AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 3, pp. 525-537
[SEL’SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)

ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 633.16:581.1:58.03/.04

doi: 10.15389/agrobiology.2023.3.525eng
doi: 10.15389/agrobiology.2023.3.525rus

INFLUENCE OF γ-IRRADIATION AND LEAD ON THE DYNAMICS OF GERMINATION OF SPRING BARLEY SEEDS

A.A. PRAZYAN ✉**, S.V. BITARISHVILI, S.A. GERAS’KIN, E.S. MAKARENKO**

National Research Centre Kurchatov Institute, All-Russian Institute of Radiology and Agroecology, 1/1, Kievskoe Shosse, Obninsk, Kaluga Province, 49032 Russia, e-mail prazyana@yahoo.com (✉ corresponding author), bitarishvili.s@gmail.com, stgeraskin@gmail.com, makarenko_ek_obninsk@mail.ru
ORCID:
Prazyan A.A. orcid.org/0000-0002-7908-1928
Bitarishvili S.V. orcid.org/0000-0002-3623-7128
Geras’kin S.A. orcid.org/0000-0001-9978-3049
Makarenko E.S. orcid.org/0000-0001-7519-9550

The authors declare no conflict of interests
Final revision received January 30, 2023
Accepted February 22, 2023

Abstract

Crops are simultaneously affected by factors of different nature; therefore, it is important to study the separate and combined effects of technogenic stressors on plants. During seed germination, there is a transition from heterotrophic to autotrophic type of nutrition, which largely determines the further development of the plant, the size and quality of the crop. The impact of biotic and abiotic factors on seeds can significantly affect the passage of germination phases. In this work, for the first time, the dynamics of development in the first phases of germination of barley variety Nur under the conditions of separate and combined action of gamma radiation and heavy metal $Pb(NO_3)_2$ was studied in detail. The antagonistic effect of preliminary irradiation on the toxic effects of lead salt during germination was revealed. The aim of the work is to evaluate the influence of separate and combined effects of gamma radiation and lead, including possible synergistic and antagonistic effects of the interaction of stressors, on the dynamics of germination of spring barley seeds. The seeds of spring barley (*Hordeum vulgare* L.) of the Nur variety of the first reproduction of 2019 were used. The germination process was assessed visually for 70 hours, with detailed observation every 2 hours from the 18th to the 38th hour and every 4 hours from the 46th to the 70th hour. The seeds were irradiated with a dose of 20 Gy (dose rate 60 Gy/h) at the GUR-120 (^{60}Co) unit (RIRAE, Obninsk). We also used the $Pb(NO_3)_2$ salt at a concentration of 2 mg/ml which inhibited the development of seedlings but did not lead to their death. In the control group, non-irradiated seeds were germinated in 7 ml of distilled water. In experimental group I, seeds irradiated at a dose of 20 Gy were germinated in the same volume of water. In experimental group II, non-irradiated seeds were germinated in water with the addition of $Pb(NO_3)_2$ at a concentration of 2 mg/ml; in experimental group III, the seeds were subjected to a combined action of γ-irradiation and lead. In total, 800 seeds were studied, 200 seeds in each group. Seeds were germinated in a MIR-254 thermostat (Sanyo, Japan) in Petri dishes (20 pieces each), on a double layer of filter paper (Belaya Lenta, Russia), in the dark, at 20±0.5 °C. The germination process was divided into 6 main phases: “point’ — pecking, the appearance of the germinal root, roots 1 (K-1), “fork” — differentiation of the germinal root into several roots 1-2 mm long; roots 2 (K-2) — the initial growth of roots, their size is less than the length of the seed; roots 3 (K-3) — mature roots larger than the length of the seed, no sprout; sprout — the appearance of a coleoptile, the seed has several roots and a sprout less than half the length of the seed; seedling — the formation of a full-fledged sprout, having at least two roots larger than the length of the seed and a sprout larger than half the length of the seed.. The nonparametric Mann-Whitney test was used to compare mean values. The coefficient of interaction Kw was used as a quantitative measure of the deviation of the observed effect from the additive effect and classification of the effects of combined action into groups of additivity, synergy, and antagonism. Under γ-irradiation of seeds, statistically significant differences from the control appeared in phases K-1 and K-3. Significant differences were noted in the “sprout” and “seedling” phases by the end of the observations. In general, γ-irradiation at a dose of 20 Gy did not significantly disrupt the passage of microphenological phases in seeds. Treatment with $Pb(NO_3)_2$ at a concentration of 2 mg/ml slowed down seed germination, which manifested itself in a delay in the transition to each subsequent microphenological phase, as well as in a decrease in the proportion of seeds at late stages of development compared to the control. In addition, lead had a negative effect on the development of the root, almost completely excluding the K-3 phase from the development of the seedling. The combined effect of γ-irradiation and lead also led to a slowdown in development, but in this variant, the proportion of seeds that reached the K-3 phase increased and approached the rate in the control, that is, γ-irradiation at a dose of 20 Gy mitigated the toxic effect of lead. Therefore, a dose of 2 mg/ml $Pb(NO_3)_2$, regardless of the effect of γ-irradiation, has an inhibitory effect on the development of seeds, but does not completely suppress it, only reducing the rate of development..

Keywords: *Hordeum vulgare*, barley, seeds, germination phases, lead, γ-irradiation, combined action of radiation and lead

Technogenic pollution limits the yield and quality of agricultural plant products. The areas of emissions from industrial enterprises reach enormous sizes. In the Russian Federation alone, the area of heavy metal (HM) contamination is more than 3.6 million hectares [1]. Lead is one of the most common agricultural pollutants. It belongs to the first hazard class [1] and is capable of influencing the morphology and physiology of plants. Lead is not an element essential for plants, and its toxic effect is largely associated with various disorders of cell metabolism and inactivation of enzymes [2]. As a result, lead inhibits germination, root elongation, seedling development, chlorophyll production, and inhibits Calvin cycle enzymes, thereby affecting plant development [2, 3].

