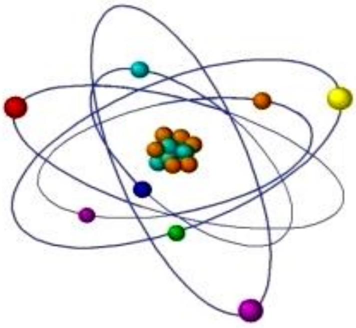
POSSIBLE ROLE OF SUPRACELLULAR MECHANISMS IN RADIOADAPTATION EFFECTS OF PLANTS



[1]Oleksandr Mikhyeyev*, [1]Liudmyla Ovsiannikova, [2]Oksana Lapan
[1]Institute of Cell Biology and Genetic Engineering, National Academy of Science of Ukraine
[2]National Aviation University, Ukraine

DOI: 10.63465/rrs6202611766 *Corresponding author: mikhalex7@yahoo.com

ABSTRACT: *The complete algorithm of research of radioadaptive response has been carried out, starting from the receipt of dose dependences of action of acute gamma-irradiation and UV-C-irradiation on seedlings of long-stalked flax with the purpose of determination of adapting and testing doses, and completing by studying actually radioadaptive response. By the example of specific and nonspecific radioadaptive response its connection has been shown with hormetic action of adapting factors and histological aspects of radioadaptation mechanism have been studied. On the basis of the obtained results, a hypothesis is based on the possible role of supracellular (in particular, proliferative) processes in providing of radioadaptation.*

Key words: UV-C-irradiation, gamma-irradiation, radiohormeis, radioadaptation

INTRODUCTION

Biological adaptation can be epigenetic (ontogenetic, physiological, phenotypic) when adaptation to environmental conditions occurs during ontogenesis, or genetic, i.e. hereditary [1]. When organisms are exposed to unfavourable factors (stressors), they experience a state of stress (tension). G. Selye identified three stages of stress: anxiety (the body's resistance decreases for a certain period of time), resistance (the body's resistance increases), and exhaustion (the manifestations of the first stage are intensified). After being exposed to small doses of a stressor, the body can be in one of the following states for some time: a) at the baseline of adaptability when its constitutive (current) level of adaptation is maintained – ordinary adaptation; b) increased resistance when the initial stability of the object increases – hyperadaptation (a state of eustress according to G. Selye); c) decreased resistance – hypoadaptation (a state of distress according to G. Selye) [2, 3]. How can the degree of adaptation of an organism be changed? Studying the mechanism of radioadaptation, researchers have mainly paid attention to intracellular processes, namely stimulation of enzymatic repair systems, synthesis of antioxidant enzymes, DNA methylation level, etc. [4-6]. At the same time, however, it is necessary to take into account the fact of structural and functional multilevelness of biological systems that leads to the assumption of the existence of multilevel mechanisms of radioadaptation. In other words, there may be supracellular mechanisms of radioadaptation.

This view is not new to radiobiology, in particular, it is believed that compensatory proliferation of cells resistant to ionising radiation can occur [7, 8]. We believe that it is not enough to claim the presence of this type of proliferation but it is necessary to identify its type. From the biocybernetic point of view, a transitional process occurs in an organism under the influence of a stressor [9] which has the character of under-restoration (hypocompensation) or over- restoration (hypercompensation) of structural and functional parameters that characterise the organism's viability (growth and development rate, cell division rate, etc.). If we consider ontogenetic adaptation, the adaptive factors actually reveal the tolerance potential (adaptive potential) of biological objects that can be achieved by applying ionising (which is considered as a universal stressor that affects all types of critical structures of biological objects) and UV radiation. We believe that certain doses of a stressor can put a biological object into a state of hypercompensation (in particular, cellular hyperproliferation) that, on the one hand, is a consequence of positive stimulation (hormesis), i.e. a state of increased viability, and on the other hand, a state of increased resistance, i.e. hyperadaptation.

Earlier we showed a direct connection between the state of hyperadaptation of plants to ionising radiation and the state of radiohormesis ("radio stimulation") in terms of growth parameters which gave us grounds to investigate the mechanisms of adaptation by studying only hormesis (radiohormesis) itself [10]. One of the possible mechanisms of hormesis (and hence adaptation) in terms of growth performance is stimulation (hypercompensation) of the proliferative activity of meristematic cells [11]. However, it would also be necessary to demonstrate an increase in the total number of meristematic cells (as responding elements) that could ensure an increase in the reliability of critical tissues [12]. The latter could be established by measuring the size of meristematic cells and the volume of the meristem.

The aim of our study was to establish the relationship between these characteristics modified by gamma and UV-C irradiation and the state of radioadaptation, i.e. increased radio resistance.

