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STUDY OF RESPONSE OF DIFFERENT COTTON VARIETIES TO PRE-SOWING GAMMA TREATMENT OF SEEDS

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Abstract: In the present study, the response of Ganja-160, Ganja-182, and Ganja-183 cotton cultivars to the treatment of seeds with different doses of γ-rays before sowing was investigated. In addition to phenological changes, the response of the plants to γ-rays was studied according to the changes in the amount of MDA and proline and the activity changes of enzymes such as SOD, CAT, and APX. The number of bolls on 30 (and 40) day-old bushes and the number of open cotton bolls showed an increasing trend up to 50 Gy radiation dose. In doses higher than 50 Gy, this number decreases significantly. What is interesting is the fact that the Ganja-160 and Ganja-182 varieties have the maximum number of bolls at the radiation dose of 400 Gy. In all three variants, it was clear that the amount of MDA, total protein, and proline as well as the activities of antioxidant enzymes were dependent on the radiation dose. It has been established that antioxidant enzymes, to some extent, function interrelatedly.

Keywords: cotton varieties (Ganja-160, Ganja-182, and Ganja-183), treatment of seeds with γ-rays before sowing, the response of plants, MDA, proline, total protein, SOD, CAT, APX.

1. Introduction

The fact that gamma rays, having the high penetrating ability, are a more effective means, as well as cost-effective, has opened the prospects of their use to accelerate the growth and development of various plants [1; 2]. It was found that gamma radiation causes several cytological, genetic [3], morphogenetic, biochemical [4], and physiological [5] changes in plant cells and tissues. For this reason, gamma ray treatment is considered one of the common methods that can induce genetic changes in plants. The sensitivity of plants to radiation, the genetic, cytological, biochemical, physiological, and morphogenetic changes in plants, as well as the changes occurring in their growth and development, were found to depend significantly on the plant variety and the applied irradiation dose [6; 7].

From the analysis of literature materials, it is clear that gamma radiation has already been recognized as a fast and reliable means capable of altering the physiological and biochemical processes occurring in plants. It has also been recognized that gamma radiation is one of the most important physical means of improving the properties and productivity of many plants. It should be noted that the use of gamma radiation technology plays an important role in breeding and genetic research aimed at obtaining many cultivated plants with desirable characteristics and increasing their productivity under both normal and stressed conditions [8]. Many studies have shown that radioactive radiation, which is ionizing radiation, in relatively small doses can increase cell proliferation, staining and development, enzyme activity, stress resistance, and productivity of plants [9].

The fact that gamma radiation can stimulate the growth and development of plants is explained as follows. It is believed that the seeds contain the necessary supply of substances and energy for initial development and that only stimulants are needed to activate the substances stored in the kernels. In this case, small doses of gamma radiation increase enzymatic activity and stimulate the embryo, stimulating the rate of cell division, which has a positive effect not only on seed germination but also on vegetative growth [10].

On the other hand, the treatment of seeds with high doses of gamma rays hurts important components of plant cells. The reason for the negative effect is that gamma radiation interacts with atoms and molecules, causing the formation of free radicals that affect protein synthesis, enzyme activity, hormonal balance, water exchange, and gas exchange in leaves [11].

2. Materials and methods

As research objects, Ganja-160, Ganja-182, and Ganja-183 cotton varieties regionalized by Agrarian Services Agency were selected.

As research methods, biometry, spectrophotometry, centrifugation, photocolorimetry, and **as research devices,** KFK-2 UHL 4.2 colorimeter (Russia), "JENWEY – 67" spectrophotometer (United Kingdom), "HIMAC - CT 15 RE" centrifuge (United Kingdom), "SDL-1" dielectric separator (Russia), "Fauna – M" humidity meter (Russia), Dry Box/Incubator PH-070A thermostat (China), Electronic Balance ABT electronic scale (Czech Republic) have been used.

Total protein content was determined colorimetrically [12], MDA content [13], proline content [14], SOD activity [15], and CAT and APO [16] activities were determined spectrometrically.