After the discovery of ionizing radiation (IR), research began on its effect on living organisms. The biological action of IR is based on the direct interaction of radiation quanta with biological macromolecules and the formation of reactive oxygen species in the process of water radiolysis [4]. Already the first experiments showed that with increasing radiation dose, damage to biological structures increases, loss of their functions is observed, inhibition of reproduction and growth and, as a result, death of the organism. However, in the low-dose region, deviations from the monotonic nature of the dose-effect relationship were found, which is associated with a qualitative difference in cell responses to irradiation at high and low doses [5]. Moreover, in the low-dose region, radiation hormesis is observed, when inhibition of physiological processes is replaced by stimulation [6].

In real conditions, plants are affected by combinations of factors that differ in toxicity and mechanisms of action. In this regard, it is important to study the combined action of factors of different nature, which can influence biochemical processes, accelerate or slow down metabolism and, accordingly, affect the rate of plant development [2, 7]. Qualitative differences between the mechanisms of biological action of factors and their targets in the cell can cause fundamentally different plant responses, from antagonism to synergism [8]. In particular, preliminary irradiation of barley (*Hordeum vulgare* L.) seeds, *Arabidopsis thaliana* L. and faba beans (*Vicia faba* L.) increased plant resistance to the toxic effects of lead and cadmium [9-11].

When a seed germinates, a transition occurs from a heterotrophic to an autotrophic type of nutrition, which largely determines the further development of the plant, the size and quality of the harvest. Exposure of seeds to biotic and abiotic factors can significantly influence the progression of germination phases [12, 13]. In the scientific literature, there is practically no data on the detailed dynamics of the germination process, both in the absence of technogenic factors and when factors of different nature act separately or together.

In this work, for the first time, the dynamics of germination in barley variety Nur under the separate and combined action of γ-radiation and $Pb(NO_3)_2$ was studied in detail. Pre-irradiation has been shown to mitigate the toxic effect of lead salt during germination.

The purpose of the work was to assess the influence of each of the studied factors, the γ-radiation and lead salt and their combination, including possible synergistic and antagonistic effects of the interaction of stressors, on the germination of spring barley seeds.

Materials and methods. We used seeds of spring barley (*Hordeum vulgare*

L.) Nur variety of the first reproduction in 2019. The germination was assessed visually over 70 hours, with detailed observations every 2 hours from the 18th to the 38th hour and every 4 hours from the 46th to the 70th hour.

The seeds were irradiated at 20 Gy (the dose rate 60 Gy/h) using a GUR-120 (^{60}Co) installation (ARRIRAE, Obninsk). In our previous experiments [14], this dose stimulated the development of barley Nur and Grace seedlings. The seeds were placed in paper bags with a surface area of 25 cm^2, which ensures an even distribution of the dose. The radiation dose absorbed by the seeds was assessed with a DKS-101 dosimeter (Politechform-M, Russia). We also used $Pb(NO_3)_2$ salt concentration 2 mg/ml, which inhibited the development of seedlings, but did not lead to their death [15].

The control was non-irradiated seeds germinated in 7 ml of distilled water. The first treatment was seeds irradiated at 20 Gy and germinated in the same volume of water. The second treatment was non-irradiated seeds germinated in water with 2 mg/ml $Pb(NO_3)_2$; the third treatment was the seeds subjected to the combined action of γ-radiation and lead salt. A total of 800 seeds were examined, 200 seeds per treatment. Seeds were germinated in a MIR-254 thermostat (Sanyo, Japan) in Petri dishes (20 seeds per each) on a double layer of filter paper (White Lenta, Russia) in the dark at 20±0.5 °C.

Microphenological stages of seed germination were assessed as described [16]. The germination process was divided into six main stages, “point” means pecking, appearance of the embryonic root; roots 1 (R-1), “fork” means differentiation of the embryonic root into several roots 1-2 mm long; roots 2 (R-2) corresponds to initial growth of roots, their size is less than the length of the seed; roots 3 (R-3) means mature roots larger than the length of the seed, no sprout; sprout means the appearance of a coleoptile, the seed has several roots and a sprout measuring less than half the length of the seed; seedlings correspond to the formation of a complete sprout that has at least two roots larger than the length of the seed and a sprout measuring more than half the length of the seed. The “point” stage determines the beginning of pecking of the rudimentary root, the length of which should not exceed 1 mm. During seed germination, shoot and root growth stages were distinguished [17], and root growth, in turn, was divided into three stages. The scale is consistent with the approaches underlying the GOST on methods for determining germination [18].

The nonparametric Mann-Whitney test was used to compare mean values. As a quantitative measure of the deviation of the observed effect from the additive one and the classification of the combined effect as additivity, synergism, and antagonism, the interaction coefficient K_W [8] was calculated:

$$K_W = \frac{\Delta A(\gamma, HM)}{\Delta A(O, HM) + \Delta A(\gamma, O)},$$

where $\Delta A(X,Y) = A(X,Y) - A(O,O)$ is the increment (the excess of the level induced by stressors over the spontaneous one) at the ionizing radiation dose X and the heavy metal concentration Y.

Since the level of an observed effect is a random variable that has a probability distribution, the value of the interaction coefficient K_W is also a random variable. To classify the response of plants to a combined effect, it is necessary to test the statistical hypothesis that Kw is equal to 1. The effect is recognized as additive if $K_W \sim 1$, as antagonistic if K_W is statistically significantly less than 1, and as synergistic if K_W is statistically significantly greater than 1.

