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The Functioning of the Antioxidant Defense System in Two Generations of *Solanum melongena* L., the Seeds of which before Sowing were Subjected to γ-irradiation

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A B S T R A C T

The growth and development of the fruits was investigated in two generations of eggplant, the seeds of which before the first sowing were exposed with γ-irradiation at different doses. Based on changes in the content of malondialdehyde (MDA), proline and activity of antioxidant enzymes was estimated the functioning of the antioxidant defense system in each generation of the plant. It is shown that the biometric parameters of fruits of the second generation are different from the fruit as the first generation and the control. Second generation typically characterized by intense development and high biomass accumulation. It was found that with increasing radiation dose increases the content of MDA, which is the main product of lipid oxidation by free oxygen radicals. It is assumed that with increasing doses is increased formation of reactive oxygen species in both generations of eggplant, which is accompanied by the large destruction in lipid membranes. It has been found dose-dependent changes of the proline content and activity of ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD). As is known, these antioxidants are actively involved in the protection of plants from the effects of radiation. It was determined that these changes are different also for the different generations. It has been established that the functioning of the antioxidant system in low doses leads to increased synthesis of proline and APX activation for the first generation of the plant. However the effect of stimulating the synthesis of proline and increasing the activity of the APX in the second generation does not occur. In contrast, at high doses, which induce large destructive action in lipid membranes, proline and APX play an insignificant role. At these doses, in both generations in the protection of plants against radiation are actively involved of SOD and CAT.

Introduction

Improving of plants resistance to unfavorable environmental factors acquires great importance in connection with the aggravation of the environmental crisis. These factors by acting on the plant can cause varied response reactions therein. Many of them, to which the plant evolutionarily does not adapted, can render a stressful effect on the plant, that undoubtedly will lead to different physical and chemical anomalies, damage their structure and metabolic functions (Levitt, 1983). One of these important environmental factors that determine the diversity and productivity of plants is ionizing radiation (Blokina *et al*., 2003).

It is known that one of the interesting features of the effect of ionizing radiation is the detection of its consequence after a certain time after irradiation. Then occur the following questions. In what form is stored a primary damage in irradiated body for this time? What processes, which occur during this time, are ultimately responsible for the appearance of negative consequences? In which form ionizing radiation will influence on the antioxidant system and what results will be obtained as a result of biochemical transformations occurring in the cells? Is it possible to prevent occurrence of undesirable effects by intervention?

Based on these considerations, and for radiobiological research of this kind, we found it useful to use the seeds of plants. Since their can be irradiated and be obtained a homogeneous irradiation of different parts. Furthermore, with the use of special techniques before irradiation is possible to change the physiological state of the embryo, which is extremely important, both to improve productivity and for the settlement of development and growth of plants.

It will be noted that for the successful solution of this problem is very important to find out the mechanisms of formation of protective responses in unfavorable conditions for plants. For this reason, in the present study we have investigated the functioning of the antioxidant defense system *Solanum melongena L.* at the level of its individual elements, both in first and second generation of the plant.

We assume that similar works allow understanding the essence of the response of protective reactions of plant organisms. Furthermore, based on the obtained results can create a base for the development of models for describing the processes functioning of the living system in conditions of low intensity ionizing radiation.

Materials and Methods

The Subject of Research – eggplant (*Solanum melongena 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). For this approximately 1g freshly picked leaves of plant samples was cut into small pieces, homogenized with 2.5 ml of 5% trichloroacetic acid, and then centrifuged at 12000 g for 10 min. at room temperature. The equal volumes of supernatant and 0,5%

thiobarbituric acid in 20% trichloroacetic acid were added in a new tube and incubated in 96°C for 30 minutes and then quickly cooled in an ice bath. 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

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

(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 mmo*l*/*l* per 1g of dry weight.

Determination of the Proline Content

The content of proline was determined by the classical method Bates *et al*. (1973). For this purpose 0,3 g of plant material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid. The homogenate filtered through 2 filter paper and was precipitated in a centrifuge for 15 minutes at 1000 g. 2 ml of filtrate was reacted with 2 ml acid - ninhdrin and 2 ml of glacial acetic acid in a test tube for 1 hour at 100^0C, and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed vigorously with a test tube stirrer for 15-20 sec.

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 (1981). 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*} 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 (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*} 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 (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 on the basis of 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, which gave results with an error of 0 to ±20%. The figures show the mean values of the measured values. 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). The differences were significant at $|t| > 2$ ($p<0.05$).

The study of Biometrical Parameters of *Solanum melongena L.* Fruits in his Two Generations, the Seeds of which before the first Sowing were Irradiated with γ - rays at Different Doses

As it is known, on the base of biological effects of ionizing radiation is worth the physical stage of energy absorption of the substance and, as a result, the formation of free radicals and oxidants. This is a prerequisite for starting the mechanism of the following chemical and biological stages of radiation damage. At present in radiobiology are accumulated extensive experimental data on the primary mechanism of biological effects of ionizing radiation. Mechanisms of disturbance of biochemical and physiological processes comprehensively investigated. In these works, most of all, attention was paid to the study of molecular mechanisms, biochemical, and cytological effects of radiation. However, practically no have been studied distant consequences of the effect of radiation in subsequent generations.
Moreover, for increase the productivity of agricultural plants, some time their seeds before sowing were exposed to irradiation of γ- rays. In so doing was taken into account the fact according to which a small dose of radiation can stimulate the growth and development of plants in certain conditions that more total appears in their biometric characteristics and reproducibility.