OBJECTS AND METHODS

The dose dependence of acute gamma-irradiation on growth parameters, histological and cytological (mitotic index, meristem volume, cell size) characteristics of long flax seedlings Linum usitatissimum L., f. elongata of Kyivsky variety was determined. The seeds were irradiated at the cobalt gamma-ray facility «Rokus» in doses from 1 to 10 Gy at a power of 1.42 cGy/s. Irradiated seeds were being soaked for 6 hours in settled water from the water supply in Petri cups. The soaked seeds were placed on glasses covered with damp filter paper. The glasses were placed in a humid chamber in a thermostat at 22°C. Every day the seedlings were photographed for the further processing of the results. Root length was measured using the tps2 computer program. The growth activity of seedlings was determined using the growth index (GI), which was calculated as the ratio of the current average length of seedling roots to their initial average length. The UV-C irradiation of seedlings was carried out using two lamps of the OBM-150m type at a power of 0.5-3.4 W/m^2.

Mitotic index (MI) was determined according to the generally accepted method. The counts were performed 3 times on different roots. The total number of cells viewed was 1000 for each root. The volume of the root meristem was determined by taking its shape as a truncated cone. The large and small radii were measured using a binocular microscope meristem that had been previously dyed with methylene blue, and the corresponding formula to calculate the volume of this type of geometric shape was used.

The adaptive response was studied according to a commonly used scheme, when the application of the test dose (TD) was preceded (24 hours) by the effect of the adaptive dose (AD). BP and TD were determined in previous experiments. The growth-stimulating dose was used as an adaptive dose. The following options were used in the experiments: 1. The «absolute» control was the variant of the experiment, which was not subjected to any experimental influence. 2. The variant whose seedlings were exposed only to the test dose (TD). 3. A variant that was exposed only to the adaptation dose (AD). 4. A variant whose seedlings were exposed first to AD and then (after 24 hours) to TD (AD+TD).

The statistical processing of the results was carried out using standard methods, and only statistically significant results ($p \le 0.05$) are discussed below.

RESULTS AND DISCUSSION

Speaking in the introduction about adaptive potential, we primarily meant one of the types of biological potential, which indicates the ability (potential) of a biological object to perform a certain amount of «biological work» in the form of ensuring (increasing) its resistance to stressors, certain doses of which can fully realise or bring to the maximum the realisation of this potential and, in particular, radioadaptation potential [13].

In order to study radioadaptation it was necessary to obtain dose dependencies in the range of hormonal and inhibitory doses, to check the identity of hormonal and adaptive doses, and to obtain the actual adaptive (hyperadaptive) effects. The latter was supposed to be done in two ways – when a certain dose of gamma radiation or a certain dose of UV-C radiation acted as an adaptive dose.

Fig. 1 shows the results of studying the dose dependence of the effect of acute gamma irradiation of flax seeds on the root length of 24-hour seedlings. It can be seen that the all applied doses caused a hormesis effect. Subsequently, the dose of acute irradiation of 5 Gy, as the one that gave the greatest hormetic (positively stimulating) effect, was used as a potentially adaptive dose (AD).

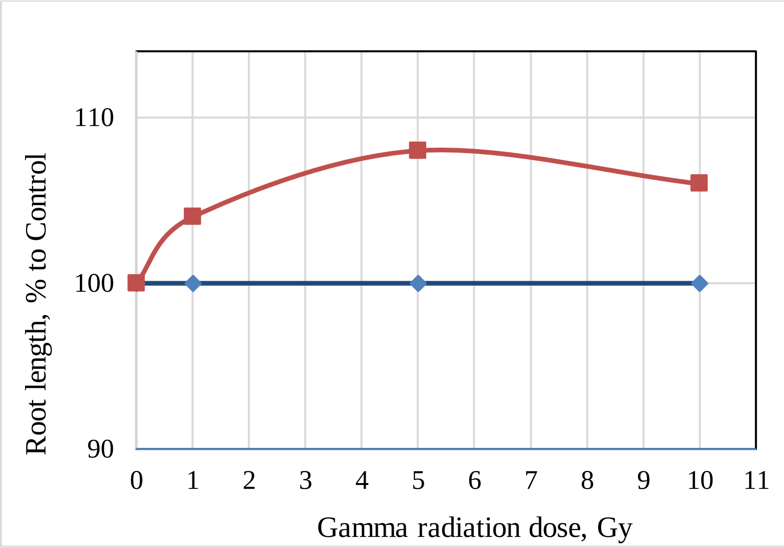


Fig. 1. Dose dependence of the effect of acute gamma irradiation of flax seeds on the root length of 24-h seedlings

Fig. 2 shows the dynamics of the growth activity of seedlings' roots obtained from acutely gamma- irradiated seeds. The approach of the root length of irradiated seedlings to the values of the control variant indicates the temporary (transient) nature of the hormetic effect of irradiation, i.e., it is observed only in a certain time interval. If we take into account the fact that at the primary (physicochemical) level gamma radiation acts absolutely destructively, in particular by ionising atoms and molecules, then we should consider the stimulation of the growth activity as a compensatory, or rather hypercompensatory process that, due to the work of homeostatic regulation eventually leads to the return of growth parameters to the corresponding values of control plants. This is the reason why the hormetic effect is transient. Surely, it can be observed only in a certain dose range and at certain values of the power of the modifying effect [14]. Probably, with earlier observation of root growth activity, its inhibition could be observed. It is because the main hormonal "events" occur under these experimental conditions within 24 hours, that we further focused on this time when studying adaptive responses.