Calculations were carried out in Microsoft Excel 2010 and Statistica v. 8.0 (StatSoft, Inc., USA). The differences were confirmed statistically with a significance level of $p < 0.05$.

Results. In the “point” phase (Fig. 1), in seeds irradiated with a dose of

20 Gy (treatment I), significant differences ($p < 0.05$) vs. control occurred only at the 20th hour. In the R-1 phase, differences vs. controls appeared at the 30th hour of germination (Fig. 2, A), while the peak of development of both groups occurred at the 32nd hour. Upon transition to the R-3 phase (see Fig. 2, B), there was a statistically significant excess vs. control in irradiated seeds from the 58th to the 62nd hour. During sprouting (Fig. 3, A), differences from the control were significant ($p < 0.05$) at the 54th and 66th hour of germination. In general, the development of irradiated seeds repeated the dynamics of control seeds, and the few deviations were not systematic.

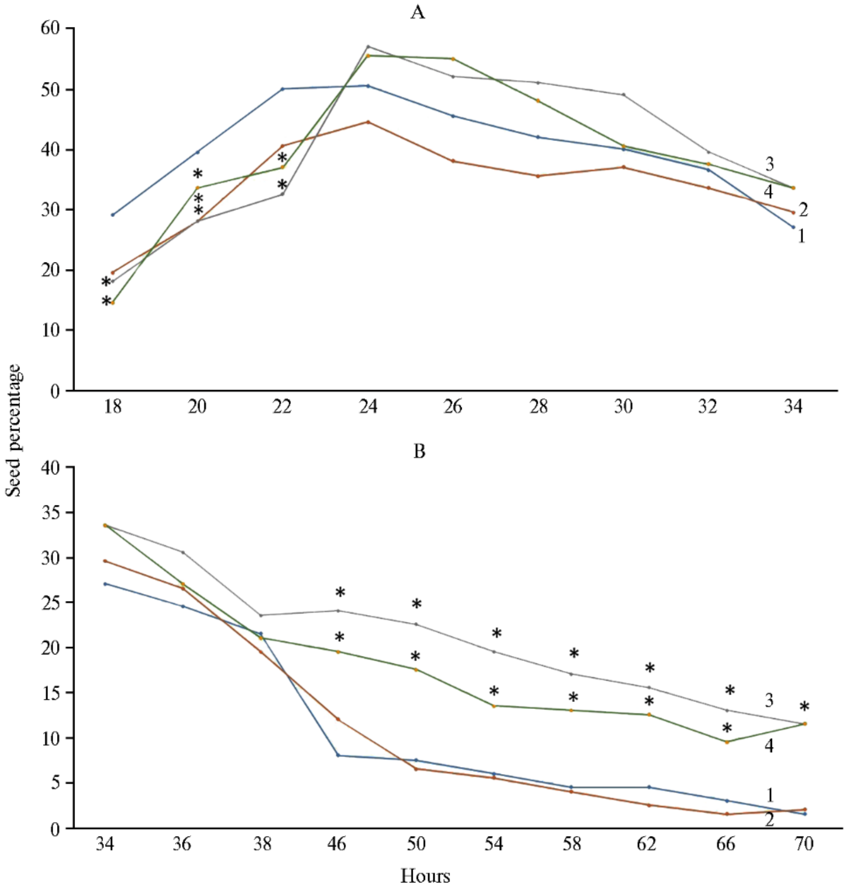

Fig. 1. The proportion of seeds of spring barley (*Hordeum vulgare* L.) variety Nur at the "point" stage from the 18th to 34th hour (A) **and from the 34th to 70th hour of germination** (B): 1 — control (no treatment), 2 — irradiated seeds 20 Gy (treatment I), 3 — seed treatment with $Pb(NO_3)_2$ (treatment II), 4 — seeds exposed to the combined action of γ-radiation and lead (treatment III) (lab tests). A total of 800 seeds were studied, 200 seeds per treatment.
* Differences from control are statistically significant at $p < 0.05$.

When treated with lead (treatment II), a slowdown in the passage of all stages of development was recorded. From the first hours, a slowdown in swelling was observed, as a result of which the proportion of seeds that entered the "point" phase was significantly lower ($p < 0.05$) compared to the control (see Fig. 1, A) up to the 22nd hour. However, by the 24th hour, the proportion of seeds treated with lead in the "point" phase reached and even slightly exceeded the control, which indicated an equalization of growth rates. A statistically significant ($p < 0.05$) increase in the proportion of lead-treated seeds that reached the "point" stage compared to control was recorded starting from the 46th hour until the end of the observations (see Fig. 1, B).

