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Antioxidant reaction of *Solanum lycopersicum* L. and its first generation to pre-sowing γ - irradiation of seeds

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Abstract
Research results show that for the parent tomato there is a certain dose-dependent change in the size and weight of the fruit. More precisely, in a dose of 50 Gy, the size and weight of a tomato fruit is about 40% higher than in the control. A dose of 50 Gy was taken as a stimulating dose for a tomato in these soil and climatic conditions. It is shown that for the first generation of tomato there are no noticeable dose-dependent changes in mass and size. In other words, the effect found in the parent plant does not occur in its first generation. At the same time, if you do not take into account the minor variations, the mass and size of the experimental and control fruits are almost the same.
For the parent plant, an increase in the radiation dose in the region (1–50) Gy leads first to a small and then to a sharp increase in the MDA content, and in the region (50 to 400) Gy, on the contrary, to a noticeable decrease. At the same time, the content of proline for the parent plant remains almost constant from 1 to 100 Gy, and above 100 Gy it slightly increases. It is assumed that at radiation doses below 100 Gy, the need for proline is low. Therefore, antioxidant enzymes play a major role in protecting plants from adverse conditions. In fact, at these relatively low doses, APX and CAT show high activity. However, in large doses, in all likelihood, along with SOD, there is a need for the protective role of proline and its synthesis is enhanced.
It was shown that APX and CAT enzymes exhibit similar dose-dependent activity and, with high activity of these enzymes, SOD exhibits low, and with low CAT and APX activity, SOD exhibits high activity. It is assumed that antioxidant enzymes, to some extent, function in a balanced manner both among themselves and with respect to proline.

Keywords: Solanum lycopersicum L., first generation, pre-sowing irradiation, proline, malondialdehyde, antioxidant enzymes, SOD, APX, KAT

Introduction
It is known that for accelerating growth and development, as well as increasing the productivity of agricultural plants, pre-sowing exposure of seeds to γ-irradiation in small doses has been successfully applied to this day [Ershov, 2013; Geraskin *et al*., 2015; Kozmin *et al*., 2015] [6, 8, 23]. However, the fact of undesirable negative effects caused by ionizing radiation in many cases remains out of sight of researchers. Undoubtedly, under any stress conditions, including the pre-sowing γ-irradiation of seeds, it is necessary to protect plants from adverse conditions, which lead to increased synthesis and accumulation of protective elements.
Considering this, the role of antioxidant enzymes such as superoxide dismutase, catalase and ascorbate peroxidase, which play a key role in protecting *Solanum lycopersicum* from the damaging effects of the stressor has been investigated. Based on the fact that proline, as a low molecular weight antioxidant, may play a role in protecting the plant from the adverse effects of ionizing radiation, its content has also been determined.
The extent of radiation damage was evaluated by the content of malondialdehyde, a product of cell membrane lipid peroxidation.
Considering that the consequence of radiation can be detected at a certain time after the exposure, sometimes even in subsequent generations of plants, an attempt has been made to evaluate the role of antioxidant protective elements also for the first generation of the plant.
We assume that the clarification of these questions will contribute to understanding the essence of the protective responses of the plant under adverse conditions.

Materials and Methods
The Subject of Research – tomato (*Solanum lycopersicum* L.).
Equipment - source of γ- radiation – Co^{60}, centrifuge - type HIMAC *CT 15 RE* (United Kingdom), spectrophotometer – type spectrophotometer Ultrospec 3300 Pro (Amersham, USA).
Determination of the Malondialdehyde Content. The malondialdehyde (MDA) content, as a product of lipid peroxidation, was determined by thiobarbituric acid reaction [Ohkawa *et al*., 1979] [21]. After recentrifugation at 12 000 g for 10 min, the absorbance of supernatant was recorded at 532 and 600 nm. The value for non-specific absorption at 600 nm was subtracted. The concentration of MDA was calculated using the formula

$$C_{MDA} = (D_1 - D_2) \cdot V_2 \; / \; \varepsilon \cdot I \cdot V_1$$

(Where - D_1 and D_2 optical densities at 532 and 600 nm, respectively; ε - coefficient of absorbance: ε - = 155 mM^{-1} cm^{-1}; V_1 - the total and V_2 - the final volume of the ditch in sm^3; I - the length of the ditch in sm).