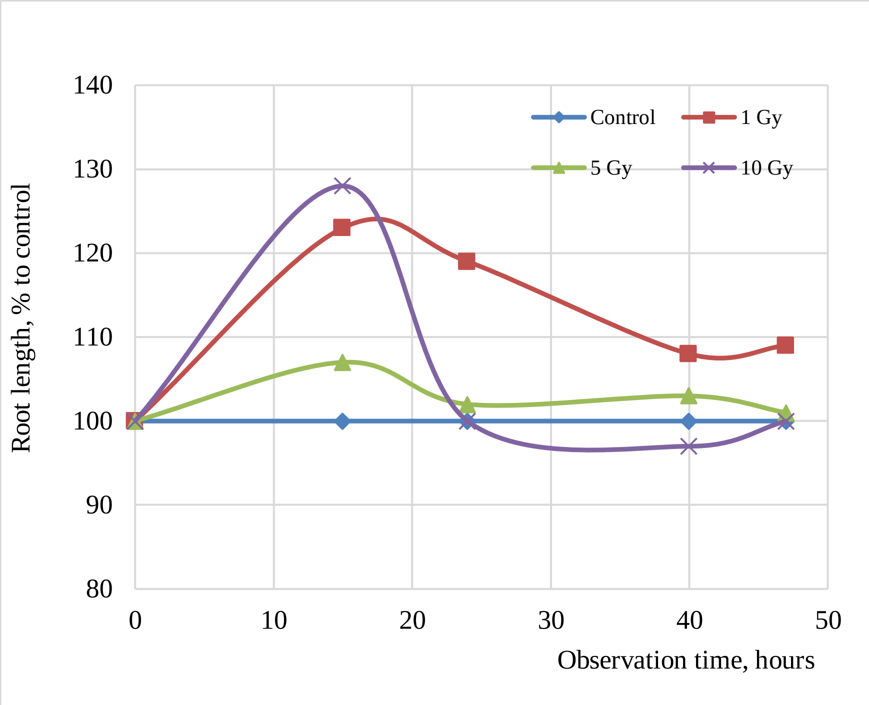


Fig. 2. Dynamics of growth activity of roots of flax seedlings obtained from acutely gamma irradiated seeds in different doses

Similar experiments were carried out with UV-C irradiation, with the only difference being that flax seedlings had already been irradiated, since the penetrating power of UV-C irradiation is not sufficient for the embryo in dry seeds to be directly affected. Fig. 3 shows that the applied dose interval allowed to detect both hormonal and growth inhibition effects. The doses in the range of 100-200 J/m2proved to be consistently hormonal. It should be noted (see also Fig. 4) that the time interval in which the hormetic effect is observed was shorter (less than 20 hours) than when hormetic effects were observed in seedlings obtained from gamma-irradiated seeds. It may have been influenced by the difference in the physiological state of plants at the time of irradiation – the state of the embryo when gamma irradiation was applied and physiologically active seedlings when UV-C irradiation was applied.

Fig. 3 also shows the dose dependence of the survival of the apical part of the embryonic roots of irradiated seedlings that was determined by the irreversible cessation of root growth and browning of its apical part. It can be seen that doses in the range of 50-100 J/m2are threshold in terms of irreversible damage to the apical meristem. Taking this fact into account, doses less than 100 J/m2were used as adaptive doses, although they were not maximally hormetic in relation to root growth activity. The data obtained in these experiments also allowed us to determine the range of test doses (TD) – 500-700 J/m2.

The dynamics of flax seedlings' response to UV-C irradiation (see Fig. 4) is similar to the dynamics of response to acute gamma irradiation of seeds. There is a gradual return of the growth activity of stimulated variants to the control level. The inhibitory doses act either reversibly (doses up to 500 J/m2), when the suppressed growth activity is restored over time, or irreversibly (during the observation period), when the degree of inhibition is constantly increasing (dose 700 J/m2).