Cotton seeds were treated with γ-rays at radiation doses of 5, 10, 50, 100, 200, 300, and 400 Gy with the help of a Co-60 radiation source in the RUXUND-20000 device at the "Isotope Sources of Radiation" scientific-experimental complex (the dose strength was 0.342 Rad /sec in all cases).

Irradiated cotton seeds were grown together with control seeds in the experimental field of the laboratory. In the experimental area, paths corresponding to each dose (1 control, 7 experimental) were opened with a distance of 60 cm between them, and 10 nests were dug on a path corresponding to each dose, with a distance of 25 cm between them, and 5 seeds were planted in each nest.

Fig. 1. Cultivation of plants in the experimental field

3. Results and their explanation

Daily phenological observations were made on the growing seedlings. It has been recorded that high doses of radiation had an inhibitory effect on the development of cotton plants.

Leafing was observed about 10 days after sowing, except for the control variants of Ganja-160 and Ganja-183 and the experimental samples of Ganja-182 corresponding to 300 and 500 Gy.

After 30 and 40 days, our results based on visual observations are presented in table 1 and table 2, respectively.

Table 1

Indicators on 30-day plants

Irradiation dose, Gy	Number of flowering bolls			Number of bolls			The number of open cotton bolls			Leaf formation		
	G-160	G-182	G-183	G-160	G-182	G-183	G-160	G-182	G-183	G-160	G-182	G-183
K	-	3	4	-	15	14	-	2	2	-	E	E
5	3	2	3	11	19	1	1	2	-	G	E	P
10	-	-	5	-	5	13	-	1	6	P	VB	E
50	4	2	7	8	30	4	1	-	1	P	G	P
100	3	3	-	6	18	7	-	-	-	P	N	P
200	6	4	-	2	5	4	-	-	-	P	N	A
300	5	-	7	5	-	12	-	-	-	G	-	G
400	-	-	-	22	23	-	-	-	-	E	E	-

E - excellent, G - good, A - average, P - poor, N - normal, B - bad, VB - very bad.

Table 2

Indicators on 40-day plants

Irradiation dose, Gy	Number of flowering bolls			Number of bolls			The number of open cotton bolls			Leaf formation		
	G-160	G-182	G-183	G-160	G-182	G-183	G-160	G-182	G-183	G-160	G-182	G-183
K	-	3	6	-	14	18	-	3	3	-	E	E
5	3	2	3	10	18	1	2	3	-	G	E	P
10	-	-	6	-	5	15	-	1	6	-	P	E
50	4	2	7	8	32	4	1	-	1	P	G	P
100	3	4	-	5	18	8	1	-	-	P	G	P
200	6	4	-	2	5	4	-	-	-	P	N	G
300	5	-	8	5	-	12	-	-	-	G	-	G
400	-	-	-	21	25	-	1	-	-	E	E	-

E - excellent, G - good, P - poor, N - normal, B - bad

Fresh plant leaves were used for biochemical analyses. The biochemical analyses aimed to clarify the response of seeds to radioactive radiation and the activity of the antioxidant defense system under radiation stress conditions.

Study of the dependence of malondialdehyde amount on radiation dose.

It is known that the first target of free radicals formed under stressful conditions is cell membranes and as a result of this effect, some disruptions occur in the structure of membranes. As a result of these disruptions, which are called peroxide oxidation reactions of membrane lipids, a substance called malondialdehyde (MDA) is formed as the final product of these reactions and based on its amount, it is possible to speculate about the extent of cell damage [17].

In view of what has been said, we thought it appropriate to clarify the level of damage caused to the cells by the treatment of seeds with γ-rays and the dependence of the degree of damage on the radiation dose.

Our results on the radiation dose dependence of the amount of MDA, a product of lipid peroxidation reactions, are presented in Figure 2.