MDA concentration was determined in mmol / l per 1g of dry weight.

Determination of the Proline Content: The content of proline was determined by the classical method Bates *et al*. [Bates *et al*., 1973] [1]. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene for a blank.
The proline concentration was determined from a standart curve and calculated on a fresh weight basis as follows: [(µg proline / ml x ml toluene) / 115.5 µg / µmole] / [(g sample) / 5] = moles proline / g of fresh weight material.

Determination of Ascorbate Peroxidase Activity: Activity of ascorbate peroxidase APX (EC1.11.1.11) was determined by the method of Nakano and Asada [Nakano, Asada, 1981] [20]. The optical density was recorded in a spectrophotometer Ultrospec 3300 Pro (Amersham, USA) at 290 nm against a control without enzyme extract. As a measure of activity, we used the APO decrease in optical density during the first 30 sec of the reaction. Enzyme activity (in mmol / (g · min) was calculated based on a molar extinction coefficient (ε = 2,8 $mM^{-1} \cdot sm^{-1}$).

Determination of Catalase Activity: For determination of catalase (CAT, EC 1.11.1.6) activity was used the method developed in the work of Kumar and Knowles [Kumar, Knowles, 1993]. Decline of optical density was measured on a spectrophotometer at 240 nm per 1 min. Enzyme activity (in mmol / (g ·min) was calculated based on a molar extinction coefficient (ε = 39, 4 $mM^{-1} \cdot sm^{-1}$).

Determination of Superoxide Dismutase Activity: The activity of superoxide dismutase (SOD, EC 1.15.1.1) also were determined by the method developed by the Kumar and Knowles [Kumar, Knowles, 1993]. The reaction was started by adding the riboflavin, followed by incubation for 20 min on a white light (4000 lux). The maximum level of formazan formation was observed in the variant without plant extract (2.65 ml starting buffer, pH 7.8). Measurements were carried out based on the control variant, which was stored in the dark. Optical density was measured spectrophotometrically at 560 nm. The unit of SOD activity was taken 50% inhibition of formazan formation.
Experiments were carried out in double biological and triple analytical replicates. Statistical processing was performed by standard methods of variation statistics. The significance of differences of control and experimental results was assessed by Student's *t* – criterion [Lakin, 1990] [15]. The differences were significant at $|t| > 2$ ($p<0.05$).

Results and Discussion

Changes dinamics in fruit size and mass of the tomato plant and its first generation depending on the radiation doses:

Low-dose radiation is known to stimulate plant growth and development under certain conditions, which is manifested in biometric and reproductive properties of plants [Kuzin, 1977; Melki, Marouani, 2010; Gressel, Dodds, 2013] [12, 17, 9]. Therefore, the method of pre-sowing irradiation is successfully used to increase productivity of some agricultural plants [Sanzharova *et al*., 2013; Geraskin *et al*., 2015; Lakhanova, Sarsembayeva, 2015] [23, 26, 14].
To identify the potential impact of ionizing radiation on the productivity of tomato plants, as well as to clarify the role of the antioxidant defense system in this process the seeds were exposed to γ-radiation of 1, 5, 10, 50, 100, 200, 300 and 400 Gy and sowed in the experimental field along with control (not irradiated) variants. The purpose of these researches was also to determine the limits of stimulating doses, depending on the soil and climate conditions of the cultivation area of tomato plants.
After the harvest, the seeds were separated, stored in a special chamber and used for the next sowing. In this case, the seeds were not exposed to irradiation.
Figure 1 demonstrates dependence between biometric indices and the irradiation doses in parental forms as well as the first generation plants.