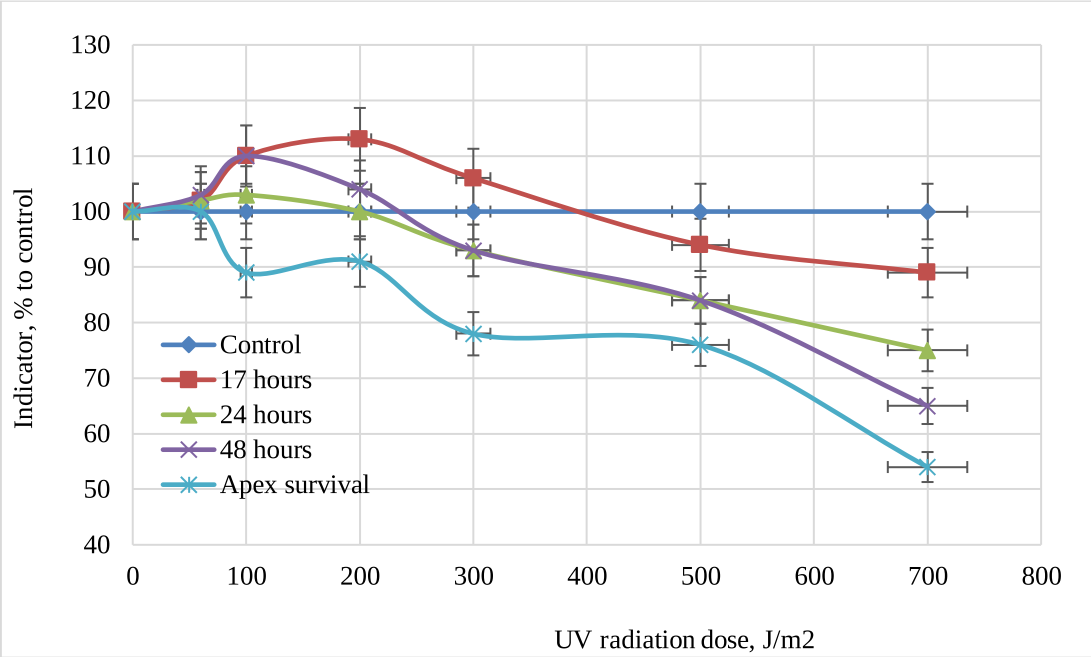


Fig. 3. Root growth index (17-48 h after irradiation) and survival of root apices (10 days after irradiation) of long-flax seedlings after UV-C rradiation

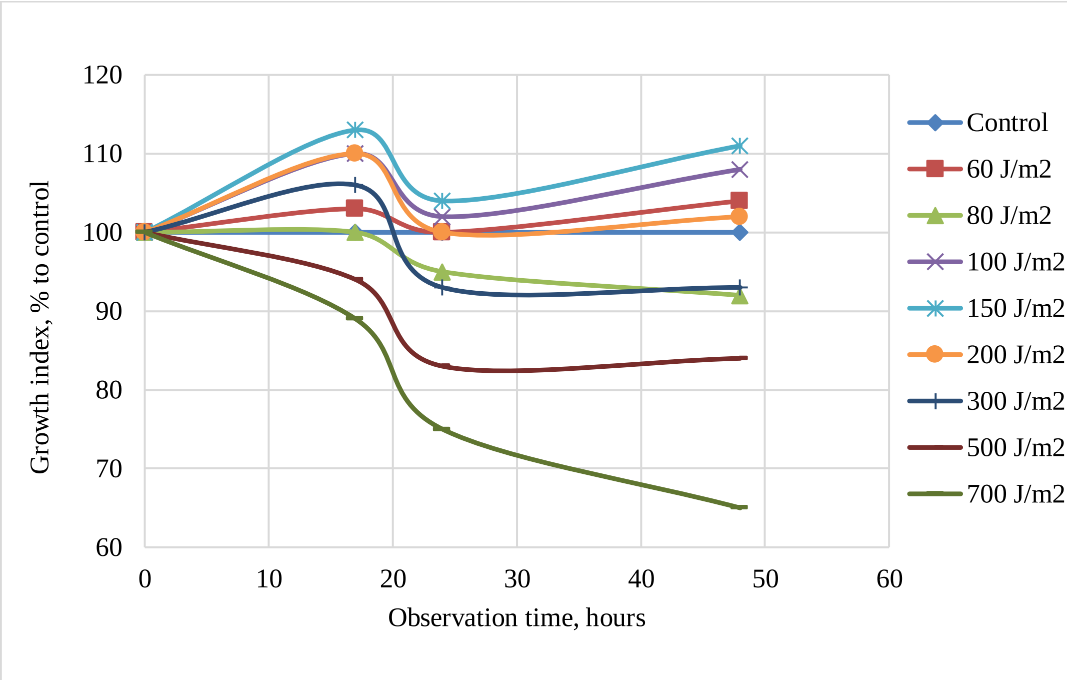


Fig. 4. The dynamics of UV-C irradiation effect on the growth index (GI) of flax seedlings roots

