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COMPLEX SYSTEMS BIOPHYSICS

Cytogenetic Effects in the Root Meristem Cells of Lettuce Seedlings after Fast Neutron Irradiation (10 Gy) of Seeds and Their Modification by Hypomagnetic Conditions during Germination

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Abstract—Seeds of lettuce *Lactuca sativa* L. were irradiated with neutrons with an average energy of 1.6 MeV at a dose of 10 Gy. Irradiation was performed at the end of the limiting time for preserving the conditioned freshness of seeds. The seeds were germinated in a hypomagnetic chamber at a magnetic induction of 1×10^3, 1.4×10^2, and 2×10^1 nT, which corresponded to the reduction of the geomagnetic field by 5×10^1, 3.6×10^2, and up to 2.5×10^3 times, as well as at laboratory conditions (5×10^1 μT). After germinating at hypomagnetic conditions, the percentage of cells with chromosomal aberrations in the root meristem of seedlings grown from irradiated seeds increased at all the considered values of the reduced geomagnetic field. The synergistic effect of factors according to the criterion of cells with chromosomal aberrations and the antagonistic interaction according to the average number of dividing cells at the stages of ana-telophase was noted.

Keywords: hypomagnetic conditions, fast neutron irradiation, *Lactuca sativa*, lettuce seeds, chromosome aberration, durability of seeds

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INTRODUCTION

The prospects of long-range and long-term space flights raise the problem of crew safety outside the Earth's magnetosphere. The hypomagnetic conditions (HMC) of space have been poorly studied. The geomagnetic field (GMF) is slightly reduced in orbital stations flying in near-Earth space. On lunar and Martian bases, as well as during interplanetary flights, the GMF will be decreased by several orders of magnitude.

There is evidence of the negative impact of HMC on various biological objects. Exposure of rats for 10 days to the HMC at approximately 100 nT led to increased intra-species aggression and memory impairment [1]. Incubation of the eggs of the Japanese quail *Coturnix coturnix japonica* in the GMF reduced by 80–100 times caused disorders in the formation of the cardiovascular system and other systems in embryos [2]. Under exposure to an ultra-weak static magnetic field in a shielded chamber made of soft magnetic material with the magnetic induction of 0.2 μT in the center of the chamber, fibroblasts of a healthy donor showed a pattern similar to that which occurred when DNA was damaged, that is, there was an increase in the content of the P53 and P21 proteins and the formation of 53BP1 foci [3]. The frequency of chromosomal aberrations in the cells of roach *Rutilus rutilus* embryos increased in the HMC [4].

The radiation conditions in space flight are formed by galactic and solar cosmic rays, as well as secondary radiation, especially neutron radiation, in the ship's material resulting from nuclear reactions under the action of high-energy protons of galactic cosmic rays. Dose equivalent rate of neutron radiation in the channels of the tissue-equivalent phantom located inside the Mini-Research Module (MRM1) during the main expeditions to the International Space Station (ISS-35/36 and ISS-41/42) ranged from 77 ± 13 to 157 ± 25 μSv/day, which corresponded to 17 to 28% of the total dose [5]. With the increase in the thickness of the passive radiation protection of the spacecraft, the contribution of secondary neutrons increases. Albedo fast neutrons from the surface of the Moon also pose a certain danger. For fast neutrons with an energy of approximately 0.45 MeV, the precised maximum coefficients of relative biological efficiency (RBE) for doses of 0.01–0.05 Gy were of 41 when considering the initial stages of lens opacity [6]. Upon total neutron irradiation of mice with energy of 1.5 MeV at doses of 2.5–25 Gy, the RBE coefficients 24 and 72 h after irradiation were in the range from 4.1 ± 0.1 to

Abbreviations: HMC, hypomagnetic conditions; GMF, geomagnetic field; RBE, relative biological efficiency.