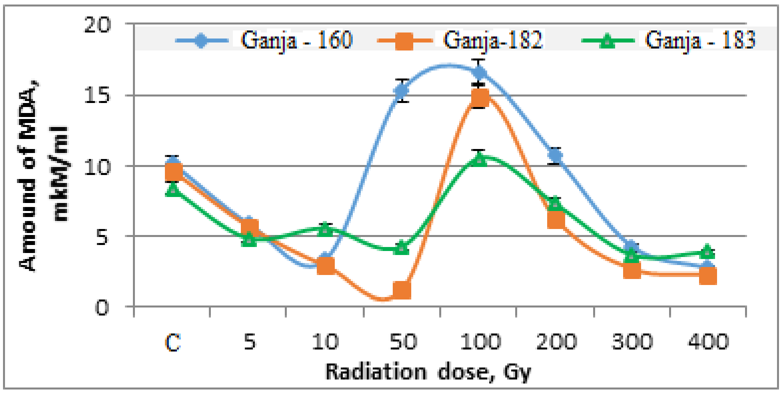

Fig. 2. Dependence of the MDA amount on the radiation dose

From the results obtained, it was understood that all three cotton varieties show similar change dynamics in dependence of MDA amount on radiation dose. Thus, the increase in radiation dose in all three options causes the MDA amount to decrease at low doses, increase at medium doses, reach a certain maximum level, decrease again at high doses and remain constant at very high doses. The response of the three cotton varieties to the effect of radioactive radiation on the lipid peroxidation reaction was almost identical.

It is difficult to understand that the amount of MDA in the Ganja-160 variety up to 10 Gy radiation dose, and in the Ganja-182 and Ganja-183 varieties up to 50 Gy irradiation dose is less than even in the control sample. Most likely, it can be considered that the absorbed energy during the irradiation of seeds at small doses is sufficient to reduce the level of MDA generated in normal metabolism even in the case of non-irradiated seeds.

Increasing the radiation dose in the dose range of 10-100 Gy causes a significant increase in the amount of MDA. Most likely, the effect of ionizing radiation in this dose range generates a large number of active oxygen forms, the primary target of which is the lipids of cell membranes. Therefore, the MDA level rises sharply.

At doses higher than 100 Gy, the regular decrease in the amount of MDA is most likely due to the fact that the products of lipid peroxidation themselves become targets of free radicals.

Study of the dependence of proline amount on radiation dose. As known, proline, as a small molecule non-enzymatic antioxidant, plays an exceptional role in protecting plants from stress factors [18].

Our results on the dependence of proline content on the radiation dose of seeds are presented in Figure 3.

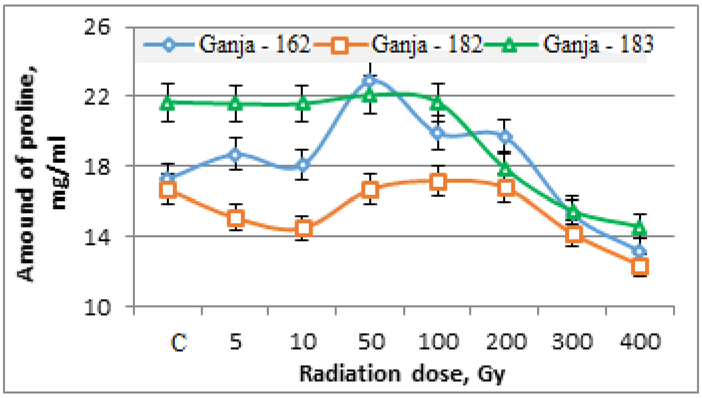

Fig. 3. Dependence of the amount of proline on the radiation doses.

The results show that the proline content of cotton samples of all three varieties has similar dynamics of change, except for small deviations within the experimental error. Thus, at low radiation doses, there is no major change in the amount of ptolin, while at medium doses a slight increase, and at high doses, a relatively large decrease is observed.

According to the obtained results, it can be said that the antioxidant proline does not have an important role in the protection of cotton seeds from radiation stress. The reason for the decrease in proline content at high radiation doses is probably due to exposure to ionizing radiation.

Study of the dependence of the amount of total protein on the radiation dose. There is information that irradiation of seeds in small doses causes an acceleration of the growth rate of the root system in the early stages of ontogenesis and an increase in the concentration of soluble sugars and proteins, as well as proline, due to an increase in the activity of the enzyme system of the organism [19]. In high doses, the nature of protein biosynthesis may change, and the metabolic process and physiological functions of the organism as a whole may be disrupted [20].

Taking into account what has been said, we have tried to clarify the amount of total protein.