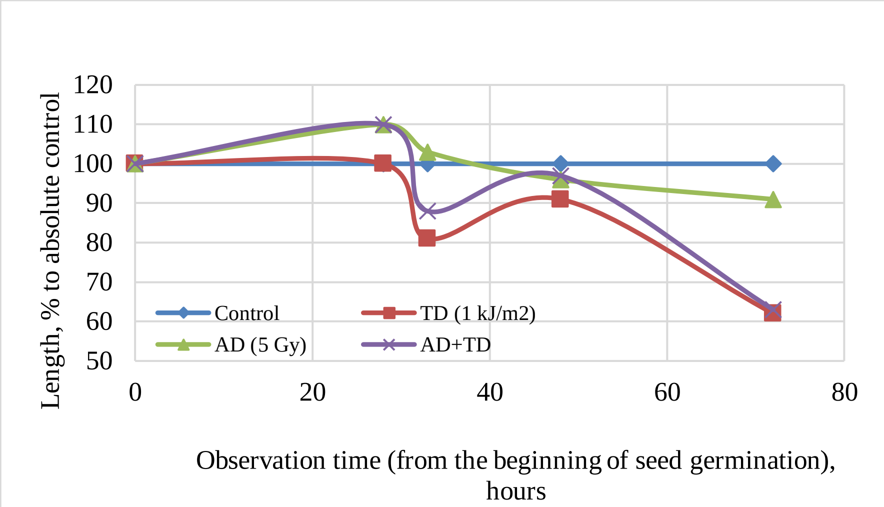


Fig. 5. Dynamics of growth activity of flax seedlings irradiated in different modes (1st calculation method – all variants as a percentage of the absolute control)

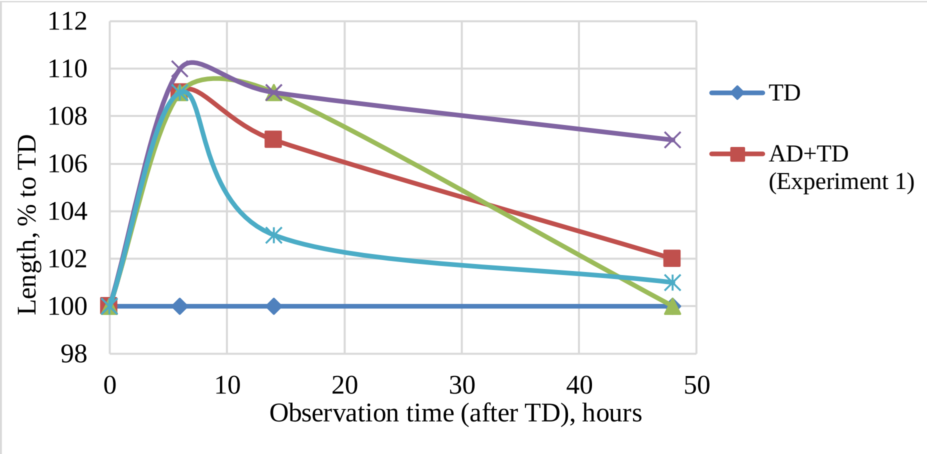


Fig. 6. Dynamics of growth activity of flax seedlings irradiated in different modes (2nd calculation method – percentage to the TD variant – 1000 J/m2) obtained from pre-irradiated flax seeds at the dose of 5 Gy

After determining the adaptive (in fact, potentially adaptive) doses of acute gamma irradiation, UV-C irradiation and test doses of UV-C irradiation a series of experiments was made to study the actual radioadaptation (hyperradioadaptation) of flax seedlings under UV-C irradiation. Fig. 5 shows the results of one of the typical experiments on the study of nonspecific (decussate) radioadaptation, i.e. when the adaptive and testing factors are of different nature (in our case, gamma radiation and UV-C radiation, respectively). As it can be seen, the test irradiation was applied at the moment when the hormonal effect on the growth parameter reached its maximum level. The fact that the curve describing the dynamics of seedling response to AD+TD is located above the curve describing the dynamics of response to TD alone indicates the presence of a radioadaptive effect. The transient effect of the adaptive response itself should also be noted. This is evidenced by the fact that the curves describing the dynamics of the response to TD and TD preceded by AD converge.

Fig. 6 shows the results of four independent experiments on the study of nonspecific (decussate) radioadaptation. If we compare the dynamics of TD and AD+TD curves, it can be stated that the resistance of flax seedlings obtained from pre-irradiated seeds increases by 10%. The dynamics of the adaptive response resembles the dynamics of the hormesis effect of irradiation – the growth parameters of adapted variants gradually converge with the corresponding parameters of non-adapted ones, i.e. the transitivity of the adaptive response is again observed.