To this day is unclear the question: stimulating effect is a common regularity or it is manifested for plants growing only under special conditions? Not clear question also, in what form the ionizing radiation influences on antioxidant system and in what will result biochemical transformations occurring in the cells, in the second generation plants?

In order to clarify these issues, we examined the reaction of *Solanum melongena L.* seeds to the effects of γ - radiation. Reaction of seeds in so doing was investigated based on the functioning of the antioxidant defense system of the plant.

Seeds of *Solanum melongena L.* before first sowing were subjected to γ - radiation at doses of 1, 5, 10, 50, 100, 200, 300 and 400 Gy using a Co^{60} source. The dose rate in all cases was 0.048 Gy / sec. The irradiated seeds, also seeds of the second generation together with their control samples were grown on the experimental plot of the Scientific-Research Institute of Vegetable Growing (Fig. 1a and 1b, respectively).

The systematic phenological observations over plant growth and development were conducted during the vegetation period. Functioning antioxidant defense system was evaluated in extracts of freshly picked leaves at the flowering stage of the plant. Estimation has carried out on the basis of changes of proline content and activity of antioxidant enzymes. In so doing parallels between these changes and changes in the level of MDA were carried out also.

At the end of the growing season were collected the fruits of the plant and were determined their biometrical parameters.

The seeds of the fruit have been stored in special conditions and were used for subsequent planting (these seeds not were irradiated). The content of proline and activity of antioxidant enzymes were determined also for the plants of the second generation. Biometrical parameters of fruits were determined at the end of the vegetative period of the second generation again.

Fig. 2 - 4 shows the results of biometric measurements of the fruit (average value of measured values) both first and second generation.

The graphs show that for the first generation of plants compared to the control there is a clearly expressed dose -dependent difference in the sizes and masses of fruit.

There is the stimulation and the inhibition of growth and fruit mass. Stimulation and inhibition manifests itself in different intervals of dose. The results indicate that ionizing radiation at low doses (doses in 1 - 5 Gy) does not cause noticeable changes in biometric indicators of fruits. However, further increase of the radiation dose leads to an increase of fruit weight and size. The graph depicting the dose-dependent changes in the mass and size of the fruit, has a maximum at a dose of 50 Gy. An exposure of seeds at high dose leads to a marked reduction of weight and size of fruit. Reduction in the size and weight of the fruits was accelerated with increasing doses. It can be assumed that the dose of ionizing radiation in the range of 10 to 50 Gy plays a role of stimulator accelerating the accumulation of biomass fruits for the first generation. However the dose of 100 to 400 Gy is an inhibitor of the development of fruits.

Stimulation of fruits development at low doses of irradiation of seeds, in all probability, is due to the acceleration of division of meristems cells. In so doing the suppression of the development of the fruits at high doses is a result of the loss of their division ability.

To note that exposure of seeds of different agricultural plants before sowing has shown that the radiation dose, which stimulates the development of plants, depends significantly on the radio sensitivity of seeds. For this reason, irradiation of seeds affects to the development of plants differently. For example, seeds of tomato the irradiated before sowing at a dose of 2 kRad (20 Gy) demonstrated a sharp increase of index rapid development (relative to 143% control) (Suess and Grosse, 1969). Increasing of the tomato productivity whose seeds before sowing were irradiated at doses of 5 - 10 Gy was shown by Maltseva too (1979).

The irradiation at doses of 100 - 200 rad (dose rate of 5 - 10 rad / min) seeds of some other agricultural plants also showed the effect of development stimulating (Suess and Grosse, 1969).

Irradiation of wheat seeds (grade of Diamand) with γ - Co^{60} rays at doses of 10, and 50 Gy has almost no influence on the biomass and productivity of plant. However dose greater than 100 Gy (in particular, 200 Gy) lead to a decrease in plant productivity (Kuzin, 1963).

Irradiation of barley seeds (grade of Chernovets) at doses of 10, 30, 50 and 200 Gy (dose rate of 10 Gy / min) also showed that 10 Gy does not influences on plant productivity. However irradiation of seeds in doses of 30 and 50 Gy leads to an increase the vegetative mass and the mass of plant fruits (Savin, 1981).

It is interesting that our results on biometric parameters of the second generation fruits differ significantly from the results of the first generation. The dose-dependent changes of biometric parameters of second generation fruits is also different from the first generation fruits. In other words, the changes observed in the first generation are not repeated in the second generation. The seeds of eggplant which showed high sensitivity to the effects of radiation at low doses in the first generation, almost did not react to such exposure in the second generation. Since the fruits of this plant both in size and weight do not differ from control plant fruits.