Table 1. The variants of germination of lettuce seeds

Irradiation of the seeds	The magnetic induction			
	5×10^1 μT, magnetic field of the Earth	2×10^1 nT, attenuation by 2.5×10^3 times	1.4×10^2 nT, attenuation by 3.6×10^2 times	1×10^3 nT, attenuation by 5×10^1 times
Nonirradiated seeds	K-K	K-1	K-2	K-3
Irradiated seeds	10-K	10-1	10-2	10-3

7.3 ± 0.1 according to the criteria of a decrease in the mitotic index and the formation of aberrant mitoses [7].

Only a few studies of the combined action of HMC and radiation have been performed. When germinating lettuce seeds were irradiated with argon ions with energy of 290 MeV/nucleon and carbon ions with energy of 400 MeV/nucleon at a dose of 1 Gy in HMCs, an increase in the severity of radiation damage was observed according to the chromosomal aberration test [8, 9]. Lettuce seeds were repeatedly used in a number of space experiments. Due to their biological characteristics, they can be exposed in space conditions from several days to a year or more [10]. The limiting time for preserving the conditioned freshness of lettuce seeds is of 3–4 years [11]. Radiation aging can be partially modeled using seeds that were stored for a long period of time. In space greenhouses, the seeds will germinate under HMC, being simultaneously exposed to cosmic radiation and secondary radiation neutrons.

The aim of this work was to study the reaction of seeds at the end of the storage period, while preserving its conditioned freshness, to the combined effects of neutron irradiation and HMC.

MATERIALS AND METHODS

The seeds of lettuce *Lactuca sativa* L. of the Moskovskii parnikovi variety of the 2012 harvest were used in the study. The seeds were produced under protected soil conditions in the Moscow region at the Experimental Production base of the Federal Scientific Vegetable Center. The seeds were irradiated with neutrons with an average energy of 1.6 MeV on the biological equipment of the Budapest Research Reactor in July 2016. The dose rate was 14.2 mGy/s; the proportion of gamma radiation was 10% and the uncertainty was at least 5%. The seeds were placed in package of tracing paper of 2 × 2 cm. The heterogeneity within one package was less than 3%. Air-dry seeds were stored in the refrigerator at 4°C.

The seeds were germinated in October 2016 in Petri dishes on filter paper moistened with distilled water at a temperature 21.0 ± 0.5°C. The variants of germination of the lettuce seeds are presented in Table 1. The number of seeds put on germination was 100 pieces per variant.

Germination was performed in a hypomagnetic chamber with a working volume of 35 L made of rolled soft magnetic material [12]. With the lid open, a GMF attenuation gradient was created in the chamber, which made it possible to put the seeds in locations with different GMF reduction. The seeds were germinated at magnetic induction of 1×10^3, 1.4×10^2, and 2×10^1 nT, which corresponded to the reduction of the geomagnetic field by 5×10^1, 3.6×10^2, and 2.5×10^3 times, as well as at laboratory conditions at 5×10^1 μT. The magnetic induction was measured with a HB0204.4A three-component type magnetometer (NPO ENT, St. Petersburg), which had a measurement range from 10 nT to 100 μT and a measurement accuracy of 10 nT.

The percentage of germinated seeds on the third day was considered as the seed vigor and the percentage of germinated seeds on the seventh day was considered as the germinating ability. Seeds with a root length of 2–4 mm were considered as germinated; this corresponded to the first mitosis in the root meristem. The seedlings were fixed and stained with acet-orcein and temporary preparations were prepared according to the standard procedure. For further cytogenetic analysis seeds that sprouted on the first day were used. The preparations were examined under a microscope with a magnification of 945 times, at 30–31 roots per variant. The ana-telophase analysis method was used, chromosomal and chromatid bridges and fragments were counted, the average number of dividing cells per a root, the number of aberrations per an aberrant cell, and the percentage of cells with chromosomal aberrations and cells with multiple aberrations were calculated.

Statistical analysis of the results was carried out using the Student's *t*-test. The synergistic effect coefficient was calculated as described in [9].

