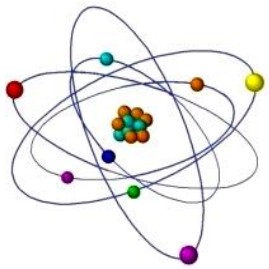
ROLE OF REGENERATION MECHANISM IN RADIOADAPTATION OF SEEDLINGS OF *PISUM SATIVUM L.*



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ABSTRACT: *The experimental dates are obtained, that at modified by decapitation of the main root of seedlings on the base of the increase of radioresistance (radioadaptations), fixed on the various parameters of growth activity of lateral roots, simultaneously a capacity is substantially reduced for the repair of sublethal damages, exposed by the method of fractionating of an acute dose of gamma-irradiation. The obtained facts allow concluding such mechanism of radioadaptive effect of decapitation, in which main part is acted by ephycell mechanisms. Additional comparative research of cyto- and histological parameters of cells of apex meristem of lateral roots of controls (intact) and experimental (decapitation) confirmed this conclusion.*

Keywords: radiobiology, regeneration, radioadaptation.

INTRODUCTION

A reaction of biological objects to the action of ionizing radiation is a comfortable model for the studies of general conformities to the law of transition in the state of stress. Special theoretical and practical interest among such states presents eustress [1], showing up in the increase of the initial level of resistance (adaptation state) a biological object under the influence of the proper doses of stressors. If ionizing radiation plays the role of stressors, appears the possibility of strictly measuring out an operating factor and expressly taking into account the consequences of his influence. Except for it, a radiation factor can be applied in the role of a testing factor.

In our and a row of other research the possible role of nonrepair mechanisms, in providing a radioadaptive answer was shown [2,3]. Obviously, the idea of the existence of the inducible/stimulation systems of recovery can be spread to a great number of other higher levels of structurally-functional integration of biological objects. Otherwise speaking, logically it was to assume that exists not only reparation and cell-repopulation mechanisms of radioadaptive answer (RA), but also organ, organism and other levels of this mechanism.

One of the necessary conditions of the study of RA is a search for such doses of radiomodifying influence, which cause passing the explored object in the post-factor period of a hyper compensate phase by values of the used parameters. From our data exactly doses, possessing such an ability can induce RA [4, 5, 6]. Exactly hypercompensate (phase of positive overshoot) processes in a biological object, showing up in the appearance of additional elements of the recovery (molecules of enzymes of repair, meristem cells and other) systems, provide the level of his radioresistance promoted in the end and in general case increase of the initial level of reliability [7].

At the level of separate organs of the plant the phenomenon of awakening of the plant buds can the example of hypercompensate processes serve under the influence of decapitation of stem apex or sped-up formation of lateral roots after the removal of the apex prevailing the apex of main root [8, 9].

Obviously, any affecting biological object, unbalancing him and inducing/stimulating thus transitional process with the phase of hypercompensation, can come forward at a certain dose and to the certain

parameters in a role of adapting.
If as an object of radiomodifying influence to choose the main root of seedlings that he can test on itself influencing of different degree – from the neutral and stimulants (hormesis) influencing to inhibitory and lethal. Obvious also, that practically any physical, chemical and/or biological factor can play this role. In this connection the measure (by a dose) of influence length of the chopped off area of the main root of seedlings was chosen. We assumed thus, that the certain dose interval of such influence will be rendered by stimulant (hypercompensate) influence on the formation of lateral counterfoils when mass (length and/or amount) of lateral roots will excel similar indexes at control seedlings. In subsequent, it was assumed to use the stimulating «doses» of decapitation as radioadapting.
Thus, the main task decided by the experiments described below – is to define the role of processes of regenerations as one of the possible mechanisms of radioadaptation.

MATERIALS AND METHODS

The pea seeds of the cultivar of Aronis (harvest 2006) were wet in plumbing water and grew in darkness at the stationary temperature of 22^0C. After 5 days of growth, the seedlings were parted into 7 groups, forming seven variants of experience. The seedlings of each group were subjected to the mechanical deletion of root apex proper length:1 – 0 mm (control); 2 - 3-4 mm; 3 – 10 mm; 4 – 20 mm; 5 - 30 mm; 6 – 40 mm; 7 – 50 mm.
Deletion of the apex was made via a blade of a safety razor, whereupon seedlings in an amount of 30 were placed in 0,5 l vessels with plumbing water and were put in the terms of permanent illumination. Further periodicity in days made measuring of the proper parameters (amounts of lateral counterfoils, their middle length, total length of lateral counterfoils, total and middle mass of lateral counterfoils).