It can be assumed that in the first generation plants under the influence of small doses of radiation are generated adaptive signs. And as a result of this is not observed significant changes in the second generation. In other words, it can be assumed that the observed positive effect in the first generation at low doses of radiation, most likely associated with the violation in DNA system, which is accompanied with an intense accumulation of organic matter. Therefore violation in the DNA system occuring in the first generation is restored in the next generation.

To a certain degree is inexpedient to link the cause of these long-term changes occurring in the body with primary disorders in the physiological and biochemical system of irradiated seeds. In all probability the reason for long-term preservation of radiation effects in the organism 50, 100 and 200 Gy (dose rate of 7 Gy / min) showed that the radiation dose of 10 is changes in the DNA system which is characterized by the low mobility.

Large doses, as in the case of the first generation, prevent the development of fruits. It can be assumed that the damage that takes place at higher doses in the first generation plants is stored in the second generation also.

It is noted that in the scientific literature regarding the processes occurring in the second generation of plants there are contradictory data. For example, it is shown that negative effect which observed in the first generation could become a positive effect in the second generation (Savin, 1981).

The results of another study showed that for barley, whose seeds before sowing were irradiated at a dose of 10,000 R negative effect observed in the first generation is further enhanced in the second generation.

In the case of large doses the negative effect may persist and in the third generation. However, the plant productivity of second generation did not differ from control in the case of low dose (Miller, 1965).

It is possible that in this process, apart from natural changes in environmental condition, an important role is also played by the physiological state of the plant seeds.

Study the Dynamics of a Dose-Dependent Accumulation of MDA in Two Generations *Solanum melongena L.*, seeds of which before the First Sowing were Subjected to the Effects of γ - rays at Different Doses

It should be noted that the study of the genetic consequences of the influence of ionizing radiation on living organisms for a variety of reasons, causes difficulties. At first, it is connected by that a solution to this problem requires a long observation. Second, defects as the newly formed both and genetically transferred have high variability. This makes it impossible to distinguish the radiation-induced defects from others. For this reason, in spite of

numerous studies on the effects of ionizing been elucidated.

Today the mechanisms of radiation action on biological objects some scientists are associate with any chemical changes in the cells (indirect effect) (Margulis and Margulis, 2005). There is a view according to which radiation affects directly on the DNA (the target theory) (Tsytsugina, Polikarpov *et al*., 2005).

The indirect effect is based on the interaction of ionizing radiation with water molecules, resulting in the formation of different oxygen free radicals. The mechanisms of direct exposure are associated with the direct influence of radiation on DNA and RNA, which plays a role of target in the cell (Tsytsugina, Flora *et al*., 2005).

At the time due to the interaction of free radicals with the membranes of cell occurs lipid oxidation also (Burlakova *et al*., 1975).

Due to the fact that lipids of membrane can be a target of reactive oxygen species (ROS) lipid peroxidation can cause to significant structural damage of the membranes. The oxidation and damage of membranes causes the formation of several end products. MDA is one of them (Montiller *et al*., 2004). In this case the degree of structural damages defined by level of the product.

ROS concentration in normal conditions is maintained at a minimum level and does not cause a danger for the organism.

ROS concentration is maintained at a minimum level in normal conditions and does not cause a danger for the body. Nevertheless, under stressful conditions their number may increase rapidly and be dangerous for the organism (Ogawa *et al*., 1996).

In Fig. 5 are presented data on dose-dependent changes of the MDA content in the leaves of *Solanum melongena L.*, seeds which before sowing were exposed with γ - rays at different doses.

From the results it is seen that for the first generation of plant at low dose radiation on living organisms until now the mechanisms of its toxic effects have not (1 to 10 Gy) with the increase of radiation dose are observed dynamics of the gradual increase in the content of MDA. In doing so increase of dose from 10 to 100 Gy does not lead to a noticeable change in the content of MDA. However, further increasing of the dose leads to a sharp increase in lipid peroxidation products. In other words, if the increase in MDA content at low doses is approximately monotonic, there is a sharp increase in MDA content in high doses.

It can be assumed that increasing of the absorption dose of ionizing radiation at low doses leads to an increase in the scale of cell membranes damage. As a consequence, the content of MDA increases.

Ionizing radiation at high doses causes large - scale damage in the membranes. As a result of this the function of membranes is disrupted and MDA content is increased greatly.

The second generation of plant by the change character of MDA content differs from the first generation. Since in this case at low doses with increasing doses the growth in MDA content are not observed. In doing so at the dose range of from 1 to 50 Gy the MDA content remains almost constant. Further increasing the dose up to 400 Gy leads to a marked increase of lipid peroxidation products as in the case of the first generation.

It can be assumed that in the seeds irradiated at low doses are formed adaptive features. Therefore, the MDA content in the next generation at low doses is not very different from the content in control sample. However, high doses for the second generation just as in the case of the first generation are damaging for the cells. In all probability, adaptive reactions which are the main factor-protecting cells from radiation exposure at low doses not are taking place at higher doses.