RESULTS AND DISCUSSION

The data on seed germination under various conditions are presented in Fig. 1. The figure shows that the number of nonirradiated seeds germinated under HMC on the first day was reduced by 9–14% depending on the variant; by the second day these differences leveled and the number of germinated seeds was 98–100%. Nonirradiated seeds of the same batch during germination 2 years earlier also showed a delay in ger-

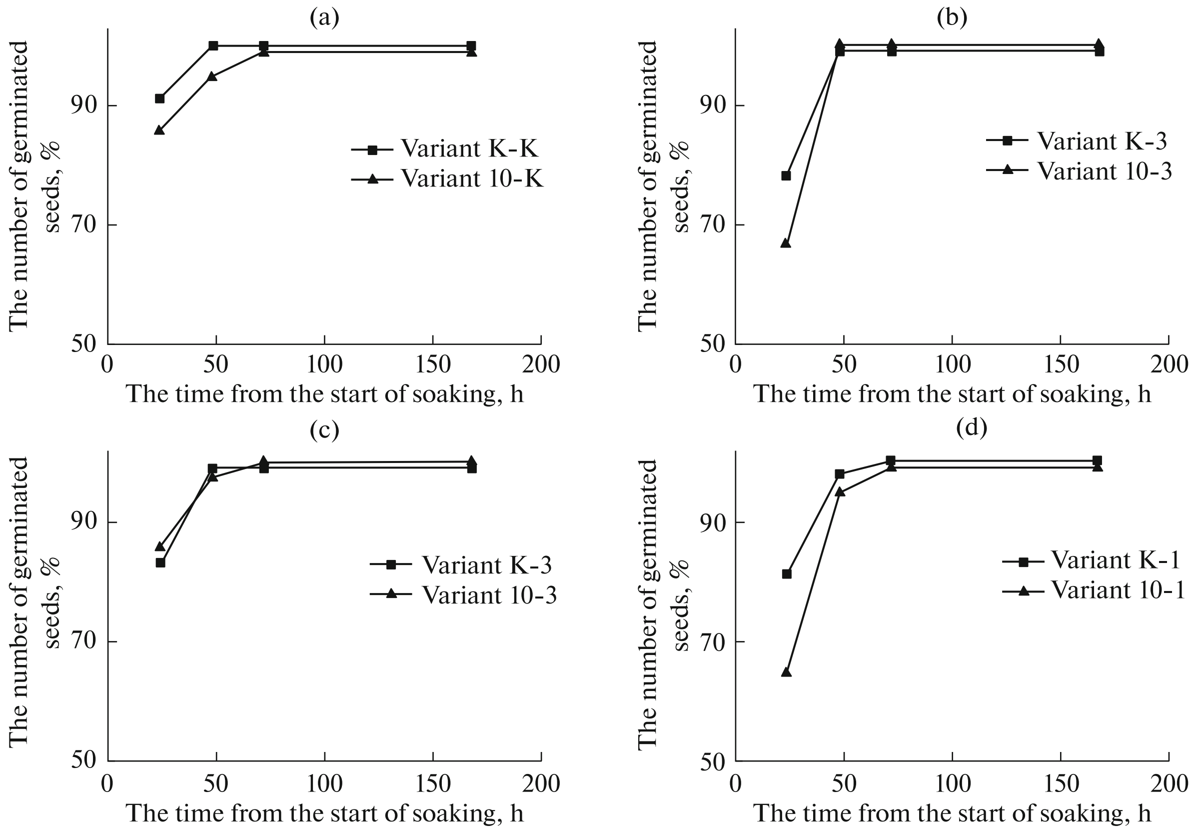

Fig. 1. Lettuce seed germination at a magnetic induction of 5×10^1 μT (a), 1×10^3 (b), 1.4×10^2 (c) and 2×10^1 nT (d).