For experiments on the research of radioadapting action of root decapitation, 4th days seedlings were parted into two groups, one of which was remote the apex of the main root length 3-4 mm. Controls and experimental seedlings (approximately for 40 things of every variant) are placed on 0,5-l vessels (for 20 things of seedlings on a vessel), filled with plumbing water and placed in a thermostat at 22^0C with permanent illumination. Thus, 4 groups of plants were formed for the realization of the next variants of the experiment: 1 – intact seedlings; 2 - decapitation plants («adapting dose»); 3–7 Gy on nondecapitation seedlings; 4–7 Gy on decapitation seedlings; («adapting dose» + 7 Gy on decapitation plants).
On 4 days after the decapitation, we looked after stimulation of root growth on the parameters of common amount of lateral counterfoils (180 % of control), middle length of lateral roots (117 % of control) and total length of lateral roots (140 % of control) and was accepted the decision to expose the proper variants of seedlings by test-dose. Irradiation was conducted on gamma-apparatus «RESEACHER» at a power of dose of 3,0 cGy/s.
For experiments with fractionating of acute dose of gamma-irradiation were used 3rd days seedlings which the followings variants were formed from: intact (control) roots: 1 – 0 Gy, 2 - 7 Gy, 3 - 3,5 Gy + 2 ÷ + 3,5 Gy, 4 - 3,5 Gy + 4 ÷ + 3,5 Gy; decapitation roots: 1 – 0 Gy, 2 - 7 Gy, 3 - 3,5 Gy + 2 ÷ + 3,5 Gy, 4 - 3,5 Gy + 4 ÷ + 3,5 Gy.

The irradiation of seedlings on the scheme that was presented higher was carried out at the age of 6 days (3 days after the decapitation). The dose of irradiation was 7 Gy. The time between equal factions of doses was 2 and 4h, which correspond to characteristic times of excision reparation.
The moment of irradiation was chosen coming from the results of looking at the sizes of growth parameters. Thus, the moment of irradiation was on stimulation of values of such parameters as the total length of lateral roots (157 % of control level on 3-day after decapitation and mass of dry matter of

lateral roots (about 200 % of control level).

The analysis of proliferate activity of cells of apex meristem of lateral roots was made on temporal preparations on the generally accepted method [10]. Determination of meristem was conducted coming from supposition, that she in some approaching is the truncated cone. Therefore, by micrometer, set on a binocular microscope, we determined length of meristem zone of root and its minimum and maximal radiuses of one. Further, was used a formula for the calculation of volume of the truncated cone:

$$V = h \cdot \pi(R^2 + Rr + r^2)/3$$

where: h - height of cone, R - large radius, r - small radius.

RESULTS AND DISCUSSION

If to estimate doses dependences by comparing absolute (total, integrate) indexes to which it is possible to deliver the common amount of lateral roots of seedling, a total length of lateral roots of one seedling, the total mass of dry matter of lateral roots of seedling, results of supervisions, presented on pictures 1 and 2. show that by the most effective dose of decapitation with point of view stimulation of number of lateral roots, their total length and total mass of dry matter (on seedling), there is a deletion of apex part of root length no more than 20 mm. The deletion of more extensive areas inhibits formation of lateral roots on the indicated parameters. If to estimate the reaction of decapitation seedlings on the specific parameters of formation of lateral roots (middle length of lateral counterfoil and mass of dry matter of one lateral root), there is diametrically an opposite picture (fig. 1-3). Stimulation on specific parameters is observed at the length of a remote area within the limits of 30 – 40 mm, that it is possible, obviously, to examine as a result of compensatory processes, i.e., diminishing of shortage of elements multiplying their single sizes and mass.