Study the Dynamics of Dose-dependent Change of Proline Content in Two Generations *Solanum melongena L.*, seeds of which before the First Sowing were Subjected to the Effects of γ - rays at Different Doses

Under normal physiological conditions, i.e. during normal cells operation the concentration of ROS and degree of lipid peroxidation induced by them are in a low stationary level, which has no toxic effect on the cells and organism as a whole (Ogawa *et al*., 1996). This effect is due to the fact that the living cell possesses a unique antioxidant system which performs the function of neutralizing free radicals. Antioxidant defense system consists of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POX), and glutathione reductase (GR) and low molecular weight antioxidants, such as ascorbate, glutathione, proline, carotenoids, etc. (Saglama *et al*., 2011).

Analysis of literature data shows that the reaction of different plants to the effects of stressors ambiguous. In many cases, in the process together with antioxidant enzymes are involved also low molecular weight antioxidants, one of which is proline (Radyukina *et al*., 2011). There is data, according to which the content of proline, having the ability to neutralize ROS, under stress increases significantly. Furthermore, it is shown that a high content of proline can inhibit the activity of antioxidant enzymes (Ozturk and Demir, 2002). However, there is a definite correlation between the activity of enzymes and proline content. It is established that the plant having the ability to accumulate proline, generally has a low activity of SOD (Kuznetsov *et al*., 2009).

It should be noted that despite multiple studies, the role of proline in different stress conditions remains unclear. Therefore, the study of the protective role of proline in unfavorable conditions for plants has both scientific and practical significance.

Considering this, we studied participation of proline in cell protection of seeds exposed to radiation at different doses. Additionally, role of proline as an antioxidant was studied in next generation of eggplants based on the change of its levels. Obtained by us data on the content of proline at different doses of radiation, for the first and for the second generation are represented in Fig. 6. As can be seen from the data presented, for the first generation the response of plant seeds to radiation exposure at low doses (1 to 10 Gy) is characterized by a gradual increase in proline content (Fig. 6 a). Apparently, this is due to the fact that the amount of the products of lipid peroxidation (LPO) increases with increasing doses in this field, which undoubtedly leads to activation of the antioxidant defense system. As a result of this, naturally, the amount of low-molecular antioxidants, including proline, will increase. Let us remind that at low doses, with increasing of dose was increased the content of MDA, as a products of LPO (Fig. 5 a). It is clear from the graph, at high doses, on the contrary, increase of dose causes decline in proline content. It can be assumed that the radioactive radiation at high doses inhibits the pathway of proline synthesis. It

is not excluded that in doing so the antioxidant enzymes can be activated and, as a consequence of this, the need of proline may decrease.

It is interesting that the nature of change of proline content for the second generation does not coincide with the nature of change for the first generation. In other words, irradiation in small doses in the seeds of second generation does not lead to a change of proline content or the change is within of the measurement errors (Fig.6, b).

It can be assumed, that in the irradiated seeds under the influence of radioactive exposure at a low dose are formed certain defense mechanisms, which are stored also in the second plant generation. The formed protective mechanisms, in all probability are conditioned increased synthesis of proline as of one important antioxidant with low molecular weight. Therefore, a high level of proline at low doses is stored and in the second generation of plant. It is interesting that inhibition of metabolic pathways of proline synthesis at high doses holds and for the second-generation plant.

We assume that a protective role in large doses takes on itself the macromolecular antioxidant enzymes and low molecular weight antioxidants such as proline, in this case, do not play a significant role.

Study the Dynamics of a Dose-dependent Changes in the Activity of Antioxidant Enzymes in the Two Generations of *Solanum melongena* L., seeds of which before the First Sowing were Subjected to the Effects of γ – rays at Different Doses

Study the Dynamics of a Dose-dependent Change in SOD Activity

It is known that in plants under the influence of a wide variety of adverse factors (including ionizing radiation) there is intense development of oxidation processes. In order to curb these processes is necessary rapid and significant increase in antioxidant cell resources. In doing so generally as a response to the increased generation of reactive oxygen species, observed a change of activity of the antioxidant enzymes (Fang and Kao, 2000; Hu and Liu, 2008). It should be noted that changes of the activity of antioxidant enzymes such as SOD, CAT and APX plays a key role in protecting of metabolism against damage under conditions of oxidative stress.

The data we obtained on SOD activity in the first and second generation of *Solanum melongena L.*, whose seeds before of the first sowing were irradiated with γ-rays at different doses, are shown in Fig. 7.

As seen from the results obtained for the first plant generation an increase in SOD activity occurs at higher doses. However at low doses (up to 10 Gy) the increase of absorption dose does not result to change the activity of SOD.

The increase of SOD activity at higher doses can link with an increase in the protective role of this enzyme. Can be assumed that in large doses in protecting of cells against the effects of ionizing radiation SOD plays a key role.