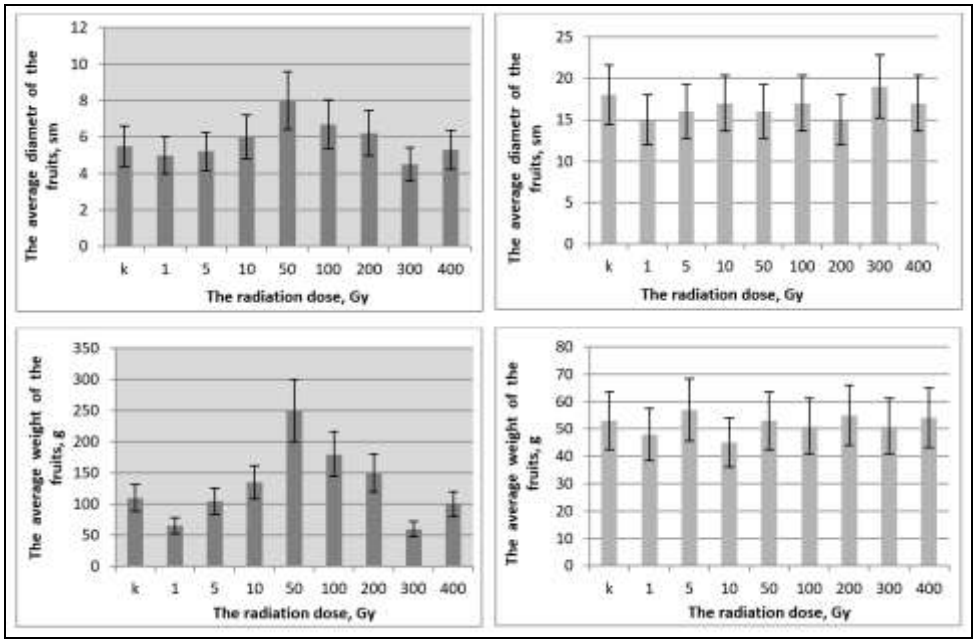

Fig 1: Changes dinamics in biometric indices of tomato fruit related to irradiation doses (black and white backgrounds indicate, respectively, the parental form and first generation).

As seen in the figure, marked differences occur in sizes and masses of fruit in the parental form. Moreover, 50Gy irradiation leads to 40% increase in the plant size compared to the control. This irradiation dose is likely to be considered as a stimulating dose of tomato plants for given soil and climatic conditions.

There were reports on the increase in productivity of tomato plants under 5-10 Gy irradiation [Maltseva,1979]. According to the results of the experiments with barley seeds (Chernovets variety) exposed to pre-sowing irradiation of 10, 30, 50 and 200 (10Gy/min), 10 Gy irradiation had no effect on the plant productivity. While, 30 and 50Gy irradiation resulted in an increase in both the plant vegetative mass and seed mass [Savin, 1981] [27].

The reason for the differences in the stimulating dose is most likely due to the radio sensitivity as well as the moisture content of the seeds. In radiation-resistant seeds, the stimulation effect occurs at higher doses and increases in the moisture content of the seeds make them more radio-sensitive [Berezina, Kaushansky, 1975; Melki, Marouani, 2010; Gressel, Dodds, 2013] [2, 17, 9].

Interestingly, the first generation of this plant is not similarly dependent on the irradiation doses. The determination of the fruit size in the first generation tomato plants showed that the effect observed in the parental form was not inherited by the next generation. If not to consider small deviations, there were not differences in the fruit mass and size compared to the control.

There were various reports on the processes occurring in the first generation plants exposed to pre-sowing irradiation. The data presented in some articles even contradict our results. Thus, the negative effect occurred in the plant can be converted to the positive effect in the next generation [Poryadkova, 1958; Glushchenko, Zakharova, 1960] [22, 10] and the negative effect observed in the parental form can be intensified in the first generation [Miller, 1965] [18]. It should be noted that the objects of these studies were not tomato plants.

Based on our results, the positive effect of low-dose irradiation is related to disturbances occurring in the DNA system, which are restored in the next generation.