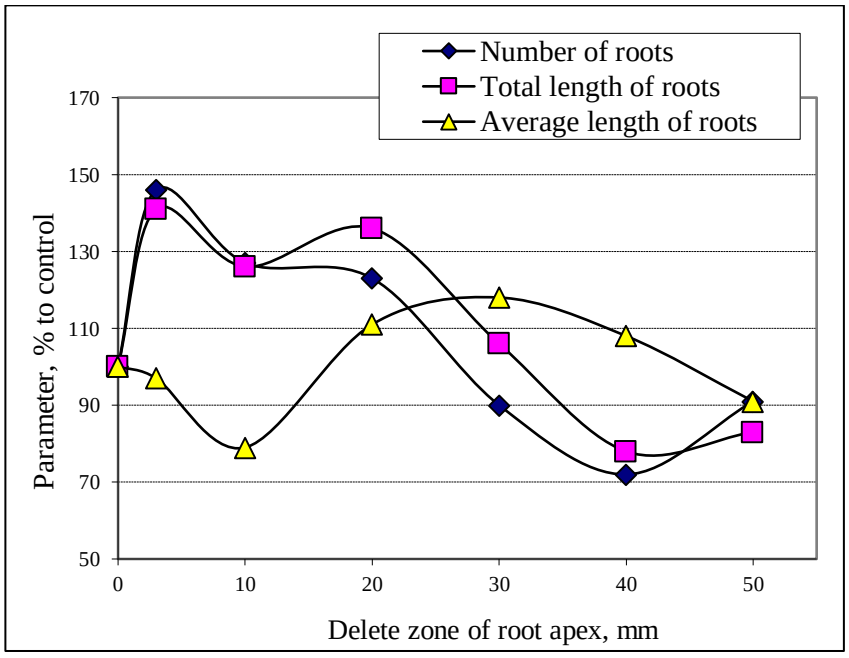

Fig. 1. Influence of length of delite zone of main root of pea seedlings on the growth parameters of lateral roots (7th day after decapitation)

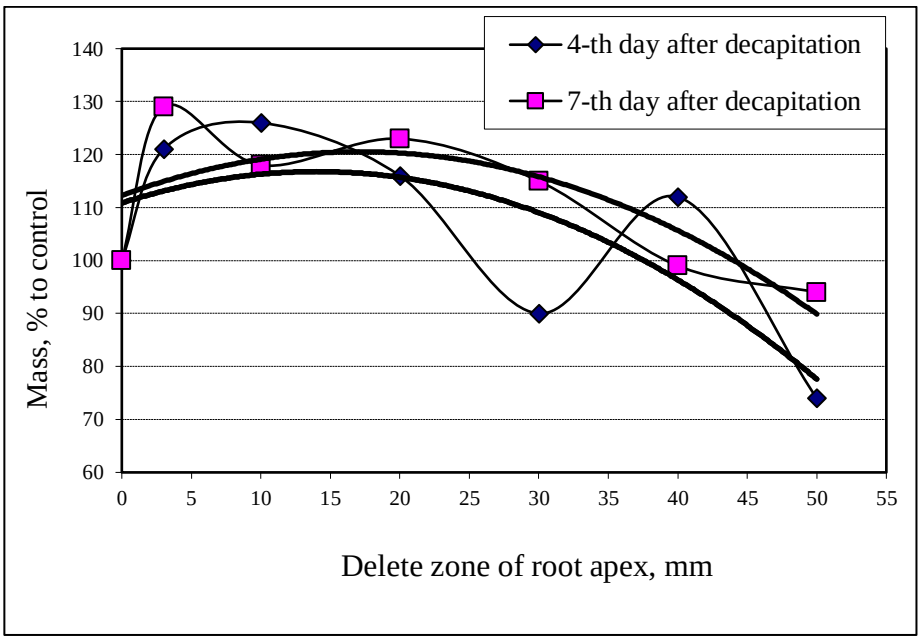

Fig. 2. Influence of length of the delited zone of main root of pea seedlingson mass of dry matter of lateral roots of one проростка (7th day after decapitation)