Analysis of data obtained by different authors shows that various stressors influence differently on the activity of this enzyme. In other words, in one case the stressors result in increased activity of SOD, and in other case they cause a decrease in enzyme activity. For example, increasing the activity of this enzyme was observed under conditions of water deficit (Kaminska - Roïek and Pukacki, 2004) and waterlogged soil (Kalashnikov *et al*., 1994), at thermal shock (Kang and Saltveit, 2001), cooling

(Kuk *et al*., 2003), salt stress (Lee *et al*., 2001) and UV irradiation (Schmitz-Eiberger and Noga, 2001). There is evidence that changes of SOD activity is dependent on the intensity and duration of exposure of stress factor (Baranenko, 2006), on the susceptibility of the organism (Wu *et al*., 2003), as well as on the stage of plant development (Jafarov *et al*., 2016).

Based on the data obtained by us it should be noted that both the first and second generation of plants is characterized by the similar dynamics of dose-dependent changes in SOD activity. In doing so if in the low doses does not take place a dose-dependent change of SOD activity, in high doses increasing the dose leads to an increase in the activity of this enzyme in both generations of the plant. In other words, the detected in the first generation effect retained and in the second generation.
Despite the similarities, the dynamics of a dose-dependent change of SOD activity at higher doses in different generations are different in scale changes. In other words, the scale of of these changes is not equal in different generations. Since, if the SOD activity in the first generation about 2.1 times greater compared with control, in the second generation the activity of this enzyme exceeds of control approximately 1.5 times.

Unfortunately, there is no data in the literature concerning the activity of SOD in the second generation of plants. Therefore we could not make a generalized opinion about the role of SOD in the second generation of this plant.

Study the Dynamics of a Dose-dependent Change in APX Activity

APX as an antioxidant enzyme performs detoxification H_2O_2 in cell by dint of oxidation of ascorbic acid. The enzyme has a high affinity for the substrate and is able to neutralize peroxide at very low concentrations (Polesskaya, 2007).

Ascorbate peroxidase reaction - it is the central process of a cycle of reactions aimed at removing the main reactive oxygen species in chloroplasts (Polovinkina and Sinitsyna, 2010). It is believed that the increase in peroxidase activity is a non-specific response of plants to biotic and abiotic stressors (Fang and Kao, 2000).

Increasing of the peroxidase activity (compared to control 116 - 120%) was shown for the potatoes, seeds which before sowing were irradiated with γ-rays at a dose of 300 rad (Serebrennikov *et al*., 1971).

Obtained by us data on the APX activity in two generations of *Solanum melongena L*. are presented in Fig. 8.

As can be seen from the results, for the first generation the dynamics of dose-dependent change of APX activity is different from the dynamics of change in SOD activity. In doing so APO activity increases with an increase of the absorption dose and at 5 Gy reaches the maximum. Further increase of the dose does not lead to an increase of APX activity, conversely, activity of this enzyme decreases with increasing doses. In the interval of dose from 200 to 400 Gy the APX activity remains almost unaltered.

Based on the literature data can assume that the increasing APX activity in small doses may be associated with increasing synthesis of new isoenzymes of this enzyme. It is not excluded that the reason for increasing the activity of the APX is the accumulation of substrates, which can induce the synthesis of this enzyme. To note that, and that, and another is quite admissible under

unfavorable conditions, including in conditions of radiation.

Can be assumed that the protective role in the low doses of radiation takes on himself namely this enzyme. Since, at low doses the activity of another of main antioxidant enzyme, i.e. activity of SOD remains virtually unchanged. In all probability, in the protection of harmful effects of radiation SOD and APX perform interconnected functions. Since, a decrease activity of one is compensated by an increase in activity of another.

As we have seen, in small doses antioxidant prolines also are played a certain role in this process. Since their content are increased considerably in small doses (Fig. 6, a). We consider that in protection of cells from the effects of radiation together with APX participates and the proline. This suggests that the antioxidant enzymes of the antioxidant defense systems operate in a coordinated manner with low molecular weight components of this system.

We assume that the decrease of APX activity in high doses are connected with the fact that the role of protecting cells against the effects of radiation in this case takes on the SOD (activity of this enzyme is greatly increased).

It is possible that the enzyme itself may be a target of ionizing radiation in these conditions.

The results of APX activity for the second-generation plant differ from the results obtained for the first generation. Since activity of APX for the second generation decreases gradually as the dose increases in the range of 1 to 200 Gy. In doing so APX activity decreases differently in different intervals of dose. Since the activity of the enzyme decreases insignificantly in the dose range from 1 to 50 Gy and considerably in a dose range from 50 to 200 Gy. Further increase of the dose does not alter the activity of this enzyme.

From the results can be concluded that the observed effect of stimulating in APX activity in the first generation at a small doses is replaced by the effect of inhibiting in the second generation.