It should be noted that the problem of the effect of low-dose ionizing radiation on plants has long been in focus of researchers. The increasing interest to this problem is related to radioactive pollution of the environment. Currently, a large amount of information available concerning negative effects of ionizing radiation on plants. Moreover, positive effects of low-dose ionizing radiation have also been observed in some researches. However, it is not clear if there is any common regularity or it is only characteristic for plants grown under certain conditions.

The resolving this problem has a great practical importance along with scientific importance in terms of clarifying the mechanism of action of small dose ionizing radiation.

Changes dinamics of malondialdehyde content depending on irradiation doses

In all processes occurring with the involvement of molecular oxygen, reactive oxygen species are formed and their interaction with nucleic acids and proteins results in the denaturation of the latter and their interaction with lipids leads to lipid peroxidation [Cheeseman, 2007] [4]. The damage can be assessed on the basis of the amount of malondialdehyde (MDA) which is the final product of this process [Montiller *et al*., 2004] [19].

Figure 2 presents MDA amounts corresponding to each irradiation dose in plant leaves.

As seen in the figure an enhancement in the irradiation dose in the range of 1 Gy– 50 Gy results first in a slight and then sharp increase in MDA content. It reaches the maximum amount under 50Gy irradiation. The subsequent increase in the irradiation dose leads to the reduction in the lipid oxidation product, rather than the increase.

It may be assumed that the increased intensity of irradiation in small doses may disturb the function of the membrane lipids, whereas large doses completely destroy the membranes.

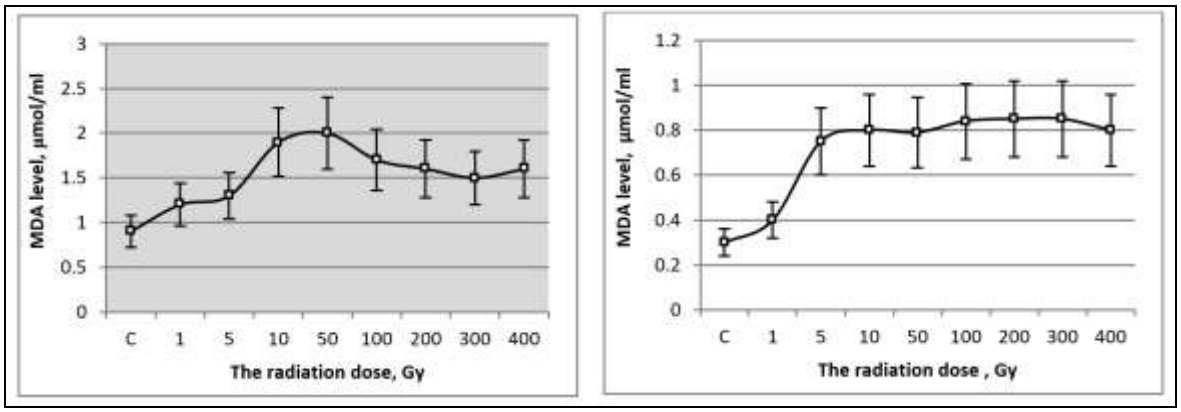

Fig 2: Changes dinamics of MDA depending on the irradiation doses (black background-parental forms, white background-the first generation).

The different pattern was observed for the first generation. Thus, dynamics of MDA depending on the irradiation dose has patterns similar to parental forms under low dose irradiation and different patterns under high doses. In other words the effects of low dose irradiation observed in parental forms maintained in the next generation. However, the decreasing tendency of the effects observed in parental plants exposed to high dose irradiation changed to stability in the next generation. It can be assumed that resistance is formed against high dose irradiation in the subsequent generations, which results in the prevention of cell membranes from large-scale disturbances due to free radicals.

Dose dependent change in proline content

One of the interesting traits of plants is their multistep protection system against reactive oxygen species (ROS). Antioxidant defense system (AODS) consists of enzymatic components, such as superoxide dismutase, ascorbate peroxidase, catalase and non-enzymatic low-molecular compounds (ascorbic acid, phenolic compounds, proline, carotenoids, glutathione, ubiquinone, vitamins D and K, prolomines), which play the role of "trap" for ROS [Saglama *et al*., 2011] [25].