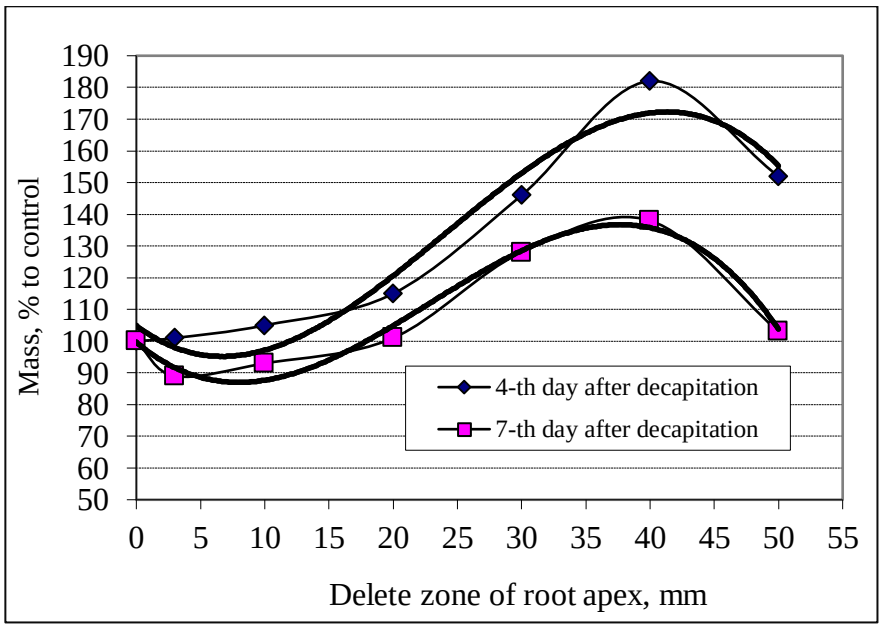

Fig. 3. Influence of length of the delited zone of main root of pea seedlings on mass of dry matter of one lateral root (7th day after decapitation)

Thus, the obtained results allow to draw conclusion about possibility of receipt of effects (and, as we supposed, radioadaptations) of stimulations in the wide range of levels (doses) of stress-factor as which used mechanical delete of root apex.It was further assumed to study connection between the stages of restoration process, fixed on the parameters of formation of lateral roots, and radioadaptive answer.The results of study of radioadaptation effect of decapitation are presented on a fig. 4-6. Evidently, that the irradiation of seedlings with decapitation roots (a test-dose 7 Gy) in the moment of

stimulation (4 day after decapitation of root apex) of formation of lateral roots (on total, middle length, mass of dry matter) exposed them more high level of radioresistance as compared to nondecapitation roots which were exposed to the rays in the same dose. Otherwise speaking, the decapitation of root at 4-day seedlings showed the radioprotective action which we interpret as radioadaptation.It is necessary especially to mark that a test-influencing is carried out in moment when the root system of experimental («adapted») variant has plenty of reactive elements and their large quantitative parameters. Thus, we once again were in a position to be satisfied of том, that one of terms of transition of the system in the state the promoted (by comparison to initial) stability there can be acquisition by her additional «reactive» elements.

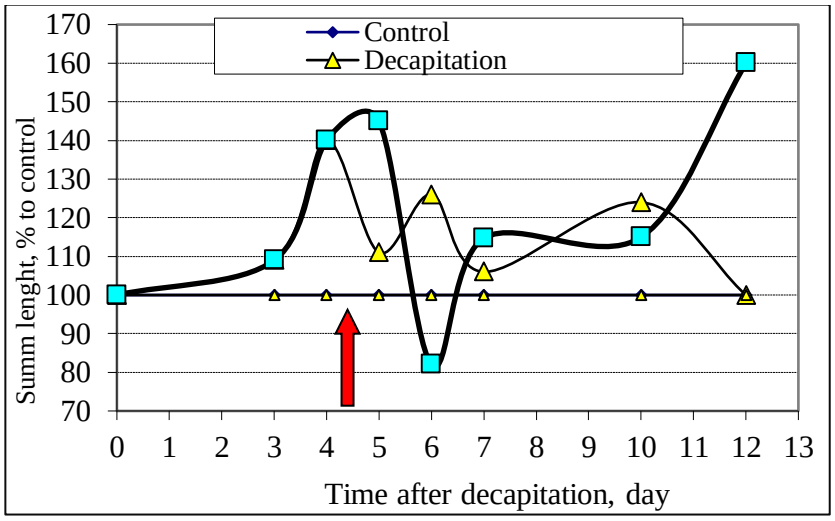

Fig. 4. Dynamics of the total length of lateral roots after the different variants of treatment

NB. A point shows the moment of irradiation in a dose 7 Gy.