Figure.1 Experimental Plot of the Scientific-Research Institute of Vegetable Growing (a - for the first generation, b - for the second generation)

Figure.2 The Average Weight of the *Solanum melongena* L. Fruit at Different Radiation doses (a - for the first generation, b - for the second generation)

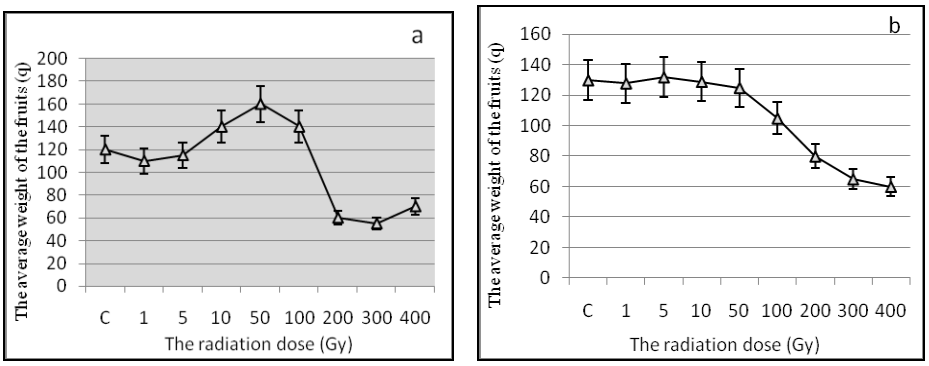


Figure.3 The Average Length of the *Solanum melongena* L. Fruit at Different Radiation doses (a - for the first generation, b - for the second generation)

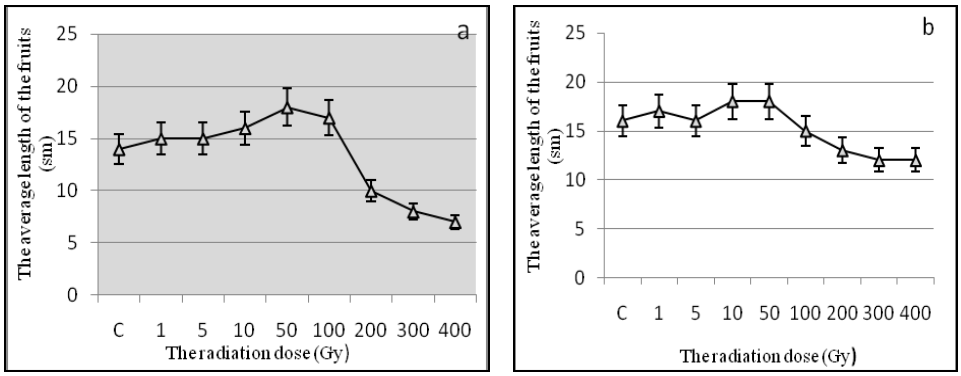


Figure.4 The Average Diameter of the *Solanum melongena* L. Fruit at Different Radiation Doses (a - for the first generation, b - for the second generation)

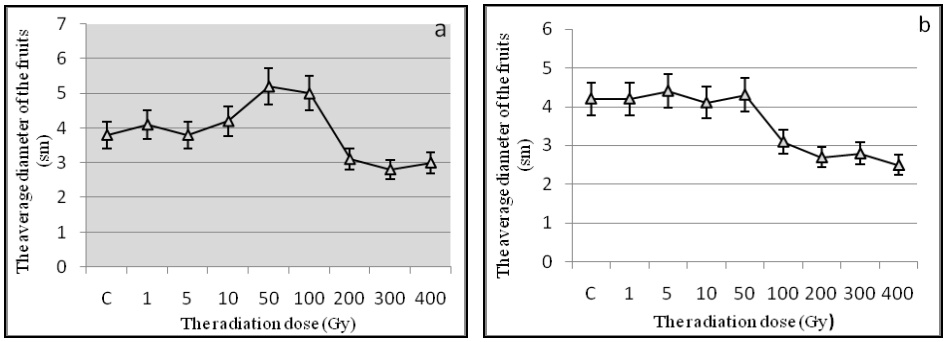


Figure.5 Dynamics of a Dose-dependent Changes of MDA Content (a - for the first generation, b – for the second generation)

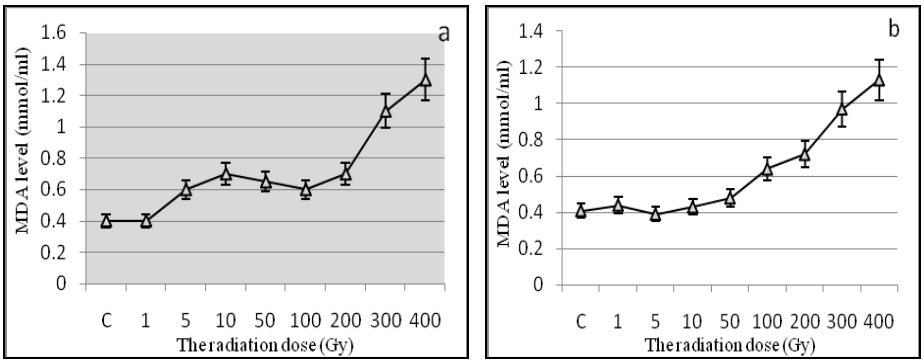


Figure.6 Dynamics of a Dose-dependent Changes of Proline Content (a - for the first generation, b – for the second generation)