mination under HMC [9]. After germination under normal conditions, the number of germinated irradiated seeds (variant 10-K) on the first day was reduced by 5% compared to nonirradiated seeds that germinated under the same conditions, while the seed vigor and germinating ability were at the control level. The same high seed vigor and germinating ability were demonstrated by nonirradiated seeds during germination 2 years earlier. When the seeds were irradiated with carbon ions with energy of 400 MeV/nucleon at a dose of 1 Gy, the decrease in the number of germinated seeds on the first day was 14% compared with nonirradiated seeds [9]. Under HMC at 2×10^1 and 1×10^3 nT, the number of neutron-irradiated germinated seeds on the first day was reduced by more than 20% compared to the same seeds that germinated under normal conditions; the percentage of germinated seeds on the first day did not change at 1.4×10^2 nT. At the same time, the seed vigor and germinating ability of all variants was 99–100%. It should be noted that we say about the laboratory germination and not about a field germination.

The maximum number of dividing cells at the stages of ana-telophase was observed in nonirradiated seeds of the K-K variant that were germinated under normal conditions (Table 2). After germination of nonirradiated seeds under HMC, there was a decrease in the number of dividing cells by 45% at 1×10^3 nT, by 41% at 1.4×10^2 nT, and by 21% at 2×10^1 nT. A decrease in the number of dividing cells was noted in HMC during germination of nonirradiated seeds at the beginning of the storage period [8]. The irradiated seeds that germinated under normal conditions (variant 10-K) also had a 65% decrease in the number of dividing cells. A decrease in the number of cells at the stages of ana-telophase was also demonstrated in the root meristem of seedlings obtained from lettuce seeds irradiated with ions of argon [8] and carbon [9]. When the seeds of the same batch were irradiated 3 years earlier with neutrons with the same beam parameters at a dose of 10 Gy, a two-fold decrease in the number of dividing cells was recorded [13]. Thus, a stronger suppression of cell division was found in seedlings obtained from irradiated seeds at the end of the conditioned shelf life. When irradiated seeds were germinated under HMC, the number of dividing cells in the root meristem of seedlings increased at 1.4×10^2 and 2×10^1 nT. This might be due to a compensatory increase under these conditions of reduced GMF.

In seedlings obtained from nonirradiated seeds, a significant decrease in the percentage of cells with chromosomal aberrations was noted at 1.4×10^2 nT(Table 2); the percentage of chromosomal bridges decreased, and chro-

Table 2. The results of cytogenetic studies of lettuce seedlings after germination under normal and hypomagnetic conditions

Variant	The number of examined dividing cells (ana-telophases)	Proportion of aberrant cells, %	Proportion of cells with multiple aberrations, %	The number of aberrations per an aberrant cell	Average number of ana- and telophases per one root
K-K	3403	1.09 ± 0.18	0.15 ± 0.07	1.14	130.9 ± 4.5
K-3	2215	0.90 ± 0.20	–	1.00	71.5 ± 3.2***
K-2	2334	0.30 ± 0.11***	0.04 ± 0.04	1.14	77.8 ± 2.6***
K-1	3108	1.00 ± 0.18	0.10 ± 0.06	1.10	103.6 ± 4.7***
10-K	1387	17.81 ± 1.03***	4.04 ± 0.53***	1.26	46.2 ± 2.8***
10-3	1274	26.96±1.23***^^^	5.57 ± 0.64***	1.23	42.5 ± 2.5***
10-2	1785	22.97±1.00***^^^	3.59 ± 0.44**	1.18	59.5 ± 3.1*** ^^^
10-1	2137	23.54±0.92***^^^	3.79 ± 0.41***	1.18	71.2 ± 1.3*** ^^^

** Differences of the variant from the laboratory control were reliable at the significance level $p < 0.01$; *** differences of the variant from the laboratory control were reliable at the significance level $p \leq 0.001$; ^^^ differences of the variant from seeds irradiated with neutrons and germinated in laboratory conditions (variant 10-K) were reliable at the significance level $p \leq 0.001$.

matid fragments were not observed (Table 3), while the number of aberrations per an aberrant cell remained unchanged. There were no cells with multiple aberrations at 1×10^3 nT. In our previous experiments [9, 14], an increase in the percentage of cells with chromosomal aberrations in the root meristem of seedlings obtained from nonirradiated seeds was observed when they were germinated under similar conditions of reduced GMF. The irradiation used in this study was carried out 2 years later with seeds of the same batch; the results we obtained were apparently due to the aging of seeds and changes that occurred as a result of storage.