The analysis of the previous reports shows that the reaction of plants to these effects is ambiguous and in most cases in addition to antioxidant enzymes this process involves proline, polyamine, phenolic compounds (anthocyanins, flavonoids), and carotenoids of terpenoid nature [Cakırlar *et al*., 2008; Radyukina *et al*., 2011] [3, 24]. There are reports on significant increases in low-molecular metabolites such as proline under stress [Kuznetsov *et al*., 2009; Radyukina *et al*., 2011] [13, 24].

It should be noted that, despite the collection of a large number of research data, the role of proline in the effect of various stressors has not yet been clarified. Therefore, the study of the protective role of proline in the effect of stress factors of different nature on plants of various ecological groups is of great scientific and practical importance. Thus, the research carried out in this direction will help to clarify the adaptation mechanisms of plants to stress conditions, protect biodiversity and open wide perspectives for agriculture. Proline amounts in leaves of tomato plants, which seeds were exposed to pre-sowing γ-irradiation are presented in figure 3.

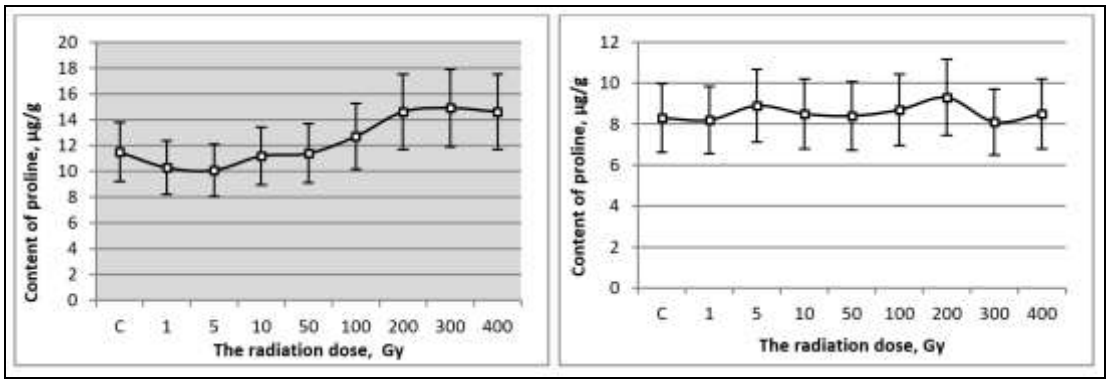

Fig 3: Changes dinamics of proline content depending on the irradiation doses (black background-parental forms, white background-the first generation).

It is noteworthy that irradiation dose below 100Gy has no effect on the proline amount which demonstrates the tendency to increase with enhancing irradiation doses above 100 Gy. This shows that there is no need for antioxidant proline in doses less than 100 Gy. In this case antioxidant enzymes are likely to play an important role. When plants are exposed to irradiation doses above 100Gy the need for the protective role of proline increases leading to the intensification of its synthesis.

The results confirm high resistance of the first-generation plants. Thus, no need for proline was observed under low as well as high irradiation doses.

Changes dynamics of the activities of antioxidant enzymes depending on irradiation doses

Dynamics of the activities of antioxidant defense system enzymes depending on irradiation doses was studied to evaluate effects of pre-sowing ionizing radiation of various doses on the antioxidant defense system functioning.

Superoxide dismutase: Figure 4 presents superoxide dismutase (SOD) activity changes depending on the irradiation doses.

Analysis of the activity results shows that for the parent plant in the dose range from 1 to 50 Gy there is no noticeable dose-dependent change in SOD activity. Existing minor changes are within the experimental error. However, increasing the radiation dose from 50 to 200 Gy leads to a significant increase in the activity of this enzyme. And in the area of relatively large doses (from 200 to 400 Gy), increasing the dose does not cause a change in the enzyme activity. In this case, the enzyme retains greater activity (SOD activity at a dose of 200 Gy is about 2 times greater than in the control).