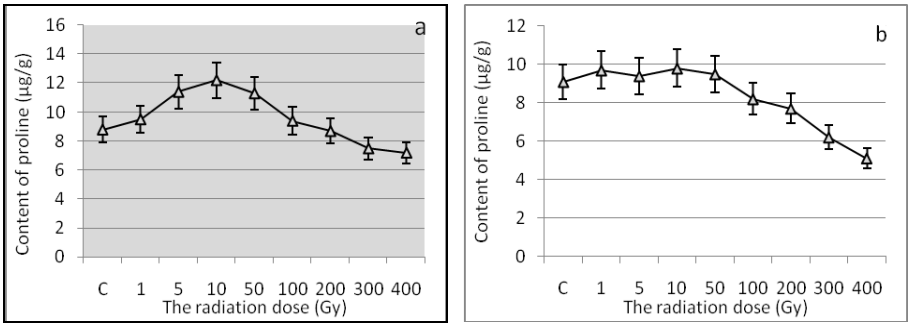


Figure.7 Dynamics of a Dose-dependent Changes of SOD Activity (a - for the first generation, b – for the second generation)

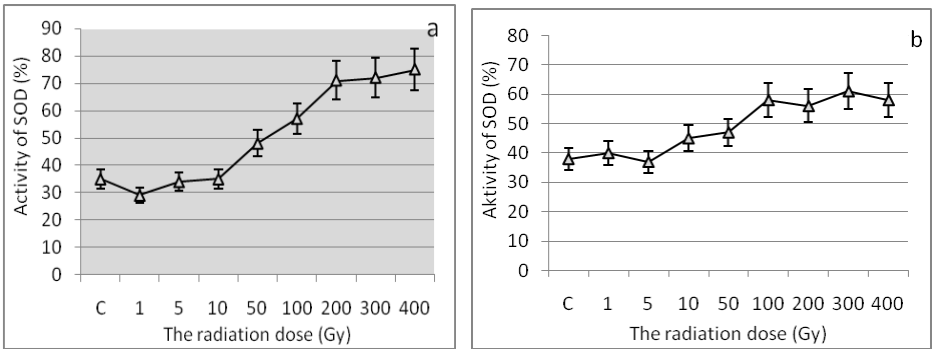


Figure.8 Dynamics of a Dose-dependent Changes of APX Activity (a - for the first generation, b – for the second generation)

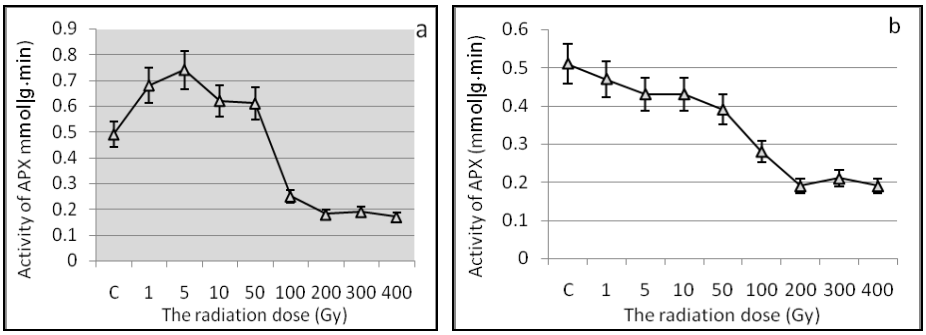
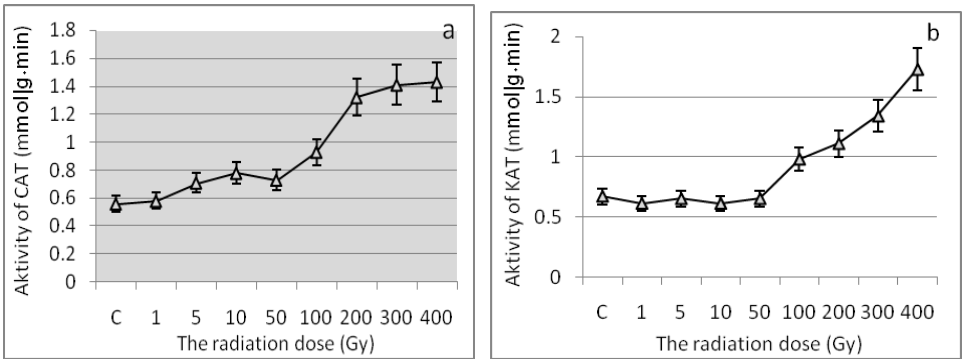


Figure.9 Dynamics of a Dose-dependent Changes of CAT Activity (a - for the first generation, b – for the second generation)



In contrast, the character of change of enzyme activity at high doses (greater than 50 Gy) is identical for both generations.

We note that the inhibition of the enzyme activity at high doses can be caused by high concentrations of the peroxidase substrates too. It is possible that in high doses can be increased the number of low molecular weight antioxidants. As a result of this may be reduced a need to the APX and consequently can be decreased of its activity.