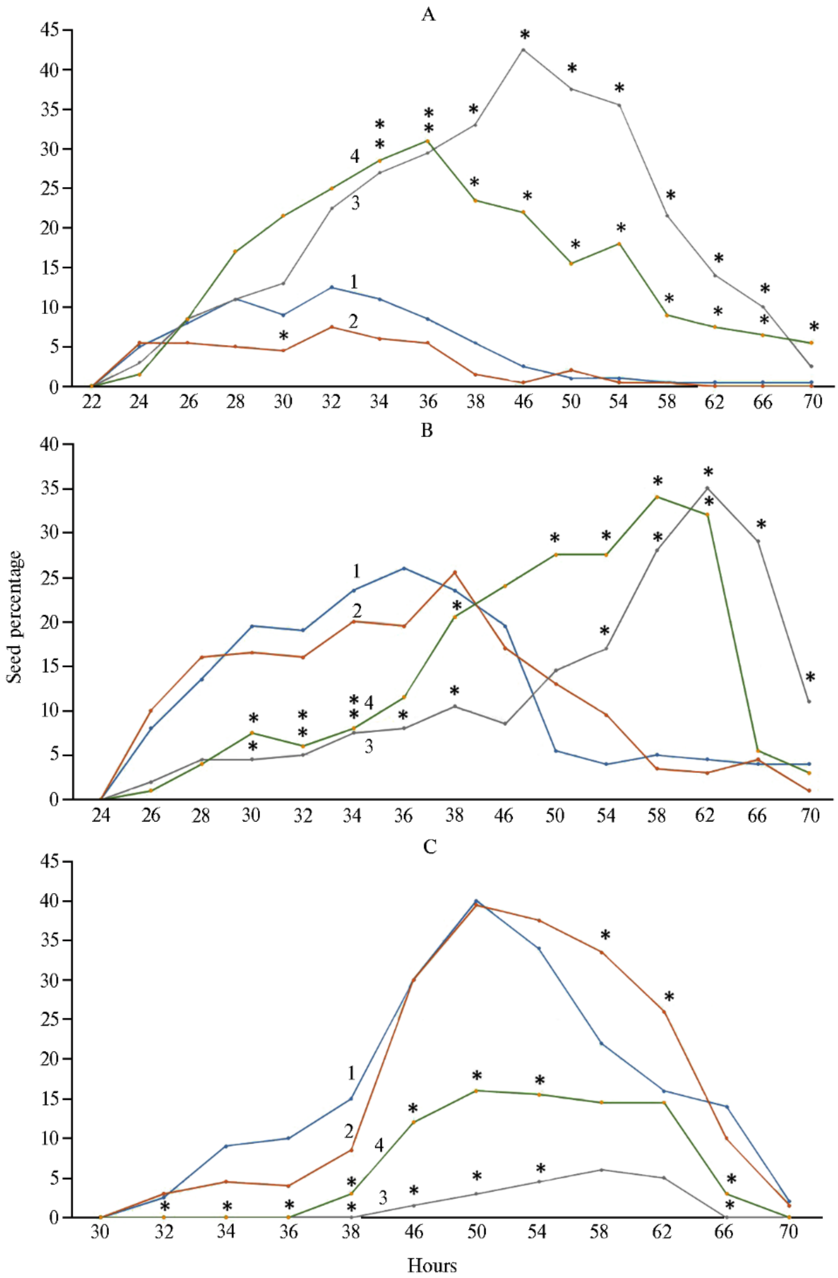

Fig. 2. The proportion of seeds of spring barley (*Hordeum vulgare* L.) variety Nur at stages R-1 (A), **R-2** (B) **and R-3** (C) **from the 30th to the 70th hour of germination:** 1 — control (no treatment), 2 — irradiated seeds (20 Gy) (treatment I), 3 — seed treatment with $Pb(NO_3)_2$ (treatment II), 4 — seeds exposed to the combined action of γ-radiation and lead (treatment III) (lab tests). A total of 800 seeds were examined, 200 seeds per treatment.

* Differences from control are statistically significant at $p < 0.05$.

At the R-1 stage, significant differences from the control ($p < 0.05$) were observed from the 34th to the 66th hour (see Fig. 2, A). The proportion of seeds that reached the R-1 stage (43%) in treatment II was the greatest at the 46th hour. This value turned out to be higher than in treatment III (31%), in the control (13%) and in treatment I (7.5%), that is, exposure to lead significantly slowed down the development at the R-1 stage. The main proportion of lead-treated seeds reached the R-2 stage (see Fig. 2, B) by the 62nd hour, which is 26 hours later than in the control group. Significant differences vs. control aooeared from the 30th hour until the end of observations. From the 46th to the 50th hour, there were no significant differences in the proportion of seeds that entered the R-2 stage, since in this interval the number of such seeds in treatment II began to increase and their number sharply decreased in the control.

The proportion of seeds in the R-3 phase (see Fig. 2, B) when exposed to lead decreased significantly compared to the control from the 32nd to the 54th hour. In the sprout phase, a statistically significant excess ($p < 0.05$) over the control was observed from the 66th hour (see Fig. 3, A). Since the proportion of seeds in the seedling stage in treatment II increased much more slowly (see Fig. 3, B), the number of such seeds in the control was statistically significantly greater. In general, lead-treated seeds showed a slow entry into the seedling phase (+1% per 4 hours, starting at 62 hours).