Our results for the total protein amount are presented in Figure 4.

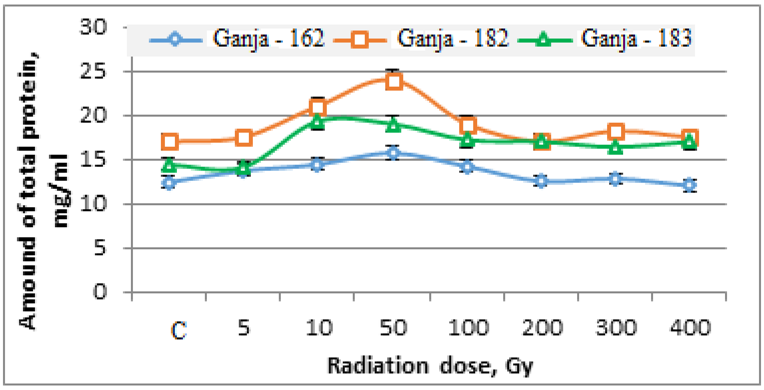

Fig. 4. Dependence of the protein amount on radiation doses.

From the results, it is clear that treating seeds with different doses of γ-rays before sowing does not cause large-scale changes in protein synthesis. Simply, in the dose range 10-100 Gy, radioactive radiation has a weak stimulatory effect on protein synthesis.

Study of the radiation dose dependence of the activities of antioxidant enzymes. As is known, the active forms of oxygen, which pose a real danger to the cell, are neutralized by a system called the antioxidant defense system (AODS) [21]. The main antioxidant enzymes of this unique system are superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) [21].

The results obtained for the radiation dose dependence of antioxidant enzyme activities of Ganja-160, Ganja-182, and Ganja-183 cotton varieties whose seeds were treated at different doses before sowing are reflected in figures 5-7.