The seedlings obtained from irradiated seeds that germinated under normal conditions (variant 10-K) had an increased percentage of cells with chromosomal aberrations and multiple chromosomal aberrations (Table 2). This was due to the pronounced damaging effect of neutron irradiation at such a dose. A similar percentage of aberrant cells (17.1 ± 1.0) was obtained after gamma irradiation of lettuce seeds at a dose of 150 Gy at a dose rate of 90.9 sGy/s [15]. Thus, the neutron RBE coefficient at this dose was 15. The average value of the RBE for fast neutrons is 10 [16]. At the same time, RBE depends on the dose, the criterion of the evaluation of the effect, and the type of biological object.

The seedlings obtained from seeds irradiated with argon ions [8], as well as from seeds that spent 231 days in the International Space Station [17], showed an increase in the percentage of cells with chromosomal aberrations and cells with multiple aberrations only after germination at 2×10^1 nT. When seeds irradiated with carbon ions were germinated under HMC, the percentage of cells with chromosomal aberrations increased at 1.4×10^2 and 2×10^1 nT [9]. When seeds irradiated with neutrons at a dose of 10 Gy were germinated under HMC, there was an increase in the percentage of cells with chromosomal aberrations ($p \leq 0.001$) with all the reduced GMF considered, while the percentage of cells with multiple aberrations did not change significantly (Table 2). This was apparently due to greater damage to the meristem after irradiation at a higher dose. The increase in the percentage of cells with chromosomal aberrations was due to an increase in the proportion of chromosomal bridges and the proportion of chromatid bridges, while the proportion of chromatid fragments did not change significantly; the proportion of chromosomal fragments decreased at a magnetic induction of 1×10^3 nT and 2×10^1 nT (Table 3).

To assess the combined effect of HMC and radiation, the synergistic effect coefficient k was calculated; this shows how many times the effect of combined exposure exceeded the effect assessed as a sum of the effects of factors acting independently (Fig. 2). For the percentage of cells with chromosomal aberrations at all modes of GMF reduction, a synergistic effect was noted ($k > 1$); whereas for seeds irradiated with carbon ions at a dose of 1 Gy, a synergistic effect was noted only with average and maximum reduction [8]. This

Table 3. The types of chromosomal aberrations

Variant	Proportion of chromosomal bridges, %	Proportion of chromosomal fragments, %	Proportion of chromatid bridges, %	Proportion of chromatid fragments, %
K-K	0.88 ± 0.16	0.15 ± 0.07	0.18 ± 0.07	0.03 ± 0.03
K-3	0.86 ± 0.20	–	0.05 ± 0.05	–
K-2	0.30 ± 0.11**	–	0.04 ± 0.04	–
K-1	0.97 ± 0.18	–	0.13 ± 0.06	–
10-K	13.63 ± 0.92***	6.56 ± 0.66***	1.80 ± 0.36***	0.36 ± 0.16*
10-3	23.63 ± 1.19*** ^^^	3.77 ± 0.53*** ^^^	4.71 ± 0.59*** ^^^	0.08 ± 0.08
10-2	18.04 ± 0.91*** ^^^	4.93 ± 0.51***	3.81 ± 0.45*** ^^^	0.22 ± 0.11
10-1	21.24 ± 0.88*** ^^^	2.71 ± 0.35*** ^^^	3.79 ± 0.41*** ^^^	0.09 ± 0.06