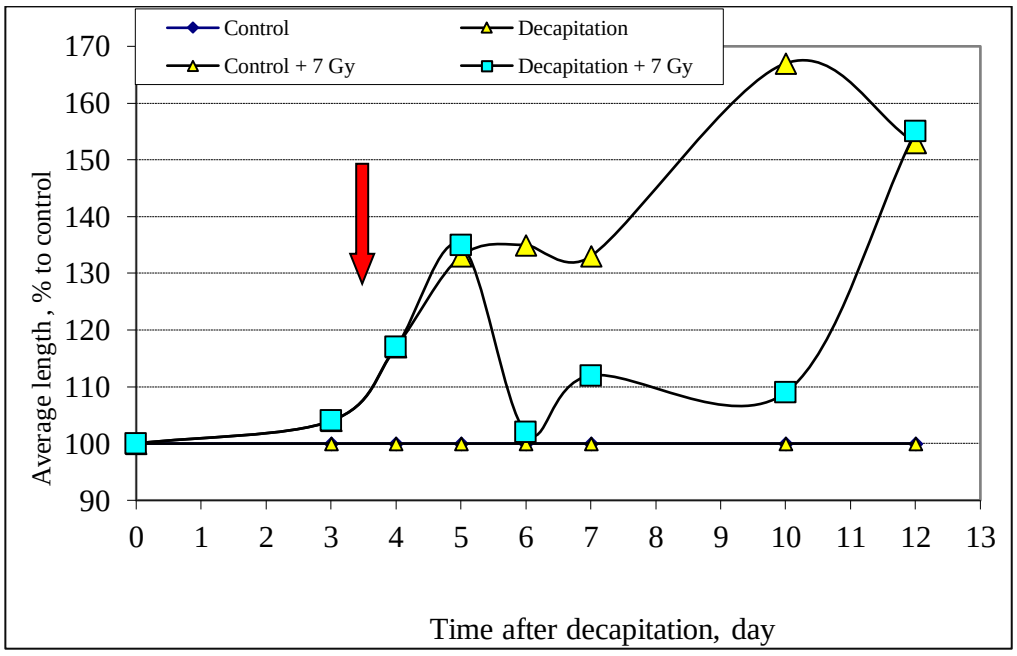

Fig. 5. Dynamics of average length of lateral roots after the different variants of treatment

NB. A point shows the moment of irradiation in a dose 7 Gy.

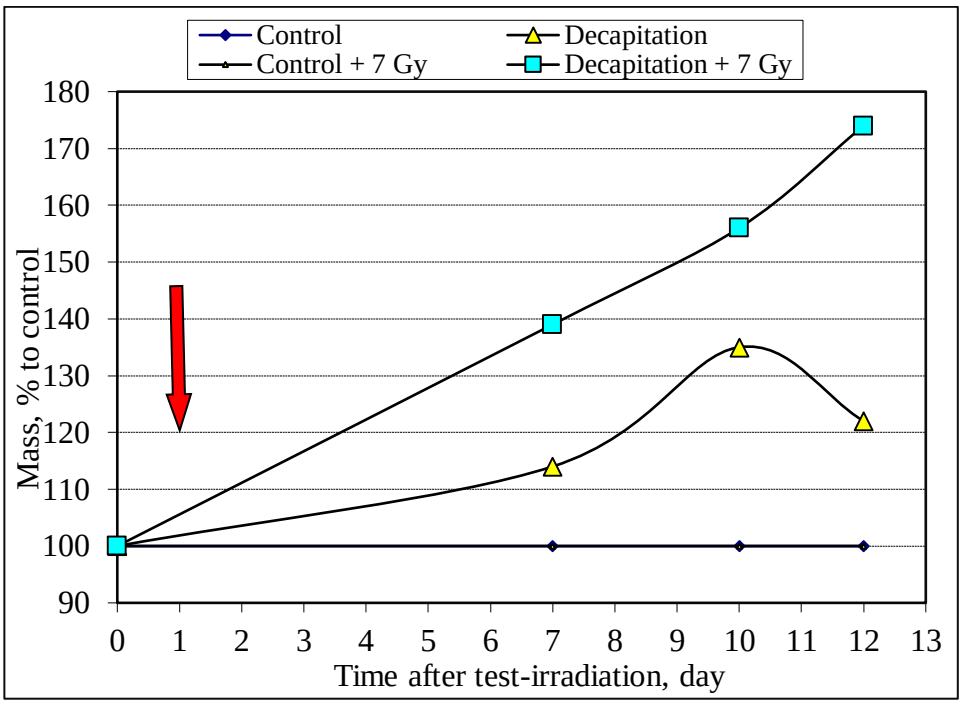

Fig. 6. The mass dynamics of dry matter of lateral roots of pea seedlings (irradiation at the age of 3rd day after decapitation) ***NB. A point is indicating the moment of irradiation in a dose 7 Gy. (3 days after decapitation).***

The large enough test-dose, applied in this experiment, allowed to study the degree of modified radiosensitivity yet on one parameter, namely on survivability of lateral roots after an irradiation. The lateral roots at which it was marked irreversible inhibition of growth activity had red apex, which testified to death of apex meristem. The count of stake (percent) of lateral roots with lost meristem in control and decapitation variants that were exposed to the rays allowed comparing their radioresistance. Appeared, that at the moment of completion of supervision the level of survivability of lateral roots of the intact variant was 45 %, and the decapitation variant – was 62 %, which also simply testifies to greater radioresistance of experimental (decapitated) variant.