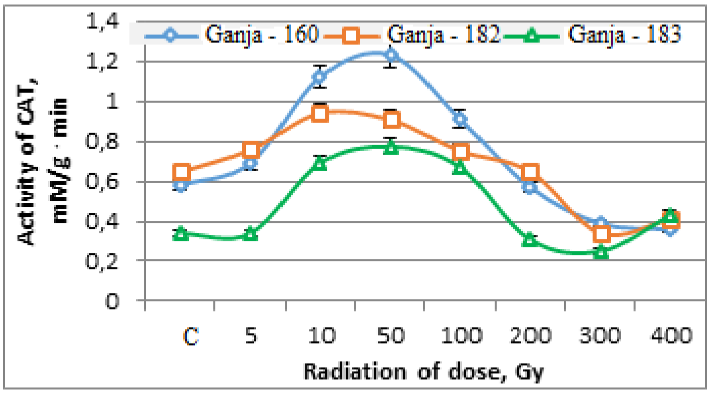

Fig. 5. Dependence of CAT activity on radiation doses

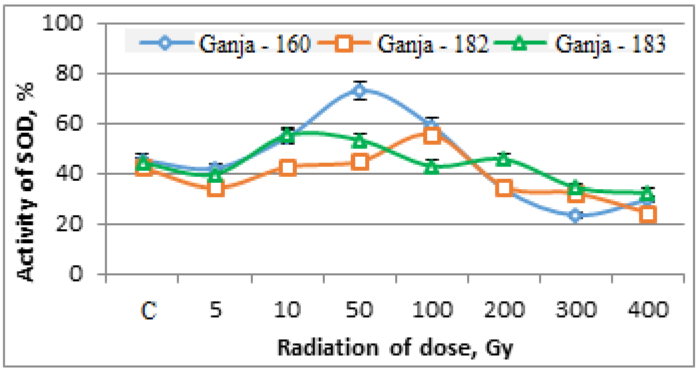

Fig. 6. Dependence of SOD activity on radiation doses

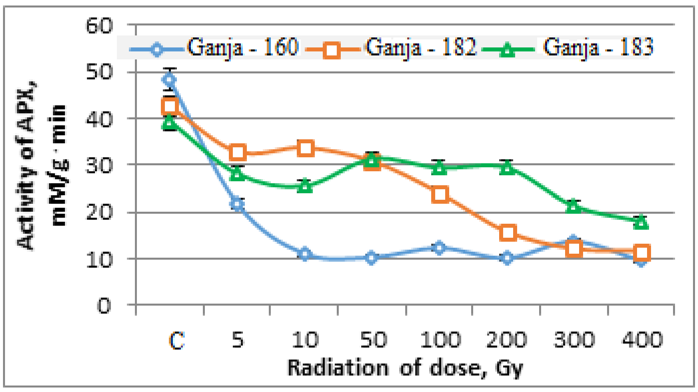

Fig. 7. Dependence of APX activity on radiation doses

It is clear from the results that the radiation dose dependence of the activity of each antioxidant enzyme for all three cotton cultivars occurs according to similar dynamics. Thus, antioxidant CAT and SOD enzymes show high activity in the radiation dose range of 10-100 Gy for all three cotton cultivars, while APX shows activity that decreases steadily with increasing radiation dose and does not change at high doses.

The CAT activity is maximum when the seeds are irradiated with a dose of 50 Gy for Ganja-160 and Ganja-183 varieties and 10 Gy for Ganja-182 varieties.

The maximum activity for SOD is observed when the seeds of the Ganja-160 and Ganja-183 cultivars are irradiated at a dose of 50 Gy and the seeds of the Ganja-182 cultivar at a dose of 100 Gy.

The radiation dose dependence of APX activity is different from the radiation dose dependence of SOD and CAT activity. In general, increasing radiation dose causes a steady decrease in the activity of this enzyme throughout the dose range.

It is known from the literature that active oxygen forms are formed under stressful conditions (including radiation stress conditions). It is stated that superoxide anion radical (O_2^{*-}) is the first active form of oxygen formed in the cell during stress reactions and this form stimulates the reactions of the second active forms of oxygen. Thus, O_2^{*-} accepts one electron and two protons non-enzymatically or enzymatically with the participation of SOD and forms hydrogen peroxide according to the following scheme [22]:

$$2O_2^{*-} + 2H^+ \rightarrow H_2O_2 + O_2$$

$$2O_2^{*-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

These chemically active radicals interact with and can "damage" the macromolecules of the cell, such as proteins, DNA, RNA, and lipids of the cell membrane, which can ultimately lead to the destruction of the cell.

The SOD enzyme is assumed to form the front line of antioxidant defense. This is because SOD performs the dismutation of superoxide anion radicals formed during stress, i.e. it acts as a catalyst in the reaction of their conversion to H_2O_2.

It should be noted that although H_2O_2 is of special importance in the life of plants, this form, which is a product of dismutation reactions, is another active form of oxygen. Although H_2O_2 is not as highly reactive as O_2^*, the hydroxyl radicals (OH^*) produced by them according to the Haber-Weiss mechanism are more reactive forms of active oxygen. Therefore, it can be considered that the cause of cell damage is not H_2O_2 itself, but free radicals created by them.

Besides H_2O_2, superoxide radicals also serve as a source for the generation of other more toxic active forms of oxygen, which occur according to the following scheme and are called hydroperoxides [23].

$$O_2^{*-} + H^+ = HO_2^*$$

Based on what has been said, the reason why SOD is activated in a certain dose range can be explained by the intense formation of superoxide anion radicals in this range. Thus, free radicals should be rapidly neutralized to avoid oxidative stress. This, as we have already mentioned, should be done by SOD, i.e. there must be a demand for the activity of SOD. The activity of SOD, as is known, should result in the formation of hydrogen peroxide.

It is noteworthy that CAT is also active in the dose range where SOD is active. Again, referring the literature materials, the increase in CAT activity can be explained in this way. It is known that CAT is an antioxidant enzyme that detoxifies H_2O_2 (hydrogen peroxide), one of the main “aggressive” active forms of oxygen, which is produced under stressful conditions [24]. For the neutralization of H_2O_2 generated by SOD activity, the need for CAT should arise and its activity should increase. From this point of view, it is completely understandable that CAT is also active in the dose range where SOD is active.

