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Using a 120-cm Cyclotron to Study the Combined Effects of Ionizing Radiation and Hypomagnetic Conditions on Lettuce Seeds

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Abstarct—The impact of ionizing radiation and hypomagnetic conditions on seeds of the lettuce *Lactuca sativa L.* is studied on the 120-cm cyclotron at the Skobeltsyn Institute of Nuclear Physics. The seeds are irradiated with α-particles with energies of around 25.8 MeV and placed to germinate in a hypomagnetic chamber under conditions of an attenuated (up to 2500 times) geomagnetic field. Chromosomal aberrations in the first mitosis of the root meristem are analyzed. Changes in the irradiated samples are observed when compared to control samples. Hypomagnetic conditions greatly magnify the negative impact of ionizing radiation.

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INTRODUCTION

The execution of plans for long-distance flights of manned spacecraft and prolonged visits to a lunar base or a near-lunar station can be limited by a number of factors not encountered during near-Earth missions. The most important of these is the ionizing effect of heavy nuclei components of solar and cosmic rays with very high linear transfers of energy [1–3]. Protecting crews from such exposure is a fairly complicated technical problem in which some biomedical aspects have yet to be resolved. Another factor that can affect the safety of space flight is the extremely weak (by several orders of magnitude) magnetic field in outer space, relative to the value of the geomagnetic field (GMF) to which we are accustomed (i.e., hypomagnetic conditions, or HMCs).

Studies of HMCs' effect on living systems under terrestrial conditions show their negative influence on organism, including humans [4–9]. Nevertheless, the level of danger from ionizing radiation (IR) and HMCs for humans and the biological life-support systems (BLFSes) of space vehicles in deep space remains poorly studied and requires further detailed investigation. In addition, there has been virtually no research on their combined impact on any biological objects, though the available data indicate the negative impact of each is growing stronger [10, 11]. It is therefore important that we simulate exposure to ionizing radiation and hypomagnetic conditions for biological objects under terrestrial laboratory conditions, both separately and in combinations.

In this work, seeds of the lettuce *Lactuca sativa*, the leaves of which could be included in the food of space vehicles' crews, were irradiated using α-particles with energies of around 25.8 MeV on a 120-cm cyclotron, and were then placed to germinate in a hypomagnetic chamber with three different modes of geomagnetic field attenuation (up to 2500 times). The chromosomal aberrations in the first mitosis of the root meristem were analyzed.

EXPERIMENTAL

Our study was performed on the U-120 cyclotron at the Skobeltsyn Institute of Nuclear Physics, allowing us to obtain accelerated helium nuclei, and using the irradiation of our biological object in vivo. A special chamber made of soft magnetic material was used to attenuate the geomagnetic field.

The general scheme of the experiment was the same as in [12]. The cyclotron's beam of α-particles with an energy of 30.4 MeV traveled along the ion guide through quadrupole lenses, a deflecting magnet, a protective wall, and through a system of permanent diaphragms to exit the ion guide window into the air. It then passed through a replaceable diaphragm to fall on a working cell containing seeds of lettuce. Aluminum foil 50 μm thick was used as a window on the flange of the ion guide. The beam was monitoring by measuring the strength of the charge landing on the diaphragm and the cell, which were isolated from the ground. The absolute accuracy of determining the amount of absorbed dose was estimated at 30%, while the relative accuracy was no worse than 10%.

Results from our cytogenetic analysis of seeds

Variant	Number of observed dividing cells (anatelophases)	% of aberrant cells	Student's *t*-criterion for the % of aberrant cells	% of cells with multiple aberrations	Student's *t*-criterion for the % of cells with multiple aberrations
KK	2027	1.18 ± 0.24	–	0.10 ± 0.07	–
K3	1756	2.5 ± 0.4**	3.02	0.97 ± 0.23***	3.62
K2	1240	1.7 ± 0.4	1.16	0.97 ± 0.28**	3.01
K1	1185	2.0 ± 0.4	1.79	1.4 ± 0.3***	3.6
20-K	1828	1.48 ± 0.28	0.81	0.4 ± 0.1	1.79
20-3	1115	4.2 ± 0.6***	4.69	2.1 ± 0.4***	4.5
20-2	874	2.9 ± 0.6**	2.76	1.1 ± 0.4**	2.84
20-1	1059	2.1 ± 0.4	1.8	1.0 ± 0.3**	2.73
100-K	1444	2.6± 0.4**	2.87	0.69 ± 0.22*	2.55
100-3	1484	4.5 ± 0.5***	5.53	2.0 ± 0.4***	5.04
100-2	979	2.3 ± 0.5*	2.03	0.31 ± 0.18	1.09
100-1	1160	2.6 ± 0.5**	2.67	1.1 ± 0.3**	3.21

* Differences from the laboratory control are reliable at significance level $p = 0.05$.
** Differences from the laboratory control are reliable at significance level $p = 0.01$.
*** Differences from the laboratory control are reliable at significance level $p = 0.001$.