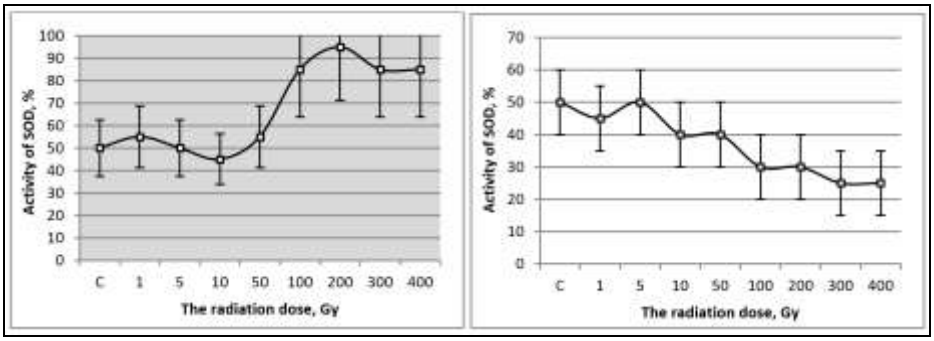

Fig 4: Changes dynamics of SOD activity depending on the irradiation doses (black background-parental forms, white background-the first generation).

Completely different dynamics of SOD activity depending on irradiation doses was observed in the first generation of the studied plants. Thus, an increase in the irradiation dose in the range of 1 Gy– 400 Gy led to a regular decrease in SOD activity.

The comparison of SOD activity in the parental plant with that in the first generation showed that the dose stimulating SOD activity in the parental plant negatively affected the first generation plants.

Ascorbate peroxidase: Figure 5 presents ascorbate peroxidase (APX) activity changes depending on the irradiation doses.

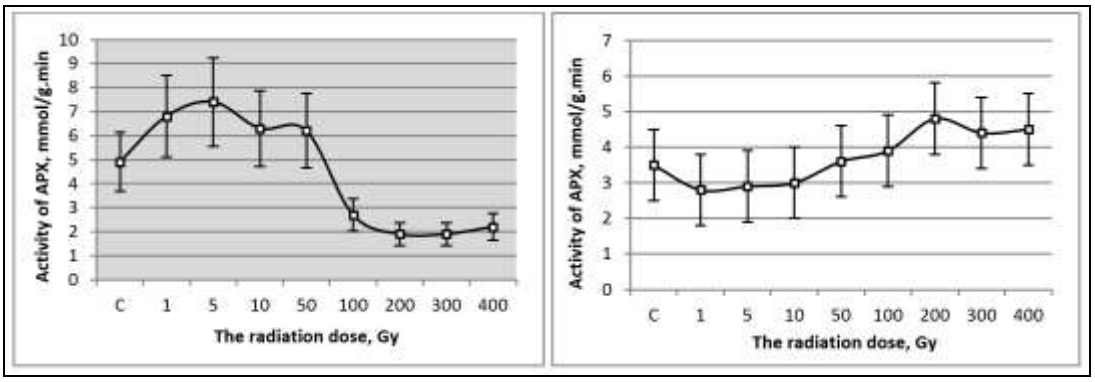

Fig 5: Changes dynamics of APX activity depending on the irradiation doses (black background-parental forms, white background-the first generation).

Thus, tomato plants have high APX activity protecting them from destructive action of free radicals at low doses (1Gy-50 Gy) of irradiation. Subsequent increases in the irradiation dose sharply decrease the enzyme activity.

The dependence of APX activity on the irradiation dose differ in the parental forms and the first generation plants. APX activity does not depend on the low dose irradiation, whereas at irradiation doses above 50Gy, the enzyme activity increases significantly with increasing doses of irradiation. Thus, a positive effect observed in the parental forms becomes negative in the first generation plants.

Catalase: Figure 6 presents catalase (CAT) activity changes depending on the irradiation doses.

It is noteworthy that the dependence of catalase and APX activity on irradiation doses is similar. Thus, CAT activity similar to APX activity, increases with increasing irradiation at low doses, reaches maximum activity at 5Gy, and further increases in the irradiation dose results in a sharp decrease of the enzyme activity. The activity remains almost unchanged at the irradiation doses above 100 Gy.