Study the Dynamics of Dose Dependent Change in the CAT Activity

Obtained by us data on the CAT activity in two generations *Solanum melongena L.* presented in Fig. 9.

The results show that in the dynamics of dose-dependent changes of the SOD and CAT activity for the first generation plants are no significant differences. If do not take into account minor changes in small doses, it is not difficult to see that CAT and SOD demonstrate almost identical dose-dependent activity. CAT, as well as SOD, exhibit low activity in small doses and high activity in high doses.

Although that the main link of antioxidant protection is SOD, which catalyses the dismutation reaction of superoxide anion radical, role CAT in the protection of sells is significant too. Since, as a result of the functioning of SOD is produced hydrogen

peroxide, and CAT is the main enzyme which removes hydrogen peroxide (Polovinkina and Sinitsyna, 2010).

This gives grounds to suggest that an intensive formation of superoxide anion radicals does not occured in small doses. Thus, there is no great need for the active SOD. Naturally, the dismutation of superoxide radicals would not play a significant role in this case, and as a consequence of this, the hydrogen peroxide content and the CAT activity not will be high.

It is known that H_2O_2 is formed not only as a result of dismutation of superoxide radicals. As a reactive oxygen species, they are formed too in other physiological processes (Kretovich, 1986). The slight increase of CAT activity in small doses probably is associated to this fact.
The sharp increase of CAT activity (as well as, the activity of SOD) at high doses are confirm that in this case predominantly formed the superoxide anion radicals. Therefore, need for SOD (and also for CAT) increases sharply.

This is confirmed too by the fact that the level of antioxidant proline is not high at higher doses. Can be assumed that if at high doses predominantly were formed other reactive oxygen species, then the level of low molecular weight antioxidants would have been high too.

Results obtained by us for the second generation of *Solanum melongena L.*, are different from the results for the first generation. In doing so in small doses does not exist dose-dependent changes of CAT activity. But in the case of large doses there is a sharp increase in the enzyme activity.

This is likely connected with the fact that in small doses in the cells of first plant generation are formed the adaptive potential and the plant peculiarly resists to the influence of adverse factors in the next generation. In doing so in high doses the radiation causes the most damage and the plant can not resist to such influence.

In conclusion, the enzymes investigated by us play a key role in the formation of endogenous background of plant resistance to stress conditions. Since, the superoxide radicals which are formed in stress conditions turns into hydrogen peroxide with participation of SOD, and further they are inactivated by catalase. The role of peroxidase in this process lies in inactivation of the organic peroxide formed by radiolysis of water as well as of unsaturated fatty acids of membrane lipids.

It is understood that the resistance of plants to various stress conditions will be determined by the total activity of the system, which neutralizes free radicals and peroxides.

Given this, we can assume that if the irradiation of the seeds, in fact, leads to the activation of the formation processes of free radical and peroxide, undoubtedly this would increase of the activity of these enzymes as one of the mechanisms of cells adaptation to radiation factor.

Our data show that in forming of the sustainability of eggplant to the radiation exposure in small doses, APX and antioxidants with low molecular weight, such as proline, play a dominant role and role of SOD and CAT in this process is insignificantly. Therefore, can be assumed that under the influence of radiation in small doses, generally, the hydrogen peroxide are formed, and not superoxide radicals. Apparently, this is due to the fact that the

presence of a minor amount of superoxide radicals leads to oxidation of the unsaturated fatty acids of the membrane lipids and APX inactivates the organic peroxides obtained in this process. For this reason, the presence of active APX reduces the need for active SOD.

A completely different pattern is characteristic for large radiation doses. Since SOD and CAT exhibit a high activity at the large radiation doses. Apparently, at these doses the radiation causes intensive formation of the superoxide radicals and not the hydrogen peroxide. Therefore, the need of peroxidase decreases, and the activity of his enzyme declines. As mentioned above the inactivation of the organic peroxides in the membranes carried out by means peroxidase.

Note that in some cases this effect is consistent with the data detected by other authors. For example, the increase of peroxidase activity which occurs in a background of decreasing the activity of superoxide dismutase and catalase were shown for *Carthamus tinctorius L.* under drought conditions (Mostafa *et al*., 2011). The increase of the extracellular peroxidases activity was observed in *Dumortiera hir-sute* too in response to dehydration and subsequent rehydration. This process was accompanied by increasing of superoxide education (Jackson *et al*., 2010).

Furthermore, it was found that an extreme environmental condition (e.g., a sharp change in temperature and humidity) significantly increases the APX activity. The activity of another enzyme of antioxidant protection, as the SOD, usually decreases under these conditions (Zhurovskaya *et al*., 1998).

Summarizing the data obtained for the eggplant, it can be concluded that if in small doses for protection of cells, mainly are taking part low-molecular antioxidants and APX, then in the large doses the anterior front of protection form the antioxidant enzymes such as SOD and CAT. To protect cells from harmful effects of radiation, in this case, the individual components (antioxidants with low molecular weight and antioxidant enzymes) of antioxidant protection operate interconnected and coordinately.

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