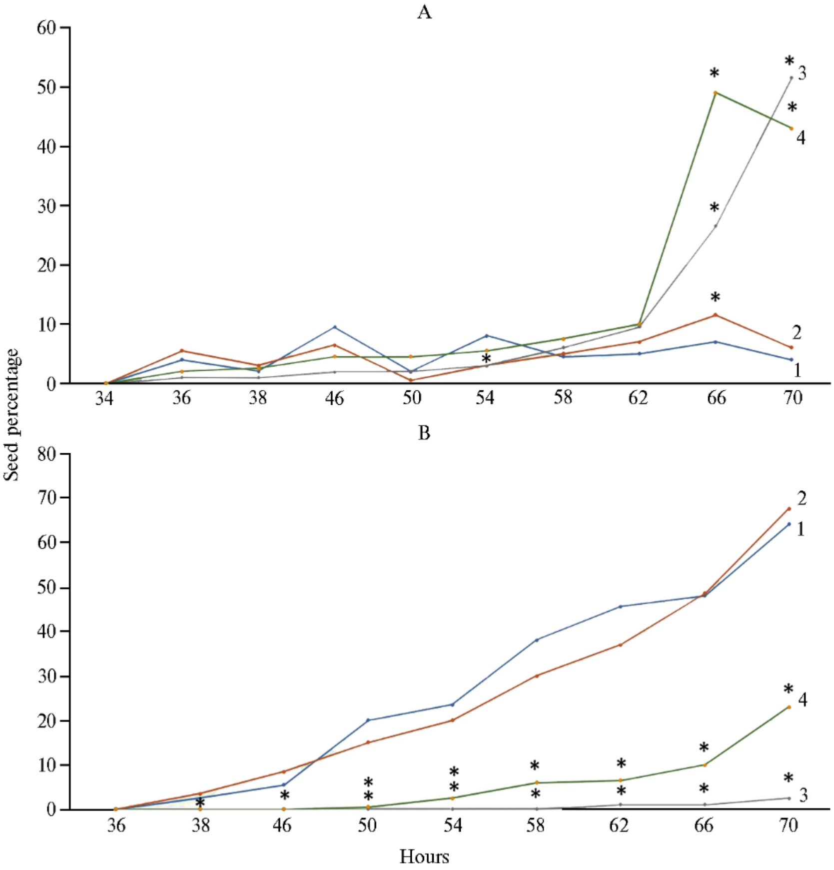

Fig. 3. The proportion of seeds of spring barley (*Hordeum vulgare* L.) variety Nur at the sprout (A) **and seedling** (B) **stages and from the 34th to the 70th hour of germination:** 1 — control (no treatment), 2 — irradiated seeds (20 Gy) (treatment I), 3 — seed treatment with $Pb(NO_3)_2$ (treatment II), 4 — seeds exposed to the combined action of γ-radiation and lead (treatment III) (lab tests). A total of 800 seeds were examined, 200 seeds per treatment.

* Differences from control are statistically significant at $p < 0.05$.

Under the combined influence of γ-radiation and lead (treatmen III) at the "point" stage, the seeds developed similarly to those treated only with lead (see Fig. 1, A, B). The largest proportion of seeds in treatmen III reached the R-1 stage at the 36th hour (31%). This value was less than with lead treatment (43%), but statistically significant ($p < 0.05$) more vs. control (13%) and treatment I (7.5%). At the R-2 stage, seeds exposed to the combined influence of two factors in the interval from 30 to 38 hours and from 50 to 62 hours demonstrated significantly less ($p < 0.05$) activity compared to the control (see Fig. 2, B).

In phase R-3 in treatment III, a significant ($p < 0.05$) effect was observed, although it was smaller than in the control and under the influence of γ-radiation. The increase occurred at the 50th hour, but the period of significant differences covered from 32nd to 54th hour (see Fig. 2, B). From the 46th to the 62nd hour in treatment III, the proportion of seeds in the R-3 phase exceeded the value in treatment II. Moreover, from the 46th to the 54th hour, the interaction coefficient K_W was statistically significantly ($p < 0.05$) less than 1, which indicates an antagonistic interaction of factors and allows us to conclude that γ-irradiation partially neutralizes the toxic effect of lead.

During the sprouting stage, significant ($p < 0.05$) differences vs. control occurred in treatment III from the 66th hour (see Fig. 3, A). The proportion of seeds that reached the seedling stage in treatment III differed significantly ($p < 0.05$) from the control, starting from the 38th hour until the end of the experiment due to a significant slowdown in development. At the 70th hour, the difference between seeds in variants II and III became statistically significant ($p < 0.05$), that is, with preliminary exposure to IR, the germination rate returns to normal faster than when treated with lead alone. In addition, the K_W values in these groups indicate the antagonistic interaction of factors.

Time during which the maximum number of seeds of spring barley (*Hordeum vulgare* L.) variety Nur reached a certain stage of development depending on the effect of γ-radiation and lead on the seeds (lab tests)

Treament	Stage	Development peak, h	Seed number
Control	«Point»	24	101
	R-1	32	25
	R-2	36	52
	R-3	50	80
	Sprout	46	19
	Seedling	70	128
I, γ-radiation	«Point»	24	89
	R-1	32	15
	R-2	38	51
	R-3	50	79
	Sprout	46	13
	Seedling	70	135
II, $Pb(NO_3)_2$	«Point»	24	114
	R-1	46	85
	R-2	62	70
	R-3	58	12
	Seedling	70	103
	Shoot	70	5
III, γ-radiation + $Pb(NO_3)_2$	«Point»	24	111
	R-1	36	50
	R-2	58	68
	R-3	50	32
	Sprout	66	98
	Seedling	70	46

Note. For a detailed description of the options, see the Materials and methods section.

From the presented results it follows that seeds treated with γ-radiation and lead develop unevenly, and the transition to each subsequent stage occurs at different times.For each treatment, the average time was determined during which most of the seeds passed a certain stage of development (Table).

In general, lead-treated seeds lagged behind in development compared to control and γ-irradiated seeds. They reached earlier stages in greater numbers than in the control and treatment I over the same periods. However, the number of seeds treated with $Pb(NO_3)_2$ in later stages of development, on the contrary, was smaller compared to the control and treatment I.

Seed is a special state of a plant in which metabolism is almost completely suppressed [12] in order to conserve resources for the development of the sprout. The entry of water into the seed initiates the swelling process, and reserve substances are converted into soluble compounds used to nourish the embryo. A cascade of events is launched aimed at transferring the cells of the embryo into an active state. Genome derepression caused by ionizing radiation or heavy metals gives rise to key metabolic processes: the synthesis of nucleic acids and proteins increases, the activity of many enzymes increases, and the content of phytohormones, the growth activators that control plant growth and development, increases [14, 19, 20].

The energy of ionizing radiation absorbed by the seeds is converted mainly into free radicals that exist for a long time in air-dry seeds. When water and oxygen enter the seed, they quickly react to form strong oxidizing agents, the hydroperoxides and hydroxyl radicals [21]. Reactive oxygen species transform the genome of embryonic cells into an active state. Previous studies [14, 19] have shown that irradiation at stimulating doses is sufficient to influence plant regulatory systems and accelerate the development program.