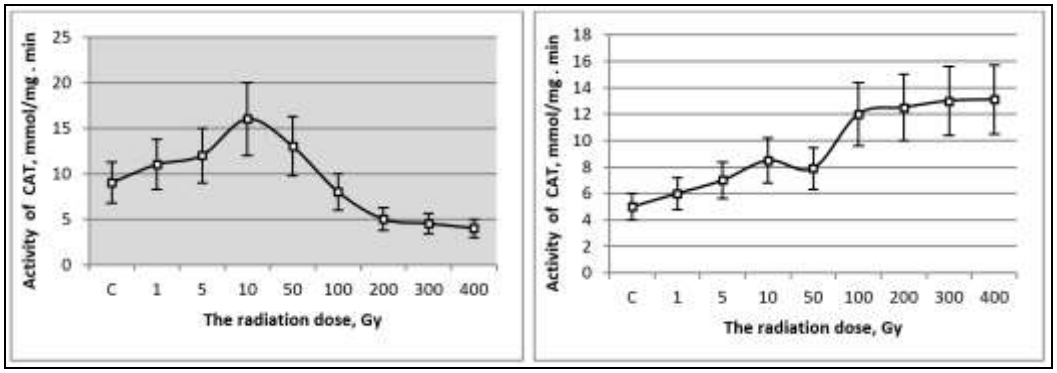

Fig 6: Changes dynamics of CAT activity depending on the irradiation doses (black background-parental forms, white background-the first generation).

CAT activity changes in the first generation plants is similar to those observed for APX too. In this case an increase in the irradiation doses in all the range leads to the CAT activity enhancement, not considering slight deviations.

An interesting fact is that in the range of small doses, the activity of APX and CAT increases against the background of low SOD activity. However, in the range of relatively large doses, an increase in the activity of APX and CAT is accompanied by a decrease in the activity of SOD. This fact is attributed to the balanced functioning of the antioxidant enzymes under stress. Thus, when SOD expresses high activity, there is no need in high activities of APX and CAT and in the case of high APX and CAT activities there is no need in SOD activity.

Conclusion

Irradiation resistance of seeds is known to be determined by the general activity of the system scavenging free radicals and peroxides. The increase in APX and CAT activities with decreasing SOD activity under low-doses of irradiation probably occurs due to the formation of H_2O_2 more than superoxide radicals. Moreover, superoxide anion radicals emerged under stress are converted into H_2O_2, in the reaction catalyzed by SOD, which is then inactivated by CAT.

According to the available literature data, the change in APX activity under the effects of stress factors may be due to the synthesis of new isoenzymes or the accumulation of substrate compounds inducing the synthesis of the enzyme [Del Rнo, 2015]. The accumulation of high substrate concentrations probably leads to the inhibition of the enzyme [Foyer, Noctor, 2015].

The studied enzymes are known to play an important role in the formation of the endogenous background of the plant resistance under high irradiation. If the formation of free radicals and peroxides is activated under stress, it is no doubt that CAT and APX activities should increase as one of the mechanisms against stress factors. According to Zhurovskaya A.N. *et al*. [Zhurovskaya *et al*., 1998] [28], peroxidase activity increases, as under extreme ecological conditions (for example, when temperature and humidity change sharply) the major chemical stress factor is H_2O_2. Under such conditions, the activity of another main enzyme of the antioxidant defense system-SOD-usually decreases. Thus, a majority of superoxide radicals oxidize unsaturated fatty acids of membrane lipids. Finally, the generated organic peroxides are inactivated by peroxidase. Therefore, in such cases, the presence of active peroxidase reduces the demand for active superoxide dismutase.

In summary, antioxidant protection system elements are likely involved in the process of pre-sowing irradiation.

The fact that ionizing radiation has a long-lasting effect allows determining the form in which the initial “damage” is maintained in the organism. Such studies may serve to prevent undesirable effects by manipulating processes occurring during the period after the exposure to the irradiation.

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