Seeds of the lettuce strain "Moskovskiy parnikoviy" were irradiated in a specially constructed ring cell with an external and an internal diameter of 60 and 20 mm, respectively.

A detailed description of the cell can be found in [12]. Here we note only that the cell was composed of two identical bolted rings 15 mm thick with a teflon ring between them. The lettuce seeds were placed in the center of the cells between two lavsan films 6 μm thick and drawn over the inner part of the cell's duralumin rings. Up to 70 seeds were placed into the cell in a single layer. Once filled, the cell was placed at a distance of 5 cm behind the window of the ion guide.

The loss of α-particle energy in the ion guide window, the air, and the lavsan film came to 4.6 MeV, so the energy of α-particles on the outer surfaces of the seeds was 25.8 MeV. The value of the linear energy transfer (LET) of α-particles with such energies is approximately 25 keV μm^{-1} of water. This value of the LET of particles on a seed's surface is close to that of the relativistic nuclei of neon and magnesium of galactic cosmic rays and grows in proportion to its flight, reaching 230 keV μm^{-1}, which corresponds to heavier nuclei (silicon) and allows us to simulate the effect the heavy nuclei of solar and cosmic rays have on an object. The average value of an absorbed dose was calculated over the entire volume by assuming the complete absorption of a particle's energy in a seed. The transverse dimension of the seeds varied from 0.4 to 0.6 mm and the flight of the α-particles in the tissue-equivalent material polymethylmethacrylate (PMMA) was approximately 0.62 mm. Each irradiation of the seeds in the cell was performed in two stages, from different sides of the cell. This resulted in a more uniform distribution of the absorbed dose over the seed volume. The total value of the dose absorbed by the seeds was 20 and 100 Gy. The seeds were irradiated at the geomagnetic field's normal magnitude of induction.

The length of exposure needed to obtain an absorbed dose of 100 Gy with our beam current of 2 nA upon exiting the ion guide window was around 30 s. The heating of the irradiated seeds was in this case negligible (no more than 2°C) and was monitored using a pyrometer.

The irradiated seeds germinated in a special cylindrical hypomagnetic chamber made of soft magnetic material with a useful capacity of 35 liters (depth, 0.5 m; diameter, 0.3 m) [13].

Petri dishes containing the lettuce seeds were placed on a table at different distances from the input to the chamber, meaning the seeds were allowed to germinate at three values of magnetic field induction: 20 nT, 140 nT and 1 μT (corresponding to attenuation of the geomagnetic field by 2.5×10^3 times, 3.6×10^2 times, and 50 times, respectively), and under laboratory conditions at a 30–40 μT level of magnetic induction, which is close to the normal terrestrial value in the Moscow region (around 50 μT). The magnetic fields inside and outside the hypomagnetic chamber were measured using a three-component HB 0204.4A magnetometer (NPO ENT, St. Petersburg, Russia) with a dynamic range of 10 nT to 100 μT and a 10 nT accuracy of measurement. When a radicle length of 2–4 mm was reached, the seedlings were fixed, stained with acetorcein, and used for cytogenetic analysis.

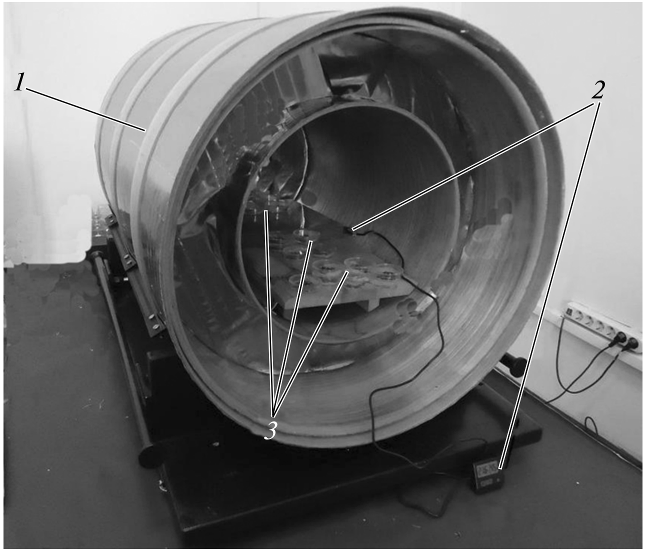

Fig. 1. Hypomagnetic chamber: (*1*) chamber body, (*2*) temperature and humidity sensor, (*3*) Petri dishes with seeds of lettuce.

The anatelophase method was used to study the chromosomal aberrations in the first mitosis of the root meristem. The chromosome and chromatid bridges and fragments were examined, and also cells with multiple aberrations. Figure 2 presents microphotographs of cells that divided normally, a cell with a chromosome bridge, and a cell with multiple aberrations.