Different doses of irradiation of barley seeds can induce qualitatively different effects, from inhibition (at 50 Gy) to stimulation (at 20 Gy) of plant development [14]. In our experiment, a statistically unreliable stimulation of the rate of irradiated seed development was observed at the R-1 stage, but the seeds soon became equal in rate of development to the control group.

Similar to the effects of ionizing radiation, exposure of seeds to heavy metals can lead, depending on the concentration, to either inhibition or stimulation of growth [20]. The main barrier to HMs is root tissues that can bind cations [22], but this is not the only way a plant can reduce HM uptake. Thus, endodermal cells, which play an important role in the development of lateral roots, are the first to be exposed to HM, which significantly inhibits their functioning and, accordingly, the overall development of the root. For this reason, when there is an excess of heavy metals in the soil, the development of the root system is primarily disrupted [22].

In our study, obvious damage to the root system occurred when assessing the stages of root development. According to the methodology we used, seeds are considered full-developed seedlings when they reach the R-3 stage and form a sprout more than half the length of the seed [16]. We have changed the germination criterion for seeds treated with lead nitrate. The germination phase was considered reached in the case of germination of the initial coleoptile, without entering the R-3 phase. This was associated with the accumulation of more lead in the root compared to other parts of the plant [23]. In the remaining parts of the seedling, at a concentration of 2 mg/ml, the development rate was close to normal. Germination of lead-treated barley seeds delayed coleoptile development by approximately 16 hours, bypassing the R-3 stage, which may pose a risk for future plant development.

Another target of HM is the plasmalemma. Exposure to lead ions changes its permeability and ion balance, and interferes with the functioning of H^+-ATPases [24]. The cause of these disorders is errors in lipid synthesis and their increased oxidation by reactive oxygen species [25]. In addition, lead can affect enzyme metabolism by binding to SH groups. As a result, thylakoids are destroyed, disruptions in the Calvin cycle and water stress occur, cell division is inhibited (impaired cytokinesis is due to a decrease in the rate of microtubule formation) and mitochondria are damaged.

In our opinion, the described features of the action of IR and HM could lead to the results obtained in this work. In wheat seeds treated with $Pb(NO_3)_2$, a decrease in root development was observed [26]. Data presented by A.V. Dikarev et al. [15] indicate significant sensitivity of barley roots to lead. The length of the roots sharply decreased even at a $Pb(NO_3)_2$ concentration of 1 mg/ml. Treatment with heavy metals resulted in a significant retardation of wheat germination [27]. After adding Cu, Cd, Ni, the length of the sprout decreased by 51, 48 and 33%, respectively, of roots by 91, 63 and 72% [27]. Growth inhibition was associated with significant metal accumulation in wheat seedling tissues. In lentil plants exposed to 0.5 mM (~ 0.56 mg/ml) and 1000 mM (~ 1.12 mg/ml) lead concentrations, germination was inhibited by 2.5 and 10% [28].

Effects of individual stressors and their combination vary markedly [8, 29-31]. The reason may be the increased level of reactive oxygen species (ROS), induced by either one of the stressors or due to their combined effect. The heavy metal lead and γ-radiation, both separately and together, cause a number of specific reactions. For example, by interacting with DNA, each of the stressors can partially suppress repair systems or induce mutations [8]. The formation of mutations is extended over time, therefore, external influences modify the proportion of potential damage recorded in the mutation. Small doses of IR can activate repair and the antioxidant systems [32].

We did not observe an effect of γ-radiation on the rate of barley seed development, while lead significantly slowed down the plant development. The combined action led to statistically confirmed antagonistic effects. Similar results were reported by H.I. Mohamed [33], that is, the combined effect of 25 Gy IR and 300 μM (~ 0.6 mg/ml) lead ions statistically significantly increased the length of the root and sprout of cowpea (*Vigna sinensis* L.). The dose load elevated to 80 Gy statistically significantly slowed down the development of the sprout, and the size of the root increased. The lead concentration of 600 μM (~ 1.2 mg/ml) and 25 Gy irradiation led to a statistically significant increase in all parameters of seedlings. Considering that when the seeds were treated only with lead in two concentrations, there was a statistically significant decrease in all parameters of seedlings while a combined effect was the opposite, it can be assumed that γ-irradiation partially neutralized the toxic effect of lead on the seeds of cowpea (*Vigna sinensis* L.). The reason for this may be an increase in the efficiency of protein and metabolite utilization, modulation of the amount of ROS, and the triggering of repair mechanisms [34]. Similarly, pre-treatment of mountain barley seeds with 50 Gy γ-radiation increased the tolerance to heavy metals in seedlings by reducing the H_2O_2 concentration [10]. The activity of antioxidant enzymes increased, which alleviated oxidative stress caused by heavy metals.

This phenomenon is also confirmed in a study on *Arabidopsis taliana* L. [9]. The authors considered the effect of the combined action of $Pb(NO_3)_2$ and 25-150 Gy γ-radiation [9]. At 50 Gy, a statistically significant stimulation of root development occurred compared to plants exposed to 500 μM (~ 1.02 mg/ml) $Pb(NO_3)_2$. When the dose was increased to 150 Gy, root growth was inhibited.

