors of [13], who determined the radiosensitivity of onion bulbs, irradiated them with doses of 0.1–2 Gy (with a ^{60}Co source) and showed that, in this range of ionizing radiation doses, the overall frequency of aberrant cells in the onion roots did not exceed the control value. Similar conclusions were obtained in [14], where the analysis of cytogenetic disorders in the root cells of irradiated onion bulbs showed the absence of dose dependence at doses less than 4–5 Gy. In our experiments, which were performed on onion seedlings, the overall frequency of chromosomal aberrations significantly increased under irradiation with a dose of 0.05 Gy, which suggests that the Allium-test with the use of onion seedlings is more sensitive than that with the use of onion bulbs.

In [10], onion seeds were used to assess the genotoxicity of soil samples from the Chernobyl exclusion zone. The authors of the cited study showed a correlation between the overall number of chromosome aberrations in seedling cells and the content of ^{137}Cs. However, data on the doses absorbed by onion seedlings (dose dependence) are absent in this work. In addi-

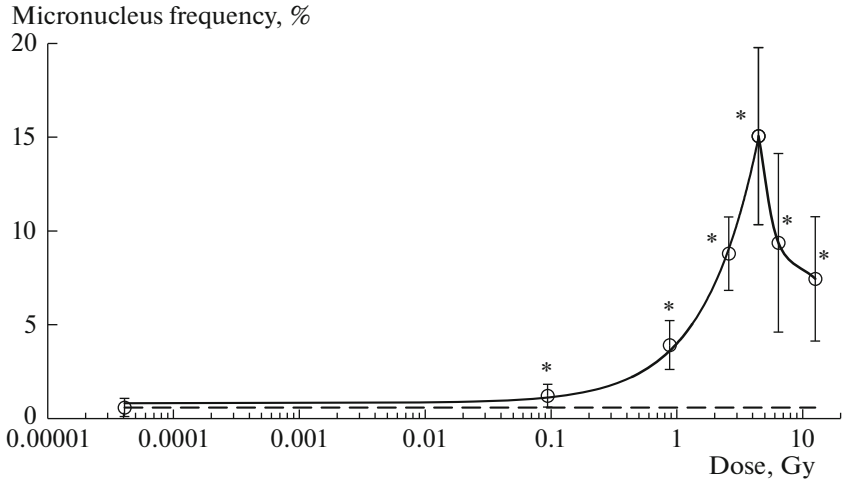

Fig. 3. Dependence of the frequency of micronuclei in onion seedling cells on the absorbed dose in the experiments performed in 2017 (%).

tion, according to [10], an increase in the content of ^{137}Cs in soil samples (i.e., increased irradiation of seeds) correlated with an increase in the proportion of chromosomal aberrations such as bridges and fragments. These results on the spectrum of chromosomal aberrations are consistent with the results of our experiments, in which bridges and fragments were also the dominant types of chromosomal aberrations. The authors of [9] investigated the effect of electromagnetic radiation (ER) on the cytogenetic and growth parameters of onion seedlings. They showed that more than a tenfold increase in the ER intensity had no effect on the length of seedlings (similarly to our experiments), which indicates the low sensitivity of the physiological parameters of seedlings to ER. However, the overall frequency of chromosomal aberrations significantly increased from 1–2% in the control to 4–7% under exposure to ER. Among the chromosomal aberrations occurring under exposure to ER, lagging chromosomes, emissions, chaotic chromosome disjunction in anaphase, and agglutination were recorded. Data on the aberration types such as bridges and fragments were not presented in that study.

The authors of [14] evaluated the level of chromosomal aberrations and micronucleus frequency after exposure of onion bulbs to γ-radiation. As the dose increased from 4 to 20 Gy, the frequency of micronuclei in cells also increased from 1 to 18%. In our experiments, the maximum frequency of micronuclei ($15.1 \pm 4.7\%$) almost coincided with that in the cited paper.

We are aware of only one paper [15] that describes the results of micronucleus assay of onion seedlings after γ-irradiation. In this paper, the level of radiation-induced micronuclei was greater than in the control at absorbed doses over 0.3 Gy for seedlings and at doses over 20 Gy for dry seeds. These data on micronuclei also confirm our previous conclusion about the greater sensitivity of onion seedlings compared to the onion bulbs under exposure to γ-radiation. However, when analyzing the data of [15] and other published data, we found no studies on the dose dependence of cytogenetic parameters of onion and comparative analysis of the levels of induction of micronuclei and chromosomal aberrations under irradiation with low doses.

Thus, the used bioassay (Allium-test) on the basis of seedlings for the first time showed an increase in both the overall frequency of chromosomal aberrations in cells in anaphase and telophase and in the micronucleus frequencies in interphase cells after low-dose γ-irradiation. However, the pattern of dose curves for these cytogenetic characteristics (the frequency of micronuclei and the frequency of chromosomal aberrations) markedly differs under irradiation at doses of 6.4 and 13 Gy. Unlike the frequency of chromosomal aberrations, the frequency of micronuclei at these absorbed doses decreases rather than increases. At the maximum dose of 13 Gy, chromosomal aberrations are detected in the majority of cells in the anaphase and telophase stages and the number of cells with multiple aberrations increases. The main contribution to the overall frequency of chromosomal aberrations, in addition to multiple aberrations, is made by three types of aberrations—bridges, fragments, and lagging chromosomes. In all experiments, γ-radiation in the range of absorbed doses from the background to 13 Gy had no significant effect on the growth of onion seedlings. The data obtained allow using cytogenetic indices of onion *Allium cepa* seedlings for assessment of the biological effects of low-dose γ-radiation.

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