RESULTS AND DISCUSSION

The results from our cytogenetic analysis are presented in the table. The following notation is used for different versions of the irradiation and germination of the seeds: KK is the control set (these seeds were not irradiated and germinated under the conditions of a nonattenuated geomagnetic field); K1, K2 and K3 denotes seeds that were not irradiated, but germinated under HMCs with maximum, average, and minimum attenuation of the GMF (here and below, the first index corresponds to the value of the absorbed dose in Gy; the second, to the value of GEM attenuation).

When the seeds not irradiated germinated under HMCs, we observed an increase in the percentage of aberrant cells with a reliable excess in version K3 ($p = 0.01$) and a reliable increase in the percentage of cells with multiple aberrations ($p = 0.001$) in all three modes of geomagnetic field attenuation.

When the irradiated seeds germinated under HMCs, we observed an increase in the percentage of aberrant cells with the maximum excess at the minimum of the considered modes of HMC attenuation (versions 20-3 and 100-3). The same versions differed reliably and by the percentage of multiple aberrations from the irradiated variants germinated under laboratory conditions (versions 20-K and 100-K).

At 140 nT, version 20-2 differed from both laboratory control KK ($p = 0.01$) and version 20-K ($p = 0.05$) in its aberrant cells and cells with multiple aberrations. Version 100-2 differed only from the laboratory control ($p = 0.05$), rather than from version 100-K.

At 20 nT, we observed no reliable difference between versions 20-1 and 100-1 from variants 20-K and 100-K germinated in the laboratory conditions. All together, it can be evidence of the polyextremal dependency of the impact of the HMT. The possibility of such dependencies was noted earlier in [14] at observing the intensity of division of Planariidae in the conditions of various attenuation of HMT and was justified in [9].

Under the same conditions of attenuation of HMT it was couched the seeds irradiated with ^{40}Ar ions with the energy of 290 MeV/nucleon and dose 1 Gy on the accelerator of HIMAC (Japan). A reliable increase of aberrant cells of seedlings was observed both in the separate and combined effects of ionizing radiation and HMT [11], and it was noted the possible polyex-

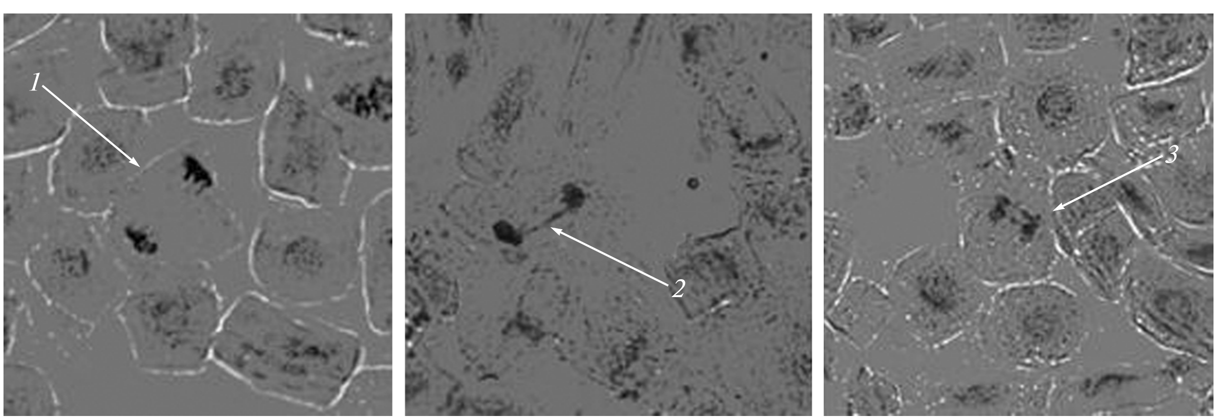

Fig. 2. Microphotographs of the apical meristem of a root stained with 1% acetoorcein, magnified 1000 times. Arrows and numbers indicate (*1*) a normal divided cell, (*2*) a cell with a chromosome bridge, and (*3*) a cell with multiple chromosome aberrations.

tremal dependence of HMT exposure on attenuation magnitude of field.

Our experiments thus showed that a combination of hypomagnetic conditions and ionizing radiation simulating the conditions of space flights considerably magnifies the impact of each, and that this must be considered to ensure the safety of crews and reliable functioning BLFSes under the corresponding conditions of outer space.

CONCLUSIONS

As a result of our experiments on irradiating seeds of lettuce with α-particles having energies of around 25.8 MeV and subsequently germinating under hypomagnetic conditions, we noted a number of changes in the irradiated samples, compared to the control samples. It should be noted that hypomagnetic conditions noticeably magnify the negative effects of ionizing radiation.

Our technique for simulating the effects the ionizing radiation of galactic cosmic rays has on biological objects using a 120-cm cyclotron combined with exposure to hypomagnetic conditions turned out to be both effective and reliable. Our results testify to the need for protection against exposure to these factors of outer space.

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