The proiliferative activity of the cells of the apex meristem of the lateral roots were also studied in the moment of application of test dose and it is set that in the moment of application of testing irradiation a mitotic index (MI) of lateral roots meristem of the control variant was 1,5 %, and decapitation – 3,4 %, that almost in 2,3 time exceeded a control value. It ensues from findings that in modified by decapitation roots are created cyto- and histological conditions on the certain stage for the increase of the level of values of growth parameters. In the final analysis, in the moment of application of test dose the modified variants can have substantially more reactive elements (cells and organs as additional lateral roots), providing them promoted radioresistance. Comparison of the efficiency of fractionating dose of acute gamma-irradiation of lateral intact and decapitated embryonic roots of pea seedlings was used for the study of the efficiency of work of the repair in the apex meristem cells of lateral roots of the indicated variants. It was assumed that efficiency of repair recovery not will be substantially modified at decapitation variants, but can be even, vice versa, reduced because there was the increase of proiliferative activity, as we showed above. The last assertion has under itself a warrant, that the actively divided cells, i.e., cells, speed-up passing a cellular cycle, have less time on realization of excision reparation. [11].

On fig. 7-10 are presented the results of the study of the effects of fractionating of dose of acute gamma-irradiation. Evidently, the positive effect of fractionating of dose of acute gamma-irradiation on the change of parameter «total length of lateral roots» (fig. 9 and10.) has a positive sign only in intact seedlings, i.e., fractionating of the dose was instrumental in the less degree of inhibition, what acute of gamma-irradiation. In decapitated variants, a fractionating effect on this parameter is not exposed. The last circumstance can be explained by the acceleration of cellular divisions, minimizing the possible deposit of modified (stimulated) enzyme repair in providing of positive radioadapting effect of decapitation.

We will remind that the effect of fractionating of dose of acute gamma-irradiation was checked up at the moment of stimulation of growth parameters of lateral roots and exactly a test-irradiation gives the proof and reproduced the effect of radioadaptation at this decapitation.

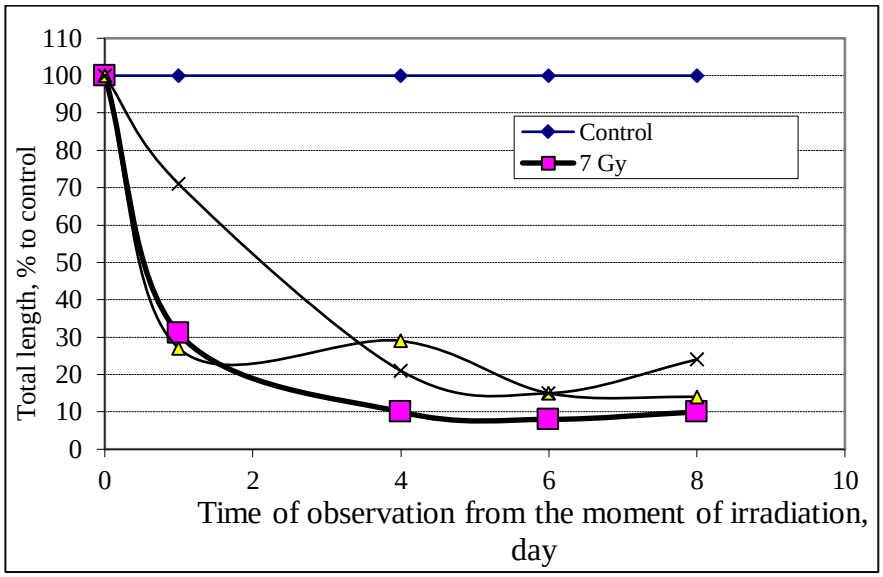

Fig. 7. Influencing acute and fractionating gamma-irradiation on total length of lateral roots of intact seedlings