*, Differences of the variant from the laboratory control were reliable at the significance level $p \le 0.05$; **, differences of the variant from the laboratory control were reliable at the significance level $p \le 0.01$; ***, differences of the variant from laboratory control were reliable at the significance level $p \le 0.001$; ^^^ differences of the variant from seeds irradiated with neutrons and germinated in laboratory conditions (variant 10-K) are significant at the significance level $p \le 0.001$.

was probably due to the degree of radiation damage to the cells. For the average number of dividing cells at the stages of ana-telophase, the antagonistic effect of factors was noted ($k < 1$). The same trend of the effect according to this criterion was noted when lettuce seeds were irradiated with carbon ions.

The observed effects might be associated with the difficulty of repair processes under HMC. Based on the analysis of the literature data, it was suggested that the biological effect is polyextremally dependent on the degree of the GMF reduction [18]. The data on the intensity of planarian division at various degrees of the GMF reduction could be well explained within this concept [19]. A 1.5-h exposure of peritoneal neutrophils of mice under HMC with magnetic shielding that gave a residual constant magnetic field of 20 nT caused a decrease in intracellular production of reactive oxygen species [20, 21]. The erythrocytes of rats exposed to a reduced magnetic field of 0.192 μT. produced more oxygen radicals than those exposed to the Earth's magnetic field [22, 23]. Magnetic fields can induce spin triplet-singlet transitions in a pair of radicals and ion-radicals and change their spin state and reactivity. There is evidence of the possibility of dependence of the ATP and DNA synthesis on magnetic fields [24]. HMC can make changes in the existing system of magnetic interactions, which may be a cause of a polyextremal dependence of the observed effects.

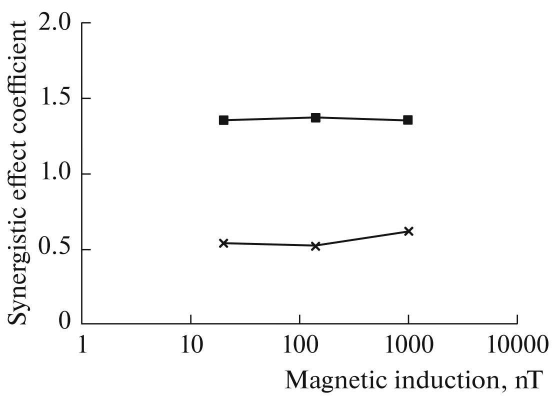

Fig. 2. The synergistic effect on lettuce seeds: squares, the proportion of cells with chromosomal aberrations; crosses, the average number (per root) of dividing cells at the stages of ana-telophase.

CONCLUSIONS

When lettuce seeds were irradiated with neutrons with an average energy of 1.6 MeV at a dose of 10 Gy at the end of the period of the limiting time for preserving conditioned freshness, the following observations were made:

(1) Seed vigor and germinating ability upon joint and separate exposure to the radiation and HMC remained at the level of 99–100%.

(2) There was a delay in germination by 5% on the first day after the irradiation. The number of nonirradiated seeds germinated on the first day under HMC decreased with all the degrees of reduction, compared to germination under normal conditions; in the case of irradiated seeds the decrease was at 2×10^1 and 1×10^3 nT.

(3) There was a decrease in the average number of dividing cells at the stage of ana-telophase per root after germination of nonirradiated seeds under HMC.

(4) HMC contributed to an increase in the mitotic activity of irradiated seeds. There was an antagonistic relationship of HMC and irradiation according to this parameter; the coefficient of synergistic effect was less than one.

(5) The percentage of cells with chromosomal aberrations decreased when nonirradiated seeds were germinated at 1.4×10^2 nT; this was not observed when fresh seeds were germinated.

(6) The percentage of cells with chromosomal aberrations increased when irradiated seeds were germinated under HMC at all modes of GMF reduction. There was a synergistic effect of HMC and irradiation according to this parameter, the coefficient of synergistic effect was more than one.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This paper does not contain a description of studies using humans and animals as objects.

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