The results of the study of a specific adaptive response, i.e. the one in which a certain factor (in our case, UV-C irradiation) causes an increase in the level of resistance to the same factor (in our case, UV-C irradiation again), are shown in Fig. 7. There can be stated the qualitative similarity of these data to those obtained in the study of nonspecific adaptation. In particular, the same transitivity of the hormonal effect of irradiation and a relatively small modifying effect (towards increased resistance) of the previous irradiation is observed.

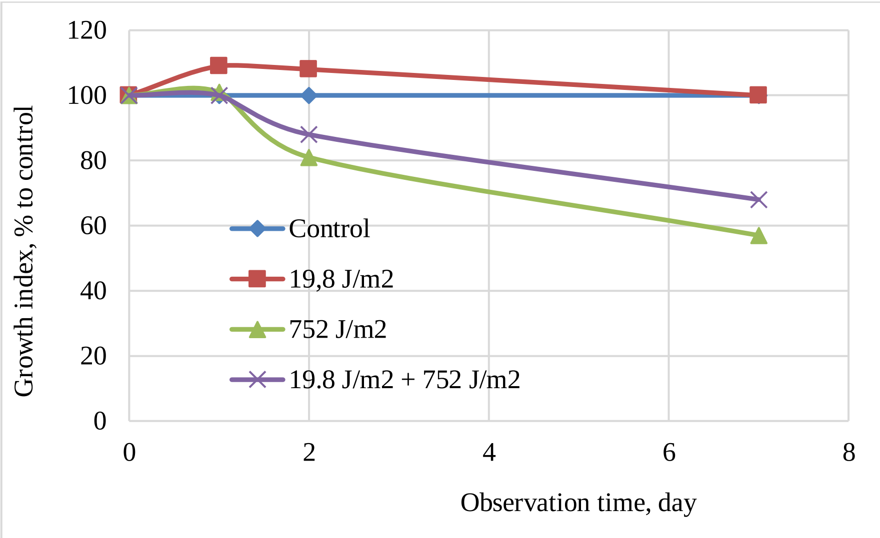


Fig. 7. Dynamics of growth activity of flax seedling roots after application of different UV-C irradiation schemes

The results obtained in the study of radioadaptation require answers to at least three questions. Firstly, what is the reason (mechanism) for the nonspecific effect of certain factors that increase resistance to factors of a different nature? This can be explained by the fact that gamma and UV-C radiation, despite the significant difference in the energy of their quanta, which makes the former (gamma quanta) capable of ionising atoms and/or molecules of the irradiated object, and the latter (UV quanta) only excite atoms, nevertheless has DNA molecules as the main target of their action [15]. In addition, there is a similarity in the mechanisms of reparation of DNA damage [16]. All of this is likely to determine the similarity of the mechanisms of response to hormonal doses of each factor, resulting in a transition to a state of increased resistance.

Secondly, is it possible to prolong the period of increased resistance or, equivalently, the hormesis state? In our experiments, plants exposed to radio modifying effects and subjected to test doses, in fact, were in the same conditions in the post-factor period and, most importantly, these conditions were not selective. Probably, if the test doses were close to irreversible inhibition, even a small difference in radioresistance acquired due to the hormesis effect of the previous (before the test exposure) irradiation could contribute to a greater survival of the modified plants, i.e. the radioadaptation effect could be observed almost throughout the entire ontogeny. Otherwise, it would be possible to record the state of increased radioresistance by creating such conditions in the post-radiation period (after the adaptive effect) when the adapted plants could survive only with stimulation of growth activity. For instance, this could be achieved when in real conditions of open ground, the roots of the stimulated plants grew in some way and were able to reach the aquifer, while the non-stimulated ones did not.

Thirdly, why is the radioadaptive (and, in fact, hormesis) effect transient? This question is related to the previous one. Indeed, the use of sublethal test doses allows both modified and unmodified plants to restore their physiological parameters to the same level sooner or later, that is, in fact, a manifestation of transitivity.

The condition that we recorded a hormesis effect on the growth parameters of the root system forced us to make an assumption about one of the intermediate links between irradiation and the final hormonal effect. Stimulation of cell division in the root apical meristem and/or stimulation (acceleration) of cell elongation in the root tensile zone could be such a link. Thus, it was significant to find out what exactly was the histological prerequisite for stimulating the growth activity of the root system. It was assumed that in the case of a radiohormesis effect on growth parameters, stimulation of mitotic activity of root meristem cells would be observed. It was supposed to precede the increase in the corresponding parameter of the control variant.

Studies using histological and cytological parameters confirmed our assumption. In particular, an increase in the mitotic activity of cells of the apical meristem of primary roots of 24-hour old flax seedlings obtained from acutely irradiated seeds was found (see Table). It was accompanied by an increase in the volume of the apical meristem as well.