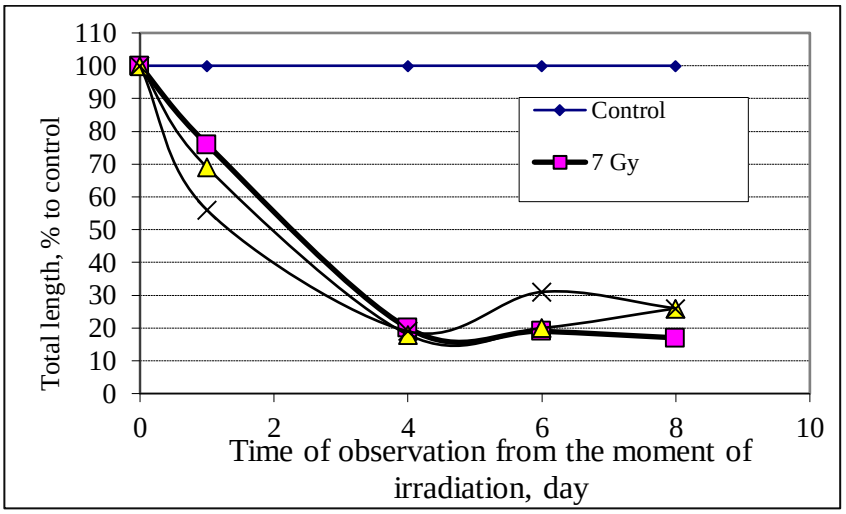

Fig. 8. Influencing acute and fractionating gamma-irradiation on total length of lateral roots of decapitated seedlings

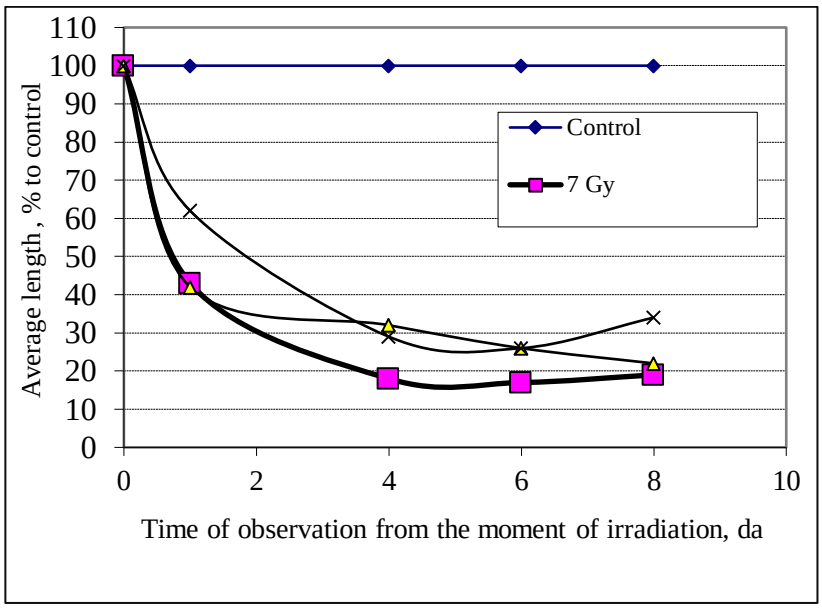

Fig. 9. Influencing acute and fractionating gamma-irradiation on average length of lateral roots of intact seedlings

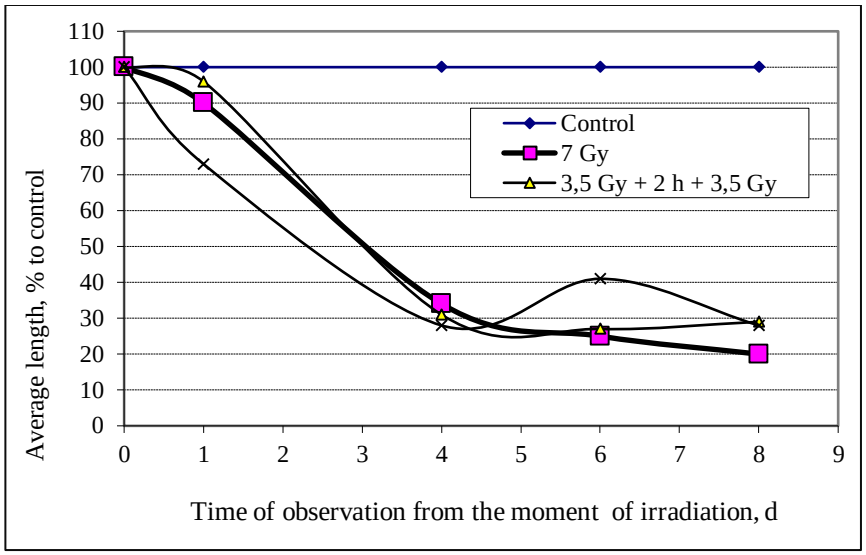

Fig. 10. Influencing acute and fractionating gamma-irradiation on average length of lateral roots of decapitated seedlings