In our experiment, the dynamics of plant development in the control and with γ-irradiation coincided (see Table 2). In both cases, the peak of the R-3 stage occurred later than the peak of the sprout stage. This may be due to polymorphism in seed germination, as well as the specificity of the development of some seeds, in which the sprout begins to develop simultaneously with the root, without reaching the R-3 phase. In the case of combined action and separate exposure to lead, there was a shift in the peak of R-2 development to a later time relative to the next stage. Due to the specificity of the effect of lead on root development [15], only a small part of the seeds reached the R-3 stage. Most of the seeds have passed this phase and entered the sprout stage. Note that the difference between the time to reach the peak development of stages R-2 and R-3 reached 4 hours with separate lead exposure and 8 hours with combined action, however, in the latter case, the sprout appeared earlier. Apparently, preliminary γ-irradiation activated the antioxidant and repair systems, which partially neutralized the effect of lead on the development of the sprout. Similar results were obtained in other works [9-11]. The peak of the seedling stage for all plants was at 70 h, since it was before this time that observations were made.

Thus, γ-irradiation of spring barley (*Hordeum vulgare* L.) variety Nur seeds at a dose of 20 Gy did not significantly disrupt the passage of microphenological phases of development. Treatment with $Pb(NO_3)_2$ at a concentration of 2 mg/ml slowed down seed germination, which was manifested in a delay in the transition to each subsequent microphenological phase, as well as a decrease in the proportion of seeds at late stages of development compared to the control. In addition, lead negatively affected root development, almost completely excluding the R-3 stage from the seedling formation. The combined effect of γ-radiation and lead also led to a slowdown in development, but the proportion of seeds that reached the R-3 phase increased and approached the control value, that is, γ-radiation at a dose of 20 Gy mitigated the toxic effect of lead. Therefore, a dose of 2 mg/ml $Pb(NO_3)_2$, regardless of exposure to γ-irradiation, has an inhibitory effect on seed development, but does not suppress it completely, only reducing the rate of development. An hourly assessment of seed germination under separate and combined action of lead and γ-radiation gives a deeper understanding of the mechanisms of plant adaptation to technogenic impacts at the early stages of development. Our findings will be used in research on genetic technologies for obtaining high-yielding barley varieties that are resistant to technogenic factors.

REFERENCES

1. Aleksakhin R.M., Fesenko S.V., Geras'kin S.A., Filipas A.S., Udalova A.A., Anisimov V.S., Selezneva E.M., Ul'yanenko L.N., Kruglov S.V., Mirzoev E.B., Belova N.V., Bakalova O.N., Dikarev V.G., Isamov N.N. *Metodika otsenki ekologicheskikh posledstviy tekhnogennogo zagryazneniya agroekosistem* [Methodology for assessing the environmental consequences of technogenic pollution of agroecosystems]. Moscow, 2004 (in Russ.).
2. Seregin I.V., Ivanov V.B. *Fiziologiya rasteniy*, 2001, 48(4): 606-630 (in Russ.).
3. Pourrut B., Shahid M., Dumat C., Winterton P., Pinelli E. Lead uptake, toxicity, and detoxification in plants. In: *Reviews of environmental contamination and toxicology*, vol. 213. D. Whitacre (ed.). Springer, New York, NY, 2011: 113-136 (doi: 10.1007/978-1-4419-9860-6_4).
4. Gudkov S.V., Grinberg M.A, Sukhov V., Vodeneev V. Effect of ionizing radiation on physiological and molecular processes in plants. *Journal of Environmental Radioactivity*, 2019, 202: 8-24 (doi: 10.1016/j.jenvrad.2019.02.001).
5. Geras'kin S.A. *Radiatsionnaya biologiya. Radioekologiya*, 1995, 35(5): 563-570 (in Russ.).
6. Calabrese E.J., Blain R.B. Hormesis and plant biology. *Environmental Pollution*, 2009, 157: 42-48 (doi: 10.1016/j.envpol.2008.07.028).
7. Sanzharova N.I., Tsygvintsev P.N., Anisimov V.S., Geras'kin S.A., Kuznetsov V.K., Loy N.N., Pimenov E.P., Panov A.V., Ratnikov A.N., Sanzharov A.I., Goncharova L.I., Sviridenko D.G., Arysheva S.P., Anisimova L.N., Dikarev A.V., Popova G.I., Perevolotskaya T.V., Suslov A.A., Frigidova L.M., Vasil'ev D.V., Kurbakov D.N., Spiridonov S.I. *Tyazhelye metally v agrotsenozakh: migratsiya, deystvie, normirovanie* [Heavy metals in agrocenoses: migration, action, control]. Obninsk, 2019 (in Russ.).
8. Geras'kin S.A., Dikarev V.G., Udalova A.A., Dikareva N.S. *Genetika*, 1996, 32(2): 279-288 (in Russ.).
9. Qi W., Zhang L., Wang L., Xu H., Jin Q., Jiao Z. Pretreatment with low-dose gamma irradiation enhances tolerance to the stress of cadmium and lead in *Arabidopsis thaliana* seedlings. *Ecotoxicology and Environmental Safety*, 2015, 115: 243-249 (doi: 10.1016/j.ecoenv.2015.02.026).
10. Wang X., Ma R., Cui D., Shan Z., Jiao Z. Physio-biochemical and molecular mechanism underlying the enhanced heavy metal tolerance in highland barley seedlings pre-treated with low-dose gamma irradiation. *Scientific Reports*, 2017, 7: 14233 (doi: 10.1038/s41598-017-14601-8).
11. El-Shora H.M., Habib H.M., Kamel H.A., Mostafa I.Y. Pretreatment with low-doses of gamma irradiation enhances *Vicia faba* plant tolerance to lead stress. *Bioscience Research*, 2019, 16(2): 1528-1537.
12. Penfield S., King J. Towards a systems biology approach to understanding seed dormancy and germination. *Proceedings of the Royal Society B: Biological Sciences*, 2009, 276(1673): 3561-3569

(doi: 10.1098/rspb.2009.0592).