Table.1. Histological parameters of cells of the apical meristem of primary roots of 24-hour flax seedlings obtained from acutely irradiated seeds

Experimental design	Root IR, % to control, 24 h after irradiation	Mitotic index, %. (x± mx)	Apical meristem volume, units.
Control	100	1.7± 0.5	1064± 490
1 Gy	119	1.4± 0.7	988± 269
5 Gy	102	2.1± 0.9	1132± 276
10 Gy	100	1.2± 0.5	1046± 301

Similar data were obtained under the influence of hormesis doses of UV-C irradiation. It should be noted that the increase in mitotic index and meristem volume (see Figs. 8 and 9) occurred at the background of virtually unchanged meristematic cell size (Fig. 10), indicating a real increase in the number of cells in the meristem, which in its turn, could make the meristem (as a critical structure) more resistant (reliable) to stressors of various nature [12]. In other words, by increasing the number of cells (elements of the critical system), the supracellular (cellular proliferative) mechanism of radioadaptation would be involved. Surely, such considerations make sense only if the time interval between the adaptive and test exposures was sufficiently long (24 hours) to induce this particular mechanism. At intervals of several hours between the AD and the TD, modified repair mechanisms, which have been mainly focused on in the study of the radioadaptive response, are likely to operate [5].

The data presented in Fig. 8 also indicate the importance of choosing the moment of application of the test effect (TE). As it can be seen, no stimulation of proliferative activity was observed on the second day of observation, and it would be difficult to hope for a radiohormesis effect.

On the second day of observation the volume of the root apical meristem at the applied doses of UV- C irradiation is restored to the control level (Fig. 9), that also confirms the transitory radioadaptive effect of the applied factor and, therefore, the existence of a rather limited time interval in which increased resistance is observed, which is manifested when applying the test effects.

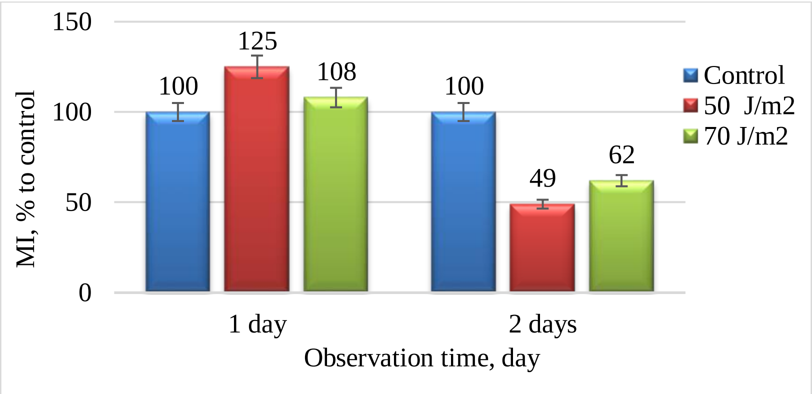


Fig. 8. Changes in the mitotic index of the root meristem of flax seedlings at different times after UV-C irradiation in hormesis doses

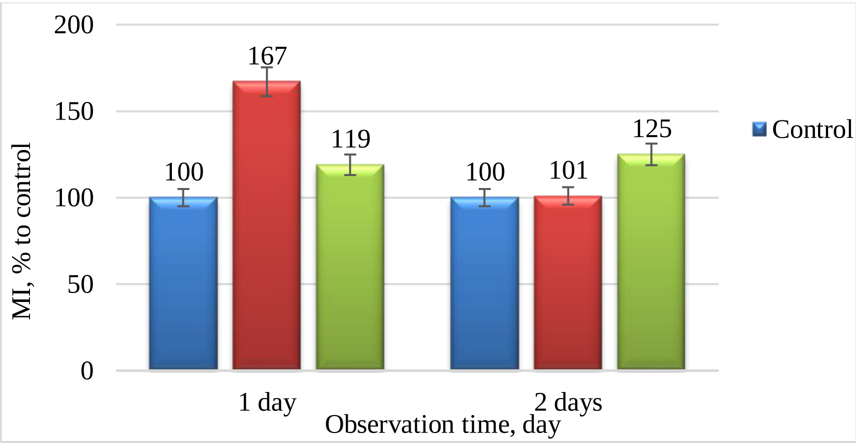


Fig. 9. Changes in root apical meristem volume of long-flax seedlings at different times after UV- C irradiation with hormesis doses