The effect of fractionating of dose of acute gamma-irradiation on the change of parameter «average length of lateral roots» has a positive sign again only in intact seedlings. In decapitated variants a fractionating effect on this parameter even has some tendency to the negative values in an initial period of observation.

Thus, we got experimental confirmation, that at modified by decapitation of main root of seedlings on a background the increase of radioresistence, which fixed on the various parameters of growth activity of lateral roots, a capacity is substantially reduced for reparation of sublethal damages, exposed by the method of fractionating of dose of acute gamma-irradiation. The gotten facts allow to draw conclusions about such mechanism of radioadaptive effect of decapitation, in which determining part is acted by supercell mechanisms. Additional comparative research of cyto- and histological parameters

of apex meristem cells of lateral roots of controls (intact) and experimental (decapitated) confirmed this conclusion once again.

As set before, modified by decapitation roots created on the certain stage cytological and, probably, histological conditions for the increase of the level of growth parameters. On the basis of it, we assumed that, in the final analysis, at the moment of application of the test dose the modified variants could have substantially more reactive elements (cells and organs as additional lateral roots), providing them promoted radioresistance. With respect to lateral roots, as we specified before, their amount at decapitation variants substantially increases as compared to nondecapitation control.

In relation to multiplying a common amount in the apex meristem of lateral roots of the modified variants, we could talk only probably. In order for such suppositions, there were more reasons we carried out the proper experiment which mainly paid attention to the histological indexes of apex meristem of lateral roots of the modified (decapitation) and controls (intact) variants. Actually, there were only two variants in an experiment – intact and decapitation. The method of growing seeds and seedlings and decapitation are repeatedly described above. Studied histological parameters of apex meristems of lateral roots on 3th and 4th days after decapitation.

To that end, at first, determined the MI of apex meristem cells of lateral roots (AMCLR) experimental and seedlings of controls. The MI of the decapitation variant was 125 % to control on the 4th day after decapitation, which, in general, was repeated before the above-described results were obtained. All of it grounded us to count on, what the common amount of AMCLR of the experimental variant will excel the similar index of control variant. In this connection and, secondly, basic attention in this experiment was spared on determining the sizes of AMBK and their geometrical volume. The comparison of these two indexes, from our point of view, would help define what amount of AMCLR dispose control and experimental (decapitation) variants in the moment of application of test-dose. The comparison of volumes of apex meristem of experimental and control variants showed that on the 4th day after decapitation the first was 114 % to control, that, in an aggregate with the fact of equality of cells of the indicated variants a size, means, more cells in apex meristem of decapitation variant.

In theory, in accordance with the general theory of the systems of Urmantseva [12] a few methods of transformation of elements of the system are possible: change of the initial number of elements, change of their quality, and change of relations between them. Obviously, as mentioned above, different combinations of these three fundamental methods are possible. With respect to the quality of elements (in this case cells), the results of experiments on the study of effects of fractionating doses of acute gamma-irradiation specify one of the principle invariability of basic quality which interests us – the quality of radioresistance.

Undoubted and obvious is a change in the amount of the elements - cells, which, can be accompanied by the change of intercellular relations (co-operations). However, what causes the increase in radioresistance? From one side, it can be conditioned greater probability of saving a minimum of the necessary number of cells for renewal of functions of meristem [13], and, from another side, more cells can provide more intensive intercellular co-operations in the post-radiation stage of renewal.

Thus, the gotten results specify, that at modified by decapitation of the main root of pea seedlings on a background the increase of radioresistance (radioadaptations), fixed on the various parameters of growth activity of lateral roots, simultaneously a capacity has substantially reduced the level of sublethal reparation of intracell damages, exposed by the method of fractionating of dose of acute gamma-irradiation. Obviously, in the mechanism of radioadaptive effect of decapitation determining part is acted by supercell processes. Additional comparative research of cyto- and histological parameters of apex meristem cells of lateral roots of controls (intact) and experimental (decapitation) confirmed this conclusion.

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