13. Sethy S.K., Ghosh S. Effect of heavy metals on germination of seeds. *Journal of Natural Science, Biology, and Medicine*, 2013, 4(2): 272-275.
14. Geras'kin S., Churyukin R., Volkova P. Radiation exposure of barley seeds can modify the early stages of plants' development. *Journal of Environmental Radioactivity*, 2017, 177: 71-83 (doi: 10.1016/j.jenvrad.2017.06.008).
15. Dikarev A.V., Dikarev V.G., Dikareva N.S., Geras'kin S.A. Analysis of spring barley intraspecific polymorphism in connection with tolerance to lead. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2014, 5: 78-87 (doi: 10.15389/agrobiology.2014.5.78eng).
16. Kazakova A.S., Kozyaeva S.Yu. Scale of microphenological phases of germination of summer barley seeds. *el'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2009, 3: 88-92 (in Russ.).
17. Strogina I.G. *Obshchee semenovedenie polevykh kul'tur* [Seed science of field crops]. Moscow, 1966 (in Russ.).
18. *GOST 12038-84. Semena sel'skokhozyaystvennykh kul'tur. Metody opredeleniya vskhozhesti* [GOST 12038-84. Seeds of agricultural crops. Germination methods]. Moscow, 1985 (in Russ.).
19. Bitarishvili S.V., Volkova P.Yu., Geras'kin S.A. *Fiziologiya rasteniy*, 2018, 65(3): 223-231 (doi: 10.7868/S0015330318030065) (in Russ.).
20. Poschenrieder C., Cabot C., Martos S., Gallego B., Barceló J. Do toxic ions induce hormesis in plants? *Plant Science*, 2013, 212: 15-25 (doi: 10.1016/j.plantsci.2013.07.012).
21. Kuzin A.M., Kaushanskiy D.A. *Prikladnaya radiobiologiya: (teoreticheskie i tekhnicheskie osnovy)* [Applied radiobiology: (theoretical and technical foundations)]. Moscow, 1981 (in Russ.).
22. Seregin I.V., Ivanov V.B. *Fiziologiya rasteniy*, 1997, 44: 922-925 (in Russ.).
23. Nishizono H., Kubota K., Suzuki S., Ishii F. Accumulation of heavy metals in cell walls of *Polygonum cuspidatum* roots from metalliferous habitats, *Plant and Cell Physiology*, 1989, 30(4): 595-598 (doi: 10.1093/oxfordjournals.pcp.a077780).
24. Ouarity O., Boussama N., Zarrouk M., Cherif A., Ghorbal M.H. Cadmium- and copper-induced changes in tomato membrane lipids. *Phytochemistry*, 1997, 45(7): 1343-1350 (doi: 10.1016/S0031-9422(97)00159-3).
25. Vodnik D., Jentschke G., Fritz E., Denayer F.O., Degen G.H. Root-applied cytokinin reduces lead uptake and affects its distribution in Norway spruce seedlings. *Physiol. Plant*, 1999, 106: 75-81 (doi: 10.1034/j.1399-3054.1999.106111.x).
26. Patra M., Bhowmik N., Bandopadhyay B., Sharma A. Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. *Environmental and Experimental Botany*, 2004, 52(3): 199-223 (doi: 10.1016/j.envexpbot.2004.02.009).
27. Gajewska E., Skłodowska M. Differential effect of equal copper, cadmium and nickel concentration on biochemical reactions in wheat seedlings. *Ecotoxicology and Environmental Safety*, 2010, 73(5): 996-1003 (doi: 10.1016/j.ecoenv.2010.02.013).
28. Kiran Y., Sahin A. The effects of the lead on the seed germination, root growth, and root tip cell mitotic divisons of lens culinaris medic. *Gazi University Journal of Science*, 2005, 18(1): 17-25.
29. Zandalinas S.I., Mittler R. Plant responses to multifactorial stress combination. *New Phytologist*, 2022, 234(4): 1161-1167 (doi: 10.1111/nph.18087).
30. Geras'kin S.A., Kim J.K, Dikarev V.G., Oudalova A.A., Dikareva N.S., Spirin Y.V. Cytogenetic effects of combined radioactive (^{137}Cs) and chemical (Cd, Pb, and 2,4-D herbicide) contamination on spring barley intercalar meristem cells. *Mutation Research*, 2005, 586(2): 147-159 (doi: 10.1016/j.mrgentox.2005.06.004).
31. Mittler R. Abiotic stress, the field environment and stress combination. *Trends in Plant Science*, 2006, 11(1): 15-19 (doi: 10.1016/j.tplants.2005.11.002).
32. Geras'kin, S.A., Oudalova A.A., Kim J.K., Dikarev V.G., Dikareva N.S. Cytogenetic effect of low dose γ-radiation in *Hordeum vulgare* seedlings: non-linear dose-effect relationship. *Radiation & Environmental Biophysics*, 2007, 46: 31-41 (doi: 10.1007/s00411-006-0082-z).
33. Mohamed H.I. Molecular and biochemical studies on the effect of gamma rays on lead toxicity in cowpea (*Vigna sinensis*) plants. *Biological Trace Element Research*, 2011, 144: 1205-1218 (doi: 10.1007/s12011-011-9058-1).
34. Volkova P.Y., Duarte G.T., Soubigou-Taconnat L., Kazakova E.A., Pateyron S., Bondarenko V.S., Bitarishvili S. V., Makarenko E.S., Churyukin R.S., Lychenkova M.A., Gorbatova I.V., Meyer C., Geras'kin S.A. Early response of barley embryos to low- and high-dose gamma irradiation of seeds triggers changes in the transcriptional profile and an increase in hydrogen peroxide content in seedlings. *Journal of Agronomy and Crop Science*, 2020, 206(2): 277-295 (doi: 10.1111/jac.12381).