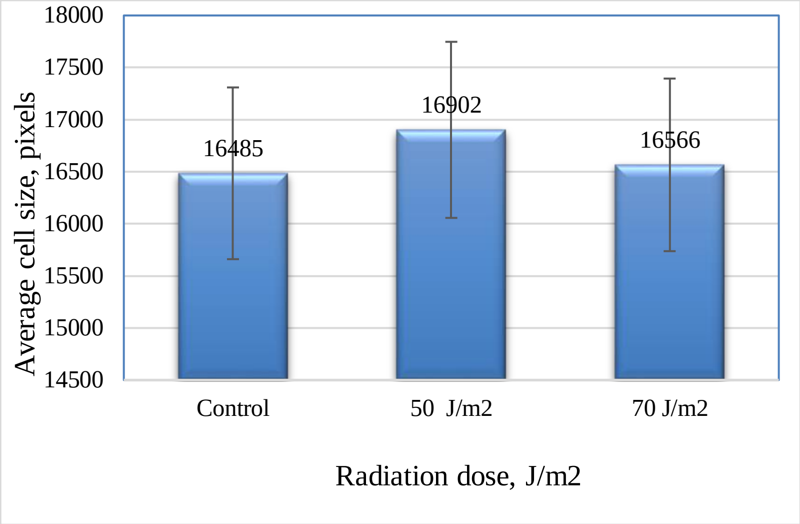


Fig. 10. Changes in cell size of the flax root meristem at the 1 day after AD irradiation

Thus, the obtained results allow to substantiate the hypothesis [17] about the existence of supracellular mechanisms of radioadaptation based on the stimulation of proliferative activity of cells in critical plant tissues.

REFERENCES

[1] Kostyuk, A. N., & Mikheev, A. N. (1997). The problem of phenotypic stress and adaptation in plants. Fiziologiya i Biokhimiya Kul'turnykh Rasteniy, (2), 81–92.
[2] Mikhyeyev, A. N., Guscha, M. I., & Shilina, Y. V. (2005). The endogenic and exogenic factors of the realization of phenotypic adaptation. In F. Brechignac & G. Desmet (Eds.), Equidosimetry – Ecological Standardization and Equidosimetry for Radioecology and Environmental Ecology (pp. 409–417). Springer.
[3] Mikheyev, A. N. (2005). Classification of radiobiological adaptations. Scientific and Technical Collection "Problems of Nuclear Power Plant Safety and of Chornobyl", (3), 36–41.
[4] Moskalev, A. A., & Shaposhnikov, M. V. (2009). Genetic mechanisms of the effects of low-dose ionizing radiation. Saint Petersburg: Nauka, 137 p.
[5] Ikushima, T., Aritomi, H., & Morita, J. (1996). Radioadaptive response: Efficient repair of radiation-induced DNA damage in adapted cells. Mutation Research, 358, 193–198.
[6] Kovalchuk, I., Abramov, V., Pogribny, I., et al. (2004). Molecular aspects of plant adaptation to life in the Chernobyl zone. Plant Physiology, 135(1), 357–363.
[7] Koterov, A. N., & Nikol'skiy, A. V. (1999). Adaptation to in vivo irradiation. Radiation Biology. Radioecology, 39(6), 648–662.
[8] Serebryanyy, A. M., & Zoz, N. N. (2002). Stimulated repopulation as the basis of antimutagenesis and adaptive response phenomena in plants. Genetika, 38(3), 340–346.
[9] Savin, V. N. (1981). Effects of ionizing radiation on the whole plant organism. Moscow: Energoatomizdat, 120 p.
[10] Mikheyev, O. M., Ovsyannykova, L. G., Sytnyk, S. V., & Dyachenko, A. I. (2007). The role of regenerative mechanisms in radioadaptation of Pisum sativum L. seedlings. Visnyk of Kharkiv National Agrarian University. Biology Series, 1(10), 76–84.

[11] Shestopalova, N. G., & Baeva, Ye. Yu. (2007). Radioadaptive response of leaf meristem cells in inbred and heterotic plants of Helianthus annuus L. during ontogenesis. Cytology and Genetics, 41(6), 44–49.

[12] Grodzinskiy, D. M. (1983). Reliability of plant systems. Kyiv: Naukova Dumka, 386 p.

[13] Mikheyev, O. N., Guscha, M. I., & Shilina, Yu. V. (2002). Endogenous and exogenous factors in the realization of plants' phenotypic adaptive potential (theoretical and experimental aspects). In Plant Physiology in Ukraine at the Turn of the Millennium, Kyiv. Vol. 2 (pp. 82–87).

[14] Geras'kin, S. A. (1995). The concept of biological action of low doses of ionizing radiation. Radiation Biology. Radioecology, 35(5), 571–580.

[15] Konev, S. V., & Volotovskiy, I. D. (1979). Photobiology. Minsk: Publishing House of Belarusian State University named after V. I. Lenin, 383 p.

[16] Grodzinsky, D. M. (2000). Radiobiology. Kyiv: Lybid, 448 p.

[17] Mikheyev, A. N., Guscha, N. I., Malinovskiy, Yu. Yu., & Grodzinsky, D. M. (1998). The role of proliferative activity of meristematic cells in providing the radioadaptive response of plants. Reports of the National Academy of Sciences of Ukraine, (10), 174–177.