Together with CAT, APX is known to play an important role in H_2O_2 neutralization. It is also known that, unlike CAT, APX exhibits a higher avidity for the substrate (H_2O_2) and can detoxify this active oxygen form even at the lowest concentrations [25]. In this case, the APX enzyme should also show high activity in the specified dose range. The reason why APX is not activated is that on the contrary, it becomes more passive as the stress increases.

Most likely, the intensive formation of H_2O_2 is due to the activity of SOD under radiation stress, and CAT can cope with their neutralization on its own. For this reason, there is no need for APX.

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ИЗУЧЕНИЕ ОТВЕТНЫХ РЕАКЦИЙ РАЗЛИЧНЫХ СОРТОВ ХЛОПКА НА ОБРАБОТКУ ИХ СЕМЯН ГАММА-ЛУЧАМИ ПЕРЕД ПОСЕВОМ

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Резюме: В представленной работе изучалась реакция сортов хлопчатника Гянджа-160, Гянджа-182 и Гянджа-183 на обработку различными дозами γ-облучения перед посевом их семян. Помимо фенологических изменений, реакцию растений на γ-облучение изучали по изменению содержания МДА, общего белка и пролина, а также по изменению активности таких ферментов, как СОД, КАТ и АПО. Установлено, что количество шишек на 30 (и 40)-дневных кустах и количество раскрывающихся шишек хлопчатника имеют тенденцию к увеличению до дозы облучения 50 Гр. В дозах выше 50 Гр это число значительно уменьшается. Интересно, что сорта Гянджа-160 и Гянджа-182 имеют максимальное количество шишек при дозе облучения 400 Гр. Показано, что во всех трех вариантах количество МДА, общего белка и пролина, а также активности антиоксидантных ферментов зависят от дозы облучения. Установлено, что антиоксидантные ферменты, в какой-то мере функционируют взаимосвязано.

Ключевые слова: хлопчатник сортов Гянджа-160, Гянджа-182 и Гянджа-183, обработка семян γ-лучами перед посевом, реакция растений, МДА, пролин, общий белок, СОД, КАТ, АПО.

MÜXTƏLİF PAMBIQ SORTLARININ TOXUMLARIN SƏPİNDƏN ƏVVƏL QAMMA ŞÜALARLA İŞLƏNMƏSİNƏ CAVAB REAKSİYALARININ TƏDQİQİ

E.S. Cəfərov, M.Z. Vəlicanova, L.V. Allahverdiyeva, N.R. Quliyeva, G.Ə. Məmmədova, N.M. Eminova

Xülasə: Təqdim olunan işdə toxumlarının səpindən əvvəl müxtəlif dozalarda γ-şüalarla işlənməsinə Gəncə-160, Gəncə-182 və Gəncə-183 pambıq sortlarının reaksiyası öyrənilmişdir. Bitkilərin γ-şüaların təsirinə reaksiyası fenoloji dəyişmələrlə yanaşı, həm də MDA və prolinin miqdar, SOD, KAT və APO kimi fermentlərin isə aktivlik dəyişmələrinə əsasən tədqiq edilmişdir. Müəyyən edilmişdir ki, 30 (həm də 40) günlük kolların üzərində olan qozaların sayında və pambıq açan qozaların sayında 50 Qr şüalanma dozasına qədər artma tendensiyası müşahidə olunur. 50 Qr-dən yüksək dozalarda isə bu say əhəmiyyətli dərəcədə azalır. Maraq doğuran Gəncə-160 və Gəncə-182 sortlarında 400 Qr şüalanma dozasında maksimal sayda qoza olmasıdır. Aydın olmuşdur ki, hər üç variantda həm MDA-nın, ümüumi zülalın və prolinin miqdarının, həm də antioksidant fermentlərin aktivliklərinin şüalanma dozasından asılılığı mövcuddur. Antioksidant fermentlərin əlaqəli fəaliyyətinə dair nəticələr alınmışdır.

Açar sözlər: Gəncə-160, Gəncə-182 və Gəncə-183 pambıq sortları, toxumların səpindən əvvəl γ-şüalarla işlənməsi, bitkilərin cavab reaksiyası, MDA, prolin, ümumi zülal, SOD, KAT, APO.